RELATIONSHIPS BETWEEN INTRAMUSCULAR COLLAGEN CONTENT AND AGE AT SLAUGHTER, GROWTH PROMOTANTS AND BREED CROSS

Heather L. Bruce[1], Isabelle Girard[1], Ivy Larsen[2], John Basarab[3] and Jennifer L. Aalhus[2]

[1]Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Alberta, Canada, T6G 2P5; [2]Agriculture and Agri-Food Canada, 6000 C&E Trail, Lacombe, Alberta, Canada, T4L 1W1; [3] Alberta Agriculture and Rural Development, 6000 C&E Trail, Lacombe, Alberta, Canada, T4L 1W1.

Abstract – Intramuscular connective tissue contributes to the toughness beef either through its content and or its cross-linking strength. Intramuscular perimysial connective tissue was isolated from the *m. gluteus medius* (GM) and *m. semitendinosus* (ST) muscles from the carcasses of 112 crossbred steers and effects of breed, growth implant, ractopamine supplementation and age at slaughter examined using a 2^3 factorial. Connective tissue was expressed on a wet and dry weight basis as a percentage of muscle wet weight. Results indicated that ractopamine tended to reduce connective tissue wet weight in the GM in steers slaughtered at 18 to 20 months of age. Pearson correlations with meat quality data collected previously indicated that the percentage dry perimysium was positively correlated with Warner-Bratzler shear force ($r = 0.29$, $P = 0.0017$) in the ST whereas there was no significant correlation between wet or dry perimysium content and shear force in the GM. Results indicated that the effect of RAC on connective tissue and the impact of connective tissue content on cooked beef toughness differ between muscles.

Key Words – beef, perimysium, ractopamine, growth implants

I. INTRODUCTION

Consumer acceptability of beef is governed by how tough the cooked product is perceived to be [1]. The toughness of beef is determined by the amounts of connective tissue or 'gristle' that is in the beef, with tough beef cuts having large amounts of connective tissue [2]. Connective tissue consists primarily of the protein collagen, which is capable of establishing trivalent intermolecular cross-links that increase the heat stability of the protein [3] and increase the toughness of meat [2]. The contribution of connective tissue amount relative to the extent of its cross-linking has not been determined, and it may differ between muscles within the beef carcass. The amount of connective tissue within muscles may also be affected by production factors such as animal age and growth rate, cattle breed or the use of growth promotants. This study investigated the effects of animal age at slaughter, breed cross, the use of steroid implants and ractopamine supplementation on the amount of connective tissue relative to muscle weight in two muscles of different toughness levels, the *m. gluteus medius* and the *m. semitendinosus*.

II. MATERIALS AND METHODS

Muscle used in the present investigation were portions of 112 *m. gluteus medius* (GM, top sirloin) and 112 *m. semitendinosus* (ST, eye of round) from a 2 x 2 x 2 factorial experiment described by Girard *et al.* [4]. Experimental treatments from this study involved steers being slaughtered at either 12 to 13 month of age (calf-fed steers, rapid growth) or at 18 to 20 month of age (yearling-fed steers, 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 typlosin 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 trenbolone 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. Data records of inherent muscle characteristics (muscle fibre type, total and soluble collagen and proximate analytes) and meat quality (L*, a*, b*, myoglobin relative contents, purge loss, cooking loss, cooking time, sarcomere length and Warner-Bratzler shear force) from these cattle were originally presented by Girard *et al.* [4, 5], respectively, and were used to investigate relationships between muscle properties, meat quality and perimysial connective tissue characteristics.

As described by Girard *et al.* [4] the left GM and ST muscles were removed from the carcasses at fabrication 24 hours post mortem. Muscles were individually labelled and weighed. Steaks were removed from the proximal to distal end for the ST and from the anterior to posterior end for the GM. The first trim steak was discarded and the second steak was cut 2.5 cm thick and used for muscle fibre type determination [4]. The remaining muscle was weighed, packaged under vacuum and aged at 4 ºC for 7 d. After ageing, muscle remaining after meat quality sampling was trimmed of fat and epimysium, chopped into cubes of approximately 2 mm^3 and retained for connective tissue studies.

Perimysium was isolated by blending muscle cubes from each muscle in 4 ^oC deionised water for 10 seconds at low speed and 10 seconds at high speed in a 1 L laboratory blender. The homogenate was filtered through a 1 mm^2 sieve and the residue retained on the sieve was deemed perimysial connective tissue. The perimysium was blended and filtered twice again in cold, deionised water and then blotted dry with Whatman No. 4 filter paper (Fisher Scientific, Edmonton) and weighed. The wet perimysium was then frozen at -70 ^oC until lyophilisation. After lyophilization, the perimysial connective tissue was weighed and its dry weight recorded.

Perimysial connective tissue data were analyzed within muscle type as a 2^3 factorial design using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC) with sources of variation including age at slaughter, hormonal growth promotant, ß-AA feed supplementation, breed cross and their interactions. Pen nested within slaughter age x implant x RAC was included as a random effect. The initial model included day of kill as a source of variation, but it was removed when it was found not to be significant ($P > 0.05$) in an analysis of covariance. Denominator degrees of freedom were calculated using the Kenward-Roger approximation and differences between treatments and interaction means were separated using the F-test protected LSD procedure ($P \leq 0.05$).

Significant Pearson correlations between perimysial connective tissue data and collagen, muscle and meat quality data presented by Girard *et al.* [4] were determined using the CORR procedure of SAS (SAS Institute Inc., Cary, NC). Thirty comparisons were made to each perimysium characteristic; therefore, a Bonferroni correction was used to correct the α significance value (30 comparisons for each perimysium characteristic, $\alpha = (0.05/30)$ and so correlations were deemed significant at $P < 0.0017$). Simple statistics for the connective tissue characteristics were also obtained during this analysis.

III. RESULTS AND DISCUSSION

Simple statistics for the perimysium content both as a wet and dry percentage of wet muscle tissue, and the moisture content of the perimysium are presented in Table 1. Factorial analysis indicated that the percentage of wet perimysial connective tissue recovered was not affected by production treatment when from the ST but there was a significant interaction between age at slaughter and RAC use ($P = 0.05$) when from the GM (Figure 1). In GM muscle, the percentage of wet perimysial connective tissue was increased by RAC in the calf-fed steers but decreased by RAC in the yearling-fed steers (Figure 1). These results are in contrast with those of Girard *et al.* [4], who found through assessment of collagen content using the hydroxyproline concentration estimated by

Bergman and Loxley [6] that there was no difference in collagen content due to RAC supplementation. Physical separation of connective tissue does not isolate solely collagen nor does it capture entirely the fine collagen fibres of the endomysium; therefore, the results of physically isolating perimysial connective tissue suggested that supplementation of yearling-fed steers with RAC may reduce the large connective tissue fibres without affecting overall collagen concentration. Girard *et al.* [4] also found that there was no significant interaction of RAC and slaughter age with Warner-Bratzler peak shear, indicating that the trend observed in the present study for perimysial connective tissue recovered to be reduced in yearling steers fed RAC was not large enough to cause an effect on cooked muscle toughness. These results agree with those of Cha and Purslow [7], who noted that RAC modified the turnover of collagen in skeletal fibroblasts and myoblasts.

Table 1. Simple statistics for perimysial connective tissue isolates

Variable	n	Mean	SD[1]	Min.[2]	Max.[3]
Gluteus Medius					
Wet perimysium (% wet muscle)	112	10.5	5.1	3.9	42.1
Dry perimysium (% wet muscle)	112	3.3	1.8	1.6	14.3
Perimysium Moisture (%)	112	76.5	4.2	65.5	87.1
Semitendinosus					
Wet perimyium (% wet muscle)	112	13.7	6.6	7.0	62.9
Dry perimysium (% wet muscle)	112	6.0	2.4	2.9	20.3
Perimysium Moisture (%)	112	67.3	3.1	60.1	76.4

[1] Standard deviation
[2] Minimum value
[3] Maximum value

Although perimysial connective tissue isolates were blotted dry similarly prior to lyophilization, moisture content of the perimysium in the ST increased with the use of steriodal implants (P = 0.0416)(67.9 versus 66.7 $\pm$ 0.6 %, implanted and non-implanted, respectively). For moisture content of the GM perimysium, CRA crossbreds had a greater mean moisture level in their perimysium than HAA crossbreds (77.5 $\pm$ 0.5 versus 75.7 $\pm$ 0.6 %, respectively). The biological implications of these differences warrant further investigation by characterizing the hydroxyproline concentration of the perimysium isolates [6] as these results may reflect a difference in glycosylation between the perimysial connective tissue due to implant use (ST) and continental breed use (GM). Increased glycosylation can provide connective tissue with increased protection from endogenous proteases [8] and contribute to the toughness of cooked meat.

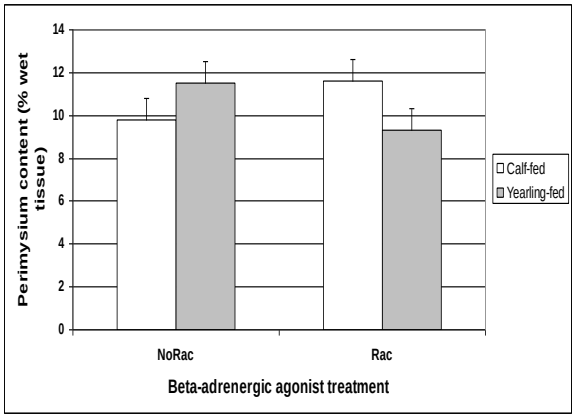

Figure 1. Mean perimysium content (% wet tissue) of the GM as affected by RAC supplementation and age at slaughter

Pearson correlations performed within each muscle type indicated that percentage dry perimysium was positively correlated with Warner-Bratzler shear force (r = 0.29, P = 0.0017) in the ST whereas there was no significant correlation between wet or dry perimysium content and shear force in the GM. In both muscles, the percentage wet and dry perimysium were positively correlated to each other (P < 0.0001) (r = 0.95 and 0.92, ST and GM, respectively). These results suggested that water content of the wet perimysial connective tissue obscured its relationship with peak shear force of the cooked ST. These results also

suggested that the cooked toughness of the ST was significantly affected by connective tissue content whereas that of the GM was not. Whether the cooked toughness of the GM is more affected by trivalent collagen cross-link content than connective tissue content remains to be tested.

IV. CONCLUSION

GM and ST muscles responded differently to production practices, with the perimysial connective tissue content of the GM muscle of yearling-fed, slow growing steers reduced by supplementation of the steers with RAC.

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