A METHOD FOR DETERMINING THE MUSCLE FIBRE LENGTH

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Background

Muscle fibres are the result of the fusions of thousands of myoblasts to form a long tube. The length of a mammalian muscle fibre is variable, and can be the length of the muscle. They are not, however, usually as long as the muscle, but still the length may even be 340 mm in long muscles (Lawrie, review 1998, p. 39). Fibres are arranged end-to-end series with their tapering ends overlapping adjacent fibres. About 50% of their length is of constant diameter, and taper at both ends by 25% of their length the angle of the taper being about 1º. (McCormick, review 1994, pp. 30-31). There is no data about porcine muscle fibre lengths easily available.

Objectives

The purpose of this study was to develop a method to estimate the fibre length of a muscle in order to count the total number of fibres in a muscle.

Materials and methods

M. longissimus dorsi muscle from three pigs of about 110 kg live weight were excised on the day following slaughter caudally to 5th *Thoracic vertebra*. Three 1 cm thick slices were cut, from both ends at 1/5 of the total length and from the middle, of each excised muscles (Figure 1A). From each slice five 1x1x1 cm cubes were usedfor fibre length measurements and five smaller pieces, of about 300 mg, for fibre diameter measurements were cut as shown in Figure 1B.

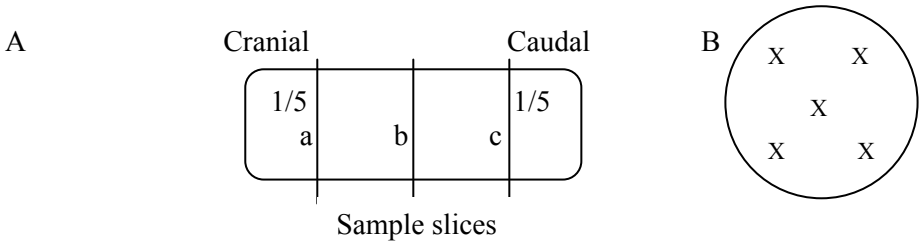

Figure 1. Sampling from *M. longissimus dorsi* muscle A: Slice locations; B: Fiber samples from a slice

Fibre length. Muscle fibres were separated by the Hooper method (1976; 1981). A sample cube was placed into a 50 ml beaker with 15 ml 4 N HNO_3. Beakers were covered with aluminium foil and kept overnight at room temperature. After the acid treatment the dimensions of the cubes were measured to determine the shrinkage (shortening) caused by the acid treatment. The cubes shrank longitudinally along the fibre axis 10%. Then each sample cube was inserted into a test tube with 5 ml Ringer-Locke solution. Test tubes were sealed and shaken vigorously by hand and by using test tube shaker, for one minute. Part of the suspension was poured on a slide and left to dry overnight.

The suspension was studied with a microscope (Olympus BH-2, Japan) at 40x magnification, with a commercial Zeiss AxioCam MRc digital camera (Zeiss GmbH, Germany) (Figure 1). Images were analysed with software package (Zeiss KS300 3.0, Germany). Individual fibre fractions were marked manually, on the images at their both ends, and the program then determined the length of the fibre. The results were stored cumulatively. Simultaneously, the number of fractions that visually seemed to be the very end of the tapered end of a fibre, was counted. From each slide sufficient fields were analysed so that the total number

of fibre fractions was at least 400. Totally over 20 000 fractions were measured (3 muscles x 3 slices x 5 cubes x more than 400 fractions).

The length of fibres was counted assuming that the tapered ends are equally distributed within the fractions (Equation 1).

$$L = \frac{\sum F}{(N/2)}$$

L= length of the fibres [mm]

F= total length of fractions [mm] (Eq. 1.)

N= number of the tapered ends.

Cross sectional area of slices. The slices were photographed, and the cross sectional area was determined using the image analysis program Zeiss KS300.

Number of fibres in a cross section. Due to an error in sample preparation, only two muscles were analysed for fibre numbers. The sample pieces were frozen in liquid nitrogen and kept then in -80 ºC. Cross sections (12 µm) were cut at -26 ºC in a cryostat (Reichert-Jung 2800 Frigocut E, Germany). The sections were not stained. From the sections, the counting area of the image and the number of fibres on the counting area were determined. The number of fibres per image was about 100. Two images per sample were analysed, totalling 10 images per slice (30 per muscle, 60 in grand total). The fibre number was counted by dividing the cross sectional area of the slice by the average cross sectional area of fibres.

Results and discussion

The fibres were well separated by the Hooper method. The fractions were clearly seen and marked for the automatic determination in the image. The tapered ends were also easy to identify, but the major obstacle was their small number. It was not possible to use a lower magnification or an automatic determination of the fractions directly from the image, because the first stage of the analysis had to be done manually. On one hand, the tapered ends of the fibres are very thin and difficult to see, but on the other hand, if this done always by the similar way, the fractions of certain size will always be counted similarly. An increase of the fields counted, however, would also much increase the work needed.

The aim of this study was develop a method for fibre length determination. Therefore, the conclusions below are only speculative, especially because the number of tapered ends was so small (varying between 4–14 within the total about 2000 fractions counted per slide). In such a small number, a change of one tapered end only has a marked effect on the results.

The fibres seem to be more than 100 mm long (Table 1). They seem to be longer in the middle of the muscle than at the end, which is in agreement with the statement of McCormick (review 1994). In this study we had actually only one end, the caudal (rear) end. The authors did not find any literature data about the length of porcine muscle fibre, and therefore no comparisons were made here. It seems, however, that the lengths obtained with this method are very long, and more analyses are needed for the validation of the method.

The fibre cross sectional areas were similar to that reported previously in Finland (Ruusunen and Puolanne, 2004) (Table 2). Because the fibres seem to be so long, theoretically there would be no need to have tapered ends at slice c, because it is closer (about 10 cm) to the caudal end of the muscle than the average length of the fibres. (On the contrary, the cranial end of the sample is not at the very end of the muscle, because a part of *M. longissimus dorsi* remained in the forepart of the carcass). Therefore, for reliable results, more slices taken at shorter spacings should be analysed, this would tremendously increase the amount of work needed.

The cross sectional area of fibres is larger in the middle of the muscle, which is a logical consequence of the observation (McCormick, review 1994) that the fibres are tapered at the end of the muscle. It must be noted, however, that the fibre axis in *M. longissimus dorsi* in not fully parallel with the muscle axis, and therefore the fibres have a slight pennate-type arrangement in the muscle. The number of fibres per muscle cross

section is about 1.1–1.3 million, on average 1.2 million. A very rough estimate of the total number of fibres in the whole muscle (length 60–70 cm, weight ca 4.0 kg) is about 4.2 million. Consequently, one fibre, 173 mm long and diameter Ø 40 µm, has a weight of about 1 mg.

Table 1. Muscle fibre lengths of porcine *M. longissimus dorsi* (n=3)

Slice (see Figure 1A on Page 1)	Muscle fibre length (mm)
a (cranial)	183 (156–298)
b (medial)	218 (186–346)
c (caudal)	137 (112–235)
Grand mean	173

Table 2. Muscle fibre cross sectional area and cross sectional fibre number in porcine *M. longissimus dorsi* (n=2)

Slice (see Figure 1A on Page 1)	Cross sectional area $x10^3$ µm^2	Number of fibres $x10^3$
a (cranial)	5.14	1147
b (medial)	5.17	1153
c (caudal)	4.67	1299
Grand mean	5.00	1198

Conclusions

A rough estimate of the muscle fibre length of porcine muscle length can be made by disintegrating muscular tissue and counting the tapering ends of muscle fibres in relation to the total length of the fibres counted. The method is, however, time consuming and hard to automatize. A fibre type staining could be included to increase the relevant data obtained from samples. The average cross sectional area of the fibres seems to vary within the muscle, being smaller at the ends of the muscle.

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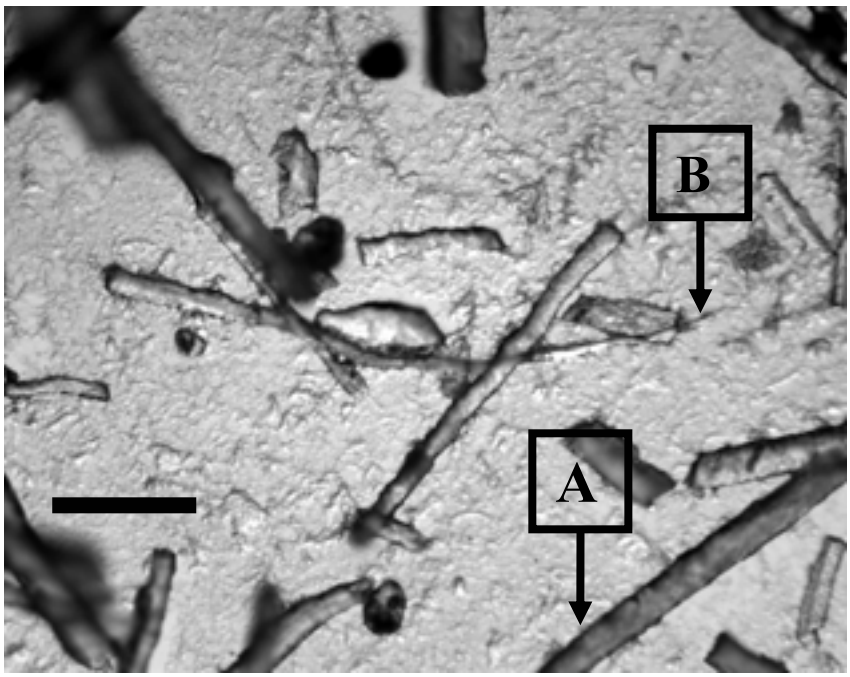

Figure 2. Fibre fractions. A: the thick middle part of a fibre; B: a tapering end. Bar: 200 μm. Microscopic magnification 100x.