Muscle Fiber Count Per Tissue Slice By Photomicrographic and Coulter Counter Techniques

J. J. Guenther and R. V. Felber

Story in Brief

Fiber numbers per tissue slice were determined on the longissimus dorsi, sartorius, semitendinosus and triceps brachii muscles taken from the left and right sides of 5 fifteen-day-old dairy calves at three locations on the long axis of the muscles. The number of fibers was determined by a photomicrographic and by a coulter counter enumeration technique developed for this experiment. No significant differences in fiber number were observed between sides by either counting method. Significant location differences in fiber count per tissue slice ($P<.05$) were noted in the longissimus dorsi and semitendinosus muscles by the photomicrographic method, but not by the coulter counter procedure. The photomicrographic technique gave consistently higher fiber counts than the coulter counter procedure, but results from both methods ranked the test muscles in the same order. Correlation coefficients between the two procedures were significant for fiber count in the longissimus dorsi and semitendinosus, but not for the triceps brachii and sartorius muscles. Both fiber enumeration techniques were sensitive in ranking the test calves according to their expected number of muscle fibers; however, the coulter counter procedure affords rapid, automated counting of many samples and eliminates the human fatigue factor inherent in the microscopic procedures.

Introduction

The number of muscle fibers comprising a muscle is believed to be genetically determined and firmly established at birth or shortly thereafter in cattle. Subsequent increases in muscle size during pre- and post-weaning development are due to the enlarging or growth of individual muscle fibers. Hence, if a procedure could be devised to determine the number of muscle fibers contained in a "representative" muscle(s) and this procedure could be imposed on live beef calves in early life, the process of identifying beef animals having the potential for heavy muscling might be accomplished more rapidly and efficiently.

The estimation of fiber numbers in small muscles of laboratory animals has usually been accomplished by microscopic procedures. However, microscopic studies are tedious, time consuming and thus self-limiting when attempt-

ing to estimate fiber numbers in the large muscles of meat animals. In recent years, however, the Coulter Corporation has developed automated equipment which may be used to enumerate particles in suspension.

The objectives of this study were to devise procedures to utilize the Coulter equipment in estimating fiber number in muscles from calves and to assess the relationship between the coulter results and those obtained by a photomicrographic technique developed in our laboratory.

Materials and Methods

The longissimus dorsi (4th rib-3rd L.T.), Sartorius, Semitendinosus and Tricepts brachii (lateral head) muscles taken from the left and right sides of five, fifteen day old dairy calves provided the experimental material for this test. Two Holsteins, one Ayrshire, and two Jersey calves were used. All muscles were removed from the freshly slaughtered calves, packaged in aluminum foil, frozen in liquid nitrogen and stored at-20 C until hisological measurements were obtained.

The muscles were sectioned, transversely, at 25, 50 and 75 percent of their long axis. Cross-sectional area at each location was determined. A core sample, ¼ inch diameter was removed at random from each of the above locations and placed in buffered saline until thaw rigor was completed. The core samples were then positioned on a microtome chuck and re-frozen with Cryokwik. Tissue sections, 20 microns thick, were obtained using a Slee Cryostat. These tissue slices were used for fiber enumeration by the two procedures.

Fiber number by the photomicrographic technique

Tissue slices were attached to glass slides, stained with thionin and covered with a glass coverslip. Photomicrographs of two random microscopic fields were taken for each tissue slice. The micrographs were made at 100 power, with a 1 mm^2 ocular grid located in the focal plane of the microscope. Muscle fibers within 5 × 5 squares of the ocular grid were counted for each field within a tissue slice. Fiber count per field was adjusted to a total count per tissue slice.

Fiber number by the coulter counter techniques

Tissue slices were placed in an accuvette vial filled with buffered saline and disrupted into individual cells by sonification. Three one-half milliliter counts were obtained per tissue slice using a ZBI Coulter Counter. The half-milliliter counts were adjusted to total count per tissue slice.

Results and Discussion

Tables 1 and 2 present the muscle fiber counts per tissue slice obtained on the left and right longissimus dorsi, sartorius, semitendinosus and triceps

Table 1. Influence of side on muscle fiber count per tissue slice in four calf muscles determined by a photomicrographic technique

Side	Muscle			
	Longissimus Dorsi	Sartorius	Semitendinosus	Triceps Brachii
Left	95.7[1]	62.2	66.9	62.2
Right	86.3	64.4	69.3	65.4
Average	91.0	63.3	68.1	63.8

[1]Mean fiber count in thousands.

Table 2. Influence of side on muscle fiber count per tissue slice in four calf muscles determined by a coulter counter technique

Side	Muscle			
	Longissimus Dorsi	Sartorius	Semitendinosus	Triceps Brachii
Left	71.6[1]	48.5	52.5	48.9
Right	67.4	46.9	54.2	48.0
Average	69.5	47.7	53.5	48.5

[1]Mean fiber count in thousands.

Table 3. Influence of location on muscle fiber count per tissue slice in four calf muscles determined by a photomicrographic technique

Location	Muscle			
	Longissimus Dorsi	Sartorius	Semitendinosus	Triceps Brachii
25%[1]	100.6[2]	62.5	68.8	63.4
50%	84.8	64.4	61.9	63.6
75%	87.5	63.0	73.5	64.3

[1]Percent of the long axis of the muscle.
[2]Mean fiber count in thousands.

brachii muscles by the photomicrographic and coulter counter procedures. The photomicrographic enumeration technique yielded consistently higher fiber counts than the coulter counter technique. No statistically significant difference in fiber count was noted between the left and right sides for any of the test muscles by either counting procedure. Moreover the test muscles were ranked longissimus dorsi>semitendinosus>triceps brachii>sartorius in fiber number by both enumeration methods. The average count was 91.0, 68.1, 63.8, and 63.3 thousand muscle fibers per tissue slice for the longissimus dorsi, semitendinosus, triceps brachii and sartorius muscles, respectively, by the photomicrographic method and 69.5, 53.5, 48.5 and 47.7 thousand fibers for these muscles, respectively, by the Coulter counter method.

Tables 3 and 4 show the number of muscle fibers per tissue slice at the three test locations along the muscles obtained by the two enumeration procedures. Significant location differences in fiber count per tissue slice ($P<.05$) were noted in the longissimus dorsi and semitendinosus muscles by

Table 4. Influence of location on muscle fiber count per tissue slice in four calf muscles determined by a coulter counter technique

Location	Muscle			
	Longissimus Dorsi	Sartorius	Semitendinosus	Triceps Brachii
25⅔[1]	73.3[2]	46.6	55.5	43.9
50%	64.8	49.2	49.5	47.5
75%	70.5	47.2	55.0	48.5

[1]Percent of the long axis of the muscle.
[2]Mean fiber count in thousands.

the photomicrographic method; however, no statistically significant differences, due to location, were observed in tissue slice fiber number with the Coulter counter procedure.

Correlations between the two enumeration methods were significant for the longissimus dorsi ($r=.864$. $P<.01$) and the semitendinosus ($r=.528$. $P<.05$) muscles, but not for the triceps brachii and sartorius muscles.

Animals, though not specifically tested, ranked Holstein>Ayrshire>Jersey in total number of fibers per tissue slice.