EFFECT OF FASCULAR ARRANGEMENT ON THE APPARENT NUMBER OF MYOFIBERS IN THE PORCINE LONGISSIMUS MUSCLE

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SUMMARY

One complete longissimus muscle was removed from each of eight Yorkshire pigs (live weight 61 to 87 kg) and divided into 11 or more subunits following fascicular arrangement within the muscle. The number of myofibers appearing in transverse sections (apparent number) at the midlength of each subunit was estimated from the subunit midlength cross sectional area (CSA) and packing density (PD) of myofibers per unit area in frozen transverse histological sections from the subunit midlength. Myofibers were delineated by silver staining of endomysial sheaths. In chops taken at the level of the second lumbar vertebrae in intact sides, the apparent number of myofibers was estimated from the longissimus muscle CSA and myofiber PD in sections cut in a plane parallel to the face of the chop. The sum of the apparent numbers of myofibers in all subunits of each muscle (total apparent number) was only poorly related (r = .35, not significant) to the apparent number in anterior lumbar chops. Weight of the whole longissimus muscle was related to the anterior lumbar apparent number (r = .59, P<.01) but not to the total apparent number (r = .02). The implication of these results is that an increase in the real number of myofibers in the longissimus muscle achieved by selective breeding will not always or automatically cause an increase in the apparent number of myofibers in individual pork chops.

(Key Words: Muscle Anatomy, Myofiber Number, Porcine Longissimus.)

INTRODUCTION

Apart from the large size of muscles in meat animals, there are two major problems which make it difficult to estimate the number of myofibers present in a whole muscle (real number). A transverse section through a muscle fasciculus will not include any myofibers which have terminated intrafascicularly before reaching the plane of sectioning (Swatland and Cassens, 1972). In the longissimus muscle, the arrangement of fasciculi makes it impossible to transect all fasciculi in any one transverse section of the muscle (Swatland, 1975b). Thus, the number of myofibers appearing in a muscle transverse section (apparent number) may be considerably less than the real number.

The purpose of this study was to determine the effect of fascicular arrangement on the fraction of the real number appearing in anterior lumbar transverse sections of the porcine longissimus muscle. Whole muscles were divided into subunits following fascicular arrangement and the sum of the apparent numbers in all subunits (total apparent number) was used as a minimum estimate of real number. Total apparent number was compared to the apparent number of myofibers occurring in the anterior lumbar region to determine if the apparent/total apparent fraction was constant or variable.

MATERIALS AND METHODS

Eight typical commercial pigs of the Yorkshire breed (live weight 61 to 87 kg) were slaughtered, dressed and hung for several days at 0 °C. The method used to calculate myofiber numbers was not affected by changes in myofiber diameters due to postmortem shrinkage since myofibers were delineated by the staining of connective tissues between myofibers. Right longissimus muscles were dissected out and weighed after subcutaneous and intermuscular fat was removed. Each muscle was divided into 11 to 16 subunits so that each subunit contained complete fasciculi running lengthwise and severed only at their periosteal or ligamentous attachments. Each subunit was bisected by a transverse planar cut at the subunit midlength. A half of each subunit was placed with its...
transected surface downwards onto tracing paper to allow the perimeter to be traced and the cross sectional area (CSA) to be measured with a planimeter. From the axis of one half of each subunit a sample of muscle (8 x 8 x 50 mm) was removed, tied to a flat plastic strip and frozen in liquid nitrogen. This simple method of freezing did not cause ice crystal artefacts in outer regions of the sample so further precautions were not needed. The ice crystals usually attributed to slow freezing in liquid nitrogen are, in fact, more likely to be caused by thawing and re-freezing of the sample during manipulation in the cryostat. An eight millimeter cube was cut from the frozen sample and sectioned transversely (8 μm). Sections were stained with silver as described by Swatland (1975b).

One chop was removed from the left side of each carcass, perpendicular to the axis of the lumbar vertebrae. The anterior face of each chop was traced for a planimeter determination of longissimus muscle CSA and then painted with an aqueous solution of eosin dye. At three points on the transected face of the longissimus muscle (dorso-medial, central and ventro-lateral) samples were removed, frozen, sectioned and stained as before. In this instance, however, blocks were sectioned in a plane parallel to their eosin marked face.

To determine myofiber packing density (PD) per unit area, a micrometer grid (10 rows of 10 squares) was calibrated in the microscope eyepiece (x 8 objective, x 7 eyepiece). On a microscope equipped with a rotatable mechanical stage, sections were positioned so that one sweep with one axis of the stage transversed the whole section. The number of myofibers appearing in a row of ten squares was recorded at predetermined regular intervals across the section. Myofibers were only counted if their central axis fell within one of the squares in the row. A total of 10 rows was recorded (area = 1 mm² on the section) in each sample. It was assumed that intramuscular adipose tissue was distributed randomly in both whole muscle and subunit cross sections and in histological sections. Over 38,000 myofibers were counted in the whole study. Linear regression lines in the figures were calculated from least squares equations.

RESULTS

When whole muscles isolated from carcasses were laid flat on a dissecting board, anterior fasciculi were at an angle of approximately 15° to the horizontal. More posteriorly, this angle steadily increased to a maximum of approximately 40°. Even though in vivo angles would have differed from these values because of the curvature of the spinal column, it was considered that not more than approximately 15% of the total number of fasciculi in the muscle were present in a cross section at any particular point along the length of the muscle. In order to transect all fasciculi at some point along their length, muscles had to be divided into at least 11 subunits. Dissection of anterior lumbar chops showed that muscle fasciculi were oriented at an angle to both antero-posterior and dorso-ventral axes of the body (figure 1). Dorso-laterally, fasciculi inserted into a strong aponeurosis covering the surface of the muscle.

In each subunit of the longissimus muscle, CSA

Figure 1. Fascicular arrangement in the porcine longissimus muscle. Top diagram shows an anterior view of the cross sectional area of the right longissimus muscle in the anterior lumbar region. A longitudinal section of the muscle (lower diagram) cut in a plane parallel to AB (top diagram) transects fasciculi longitudinally. Dorso-laterally, fasciculi attach to an aponeurosis covering the surface of the muscle.
was multiplied by myofiber PD to estimate the apparent number. The apparent numbers for all subunits were totaled (total apparent number). In each chop, the longissimus muscle CSA was multiplied by the mean myofiber PD of the three areas sampled (dorso-medial, central and ventro-lateral) to estimate the anterior lumbar apparent number. It is important to note that total apparent number was determined with \( \theta = 90^\circ \) (angle of transection of a myofiber relative to the longitudinal myofiber axis; Swatland, 1975b) while the anterior lumbar apparent number was estimated with \( \theta = 40^\circ \), approximately (Swatland, 1975b). Thus, total apparent number (determined in subdivided muscles) was independent of fascicular arrangement while anterior lumbar apparent number (determined in chops) was dependent on fascicular arrangement. The total apparent number was only poorly related \((r = .35, \text{not significant})\) to anterior lumbar apparent number (figure 2). Longissimus muscle weight was related to anterior lumbar apparent number \((r = .59, P<.01, \text{figure 3})\) but not to total apparent number \((r = .02, \text{figure 4})\).

**Discussion**

To determine whether the anterior lumbar apparent number was a constant or a variable fraction of the total apparent number, two null hypotheses were tested. Firstly, anterior lumbar apparent number was compared with total apparent number (figure 2). The null hypoth-
esis that the former was independent of the latter could not be rejected on the basis of these data. If the number of experimental animals had been much larger, it might have been possible to reject the null hypothesis. However, any conclusion formed on this basis would then only hold true (at any detectable level) if applied to similarly large numbers of animals. The purpose of the present study was to identify anatomical relationships of sufficient magnitude and practical importance to be readily identified in a small group of animals. Inability to reject the null hypothesis implied that animals with a large anterior lumbar apparent number of myofibers possessed this feature because they had a large fraction of their total apparent number appearing in the anterior lumbar region and not because they had a greater total apparent number of myofibers. This implication was tested by comparing the anterior lumbar apparent number with the fraction of the total apparent number that appeared in the anterior lumbar region (figure 5). The null hypothesis that the latter was independent of the former was rejected (P<.005).

![Figure 5](image)

Figure 5. Data of figure 2 arranged to show that the anterior lumbar apparent number is determined by the fraction of the total apparent number of myofibers that appear in the anterior lumbar sections.

The objective of the research was to determine whether the fascicular geometry within the longissimus muscle was constant (apparent/real fraction constant) or whether it was variable (apparent/real fraction variable). The total apparent number was used as a minimum estimate of the real number. The validity of this assumption rests on the following argument. There are two variables which determine the apparent/real fraction, length of intrafascicularly terminating myofibers compared to fascicular length and fascicular arrangement. To substantiate the hypothesis that the apparent/real fraction resembles the apparent/total apparent fraction and is variable rather than constant it is necessary to consider the feasibility of the antithesis, i.e., that although the apparent/total apparent fraction is variable (proven), the apparent/real fraction is still constant. The only anatomical basis for the antithesis is that animals with a high apparent/total apparent fraction might have shorter intrafascicularly terminating myofibers relative to fascicular length in thoracic, posterior lumbar and sacral parts of the longissimus muscle and vice versa. The existance of an intrafascicular growth process capable of compensating accurately and completely for variation in the apparent/total apparent fraction is extremely unlikely, especially since increases in the apparent/real fraction due to growth of intrafascicularly terminating myofibers have only been identified in, and are probably restricted to fetal and neonatal animals (Swatland, 1973, 1975a). The occurrence, in adult animals, of decreases in the apparent/real fraction due to slow growth or terminal degeneration of intrafascicularly terminating myofibers has been postulated (Swatland and Cassens, 1972) but not yet proven. At the present time, therefore, the antithetical hypothesis that the apparent/real fraction is constant appears to be extremely unlikely.

It has been suggested that the meat yield of animals could be improved by selectively breeding animals with an increased real number of myofibers in their muscles (Stickland and Goldspink, 1973). Since the apparent/real fraction varies between animals, a genetically produced increase in the real number of myofibers will not always or automatically result in an increase in the apparent number. Since it is the apparent number of myofibers not the real number of myofibers which ends up in a pork chop on the dinner plate, evaluation of progeny for anterior lumbar apparent number (Staun,
Figure 6. Repeat of figure 1 showing how an increase in length of fasciculi (stippled area, lower diagram) would increase the anterior lumbar apparent number of myofibers (stippled area, top diagram) as well as adding weight to the muscle.

1963, 1972) might still be the best basis for selection despite the economic limitations of progeny testing.

One of the first agricultural researchers to recognize the problems caused by intrafascicular anatomy and fascicular arrangement was Meara (1947). Meara showed that the rabbit psoas grew in length by increments to fascicular length while lengthwise growth of the rabbit gastrocnemius (after 2 months postnatal-ly) was due to an increase in the diameter of fasciculi inserted at an angle to the muscle-tendon axis. In the porcine longissimus muscle, variability of the apparent/real fraction indicates that the anterior lumbar apparent number is also affected by fascicular arrangement. Growth of the longissimus muscle is to some extent dependent on the growth and anatomy of the axial skeleton but considerable independence is allowed by the fact that, dorso-laterally, fasciculi insert into an aponeurosis. For example, in an animal with long fasciculi, the fasciculi would extend posteriorly to overlap (dorso-laterally) the more posterior fasciculi. The degree of overlap would increase the longissimus muscle CSA and the apparent number of myofibers (figure 6). Since the increased fascicular length would add weight to the muscle mass as well as increasing the apparent number of myofibers, variation in fascicular length might be the shared variable resulting in the correlation between muscle weight and anterior lumbar apparent number (figure 3). Further research is needed to test this hypothesis and to explore other effects that may result from variation in the apparent/real fraction.

LITERATURE CITED


