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Callipyge genotypic effects on meat quality attributes and oxidation stability of ovine *M. longissimus*

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ABSTRACT

The objective of this study was to evaluate the effects of *callipyge* (*C*) genotypes on quality attributes and oxidation stability of aged lamb loins. Only lambs possessing a wildtype maternal allele and a paternal *callipyge* allele (*+/C*) express a muscle hypertrophy phenotype. Seventeen lambs (7 months), consisting of all four possible *callipyge* genotypes (*C/+*, *C/C*, *+/C* and *+/+*), were harvested. Loins (*M. longissimus thoracis*) were separated from each carcass at 3 days postmortem, cut into 3 sections, and randomly assigned into three aging periods (3, 6 and 9 days). Genotype had no impact on proximate composition, mineral content, and pH of lamb loins ($P > 0.05$). In terms of water-holding capacity, slightly increased purge/thaw loss of *+/C* lamb loins was observed ($P < 0.05$), but cooking, drip and display weight losses were unaffected by callipyge genotype ($P > 0.05$). The highest shear force was found at *+/C* lamb loins ($P < 0.05$). *+/C* lamb loins showed initially less redness, but greater color stability during 7 days of display compared to loins from the other genotypes. Heme iron content of *+/C* lamb loins was a significantly lower than the other genotypes. Genotype had no impact on lipid oxidation stability ($P > 0.05$). Our results indicate similar quality attributes of lamb loins between non-callipyge phenotypes (*C/C, C/+* and *+/+*) and suggest that callipyge lamb loins have superior color stability, while exhibiting lower tenderness compared to loins from non-callipyge phenotype lambs.

1. Introduction

The *callipyge* allele is an autosomal mutation on the distal chromosome 18, and causes postnatal muscle hypertrophy on specific muscles in hindquarter and loins in paternal heterozygous lambs (Cockett et al., 1994; Koohmaraie et al., 1995). The inheritance of the callipyge phenotype is known as polar overdominance, as only *+/C* genotype lambs (*C* = mutant allele, *+* = wildtype allele) express the muscle phenotype. In contrast, *C/C*, *C/+* and *+/+* genotypes result in normal phenotypes (Bidwell et al., 2004). This mutation increases the diameter and proportion of fast-twitch glycolytic type IIB fibers in *+/C* lambs, while decreasing fat content (Carpenter et al., 1996; Koohmaraie et al., 1995). As a result, callipyge lambs have heavier carcass weight compared to normal lambs, despite comparable live weight (Koohmaraie et al., 1995). Due to these unique phenotypes, callipyge lambs had received attention from the sheep industry as a means of improving carcass cutability, while producing leaner meat for consumers who desire less fat consumption (Abdulkhaliq et al., 2007; Busboom et al., 1999).

However, in respect of meat quality attributes, it has been well documented that callipyge lambs produce meat with inferior tenderness (particularly in the middle region). The toughness issue of meat from callipyge lambs is mainly associated with the inferior aging impact on tenderization, whereby overly-expressed calpastatin activity inhibits calpain-mediated proteolysis of myofibrillar proteins during postmortem aging (Abdulkhaliq et al., 2002; Duckett et al., 1998a; Koohmaraie et al., 1995). Multiple studies focused on determining the causative biochemical mechanisms for the delayed/limited tenderization in callipyge lambs, and possible strategies to alleviate the toughness problem (Clare et al., 1997; Duckett et al., 1998a; Kerth et al., 1999; Koohmaraie et al., 1995, 1998; Shackelford et al., 1997). For example, several post-harvest intervention strategies, such as carcass electrical stimulation, injection/margination enhancement, freezing/thawing and/or Hydrodyne process, have shown to improve tenderness of meat from callipyge lamb carcasses (Duckett et al., 1998b; Kerth et al., 1999; Solomon et al., 1998).

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While tenderness is one of the most important quality attributes affecting consumers' eating satisfaction (Smith et al., 2008), there are also other important meat quality attributes of lamb, such as color, water-holding capacity, flavor, shelf-stability (oxidation and microbial stability), and/or nutritional value (Hopkins and Fogarty, 1998; Sañudo et al., 1998). However, the majority of research regarding genotypic effects of the *callipyge* mutation on meat quality attributes of lambs has centered on tenderness, while little to no information is available on other meat quality attributes. This leaves a significant gap in our understanding and justifies a need for more studies considering *callipyge* genotypic effects on meat quality attributes in order to precisely assess the marketability and potential of this unique lamb breed as a meat-producing animal. Therefore, the objective of this study was to evaluate the effects of four *callipyge* genotypes on chemical composition, water-holding capacity, tenderness, color stability, and lipid oxidation stability of aged lamb loins.

2. Materials and methods

2.1. Animal and sample preparation

Seventeen lambs (wethers and ewes; average live weight 48.1 kg; 7 months old) of all four possible callipyge genotypes *+/C* (n = 4), *C/+* (n = 4), *C/C* (n = 4) and *+/+* (n = 5), which were fed with basal diet based on the nutrient requirements of small ruminants (NRC, 2007) *ad libitum* in a single pen, were harvested at the Purdue University Meat Laboratory. This procedure was approved by the Purdue Animal Care and Use Committee (PACUC Number: 1112000493). The callipyge genotype of all lambs was decisively verified using the single nucleotide polymorphism and several markers that flank the *callipyge* region on chromosome 18 (Bidwell et al., 2004). The lamb carcasses were chilled in a 1 °C carcass cooler for 3 days after slaughter, and right side loin muscles (*M. longissimus thoracis*) were collected from each carcass at 3 days postmortem. The excessive subcutaneous fat was trimmed from the loin muscles, and each loin was sectioned into three equal portions. The sections were weighed to determine purge/thaw loss, individually vacuum-packaged, and randomly assigned to three different postmortem aging periods (3, 6 and 9 days). The sections assigned to 3 days of postmortem aging were immediately placed in −80 °C freezer, and other samples for 6 and 9 days of postmortem aging were frozen in the same condition after each assigned aging period. After three months of frozen storage, the frozen lamb loins were thawed in a 2 °C cooler overnight. The frozen/thawed lamb loins were blotted with a paper towel and re-weighed. After measuring pH, one chop (2.54 cm thickness) was cut from each sectioned loin and weighed to determine display weight loss. Each chop was placed on a Styrofoam tray, wrapped with a commercial PVC film, and displayed in the 2 °C cooler under continuous fluorescent natural white light for 7 days (1450 Lx, color temperature = 3500 K, color rendering index = 85, OCTRON® T8 Lamps, Osram Sylvania Ltd., Canada). The remaining portion of the loins were used for further analyses.

2.2. Analyses

2.2.1. Chemical composition

The proximate composition (moisture, protein and ash) of raw lamb loin samples was measured in triplicate in accordance with the AOAC method (AOAC, 2000). Lipid content of the samples was determined in duplicate according to the chloroform/methanol solvent (2:1, v/v) extraction method described by Soyer et al. (2010). Mineral content of raw lamb loins was analyzed in triplicate by inductively coupled plasma (ICP) emission spectroscopy after a nitric acid digestion (Havlin and Soltanpour, 1980). The pH of lamb loins was measured in triplicate using an inserting type electronic pH meter (HI 99163, Hanna Instruments Inc., USA).

2.2.2. Water-holding capacity

Purge/thaw loss was measured by calculating differences in sample weight before vacuum-packaging and the weight after aging/freezing/thawing process. Drip loss was determined in duplicate in accordance with the Honikel method (Honikel, 1998). Two chops (2.54 cm thickness) were cut from each sectioned loin and cooked on an electric griddle (surface temperature of 135 °C) until the targeted core temperature reached at 71 °C. The internal core temperature of each chop was monitored by using a digital temperature logger (OctTemp2000, MadgeTech, Inc., USA) with a thermocouple (T-type, Omega Engineering, USA). Cooking loss was calculated as the following equation: cooking loss (%) = [(weight of raw sample (g) – weight of cooked sample (g))/weight of raw sample (g)] × 100. Display weight loss (%) was determined by calculating weight differences between 1 (initial) and 7 (final) days of simulated retail display in the same sample.

2.2.3. Shear force

Six cores parallel to the muscle fiber orientation were obtained from the each cooked chop sample using a hand-held coring device (1.27 cm diameter). The shear force was analyzed using Warner-Bratzler shear attachment on the TA-XT Plus texture analyzer (Stable Micro System Ltd., UK), and the peak shear force (kg) of six replicates per chop was recorded and averaged.

2.2.4. Instrumental color evaluation

Surface color of loin chops was measured using a Hunter MiniScan EZ colorimeter (Hunter, Reston, VA, USA) equipped with a 25 mm (diameter) measuring (illuminant, D_{65} source and observer, 10°). Calibration was performed according to manufacturer's standard manual. CIE L^* (lightness), a^* (redness) and b^* (yellowness) values were taken from five random locations on each sample. Hue angle (discoloration) and chroma (color intensity) were calculated using the following expression; Hue angle = $\tan^{-1}(b^*/a^*)$ and Chroma = $[(a^{*2} + b^{*2})^{1/2}]$ (AMSA, 2012).

2.2.5. Visual color evaluation

Visual color characteristics of lamb loin chops during simulated retail display were evaluated by trained sensory color panelists (n = 8). The trained panels had previously passed the Farnsworth-Munsell 100 Hue Test and trained and participated multiple times for similar fresh meat color trials. The visual color evaluation was performed in accordance with the meat color guidelines (AMSA, 2012). The evaluating scale of lean color was 8 points (1 = extremely dark red, 2 = dark red, 3 = moderately dark red, 4 = slightly dark red, 5 = slightly bright red, 6 = moderately bright red, 7 = bright red, 8 = extremely bright red), and that of discoloration was 7 points (1 = no discoloration, 0%), 2 = slight discoloration (1–19%), 3 = small discoloration (20–39%), 4 = modest discoloration (40–59%), 5 = moderate discoloration (60–79%), 6 = extensive discoloration (80–99%), 7 = total discoloration (100%). The average means from the panelists were used for further statistical analysis.

2.2.6. Heme iron content

Heme iron content of raw lamb loins was determined in duplicate according to the method described by Ramos et al. (2012). One gram of sample was homogenized with 4.5 mL of acidified-acetone solution (acetone:HCl:water = 90:2:8), and the mixture was incubated in a dark room at room temperature. After 1 h, the mixture was filtered through glass filter paper (Whatman GFA, USA), and