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Study of the influence of genes related to muscle oxidative processes on beef color

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ABSTRACT

The biochemical bases of meat color are determined by the concentration and redox state of myoglobin, hemoglobin, cytochromes, and other pigments. Post-mortem depletion of cellular oxygen results in oxidative stresses that consume NADH and affects reducing activity, while enzymatic detoxification influences the cellular oxidative processes, both affecting meat color. The aim of this work was to study the influence of several genes related to cellular oxidative processes that could affect CIELAB meat color parameters. The study was performed in steers that received a grass-based diet combined with grain, hays and silages. Results suggest a possible link between colorimetric parameters (a*, b* and chroma) and SNPs in the GSTP1 gene ($P < 0.05$). Although the influence of the enzymes, encoded by GSTP1 gene, on meat color has been proposed previously at biochemical level and protein expression level, further association studies in different populations and functional studies of proteins are needed to confirm the genetic determination of that gene on meat color.

1. Introduction

Meat color plays a critical role in determining consumers' purchasing decision, since it is the only characteristic perceived by the consumer in the market (Andrés et al., 2008) and is frequently used as an indicator of freshness and wholesomeness. In this sense, beef-steak quality has been equated to a bright red color (Behrends, Mikel, Armstrong, & Newman, 2003). Furthermore, it was estimated that around 15% of produced beef lose commercial value due to surface discoloration (Smith, Belk, Sofos, Tatum, & Williams, 2000). The red color of meat is the result of a number of intrinsic and extrinsic factors that have been widely studied (Mancini & Hunt, 2005).

The impact of oxidative processes on food components will affect the nutritional value, functional properties, texture, and perceived keeping quality of food (Bekhit, Hopkins, Fahri, & Ponnampalam, 2013). The biochemical bases of meat color are determined by the concentration and redox state of myoglobin, hemoglobin, cytochromes, and other pigments. Myoglobin is the primary protein pigment associated with meat color and the amount of deoxymyoglobin (DOMb, purple) and its derivates oxymyoglobin (OMb, cherry-red) and metmyoglobin (MetMb, brown) on the surface determine the final observable color (Bekhit, Cassidy, Hurst, & Farouk, 2007; Bekhit & Faustman, 2005). The presence of reduced nicotinamide adenine dinucleotide (NADH) as a coenzyme is needed in reactions involved in the conversion of MetMb in its ferrous form DOMb, suggesting that this compound could also be responsible for color stability (Bekhit, Geesink, Ilian, Morton, & Bickerstaffe, 2003). Post-mortem depletion of cellular oxygen results in turn to piruvate degradation to lactate by lactate dehydrogenase (LDH); this consumes NADH and consequently affects MetMb reducing activity and meat color (Kim, Keeton, Smith, Berghman, & Savell, 2009).

Glutathione (GSH) is an important reductant tripeptide that interacts with free radicals and acts as a cofactor in H_2O_2 degradation by peroxidase (Nelson & Cox, 2000), hence affecting intracellular oxidative processes. Glutathione-S-Transferase Pi 1 (GSTP1) and Glutathione Peroxidase 4 (GPX4) play a key role in cellular detoxification and resistance to oxidative stresses, regulating the conjugation of GSH with different hydrophobic and electrophilic compounds (Lo Bello et al., 2001), and reducing organic peroxides at the expense of reduced glutathione (Nair et al., 2014), respectively. Superoxide dismutase is another important enzyme related to radical removal that catalyzes the dismutation of superoxide (O_2^-) into O_2 and H_2O_2. Copper Chaperon for Superoxide Dismutase (CCS) interacts and activates copper/zinc superoxide dismutase (Rothstein et al., 2002). All influence cellular oxidative processes and could help to maintain the reducing activity of muscles post-mortem.

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As already mentioned, there are extrinsic and intrinsic factors affecting meat color, such as breed, age, pH, slaughter procedures and transportation (Mancini & Hunt, 2005). However some variability remains among individuals that could be controlled by other factors. Therefore, genetic differences might be one possible source for such variations. In this sense, several QTL that affect meat color in cattle have already been reported (Esmailizadeh et al., 2011; Reardon, Mullen, Sweeney, & Hamill, 2010); furthermore some authors found an association of CAPN1 markers with meat color parameters (Pinto et al., 2011), this gene is located in Chromosome 29 close to GSTP1 location. Additionally, the influence of LDHA in porcine meat color parameters (Otto et al., 2007) and the effect of GSH on bovine OMb reduction (Tang, Faustman, Lee, & Hoagland, 2003) have been established.

The objective of this work was to study the influence of several genes related to meat color and/or the cellular oxidative processes that could affect meat color parameters. Angus, Hereford and crossbred animals were used to test the association of SNPs in CCS, LDHA, Mb, GPX4, and GSTP1 genes with CIELAB meat color parameters.

2. Materials and methods

2.1. Animal resources

A cattle population previously used in various studies to evaluate supplementation strategies and/or crossbreeding systems at the Experimental Station of the National Institute of Agricultural Technology (INTA, Balcarce, Argentina) was used. Throughout the fattening period, animals grazed a *Lolium multiflorum*, *Dactylis glomerata*, *Bromus catarthicus*, *Trifolium repens* and *Trifolium pretense* dominated semi-natural pasture. Whenever seasonal fluctuations on pasture growth or quality threatened a steady body weight gain over the period, animals were supplemented with either maize silage, maize grain or grass hay to meet their nutrient requirements, according to NRC (NRC, 2000). A total of 179 steers (15–18 months old and born between 2006 and 2009) were sampled. They belonged to different genetic groups and included purebred Angus (A) and Hereford (H), crossbreeds F1, F2, ¾ A–¼ H, and ¾ H–¼ A and a group produced by mating Limousin (L) sires to F1 cows (see online Supplementary material for details). All experimental procedures involving the use of animals were conducted in accordance with the Manual de Procedimientos sobre el Bienestar Animal (Handbook of Procedures for Animal Welfare), SENASA (National Service of Animal Health) of Argentina.

2.2. Meat sampling and physical determinations

After slaughter, a joint including the longissimus dorsi muscle (LD) between the 11th and 13th ribs was removed from the left half carcass, deboned before freezing until processing for analytical determinations at the University of Buenos Aires Meat Laboratory (Papaleo-Mazzucco et al., 2010). Meat color parameters (L^*: lightness; a^*: redness; and b^*: yellowness) were measured on the exposed LD muscle in the CIELAB system (CIE, 2004) using a Minolta Chroma Meter CR-300 (Minolta Camera Co. Ltd., Osaka, Japan). Calibration was performed against a white plate supplied by the manufacturer. The colorimeter has an 8 mm diameter measurement area and uses a light source of D_{65} and 0° standard observer. Determinations were done in 2.5 cm thick steaks after blooming for 1 h at 4 °C. The color of the fat-free surface of each sample was assessed using the mean value of three color determinations. The parameters Chroma (C^*_{ab}), related to the intensity of color (higher when a^* of b^* are high), and Hue angle (h_{ab}), related to the change of color from red to yellow, were also calculated with the following equations:

$$C^*_{ab} = \sqrt{a^{*2} + b^{*2}} \qquad h_{ab} = \arctan\frac{b^*}{a^*}.$$

Total lipids of muscle samples were extracted according to Folch, Lees, and Sloane-Stanley (1957), purified by alkaline methylation, and recovered on hexane. Fatty acid composition was measured using a gas chromatograph Shimadzu GC14B (Shimadzu Corp., Tokyo, Japan). Fatty acid methyl ester (FAME) separation was performed on a 100 m × 0.25 mm capillary column (ResteK, Bellefonte, Pennsylvania, USA). The chromatograph was set at 140 °C for 1 min, increased from 140 to 240 °C at 4 °C per min, and then held constant at 240 °C for 20 min. The injector and detector were kept at 260 °C. Data was recovered using the GCSolution Software (Shimadzu Corp.) and the amount of each fatty acid was quantified by the internal standard technique (Supelco 37 FAME MIX, Sigma-Aldrich Co. LLC.) and expressed as percentage of total fatty acids. Total ether extract was determined measuring the amount of fat (extracted as ether) present in 100 g of fresh muscle without the external adipose tissue.

2.3. Markers and genotyping

DNA was extracted from meat samples with the method previously reported by Giovambattista et al. (2001). For this study, 11 SNPs were selected in five candidate genes that could influence meat color: Copper Chaperon for Superoxide Dismutase (CCS), CCS-1 (rs134083963) and CCS-2 (rs110015162); Lactate Dehydrogenase A (LDHA), LDHA-1 (rs41600268) and LDHA-2 (rs208422143); Myoglobin (MB), MB-1 (rs110676666) and MB-2 (rs136397633); Glutathione-S-Transferase Pi 1 (GSTP1), GSTP1-1 (rs110594162), GSTP1-2 (rs109060695) and GSTP1-3 (rs42188145); and Glutathione Peroxidase 4 (GPX4), GPX4-1 (rs134495045) and GPX4-2 (rs133501979) (see on line Supplementary material for further information of each SNP). SNPs were selected from the reported SNPs at NCBI database (http://www.ncbi.nlm.nih.gov/snp) using the following criteria: i) the SNP must be located within the gene or in the close 5′ UTR or 3′ UTR regions, ii) polymorphism must be reported in either Angus or Hereford breeds. Genotyping was performed with the Sequenom platform (www.sequenom.com), Neogen genotyping service (USA, www.neogen.com).

2.4. Statistical analysis

2.4.1. Genetic variability

Allele frequencies, unbiased expected heterozygosity (he) and Hardy–Weinberg equilibrium (HWE) for each SNP were estimated using exact test implemented in GENEPOP 4 (Rousset, 2008) and ARLEQUIN 3.5 (Excoffier & Lischer, 2010) softwares. Linkage disequilibrium between SNP in the same gene and within genes was tested using HAPLOVIEW (Barrett, Fry, Maller, & Daly, 2005). Phases were reconstructed with Phase v2.1.1 (Li & Stephens, 2003) using default options. Phases were considered reliable when P value estimated by Phase was higher than 0.8.

2.4.2. Association test

The association analysis between genetic markers and meat color parameters was conducted using the following model:

$$y_{ijk} = SG_j + GE_k + AGE_i + pH_i + TEE_i + PUFA_i + e_{ijk}$$

where y_{ijk} is each studied trait ($L^*, a^*, b^*, C^*_{ab}, h_{ab}$) and considers the fixed effects SG = Slaughtering group (that includes year, feeding group and slaughtering date) and GE = Genotype effect, and includes the covariables: pH, AGE = age at slaughtering, TEE = total ether extract, and $PUFA$ = percentage of total polyunsaturated fatty acids (PUFA), while e_{ijk} was the error of the model. Breed group was not considered in the model as it was tested and was not significant for the studied traits. The tests were performed using a GLM procedure of SAS 9.2 (Statistical Analysis System, SAS Institute Inc., Cary, NC, USA). Additionally, as 5 traits were tested, the adjusted P-values were calculated multiplying by 5 the obtained P-values.

3. Results and discussion

From the 11 genotyped SNPs, five were monomorphic or had low minimum allele frequencies (MAF < 0.05): one in LDHA gene (LDHA-2), one in MB gene (MB-2), one in GSTP1 gene (GSTP1-2) and two in GPX4 gene (GPX4-1, GPX4-2); the rest were used to estimate allele frequencies, unbiased expected he and HWE (Table 1).

The linkage test between SNPs located in chromosome 29 (see on line Supplementary material) showed two blocks that corresponded with the genes and reflected the distance between markers. The distance between genes (800 Mb) was not large and we detected a linkage between them. Within SNPs in the GSTP1 gene, the linkage was complete and there were only two possible haplotypes detected with the phasing test in the studied samples. There was a co-segregation of alleles in each SNP that could be explained by genetic drift, natural selection or any other genetic force and which was generated before the breeds (Hereford and Angus) were established, as both haplotypes are present in the two breeds. Furthermore, no recombination was detected, consistent with the distance between the markers (only 406 bp). The block comprised by SNPs in the CCS gene showed three possible haplotypes, indicating some recombination between markers (the distance is 13,051 bp) but not an independent segregation.

The association study results in a non-significant association for the SNPs in LDHA, CCS and Mb genes and evidenced a possible link between the polymorphisms in GSTP1 gene on meat color CIELAB parameters (Table 2). In particular, GSTP1 signals were found with a^* parameter, that measures redness and correlates with myoglobin color and amount (see Table 2). C_{ab}^* calculated parameter, related to the color of the sample, was also related with all SNPs in GSTP1. The influence of the enzyme, product of that gene, on meat color was suggested in previous researches performed at biochemical and protein expression levels. On the other hand, the reported genetic association of CAPN1 markers with meat color parameters (Papaleo-Mazzucco et al., 2010; Pinto et al., 2011) could be related to the actual reported results, as that gene is 2 Mb up-stream to the GSTP1 gene. Even though, the effect of GSH over meat color has been demonstrated (Tang et al., 2003) and as GSTP1 uses GSH as substrate, it is also a good candidate and positional gene to explain the associations of the chromosome region.

As previously stated, GSTP1 takes part in cellular detoxification and resistance to oxidative stress (Lo Bello et al., 2001), favoring pigment stability post-mortem. GSTP1 catalyzes the addition of reduced GSH to electrophilic species, such as the products of lipid peroxidation (Vasieva, 2011). The enhancement of OxyMb oxidation to MetMb by lipid oxidation has also been studied (Faustman, Sun, Mancini, & Suman, 2010). Both GSTP1 SNPs investigated are located in exons and are synonymous mutations, thus suggesting that they are in linkage disequilibrium with the causal mutation. Even though they could directly affect translation speed and protein secondary structure due to the differential availability of each specific tRNA. This effect was reported

Table 1
Allele frequencies, unbiased expected heterozygosity (he) and Hardy–Weinberg Equilibrium (HWE) for the six polymorphic markers.

Gen	SNP	Allele	frequency	h_e	HWE (P-value)
CCS	CCS-1	C	0.254	0.379	1.000
		T	0.746		
	CCS-2	C	0.109	0.195	0.758
		T	0.891		
LDHA	LDHA-1	A	0.039	0.075	0.348
		G	0.961		
GSTP1	GSTP1-1	A	0.422	0.489	0.397
		G	0.578		
	GSTP1-3	C	0.429	0.491	0.627
		G	0.571		
MB	MB-1	C	0.765	0.360	0.622
		T	0.235		

Table 2
Means and standard deviations of CIELAB parameters associated for each genotype of GSTP1 studied SNPs. The numbers of animals for each genotype are presented in brackets. Model adjusted P-values are detailed and superscript letters indicate least square significant differences between genotypes.

	GSTP1-1			Model adjusted	GSTP1-3			Model adjusted	GSTP1 haplotype			Model adjusted
CIELAB parameter	A/A	A/G	G/G	P-value	C/C	C/G	G/G	P-value	AC/AC (42)	GG/AC (86)	GG/GG (48)	P-value
	(41)	(85)	(49)		(42)	(87)	(46)					
a^*	$22.87^{a} \pm 0.25$	$22.69^{a} \pm 0.18$	$21.94^{b} \pm 0.23$	0.038	$22.85^{a} \pm 0.25$	$22.66^{a} \pm 0.18$	$21.85^{b} \pm 0.24$	0.024	22.84 ± 0.25	22.66 ± 0.18	21.93 ± 0.23	0.053
b^*	12.26 ± 0.19	12.11 ± 0.14	11.69 ± 0.18	0.27	12.24 ± 0.19	12.09 ± 0.14	11.61 ± 0.18	0.17	12.24 ± 0.19	12.08 ± 0.14	11.67 ± 0.18	0.34
C_{ab}^*	25.96 ± 0.31	25.72 ± 0.22	24.86 ± 0.28	0.059	$25.93^{a} \pm 0.30$	$25.69^{a} \pm 0.22$	$24.75^{b} \pm 0.29$	0.037	25.92 ± 0.31	25.69 ± 0.22	24.84 ± 0.28	0.082

by Kimchi-Sarfaty et al. (2007) who proposed that the cumulative number of mutations in the coding regions of the gene could lead to a differential structure of the protein because of the folding available time of each specific codon sequence. In this study, the observed differences between GSTP1 haplotypes could have a similar explanation; further research must be done to confirm this hypothesis. In such case the AC haplotype would produce a GSTP1 enzyme with higher activity, leading to lower concentrations of the reactive species produced during lipid oxidation, preventing Mb oxidation and consequently maintaining color stability of muscle.

The remaining candidate genes were selected based on their biological function such as: i) being a pigmented protein of muscle (Mb) or affecting its redox stability through NADH availability (LDHA) and ii) being involved in cellular oxidative processes through GSH (CCS and GPX4). Previous reports suggest that myoglobin and color stability could be regulated by different NADH rates of supply via different LDH flux of muscles (Kim et al., 2009). The LDHA SNP investigated in this study is located at the 5′ UTR region, the reason why it could affect gene transcription and consequently the concentration of the LDHA monomer and the ratio of the isomeric tetramers. The effect of GSH over cellular oxidative process and then through color stability was also reported (Lo Bello et al., 2001) and GSTP1 results are related to those. Even though, the results of the other genes related to GSH availability were different: CCS had non-significant results and GPX4 SNPs could not be evaluated because they were monomorphic in the studied sample. Considering only these results we cannot discard an influence of those genes over meat color.

4. Conclusions

The results suggest a possible link between the GSTP1 gene, related to oxidative processes, and meat color measured through CIELAB parameters. Interestingly, the haplotype study using two SNPs in the gene also detected a significant association and could be related to the QTL previously reported in the chromosome 29 region where GSTP1 is located. Further association studies in different populations and functional studies of the proteins are needed to confirm these results. This is one of the first studies linking polymorphisms in genes related to oxidative processes with meat color in cattle.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.meatsci.2015.05.005.

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