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CHARACTERIZATION OF BEEF MUSCLES STRUCTURE BY HISTOLOGY AND IMAGE ANALYSIS IN DOUBLE-MUSCLED AND NORMAL ANIMALS

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Introduction

Double-muscled (hereafter referred to as culard) cattle have been studied for many years as a model of the relationship between hyperplasia, collagen and lipid content, and meat quality (Ashmore & Robinson 1969, Bouton et al 1982, Boccard & Dumont 1974, Fiems et al 1995). It is well known that there is an increase in fiber number and a decrease in both collagen and lipid content in these cattle when compared with normal cattle. The decreased collagen content, increased collagen solubility (Ngapo et al 2002, Uytterhaegen et al 1994) and decreased collagen cross-links (Ngapo et al 2002) are reputed to yield extremely tender meat, but several studies have failed to find significant differences between cooked shear force values of culard and normal cattle (Bouton et al 1982, De Smet et al 2000, Wegner et al 2000), or even have found higher cooked shear force values for culard cattle (Uytterhaegen et al 1994, Fiems et al 1995). These discrepancies may be partly explained by differences in post mortem tenderization and myofibrillar toughness (Uytterhaegen et al 1994, Steen et al 1997, De Smet et al 2002), and by differences in the cooking procedures used. However, raw shear force is always significantly lower in culard cattle (Bouton et al 1982, De Smet et al 1998, Ngapo et al 2002), which usually indicates decreased connective tissue content. Taste panel tenderness is also consistently better in culard animals (De Smet et al 2002). Another possible explanation for the tenderness variability is that other connective tissue parameters, especially organization, are also important in determining the role of connective tissue in tenderness, and that the relationship of quality to connective tissue parameters is only detected when collagen is not extensively solubilized at high cooking temperatures. In fact qualitative studies have shown differences in perimysial connective tissue organization between normal and culard cattle (Boccard 1981, Dumont & Schmitt 1973).

Objectives

To better characterize the meat quality of culard cattle we examined by histology the perimysial connective tissue organization in different muscles of culard and normal

Belgian Blue cattle and performed quantitative measures of its organization by image analysis. The results were related to muscle mechanical properties as an index of tenderness.

Methodology

Thirteen bulls of normal conformation (mean age 19.4 months) and ten culard bulls (mean age 21.2 months) of the Belgian Blue breed were slaughtered. Genotyping for the mutation nt821 (del 11) in the myostatin gene responsible for the double-muscling phenotype in the Belgian Blue breed was done according to Grobet et al. (1998) (culard, mh/mh; normal, +/+). At 1 day *post mortem* three muscles: *Gluteobiceps* (GB), *Pectoralis profundus* (PP) and *Semitendinosus* (ST) were excised and samples (1x1x1 cm^3) were frozen in isopentane chilled by liquid nitrogen (-160°c) and stored at -80°C. Frozen sections (10 µm thick) were stained using picro-Sirius red coloration (Flint and Pickering, 1984) which reveals the collagen of perimysium and endomysium (Fig. 1).

Histological sections were studied with a Polyvar Reichert microscope, Sony CCD video camera, computer with a Matrox image acquisition card and Visilog 5.4 software (Noesis, France). Automatic thresholding on grey level allowed segmentation of the thickest elements of the perimysium network, thus eliminating the endomysium and the thinnest elements of perimysium (Fig. 2). Measurements of this segmented network allowed calculations of the % area of perimysium. An additional step of skeletization of the segmented network reduced it to a thickness of a pixel allowing the measure of the length of the network (Fig. 2c)

This segmentation only allowed the measurement of length and area occupied by the perimysium and not measures of fascicle size. The fascicles were manually traced (Fig. 3). Measurement of the area of the primary, or smallest, perimysium fascicles of the *Semitendinosus* muscle was performed on all bulls, using Visilog software. Primary fascicles are identified as the smallest group of fiber bundles and are delimited by a continuous connective tissue which is thicker than the endomysium (Fig. 3c).

On images of ST and GB muscles obtained at higher magnification the endomysium was segmented automatically to measure fiber size within the fascicles. Fiber boundaries were manually corrected when necessary.

The shear force and sensory measurements on these animals have been reported in a previous study (De Smet et al 2000). Warner-Bratzler shear force was measured with a Lloyd TA 500 Texture Analyser on cooked (water bath heating at 75 °C) and grilled (until an internal temperature of 80°C) steaks. A 12 members sensory panel evaluated samples of each muscle for tenderness.

Statistical analysis of the measured perimysial parameters was done using the ANOVA "General Linear Model" procedure of SAS. Comparison of fascicle size was done using the Student’s T-test.

Results & Discussion

Perimysium network length and area

We found significant differences ($P<0.001$) in the perimysial surface area and length (Fig. 4) of culard and normal Belgian Blue cattle, with culard cattle having less area and total length of perimysium. The ST and GB were significantly different for both parameters whereas the PP varied slightly for length and not for area. Boccard (1981) found that the total muscle weight of PP actually was more effected by the culard phenotype than ST and Gluteus muscles weights so it is not readily evident why PP perimysium changes much less than GB and ST. As previously reported (Dumont & Schmitt 1973), we also observed that culard perimysium is thinner and has less branching.

Primary fascicle area

The area of primary fascicles in ST muscles was measured for 48 fields and 39 fields for normal and culard animals respectively. This gave a total of 1799 fascicles for normal and 835 for culard cattle with respective means of 0.31 ± 0.2 mm^2 for normal cattle versus 0.59 ± 0.44 mm^2 for culard. These differed significantly ($P<0.001$) with culard fascicles being twice as large. This is the first report of fascicle size for double muscled animals and supports that the tender raw (De Smet et al 1998) and cooked (De Smet et al 2000) meat in these animals is associated with larger fascicles which have thinner perimysium.

Fiber size

Several previous reports have shown differences in fiber size and type between culard and normal cattle (Fiems et al 1995, Holmes & Ahsmore 1972, Wegner et al 2000), with the tendency being slightly smaller fibers in culard animals and more glycolytic IIB fibers. We observed when tracing the perimysium bundles that the endomysium size and shape varied by muscle and animal type (Fig. 5). Therefore were quantified the size distribution of fibers, defined by endomysial area, for GB and ST muscles because they appeared to differ markedly. The fiber size distribution is shown in figure 6 and it is evident that ST fibers are homogeneous in normal and culard with an average size of $4136\pm2451\mu m^2$ and 5071 ± 2997 μm^2 respectively. However, GB fibers have a heterogeneous distribution in culard animals with peaks for small and large fibers. GB normal and culard fibers had a mean size of $3451\pm1581\mu m^2$ and 4283 ± 2954 μm^2 respectively. This has not previously been reported and is not readily explained by the known effects of myostatin mutations (Kambadur et al 1997). Since fiber size is associated with meat texture (Taylor 2004) these differences could influence quality. These fiber sizes are in the range of previous reports but our tendency is slightly larger culard fibers, which may be due to differences in techniques, because we measured endomysium delimited fiber size and not specific myofiber stains such as azorubin.

Conclusions

A recent review by Purslow (ICoMST 2004) discussed the importance of connective tissue organization in meat quality. Our current study is the first to show that primary fascicles are larger in culard ST muscles, which may be associated with the tenderness of meat. This is related to the so-called muscle grain which is a visual image of the coarseness of meat. However, visual inspection would only discriminate the larger secondary fascicles and not the primary as measured herein. We are developing techniques to measure the larger fascicles (Sifre-Maunier et al 2004) which are highly variable. Preliminary results in normal Charolais cattle show that tough muscles have greater proportions of small secondary fascicles.

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Tables and Figures

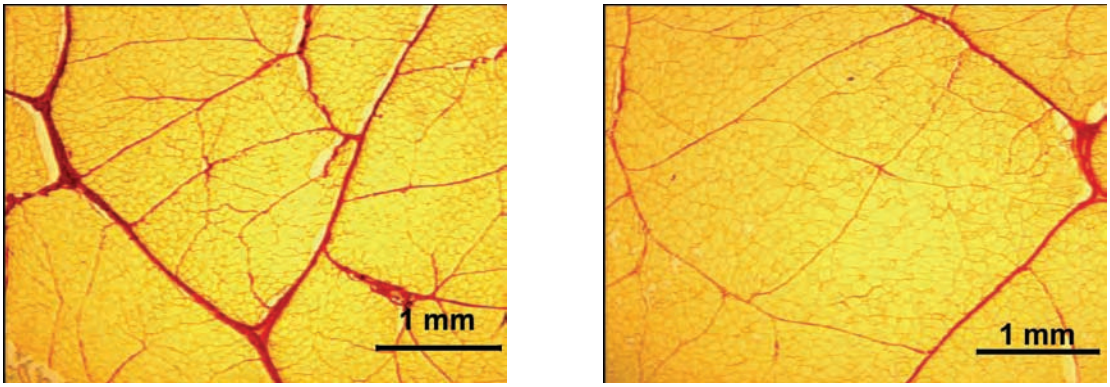


Fig. 1a Normal ST muscle **Fig. 1b Culard ST muscle**

Figure 1. Picro_Sirius red staining of ST muscle to show perimysium and endomysium organization.

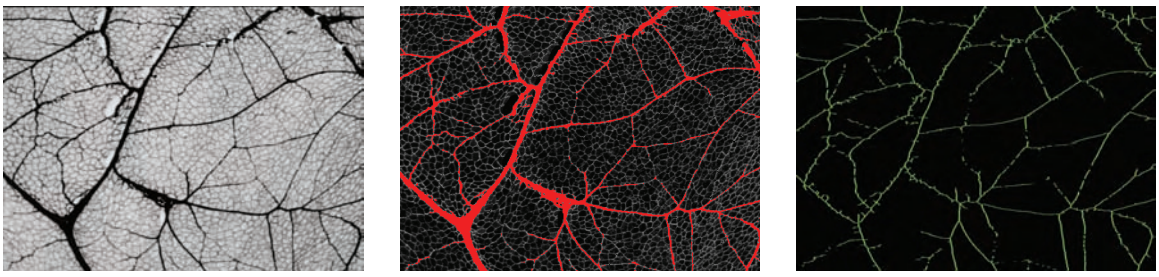
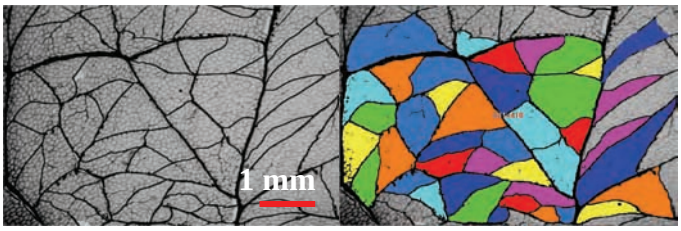


Fig. 2a **2b** **2c**

Figure 2. Segmentation of normal image (2a) to measure perimysium area (2b) and length (2c).



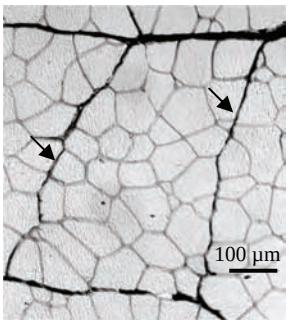
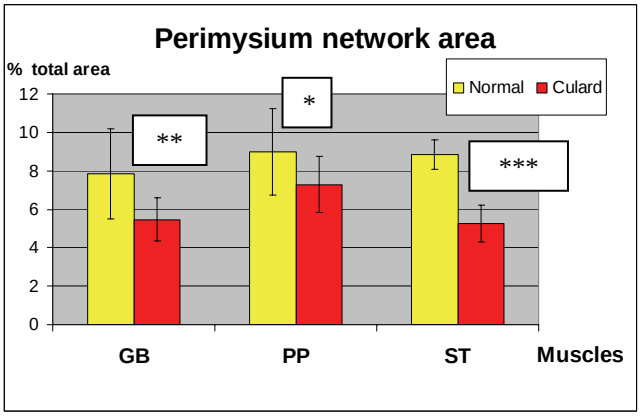


Fig. 3a **Fig. 3b** **Fig. 3c**

Figure 3. Segmented images (3a) were manually traced to give precise boundaries of primary fascicles (3b). Fascicles were identified by thick boundaries (arrows in 3c).



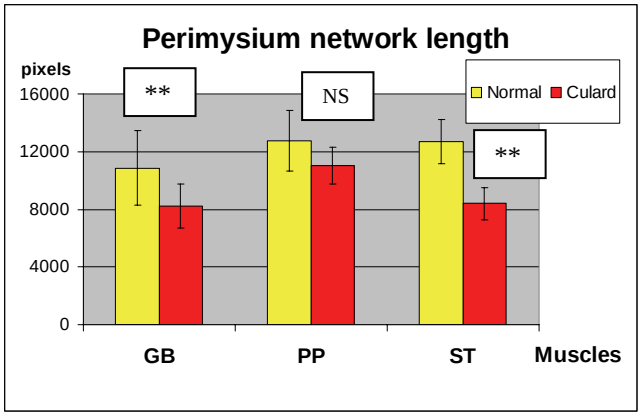
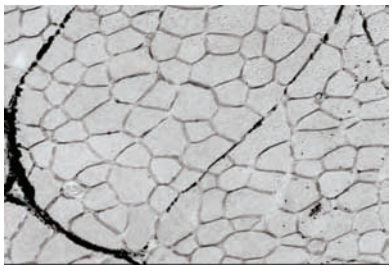


Fig.4a **Fig. 4b**

Figure 4. The area of perimysium differs between culard and normal cattle for all three muscle types (4a), and length differs for GB and ST (4b).



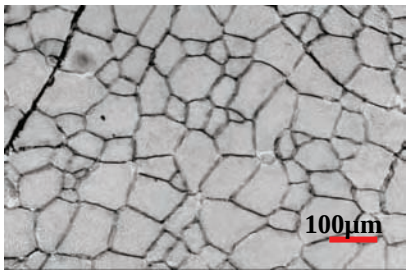


Fig 5a Normal **Fig 5b Culard**

Figure 5. Endomysial size in GB muscles was homogeneous in normal animals (5a) and heterogeneous in culards (5b)

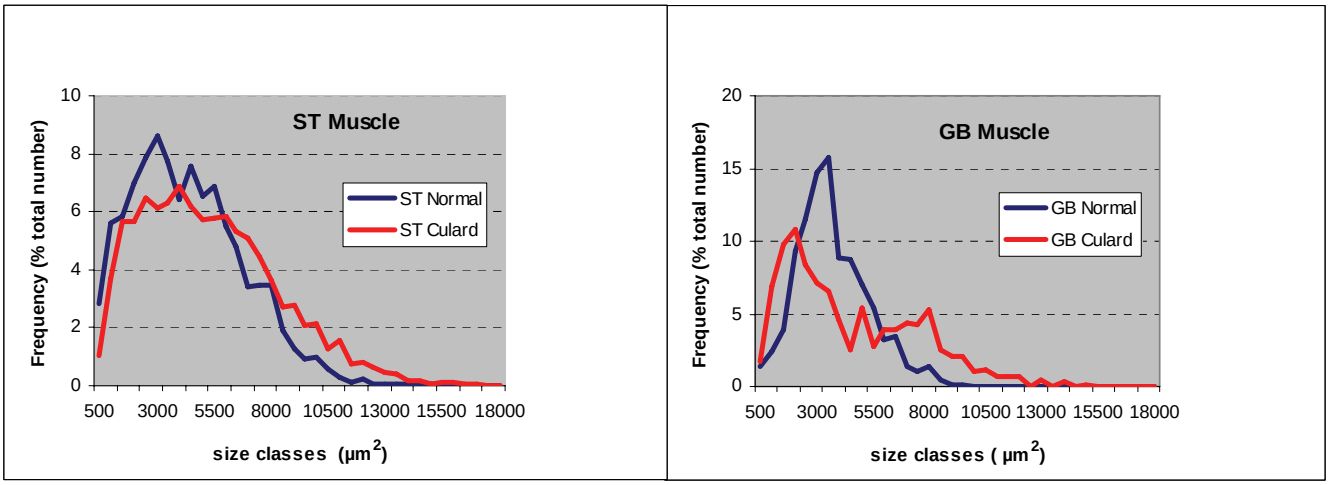


Fig. 6a **Fig. 6b**

Figure 6. Fiber size distribution was similar for normal and culard ST (6a), but heterogeneous for culard GB (6b).