COMPARISON OF METHODS FOR MEASUREMENT OF FIBER DIAMETER IN SKELETAL MUSCLE

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INTRODUCTION

Meat scientists have used various methods to measure fiber "width" or "diameter" when studying tenderness characteristics of muscle. Methods used may be divided into two groups: (1) measurement on a longitudinal plane and (2) measurement on cross sections. Because fibers occur in a variety of shapes, values obtained by measurement of width across fibers from a longitudinal plane will depend on the plane on which the muscle was sectioned. Moreover, Swanson et al. (1965) pointed out that deviation from a true cross section will increase the area exposed for measurement. Also, they stated that fibers are not uniformly round, which makes it difficult to select a particular area for measurement. Fedde (1970) found a high correlation between fiber "diameter" obtained by using a polar planimeter to determine the area of a fiber, then converting the area measurement to "diameter" and "diameter" obtained by averaging the largest and smallest "diameter" of a muscle fiber. Since fiber "diameter" is used frequently in meat research as an indication of tenderness, information relative to differences obtained by different methods of measuring "diameter" is needed to help select a method of measuring this characteristic of muscle fibers.

The purposes of this study were to: (1) compare values obtained by three methods of measuring fiber "width" or "diameter"; (2) study differences in fiber "width" or "diameter"

between the anterior and posterior positions of the longissimus dorsi (LD) muscle; and, (3) study the effect of heat on fiber "width" or "diameter".

REVIEW OF LITERATURE

Nature of Skeletal Muscle Tissue

Skeletal muscle constitutes the whole of the muscular apparatus attached to the bones. A muscle has many divisions, the fundamental histological unit being the fiber. Each muscle fiber is composed of many myofibrils. Lowe (1955) stated that the outer membrane of the muscle fiber is known as the sarcolemma. The fibers are arranged parallel to each other and grouped together into bundles called fasciculi. A fine fabric of connective tissue, endomysium, penetrates the fasciculus and gives support to the individual muscle fibers. The connective tissue that surrounds a fasciculus is called the perimysium. A group of fasciculi is surrounded by a thick layer of connective tissue, the epimysium.

The structural arrangement of fibers is responsible for muscle contractile function. Under the light microscope, both longitudinal and cross striations can be seen on a properly fixed and stained fiber. The shape of the fibers of striated muscle has been studied by many workers, who by dissecting individual fibers established that they were elongated elements, more or less tubular, but of varying appearance (Bourne p 25,

1960). Bourne (p 26, 1960) stated that when muscle fibers are sectioned transversely, the shape of their cross section is oval or spherical if cut when fresh, but irregularly polyhydral if cut after fixation. He attributed the difference in shape to shrinkage of the muscle fibers themselves and their surrounding connective tissue during fixation. He also reported the work of Buchthal and Knappeis who measured the diameter of isolated, freely suspended muscle fibers from the semitendinosus muscle of frog in two planes at right angles to each other. They found that the cross section of the fibers was usually somewhat oval.

Fiber "Diameter" as Affected by Selected Parameters

There is a considerable range in the "diameter" of fibers in different voluntary muscles, 10-100 μ commonly being accepted (Bourne p 26, 1960). Many attempts have been made to establish the factors that may be correlated with the varying "diameters" of muscle fibers. Some of the main factors affecting fiber "diameter" are age of the animal, sex, nutrition and exercise (Bourne p 28, 1960).

Age and sex. Hiner et al. (1953), Joubert (1956), Tuma et al. (1962), Carpenter et al. (1963) and Siddiqi (1970) found an increase in muscle fiber "diameter" with age as muscle growth progressed. Also, Bourne (p 28, 1960) reported that there is a gradual increase with increase in age, in absolute size of muscle fibers as measured by their "diameters". In

addition, Lowe and Kastelic (1961) and Doty and Pierce (1961) showed that muscles from young animals had fibers of smaller "diameter" than those from old animals.

Sex of the animal has been shown to affect muscle fiber "diameter". Bourne (p 28, 1960) referred to the work of Bowman in 1840 and Schwalbe and Mayeda in 1891, who found that skeletal muscle of human males had larger fiber "diameter" than that of females. Hammond and Appleton (1932) found that rams had larger fibers than ewes. Patel (1967) measured the fibers from the muscles of ewes, wethers and rams and found that rams had the largest fiber "diameter", then the wethers and the narrowest fibers came from the muscles of ewes. Elliot et al. (1943), however, did not detect any difference in fiber size between male and female rats kept under identical conditions.

Nutrition and exercise. Robertson and Baker (1933) studied the relationship of fiber "diameter" to level of feeding, and they found that fully fed animals had fibers with the largest "diameters"; half fed animals had fibers that were intermediate in "diameter"; and smallest fiber "diameters" were found for those on roughage only.

McMeeken (1941) and Joubert (1956) also reported that a low plane of nutrition produced an atrophy of striated muscle fibers; whereas, a high plane of nutrition resulted in an enlargement of muscle fiber diameter.

Yeates (1964) concluded that there was a decrease in the cross sectional area of muscle in starved cattle, and this was

associated with a reduction in the mean "diameter" of muscle fibers. With regain of live weight, he observed a complete recovery of both the cross sectional area of the muscle and its fiber "diameter".

Evans (1966) found an increase in raw fiber "diameter" with supplementation of 2000 I. U. of Vitamin A followed by a decrease in "diameter" with 4000 I. U. of Vitamin A, and a marked increase with further Vitamin A, but differences were not significant. The effect was not as great with cooked fibers.

According to Hiner et al. (1953), fibers from less active muscles increased in "diameter" more than those from active muscles as the growth of the animal progressed. Bourne (p 28, 1960) stated that muscle fiber increases in "diameter" in response to exercise.

Methods of Measuring Fiber "Diameter"

Meat scientists have used various methods to measure the "diameter" of muscle fibers. Reid and Harrison (1971) described several of those methods, and suggested that variations in results reported by different investigators may be attributed, in part, to variation in methodology.

Farrell and Fedde (1969) placed specimens from chicken muscle on corkboard and froze them in 2-methylbutane cooled to -125°C in liquid nitrogen. Specimens were mounted on a microtome chuck and surrounded by an embedding media (Cryoform). The blocks were sectioned at 10μ with a cryostat microtome at -20°C

and stained with Hematoxylin and Eosin (H & E). Images of transverse sections of H & E stained material were projected to achieve an enlargement suitable for measurement. The outlines of muscle fibers were traced and planimetric measurements were made directly on the tracings to give area in sq. cm. From the cross-sectional area of the fibers, fiber "diameter" was calculated. Gauthier and Padykula (1966) used almost the same procedure.

Doty and Pierce (1961) used transverse sections from the longissimus dorsi and the semitendinosus muscle to determine the size of primary and secondary muscle bundles and individual muscle fibers. At 20X magnification, the boundaries of several secondary muscle bundles were traced on a piece of lens paper spread over ground glass. The number of primary muscle bundles in a representative secondary muscle bundle was determined at a magnification of 64X. The area of a representative bundle was traced and the number of muscle fibers enclosed in it was recorded. The "diameter" of individual muscle fibers was determined by using an eye piece micrometer and by counting the number of muscle fibers in a given distance in longitudinal sections. This latter method gave low values, because not all fibers were cut at their "diameter". The calculated "diameter" from cross-sectional area measurements gave high results because the area occupied by the endomysium within a primary muscle bundle was not subtracted. For this reason, the mean

value from the two methods of calculation was reported as the final value.

Evans (1966) measured "diameter" of fibers teased from a core of muscle in distilled water. A drop of the suspension was placed on a glass slide and examined under a light microscope. Measurements were made in microns with an ocular micrometer in the eye piece of the microscope.

Tuma et al. (1962) blended muscle samples with 50 ml physiological saline solution for 30 seconds in a Waring blender with the blades reversed. Part of the blended material was poured into a petri dish, and fibers were measured by using an ocular micrometer in the eye piece of a phase contrast microscope at 100X magnification.

A thin slice of raw muscle tissue fixed in 10% formalin was used by Hiner et al. (1953) to determine fiber "diameter". Thin sections of muscle tissue were sliced from the fixed sample and floated in clear beef blood serum on a clean glass slide. With the aid of a dissecting microscope and a pair of dissecting needles, 30 to 40 fibers were teased from the slice of muscle. The average "diameter" of fibers was determined by measuring 12 muscle fibers in three locations. Any fiber that appeared to be split or damaged was avoided as well as the ends of fibers that might be pointed or flat. The 36 measurements were averaged and the average considered the "diameter" of fibers in that muscle.

Lowe and Kastelic (1961) took samples near the surface of both raw and cooked muscles and preserved them in a 10% formalin and physiological saline solution. Longitudinal sections were prepared on a freezing microtome, mounted on slides and stained. Fiber "diameter" was estimated using a 10X eye piece and a 43X objective. An area was located in the microscopic field of the sections in which the fibers lay side by side and the number of fibers in the full microscopic field was counted; the larger the number of fibers in the field, the smaller the fiber "diameter".

Ely (1967), Norris (1968), Patel (1967) and Reid and Harrison (1971) measured the "width" of fibers in longitudinal sections with an ocular micrometer in the eye piece of the microscope.

Brady (1937) stained sections about 25μ thick with Sudan IV and counterstained them in Harris Hematoxylin. The "diameter" of 50 fibers from each muscle was measured with a filar micrometer after microdissection.

Changes in Fiber "Diameter" with Heating

Satorius and Child (1938) studied the effect of heat on beef muscle fiber "diameter" and found that the fiber "diameter" decreased 12 to 16% during heating of the muscle to 58°C, and continued to decrease up to 67°C, but there was no difference in fiber "diameter" between 67°C and 75°C.

Hostetler and Landmann (1968) found a gradual, but small, decrease in "width" of bovine longissimus dorsi muscle fiber

fragments heated to between 45° and 62°C. The process appeared to be completed at 62°C, since little further decrease in width was noted with increased temperature. Ramsbottom et al. (1945) stated that heat denaturation and coagulation of beef fiber proteins cooked for short periods was accompanied by shrinkage and hardening of the fibers.

Relationship of Fiber "Diameter" to Tenderness

While a complex of factors is known to influence tenderness (Harrison et al., 1959; Palmer, 1963), the "diameter" of muscle fibers is regarded as being partly responsible for affecting this characteristic of meat (Hiner et al., 1953). The work of Moran and Smith (1929) as reported by Ramsbottom et al. (1945) first indicated that a small muscle fiber "diameter" and small primary and secondary bundles were associated with tender texture. Brady (1937) reported that muscle fiber bundle size is related to texture; the larger the bundle, the finer the texture, and the more tender the meat. Hiner (1953) studied the relationship of fiber "diameter" in beef muscle to tenderness. Regression analysis showed the relationship between tenderness and fiber diameter was curvilinear; the curvilinear correlation being 0.83. Tuma et al. (1962) studied the relationship of fiber diameter to tenderness as influenced by age of animal, and found that with increasing animal age fiber diameter increased, and tenderness of muscle decreased. Several other authors also reported that muscle

fiber "diameter" increased with increase in animal age (Bourne, p 28, 1960; Lowe and Kastelic, 1961; Hiner, 1953; Carpenter, et al. 1963).

EXPERIMENTAL METHODS

Muscle Used and Sampling Plan

A beef longissimus dorsi (LD) rib section was purchased from the Department of Animal Science and Industry, Kansas State University, Manhattan. The plan for sampling the muscle is presented in Fig. 1. The LD (left) was divided into two pieces, the posterior (X) and the anterior (Y). Each of those two pieces was divided into two portions, the left portion of both posterior and anterior pieces was used for raw samples, and the right portion of both pieces was cooked. Positions within raw portions were named Xr1, Xr2 to Xr15, and positions within cooked portions were named in the same way; i.e., Xc1, Xc2 to Xc15. The same plan was used to name the positions within the Y piece. Samples for measurement of fiber "diameter" or "width" were selected at random at various positions within each portion of the muscle. Cooking was done to an internal temperature of 70°C by oven roasting at 300°F. Two methods (A & B) of measuring the "width" of fibers in a longitudinal plane were used. One method (C) of measuring the "diameter" of fibers in cross sections was used. Details of all three methods are given in the Appendix, pp 32-40.

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Figure 1. Plan for sampling longissimus dorsi muscle

A, B "width" of 25 fibers measured at each sampling position by method A or B

C "diameter" of 25 fibers measured at each sampling position by method C

r raw samples

c cooked samples

X posterior piece

Y anterior piece

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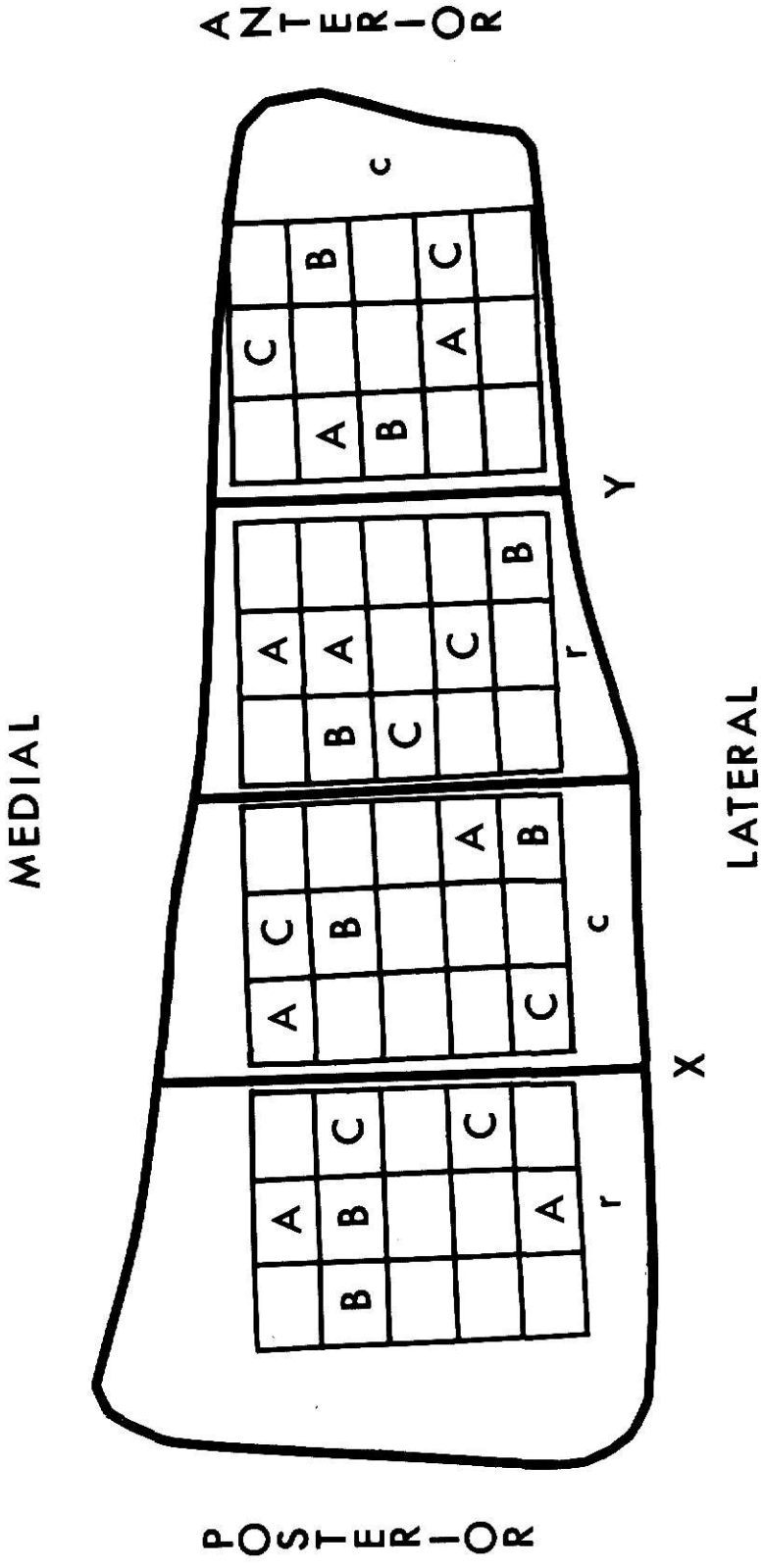
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Measurement of Fibers in a Longitudinal Plane

Method A (Tuma et al., 1962). Samples (approximately 1½ X 1½ X 2 cm) were fixed in 10% formalin and physiological saline solution for 48 hours, then sliced about 1/8 inch from the end of each sample. The slices were placed in a Waring blender with the blades reversed and blended with 50 ml physiological saline solution at low speed (about 50 on a rheostat) for 30 seconds. Part of the blended material was poured into a clean petri dish and observed through a phase contrast microscope at 100X magnification and the "width" of 50 randomly selected fibers was measured using an ocular micrometer in the eye piece of the microscope as described in the Appendix, p 32.

Method B (Food Research Laboratory, K.S.U). Samples (approximately 1½ X 1½ X 2 cm) were fixed in 10% formalin and physiological saline solution for approximately 2 to 3 weeks. Specimens (approximately 1 X 1 X ½ cm) were cut parallel to the muscle fibers. A small amount of Cryoform, an embedding matrix, was placed on the tissue holder of a cryostat microtome, and the specimen was placed on the Cryoform. The tissue holder was inserted into the microtome, and the specimen was frozen rapidly with Cryokwik, a commercial preparation of freon gas. Sections were cut 10μ thick and transferred immediately, by means of a small camel hair brush, to a glass slide containing a drop of albumin-water mixture. The glass slide was warmed at low temperature (approximately 90° - 100°F) to evaporate the albumin

water mixture. The section was then stained with picric acid and picro-ponceau stain, cleared in xylene and mounted with permount. Muscle fibers stained yellow and connective tissue stained pinkish red. The slides were evaluated by three persons using a Bausch and Lomb Dynazoom Microscope. Fiber "width" was measured with an ocular micrometer in the eye piece of the microscope as described by Norris (1968; Appendix, p 34) using 430X magnification.

Measurement of Fibers in Cross Sections

Method C (Farrell and Fedde, 1969). Specimens, approximately ½ X ½ X ½ cm, were cut with a sharp razor blade and placed on a corkboard (about 1½ X 1½ cm) that was soaked in 10% formalin and physiological saline solution. Specimens were frozen by holding the corkboard square containing the specimen in 2-methylbutane cooled to -125°C in liquid nitrogen. The frozen specimen was heat sealed in plastic tubing and kept on dry ice until sectioned (approximately three weeks). At the time of sectioning, the specimen (on the corkboard) was secured to the microtome platform with Cryoform. The specimen cube was trimmed so that the top was slightly smaller than the bottom and oriented on the platform so that the cross section could be cut with a sharp razor blade in the microtome knife holder. Sections were cut 10 u thick using the CTD International Harris Cryostat Microtome set at -20°C. The section was transferred to a glass slide by lightly touching the slide to the section while it

was still on the knife blade. About 10 slides were prepared from each specimen and stained with Hematoxylin and Eosin stain (Appendix, p 40). A microprojector was calibrated to determine magnification by projecting a 2-mm micrometer on the wall and measuring the projected line. Slides were projected and the areas of 25 muscle fibers per specimen at each sampling position (total of 50 fibers) were traced onto plain white paper. Using a polar planimeter, the area of each fiber was determined in sq cm, and recorded on a score sheet (Appendix, p 38). Assuming the fibers were round, the area in sq cm was converted to diameter by the formula:

$$D = 2\sqrt{A/\pi}$$

Diameter was converted to microns, and the necessary correction for magnification was made by dividing the diameter, in microns, by its magnification.

Statistical Analyses

Bartlett's test for homogeneity of variance was applied to the data to test the significance of differences among the observations within each method of measuring fiber "width" or "diameter". Bartlett's test also was used to estimate variance among the three methods, and the coefficient of variation was calculated for each method by position (anterior or posterior) and treatment (raw or cooked tissue). Data were analyzed by

analysis of variance to determine the significance of differences in values for fiber "width" or "diameter" obtained by the three methods of measurement.

RESULTS AND DISCUSSION

Effect of Method of Measurement on Fiber "Width" or "Diameter"

Values in the literature for mean fiber "width" or "diameter" of fibers in a given muscle vary greatly. Reid and Harrison (1971) postulated that the variation could be attributed, partially, to method of measurement. This study measured fiber "width" or "diameter" of bovine LD by three methods, and estimated the variance among observations within each method and variances among the three methods.

Mean Values. Means (Table 1) show wide differences among values for fiber "width" or "diameter" obtained by the three methods of measurement. For raw fibers, the difference between method A and B was 31.74μ; the difference between method A and C was 13.51μ; and the difference between methods B and C was 18.23μ. For cooked fibers, the difference between methods A and B was 18.20μ; the difference between A and C was 13.25μ; whereas, the difference between methods B and C was only 4.95μ.

When values for methods A and B are compared, the difference between the two methods was approximately 57% greater for raw than for cooked fibers. When values for methods A and C are compared, the difference between the two methods was

Table 1. Effect of method of measurement on fiber "width" or "diameter" of raw and cooked bovine longissimus dorsi muscle.

Method	Mean, μ	Range, μ	
		Low	High
A			
Raw	67.34	38.23 (65.39)[a]	103.62
Cooked	51.39	27.66 (54.33)[a]	81.99
Change with heating	-15.95 (-23.7%)		
B			
Raw	35.60	24.08 (25.63)[a]	49.71
Cooked	33.19	23.30 (20.19)[a]	43.49
Change with heating	-2.41 (-6.8%)		
C			
Raw	53.83	33.87 (42.43)[a]	76.30
Cooked	38.14	19.00 (37.47)[a]	56.47
Change with heating	-14.69 (-29.1%)		

A, Tuma et al. (1962) - "Width" of separated fibers measured from a longitudinal plane.

B, FN - KSU - "width" of fibers measured from longitudinal sections.

C, Farrell and Fedde (1969) - "diameter" of fibers calculated from the area of fibers measured from cross sections.

[a] Difference between low and high values.

the same for raw and cooked fibers. The difference between methods B and C was approximately 21% greater for raw than for cooked fibers.

For both raw and cooked fibers, the widest range of fiber "width" occurred with method A (Table 1). Although range can be used as a "rough and ready" measure of variability, it is not generally a satisfactory measure of variation. Variance, and not range, reveals the manner in which the bulk of observations are dispersed within the interval bounded by the smallest and largest values (Huntsberger, 1961).

Variance within methods. Bartlett's test for homogeneity of variance indicated that differences among observations within methods B and C were not significant; whereas, the variance among observations within method A was significant ($P < 0.05$; Appendix, p 43). Therefore, values for fiber "width" or "diameter" obtained by method B and method C were precise, and either would be acceptable to study effect of treatment on fiber "width" or "diameter". Both methods B and C would be preferable to method A, which was less precise. Also, variance among the three persons who measured fibers by method B was not significant (Appendix, p 44). This indicates that only one person is needed to measure fibers by this method.

Change with heating. Mean muscle fiber "width" or "diameter" for heat treated samples were smaller than those for raw fibers (Table 1). Change with heating in method A was -23.7%.

Data from studies reported in the literature, in which "width" of separated fibers was measured by a technique similar to that of method A, varied in percentage change in "width" with heating. Similar to this study, Hostetler and Landmann (1968) observed decreases of 23 to 27% in "width" of LD muscle fiber fragments heated on a microscope stage at 53°, 61°, 69° and 77°C. Evans (1966) and Satorius and Child (1938) reported smaller similar decreases in fiber "width" when muscle was heated to 58°C (12.3%, Satorius and Child) or to 65°C (11.9%, Evans). However, Satorius et al. (1938) reported further decrease in fiber "width" between 58° and 67°C.

In this study, muscle fiber "widths" measured by method B decreased only 6.8% with heating. Reid and Harrison (1971) found a decrease of 8.4 to 11.4% in mean muscle fiber "width" when bovine semimembranosus muscle was heated to 70°C. Patel (1967) measured the "width" of fibers in ovine LD muscle by method B. She found a decrease in mean fiber "width" of 5.2% for ewes; 6.5% for wethers and 6.6% for rams when muscle was heated to 75°C.

In this study fibers measured by method B showed 6.8% decrease in mean "width" between raw and cooked fibers, whereas by method A and C the percentage decrease was 23.6% and 29.1%, respectively. The small decrease by method B may be attributed, partially, to the fact that the sections of muscle on the slides were warmed to evaporate the albumin-water mixture used to make the sections adhere to the slides. The approximate temperature

of the slides ranged from 90° to 100°F. Therefore, it is possible that raw fibers decreased in width during the warming process; whereas, cooked fibers were not affected much. However, in other studies using method B (Patel, 1967; Reid and Harrison, 1971), in which slides were not warmed, results similar to those of this study were obtained.

When fibers were measured by method C, there was a decrease of 29.1% in mean fiber "diameter" when the raw samples were heated to 70°C. Reid (1971) used method C to study the effect of end point temperature on muscle fiber "diameter", and found that as the end point temperature increased from 25° to 75°C, mean fiber "diameter" decreased from 47.6μ to 32.93μ. The greatest decrease in "diameter" occurred between 25° and 45°C (15.1%). Between 45° and 55°C there was an additional decrease of 9.0% of the original "diameter". Continued decrease in fiber "diameter" during heating from 55° to 65°C and from 65° to 75°C (3.3 and 3.5%, respectively) resulted in an over-all decrease of 30.9%.

Effect of Position of Sample on Fiber "Width" or "Diameter"

Mean fiber "width" or "diameter" of fibers in raw samples from the posterior position was higher than that for the anterior position when measured by all three methods. The opposite was true for cooked fibers; i.e., regardless of the method of measurement, the mean fiber "width" or "diameter" was higher

in cooked samples from the anterior position than the mean "width" or "diameter" in cooked samples from the posterior position (Table 2).

In method A, mean fiber "width" for the raw posterior samples was 11.1% greater than the raw samples taken from anterior position. However, for cooked samples, mean fiber width for the anterior position was greater than posterior samples by only 1.1%.

When raw fibers were measured by method B, mean width for the posterior position was greater than for the anterior position by 1.9%. For cooked samples, mean fiber width was greater in samples taken from the anterior position than the posterior position by 6.5%.

In method C, mean fiber width for raw samples taken from the posterior position was higher than the anterior position by 8.7%, but for cooked samples, mean fiber width was greater in the anterior position than in posterior position by 17.7%.

The coefficient of variation (standard deviation/mean X 100) was used to study the precision with which treatment effects could be estimated. When the coefficients of variation for different methods, positions and treatments are compared, they varied only by 6.0%. The lowest coefficient of variation was 15.2% for the raw fibers taken from the posterior position and measured by method B. The highest was 21.2% for raw fibers taken from anterior position and measured by method A (Table 2).

Table 2. Effect of position of sample on mean fiber "width" or "diameter" of raw and cooked bovine longissimus dorsi muscle.

Method	Treatment	Position	Mean, u		Coefficient of variation, %
A					
	Raw				
		Anterior	63.81		17.2
		Posterior	70.88		19.5
		Difference	7.07	(11.1%)	
	Cooked				
		Anterior	51.67		21.2
		Posterior	51.11		18.2
		Difference	0.56	(1.1%)	
B					
	Raw				
		Anterior	35.26		17.4
		Posterior	35.94		15.2
		Difference	0.68	(1.9%)	
	Cooked				
		Anterior	34.31		17.0
		Posterior	32.08		18.0
		Difference	2.23	(6.5%)	
C					
	Raw				
		Anterior	51.58		19.5
		Posterior	56.09		20.6
		Difference	4.51	(8.7%)	
	Cooked				
		Anterior	41.85		19.0
		Posterior	34.42		20.2
		Difference	7.43	(17.7%)	

A, Tuma et al. (1962) - "Width" of separated fibers measured from a longitudinal plane.

B, FN - KSU - "Width" of fibers measured from longitudinal sections.

C, Farrell and Fedde (1969) - "Diameter" of fibers calculated from the area of fibers measured from cross sections.

Samples with least coefficient of variation have the lowest variance relative to their mean values.

Variance Among Methods

Bartlett's test for homogeneity of variance showed that the estimates of variance among methods for measurement of muscle fiber "width" or "diameter" by position (anterior or posterior) and treatment (raw or cooked) can be placed into four groups (Table 3). Variances within a group are not significantly different from one another, but variances are significantly ($P < 0.05$) different among the four groups.

All position-treatment variances for method A were greater ($P < 0.05$) than all variances for method B. All but one of the position-treatment variances for method A were greater ($P < 0.05$) than two of the variances for C, but did not differ significantly from two variances for C.

Results of this study do not indicate which method gives values most nearly correct for "width" or "diameter" of fibers in bovine LD muscle. Variances within each method and among the three methods indicate degree of precision only. Methods B and C were more precise than method A, and either B or C would be acceptable and superior to A for measuring treatment effects.

SUMMARY

A beef longissimus dorsi was used to compare three methods for measurement of muscle fiber "width" or "diameter". The LD

Table 3. Variance among methods for measurement of fiber "width" of "diameter" by position and treatment of LD muscle.

Group and	Variance[a]	Method	Position	Treatment
I	191.1	A	Posterior	Raw
II	127.1	A	Anterior	Cooked
	121.1	A	Anterior	Raw
III	86.7	A	Posterior	Cooked
	80.8	C	Anterior	Raw
	73.5	C	Posterior	Raw
IV	55.3	B	Posterior	Raw
	50.8	C	Anterior	Cooked
	47.6	B	Anterior	Raw
	42.8	B	Anterior	Cooked
	42.2	B	Posterior	Cooked
	38.7	C	Posterior	Cooked

[a] Variances within a group are not significantly different from one another.

left rib portion was divided into two pieces, the posterior (X) and the anterior (Y). Each piece was divided into two portions; the left portion of both pieces was raw, and the right portion was cooked by dry heat to an internal temperature of 70°C at 300°F. "Width" or "diameter" of fibers from samples taken from each position and treatment were measured by three methods: A, "width" of separated fibers measured from a longitudinal plane; B, "width" of fibers measured from longitudinal sections; and C, "diameter" of fibers calculated from the area of fibers measured from cross sections.

Mean muscle fiber "width" or "diameter" for raw samples was 67.34, 35.60 and 53.83 μ for methods A, B and C, respectively. For cooked samples "width" or "diameter" was 51.39, 33.19 and 38.14 μ for methods A, B and C, respectively.

For all samples, the mean fiber "width" or "diameter" decreased on heating. Mean fiber "width" or "diameter" for raw samples from the posterior positions was higher than that for the anterior position when measured by all three methods. The opposite was true for cooked fibers; i.e., regardless of the method of measurement, mean fiber "width" or "diameter" was higher in cooked samples from the anterior position than the mean "width" or "diameter" in cooked samples from the posterior position.

Bartlett's test for homogeneity of variance indicated that differences among observations obtained by methods B and C

were not significant, but differences among observations obtained by method A were significant ($P < 0.05$). The coefficient of variance (standard-deviation/mean X 100) was least (15.2%) for the raw fibers taken from the posterior position and measured by method B. The highest coefficient of variance was 21.2% for raw fibers taken from the anterior position and measured by method A.

Bartlett's test for homogeneity of variance also was applied to the data to study differences among observations, in terms of position and treatment obtained by the three methods. In general, variances for method A were largest, and significantly ($P < 0.05$) different from those for methods B and C; whereas, variances for methods B and C were similar. Hence methods B and C are more precise than and preferable to method A for measuring treatment effects.

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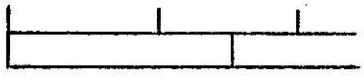
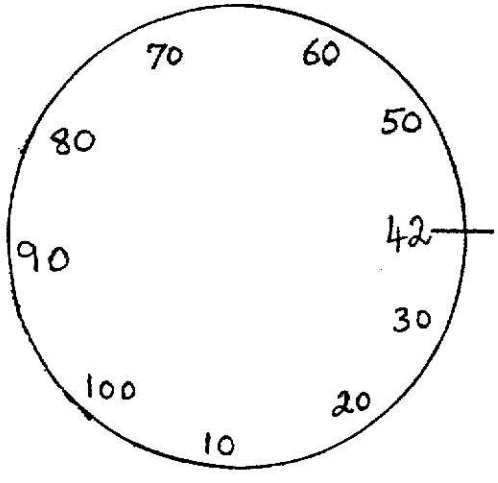
APPENDIX

CALIBRATION BY MICROMETER FOR MEASUREMENT OF FIBER WIDTH (METHOD A)

In the light microscope used, the ocular micrometer is fixed in the eye piece. This ocular micrometer has divisions that are engraved on it. A circular disc with 100 divisions at the side of microscope turns the scale on the ocular micrometer. One complete round of the circular disc (100 divisions) is equal to one division on the ocular micrometer.

Example: 

"Width"of the fibers in this example is between the first and second division on the ocular micrometer. Since one division on the ocular micrometer is equal to 100 divisions on the disc, the fiber "width" is somewhere between 100 and 200 divisions on the disc. To be exact, it is 100 plus the division on the disc, which is 42; so 100 + 42 = 142 divisions.



To determine the value of 142 divisions in microns, a stage micrometer is used that has one major division of 0.1 mm and one small division = 0.01 mm.

0.1 mm = 100 μ

One major division of stage micrometer (0.1 mm) = two divisions of ocular micrometer

so 100 μ of stage micrometer = 200 division of the ocular micrometer (approximately)

Take 10 readings of 100 μ (1 division or 0.1 mm) of stage micrometer and note if they are exactly 200 divisions or vary little. Take the mean of these 10 readings; suppose it is 198.6 divisions, the 198.6 divisions on the ocular micrometer = one division on the stage micrometer (100 μ).

$$\text{So 1 ocular division} = \frac{100}{198.6} = 0.503.$$

Fiber "width" in the example was 142 divisions.

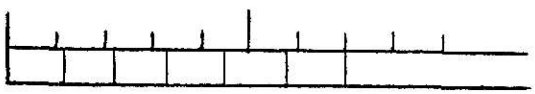
1 division = 0.503 μ

142 divisions = 0.503 X 142 = 71.42 μ

MICROSCOPIC MEASUREMENT OF MUSCLE FIBER "WIDTH" (METHOD B; Norris, 1968)

An ocular micrometer is a clear disc on which a tiny scale is engraved. Insert this disc into the eye piece by unscrewing the top lens and inserting the disc onto the shelf within the eyepiece. In order to measure the magnified image, the units on the scale of the ocular micrometer are compared to a stage micrometer, a slide with a measurement line divided into 0.01 mm units. To calibrate the ocular micrometer, insert the stage micrometer on the stage of the microscope under high power (43X objective, 10X eyepiece and Dynazoom setting 1 = 430X magnification). Match a line of the scale on the stage with a line on the squared scale of the ocular micrometer. Count the number of ocular and stage units until another line on the ocular matches a line on the stage micrometer. To find the value of each ocular unit, the distance covered by the stage units is written in its numerical value (each stage unit = 0.01 mm) and divided by the number of ocular units.

Example:



7 stage units
6 ocular units

7 stage units = 7 X 0.01 or 0.07 mm

$\frac{0.07}{6}$ = 0.012 mm/ocular unit or 1 ocular unit = 0.012 mm

Replace the stage micrometer with the slide to be studied. The "width" of the muscle fibers can be obtained by counting

the number of units that correspond to the "width" of a fiber and multiplying the number of units by the size of the unit of measure.

Example:

muscle fiber "width" = 3 ocular units

3 X 0.012 mm = 0.036 mm for that fiber's width

Convert the mm value to μ by multiplying by 1000

0.036 mm X 1000 = 36 μ

Notes. Through the center of the eyepiece, the ocular units are further divided into 5 parts. These may be used in measurements for greater accuracy.

The eyepiece can be turned in the tube, thus turning the ocular scale. In this way, fibers can be measured even though they do not lie in a perfectly vertical or horizontal direction.

Once the ocular micrometer has been set up, it should not be removed. If the disc is removed from the eyepiece, the calibrations for unit determinations need to be repeated for each magnification used as turning the disc changes the calibration readings.

SCORE SHEET FOR MEASUREMENT OF MUSCLE FIBER "WIDTH"
OF BEEF MUSCLE (METHOD A)

Sample code_____

Fiber no	Fiber "width" (μ)	Fiber no	Fiber "width" (μ)
1		26	
2		27	
3		28	
4		29	
5		30	
.		.	
.		.	
.		.	
.		50	

TOTAL ÷ 50 =
Average

SCORE SHEET FOR MEASUREMENT OF MUSCLE FIBER "WIDTH"
OF BEEF MUSCLE (METHOD B)

Sample code_____

Fiber no	Fiber "width" in microns			Average of 3 readings
	Readers			
	1	2	3	
1				
2				
3				
4				
•				
•				
•				
•				
•				
50				

Width of 150 fibers (50 fibers per each of 3 readers) ÷ 150 = mean "width"

SCORE SHEET FOR MEASUREMENT OF MUSCLE FIBER "DIAMETER" OF BEEF MUSCLE (METHOD C)

Sample code_____

Magnification_____

Fiber no	Area in sq cm	"Diameter" in microns[a]
1		
2		
3		
4		
5		
6		
.		
.		
.		
50		

Diameter in μ for 50 fibers ÷ 50 = mean "diameter"

[a]1). "Diameter" in cm was calculated by the following formula

$$D = 2\sqrt{A/\pi}$$

2). D in cm was converted to D in microns (10,000 μ = 1 cm)
3). D in microns was divided by magnification.

STAINING PROCEDURE (METHOD B)

The following staining procedure was used to stain the sections prepared by Method B

1. Rinse in tap water (2 changes)
2. Stain in a saturated solution of picric acid (2 minutes)
3. Rinse in tap water (2 changes)
4. Stain in picro-ponceau (15 seconds)
5. Dip in 70% alcohol
6. Dip in 95% alcohol
7. Dip in absolute alcohol
8. Dip in absolute alcohol + xylene (1:1)
9. Clear in xylene (2 changes)
10. Mount with permount

The formula for preparation of picro-ponceau stain was taken from Humason (1962):

Ponceau S, C.I. 27195, 1% aqueous	10 ml
Picric acid, saturated aqueous	86 ml
Acetic acid, 1% aqueous	4 ml

Muscle fibers stained yellow and connective tissues stained pinkish red.

STAINING PROCEDURE (METHOD C)

The following staining procedure was used to stain the sections prepared by Method C

1. Rinse in tap water (2 changes)
2. Stain in hematoxylin (Paragon PS 1101) for 3 to 4 minutes
3. Rinse in tap water (2 changes)
4. Rinse in 70% alcohol
5. Rinse in 95% alcohol
6. Stain in Eosin (Paragon PS 1201D) for 1 to 2 minutes
7. Rinse in 95% alcohol
8. Dip in 100% alcohol
9. Dip in 100% alcohol + xylene (1:1)
10. Clear in xylene (2 changes)
11. Mount with permount

Fibers stained bluish purple.

Table 4. Muscle Fiber "Widths" and "Diameter" of different position-treatment samples by three methods.

Method A				Method B				Method C			
Raw		Cooked		Raw		Cooked		Raw		Cooked	
Post	Ant	Post	Ant	Post	Ant	Post	Ant	Post	Ant	Post	Ant
93.05	61.37	58.35	27.66	34.17	39.61	35.73	40.39	60.48	41.52	32.83	36.13
93.56	45.77	65.89	41.75	49.71	31.84	34.95	34.17	55.22	48.69	28.12	43.44
97.58	66.90	46.78	48.79	38.83	29.51	34.17	31.84	61.73	51.59	25.42	29.50
85.00	66.40	46.28	41.25	34.95	37.28	38.83	28.74	69.83	50.93	33.87	34.03
60.86	72.94	50.30	41.75	37.28	38.06	36.50	29.51	60.48	47.17	43.23	42.58
103.62	68.91	51.31	46.78	37.28	28.74	26.41	35.51	60.48	50.02	34.92	20.86
59.86	95.57	57.34	44.26	34.95	34.17	35.73	34.17	72.87	48.69	42.37	40.81
90.04	43.76	42.75	50.30	43.49	29.51	27.96	37.28	44.50	53.54	26.79	56.47
97.08	59.35	35.71	53.82	36.50	45.05	34.17	31.07	57.92	39.74	31.73	40.81
95.57	61.87	57.84	63.38	29.51	32.62	31.84	31.07	59.23	42.37	32.83	37.14
59.35	42.76	62.87	36.72	32.62	32.62	37.28	29.51	60.48	51.59	42.37	47.40
65.39	61.87	49.80	42.25	32.62	35.73	43.49	31.07	50.93	45.63	24.00	39.95
62.87	49.80	52.81	44.26	34.95	35.73	34.95	38.06	56.54	47.98	31.73	48.16
61.37	70.92	63.38	41.25	38.83	32.62	36.50	40.39	49.34	58.19	34.92	53.13
63.88	85.01	50.30	52.31	34.17	36.50	31.07	29.51	60.48	50.74	30.55	41.72
62.37	52.82	60.36	66.40	37.28	32.62	34.17	29.51	61.73	45.63	44.03	45.87
71.43	69.92	52.81	58.85	43.49	40.39	31.84	27.96	61.73	43.23	42.38	40.81
66.90	58.35	36.22	40.24	43.49	38.06	29.51	34.17	69.83	47.17	32.83	39.95
73.94	69.41	56.84	53.82	38.06	34.17	35.73	34.95	59.23	45.63	25.41	39.00
57.84	56.34	52.31	72.94	35.73	35.73	27.96	32.62	69.83	38.81	28.12	42.58
55.83	75.95	52.81	81.99	37.28	24.08	28.74	31.07	42.77	51.59	29.36	45.87
65.39	62.87	51.81	56.84	35.73	31.07	26.41	37.28	50.93	58.19	44.04	37.14
56.84	62.87	44.77	36.72	32.62	34.95	35.73	33.40	59.23	33.87	44.04	44.25
52.31	63.38	50.80	62.88	38.83	38.83	33.40	32.62	56.54	39.74	30.55	49.60
55.83	72.43	56.34	46.28	38.06	38.83	34.17	38.06	72.87	52.26	37.91	39.00
87.52	50.30	49.29	39.23	41.94	29.51	35.73	34.17	54.03	61.09	36.05	55.22
68.91	53.32	76.46	40.74	40.39	29.51	33.40	34.17	59.37	54.66	36.05	52.39
64.89	63.38	46.28	55.33	38.06	41.16	31.07	33.40	60.69	62.33	29.43	46.23
61.37	70.92	53.32	62.57	40.39	38.06	33.40	37.28	58.12	40.54	43.33	47.82
69.41	62.87	43.26	54.83	29.51	29.51	31.07	33.40	41.11	50.34	31.80	40.97

Table 4. (Concluded)

Method A				Method B				Method C			
Raw		Cooked		Raw		Cooked		Raw		Cooked	
Post	Ant	Post	Ant	Post	Ant	Post	Ant	Post	Ant	Post	Ant
58.35	63.88	43.76	65.39	34.95	40.39	31.07	34.95	39.17	50.34	30.62	39.03
81.99	64.38	64.38	43.26	27.96	40.39	26.41	32.62	61.94	50.34	29.43	40.97
69.41	38.23	42.25	50.80	27.96	34.17	31.07	40.39	51.04	59.86	42.48	30.24
81.99	55.83	47.28	50.80	32.62	33.40	31.07	40.39	56.74	55.96	29.43	46.23
58.35	65.89	43.26	47.28	34.17	37.28	34.17	32.62	44.65	34.59	35.00	30.24
80.48	67.90	69.91	58.85	35.73	34.95	31.07	34.17	52.57	61.09	38.00	40.97
82.99	66.90	67.90	68.41	29.51	34.95	30.29	43.49	59.37	55.96	33.95	42.77
79.98	60.36	57.34	43.76	34.95	35.73	29.51	40.39	49.51	54.66	33.95	37.02
58.85	69.92	41.25	37.72	24.85	35.73	33.40	41.94	65.56	53.29	44.95	40.97
53.82	72.43	57.84	47.78	31.84	33.40	26.41	31.07	49.51	57.33	30.62	30.24
58.85	64.38	45.27	45.77	30.29	38.83	23.30	40.39	51.04	38.63	41.62	52.39
57.84	70.42	47.78	53.82	39.61	37.28	34.17	34.95	39.17	45.75	33.95	32.66
69.92	56.84	34.71	50.30	45.05	34.17	27.18	28.74	59.37	53.29	28.19	49.34
67.40	78.47	42.76	44.77	37.28	42.72	31.07	31.07	61.94	66.92	32.90	47.82
54.32	78.47	52.81	60.86	41.94	38.06	27.18	32.62	49.51	58.56	35.00	49.34
61.87	70.92	41.25	75.95	33.40	35.73	31.84	34.17	56.74	53.29	44.14	34.95
88.53	81.99	37.22	57.84	31.84	31.07	29.51	34.17	51.04	71.16	19.00	40.97
76.96	60.36	40.74	68.41	32.62	36.50	31.07	35.73	39.17	76.30	38.00	39.03
65.39	53.82	43.76	46.78	35.73	34.95	32.62	32.62	44.65	62.33	30.62	44.50
76.96	50.80	58.85	58.85	34.17	37.28	29.51	32.62	63.19	65.82	38.00	44.50

Method A, Tuma et al. (1962) - "Width" of separated fibers measured from a longitudinal plane.
Method B, FN - KSU - "Width" of fibers measured from longitudinal sections.
Method C, Farrell and Fedde (1969) - "Diameter" of fibers calculated from the area of fibers measured from cross sections.
Post, posterior
Ant, anterior

VARIANCES AMONG OBSERVATIONS (METHOD A)

Position	Treatment	Replication[a]	Variance
Anterior			
	Raw		
		1	147.21
		2	100.04
	Cooked		
		1	154.61
		2	99.92
Posterior			
	Raw		
		1	270.16
		2	115.73
	Cooked		
		1	57.53
		2	116.73

a Replications 1 and 2 represent 25 fibers each from two different locations within same position-treatment.

VARIANCE AMONG OBSERVATIONS (METHOD B)

Position	Treatment	Replication[a]	Person	Variance
Anterior				
	Raw			
		1		
			1	66.96
			2	30.26
			3	36.78
		2		
			1	34.89
			2	27.92
			3	51.53
	Cooked			
		1		
			1	48.35
			2	31.98
			3	43.43
		2		
			1	46.11
			2	25.33
			3	43.94
Posterior				
	Raw			
		1		
			1	45.10
			2	45.93
			3	49.37
		2		
			1	60.30
			2	32.27
			3	61.31
	Cooked			
		1		
			1	25.91
			2	32.88
			3	22.58
		2		
			1	32.71
			2	39.31
			3	71.88

a Replications 1 and 2 represent 25 fibers each from two different locations within same position-treatment.

VARIANCES AMONG OBSERVATIONS (METHOD C)

Position	Treatment	Replication[a]	Variance
Anterior			
	Raw		
		1	93.23
		2	35.08
	Cooked		
		1	52.81
		2	50.53
Posterior			
	Raw		
		1	64.96
		2	62.11
	Cooked		
		1	35.37
		2	43.43

a Replications 1 and 2 represent 25 fibers each from two different locations within same position-treatment.

COMPARISON OF METHODS FOR MEASUREMENT OF FIBER DIAMETER IN SKELETAL MUSCLE

by

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AN ABSTRACT OF A MASTER'S THESIS

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The purposes of this study were to: (1) compare values obtained by three methods of measuring fiber "width" or "diameter", (2) study differences in fiber "width" or "diameter" between the anterior and posterior positions of the longissimus dorsi muscle, and (3) study the effect of heat on fiber "width" or "diameter".

A beef longissimus dorsi left rib portion was divided into two pieces, the posterior (X) and the anterior (Y). Each piece was divided into two portions; the left portion of both pieces was raw, and the right portion was cooked by dry heat to an internal temperature of 70°C at 300°F. "Width" or "diameter" of fibers from samples taken from each position and treatment were measured by three methods: A, "width" of separated fibers measured from a longitudinal plane; B, "width" of fibers measured from longitudinal sections; and C, "diameter" of fibers calculated from the area of fibers measured from cross sections.

Mean muscle fiber "width" or "diameter" for raw samples was 67.34, 35.60 and 53.83 μ for methods A, B and C, respectively. For cooked samples "width" or "diameter" was 51.39, 33.19 and 38.14 μ for methods A, B and C, respectively.

For all samples, the mean fiber "width" or "diameter" decreased on heating. Mean fiber "width" or "diameter" for raw samples from the posterior positions was higher than that for the anterior position when measured by all three methods. The opposite was true for cooked fibers; i.e., regardless of

the method of measurement, mean fiber "width" or "diameter" was higher in cooked samples from the anterior position than the mean "width" or "diameter" in cooked samples from the posterior position.

Bartlett's test for homogeneity of variance indicated that differences among observations obtained by methods B and C were not significant, but differences among observations obtained by method A were significant ($P < 0.05$).

The coefficient of variance (standard deviation/mean X 100) was least (15.2%) for the raw fibers taken from the posterior position and measured by method B. The highest coefficient of variance was 21.2% for raw fibers taken from the anterior position and measured by method A.

Bartlett's test for homogeneity of variance also was applied to the data to study differences among observations, in terms of position and treatment, obtained by the three methods. In general, variances for method A were largest and significantly ($P < 0.05$) different from those for methods B and C; whereas, variances for B and C were similar. Hence, methods B and C are more precise than and preferable to method A for measuring treatment effects.