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Modification of mature non-reducible collagen cross-link concentrations in bovine m. *gluteus medius* and *semitendinosus* with steer age at slaughter, breed cross and growth promotants

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ABSTRACT

Increased meat toughness with animal age has been attributed to mature trivalent collagen cross-link formation. Intramuscular trivalent collagen cross-link content may be decreased by reducing animal age at slaughter and/or inducing muscle re-modeling with growth promotants. This hypothesis was tested in m. *gluteus medius* (GM) and m. *semitendinosus* (ST) from 112 beef steers finished at either 12 to 13 (rapid growth) or 18 to 20 (slow growth) months of age. Hereford–Aberdeen Angus (HAA) or Charolais–Red Angus (CRA) steers were randomly assigned to receive implants (IMP), ractopamine (RAC), both IMP and RAC, or none (control). RAC decreased pyridinoline (mol/mol collagen) and IMP increased Ehrlich chromogen (EC) (mol/mol collagen) in the GM. In the ST, RAC increased EC (mol/mol collagen) but decreased EC (nmol/g raw muscle) in slow growing CRA steers. Also, IMP increased ST pyridinoline (nmol/g raw muscle) of slow-growing HAA steers. Results indicated alteration of perimysium collagen cross-links content in muscle in response to growth promotants.

1. Introduction

Beef tenderness is a complex trait and is considered the main quality attribute of beef (Morgan et al., 1991). Consumers rate tenderness as one of the most important organoleptic characteristics of meat when they are making purchasing decisions (Mennecke, Townsend, Hayes, & Lonergan, 2007) and are willing to pay a premium for beef products guaranteed to be tender (Miller, Carr, Ramsey, Crockett, & Hoover, 2001). Tenderness shows great variability due to both inherent biology and production technology and, as a result, production strategies are being sought to improve meat tenderness and reduce its variability (Got et al., 1999). Solving the problem of inconsistent meat tenderness has become a top priority for the beef industry due to consumer demands and preferences, as evidenced by the emergence of guaranteed tenderness programs. To improve meat tenderness, meat producers and processors will need to work together to understand and control the impact of both biology and technology.

Although the tenderness of beef is affected by all components of muscle such as lipid and proteoglycans, it primarily depends upon the structural integrity of the proteins of the myofibrils and of the connective tissue that surrounds individual (endomysium) and bundles (perimysium) of muscle fibers (Bailey, 1972). These two main structural elements of muscle have profound effects on cooked meat tenderness and shear force (Girard, Bruce, Basarab, Larsen, & Aalhus, 2012b). The contribution of myofibrils to beef tenderness has been found to be particularly dependent on pre-slaughter stress and early post-mortem carcass processing technologies and management (Tornberg, 1996). The influence of connective tissue appears to be determined by the amount of collagen (Cross, Carpenter, & Smith, 1973), which is the major protein of connective tissues, as well as by the collagen heat solubility (Hill, 1966) and the number of heat-stable collagen cross-links (Bailey & Shimokomaki, 1972).

Collagen fibers are stabilized by both divalent and trivalent cross-links, with newly-synthesized collagen of young animals containing primarily divalent cross-links that are heat-labile (Bailey & Sims, 1976). The divalent cross-links become trivalent and heat-stable with animal age and the toughness of meat increases (McCormick, 1994) while the muscle collagen heat solubility decreases (Hill, 1966). The trivalent cross-links include the pyridinolines (PYR), hydroxylysylpyridinoline and lysylpyridinoline, and the Ehrlich chromogen (EC), all of which produce a link between two telopeptides and the helix of another collagen molecule (Eyre, 1987). Increased meat toughness and decreased collagen heat solubility have been related to increased concentration of pyridinoline (PYR) (Bosselmann, Möller, Steinhart, Kirchgessner, & Schwarz, 1995; Steinhart, Bosselmann, & Möller, 1994). Consequently,

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mature cross-link concentrations are considered key factors in collagen-related toughness (Weston, Rogers, & Althen, 2002). The importance of their influence increases particularly if total muscle collagen is constant across animals of various ages (Smith & Judge, 1991). The measurement of collagen cross-links is therefore considered to be a predictor of meat toughness (Lepetit, 2007).

The potential exists to modify production practices to increase collagen heat solubility and reduce connective tissue contribution to beef toughness. Strategies that may be useful include reducing animal age at slaughter (Bailey & Shimokomaki, 1972), decreasing animal physical activity during growth (Petersen, Berge, Henckel, & Soerensen, 1997), and increasing animal growth rate (Sylvestre, Balcerzak, Feidt, Baracos, & Bellut, 2002). Girard, Aalhus, Basarab, Larsen, and Bruce (2011) found that the mean total collagen content and mean shear force value of the m. *gluteus medius* (GM) of steers increased with animal age from 12–13 to 18–20 months and these changes were accompanied by a decrease in heat soluble collagen. This is not unexpected because the toughness of beef increases with age (Shimokomaki, Elsden, & Bailey, 1972) and this increase in toughness occurs only in muscles with moderate to high connective tissue content (Shorthose & Harris, 1990). Reducing the age of slaughter of beef cattle may decrease the contribution of collagen to meat toughness by shortening the period during which the heat-stable trivalent collagen cross-links can form. With increased growth rate, the heat solubility of collagen in meat appears to be greatest after rather than during the period of rapid growth (Boleman, Miller, Buyck, Cross, & Savell, 1996; McCormick, 1994). This increase in collagen heat solubility following rapid animal growth may be due to reduced collagen cross-link valency with muscle connective tissue re-modeling (McCormick, 1994), increased post-mortem activity by endogenous, collagen degrading enzymes (matrix metalloproteinases, MMPs) (Sylvestre et al., 2002), or a combination of both. Slow growth may decrease levels of endogenous connective tissue proteases (Harper, 1999) and increase the fascicular width of connective tissue in muscle (Allingham et al., 2009), both of which may increase meat toughness. Increased growth rate can be accomplished through the use of hormonal implants or beta-adrenergic feed supplements, which are used in the commercial cattle industry to increase weight gain and gain efficiency, and decrease the production cost per animal (Lawrence & Ibarburu, 2007). The effects of these technologies on collagen crosslinking are contentious, however, with implants potentially increasing the contribution of collagen to beef toughness (Girard, Aalhus, Basarab, Larsen, & Bruce, 2012) or decreasing it (Cranwell, Unruh, Brethour, & Simms, 1996a; Cranwell, Unruh, Brethour, Simms, & Campbell, 1996b). Fishell, Aberle, Judge, & Perry (1985) speculated that increased growth rate accompanied by muscular hypertrophy resulted in a reduction in intermolecular collagen cross-links and an increase in tenderness. The potential exists then for increased growth rate in cattle to reduce the formation of trivalent collagen crosslinks in moderate and high connective tissue content bovine muscles. Understanding the mechanisms of tenderness in relation to collagen cross-linking in these muscles may assist beef producers with enhancing beef tenderness. The objective of this study was to characterize the effect of growth rate, breed crosses, hormonal growth implants and ractopamine hydrochloride feed supplementation on the trivalent intramuscular collagen cross-links in GM and ST muscles.

2. Materials and methods

2.1. Animals and treatments

A complete description of animal management, experimental diets, experimental treatments and animal slaughtering procedure was detailed by Girard et al. (2011). Experimental treatments of this study involved steers being slaughtered at either 12–13 months of age (rapid growth) or at 18–20 months of age (slow growth). Hormonal growth implants were also administered (IMP) or not (NOIMP), and ractopamine hydrochloride was supplemented (RAC, 200 mg head^{-1} day^{-1}) or not (NORAC). The 28 steers in the calf-fed group that were implanted received Component E-S (200 mg progesterone and 20 mg estradiol benzoate with 29 g tylosin tartrate, Elanco Animal Health, a Division of Eli Lilly Canada Inc., Guelph, ON) at about 200 days of age. The 28 steers in the yearling-fed group that were implanted received the same implant as the calf-fed implanted steers at 200, 280, 350 and 440 days of age and were also implanted with Component TE-S (120 mg trenbolene acetate and 24 mg estradiol, Elanco Animal Health, a Division of Eli Lilly Canada Inc., Guelph, ON) at about 30 days before slaughter. Cattle within this study were either crossbred Hereford–Aberdeen Angus (HAA; n = 64) or Charolais–Red Angus (CRA; n = 48) and were cared for under the guidelines provided by the Canadian Council for Animal Care.

2.2. Muscles and muscle data

The left GM and ST muscles were removed from the carcasses at fabrication after 24 h *post mortem* (Girard et al., 2011). Muscles were individually labeled and weighed. Steaks were removed from anterior to posterior. The first trim steak was discarded and the second steak was cut 2.5 cm thick and used for muscle fiber typing (Girard et al., 2011). Subsequent 2.5 cm thick steaks were used to describe meat quality (Girard, Aalhus, Basarab, Larsen & Bruce, 2012a). The remaining muscle was weighed, packaged under vacuum and aged at 4 °C for 7 days. After aging, the vacuum packed muscle was thawed and trimmed of external fat and epimysium, chopped into cubes of approximately 2 cm^3 and retained for isolation of connective tissue. Data records of inherent muscle characteristics (muscle fiber type, total and soluble collagen) and meat quality (Warner-Bratzler shear force) from the muscles of these cattle were originally presented by Girard et al. (2011, 2012a), respectively, and were used to investigate relationships between muscle properties, meat quality and perimysium connective tissue characteristics of the GM and ST muscles.

2.3. Isolation of intramuscular connective tissue (IMCT)

Intramuscular perimysium connective tissue was isolated by blending cubed muscle (about 100 g) in 5 volumes (w/v) of deionized water (4 °C) for 10 s at low speed and 10 s at high speed in a laboratory blender. The homogenate was filtered through a metal sieve (pore size 1 mm^2) and the material retained on the sieve was deemed IMCT. The IMCT was re-blended in 4 °C deionized water and filtered again. This step was repeated twice and then IMCT blotted dry with Whatman No. 4 filter paper (Fisher Scientific, Fisher Scientific, Mississauga, Ontario). Visible blood vessels were removed from the retained material prior to blotting with filter paper. The wet IMCT was then weighed and frozen at −70 °C and lyophilized. After lyophilization, the IMCT was weighed and its dry weight was recorded. The lyophilized IMCT was stored at −20 °C and protected from light and was deemed perimysium (Kuypers, Tyler, Kurth, & Horgan, 1994).

2.4. Ehrlich chromogen crosslink concentration

Ehrlich chromogen (EC, pyrrole) cross-link concentration in the lyophilized IMCT was determined by tryptic digest according to the method of Horgan, Jones, King, Kurth, and Kuypers (1991). The pyrrole crosslink concentration was expressed as mol/mol collagen and nmol/g wet muscle using a molar extinction coefficient of 25000 (Kemp & Scott, 1988). The extinction coefficient was used to calculate the molar concentration of the EC in the tryptic digest from its absorbance using A/ε = molar concentration, where A = absorbance and ε = the molar extinction coefficient. The remainder of the tryptic digest was retained at −20 °C for determination of hydroxyproline content as an estimate of total collagen content of the tryptic digest. The assay was performed in duplicate.