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Physical and Chemical Properties of 39 Muscles from the Beef Chuck and Round.

Drew D. Von Seggern
Chris Calkins¹

Variation among the muscles of the beef chuck and round are profound. Physical and chemical properties of these muscles were shown to be affected the most by quality grade.

Summary

Twenty-seven and 12 muscles, respectively, from the chuck and round were analyzed for objective color, expressible moisture, emulsion capacity, pH, total collagen content total heme-iron content, and proximate composition. Observations of these physical and chemical properties showed a vast range of results. The range in data reveal the variation within and among muscles. Knowledge of this variation can lead to proper usage, thereby increasing value of the beef chuck and round. Quality grade had the most pronounced effects, whereas yield grade and weight showed fewer effects on these traits across all 39 muscles.

Introduction

With the increasing popularity of value-added products and the decline in value of the beef chuck and round (20 - 25% over a five-year period), it's necessary to characterize the muscles from these two primals. Muscle has unique physical and chemical properties, which when known and understood can allow for development of value-added products. Information (physical

and chemical properties) of many muscles within the chuck and round was lacking until this study was undertaken. It is also important to describe the effects of quality grade, yield grade, and weight of carcass on these properties. Therefore, the objectives of this study were to determine the physical and chemical properties of 39 muscles from the beef chuck and round and the effects of quality grade, yield grade, and weight on these properties.

Procedure

Ninety-four chucks and 94 rounds were selected at the IBP Inc., Dakota City, Neb. plant based on quality grade (upper 2/3 Choice, low Choice, and Select), yield grade (1, 2, 3, and 4 and 5 together), and carcass weight (550 - 650 lb and 850 - 950 lb). Twenty-seven and 12 muscles, respectively, were dissected from chucks and rounds. Each individual muscle was then trimmed of all external fat and connective tissue. Objective color (L^* , a^* , and b^* values) was observed using a Hunter Lab Mini Scan device with a 1-inch port. Expressible moisture, a method of measuring water holding capacity, was measured as the percentage of moisture lost due to centrifugation. Muscle pH was determined using a pH meter with a spear tip combination electrode. Emulsion capacity, which determines the amount of oil a specific muscle/protein system can bind, was expressed as mL oil bound/2.5g of lean tissue. Total collagen measures the amount of connective tissue within a given muscle. It was quantified by measuring the total content of hydroxyproline in a sample, which is related to collagen. Total collagen was expressed

as mg of collagen/g of lean tissue. Total heme-iron measures the amount of myoglobin and hemoglobin within a given muscle. Acetone and hydrochloric acid were used to separate myoglobin and hemoglobin from the sample. This solution then was read using a spectrophotometer; results were expressed in parts per million (ppm). Proximate composition (fat, moisture and ash) was determined using Soxhlet ether extraction procedures and a LECO Thermo-gravimetric Analyzer (a continuous weighing and heating device). Fat, moisture and ash were expressed as mg/g (%) of lean tissue. Data were analyzed statistically using mixed and least square means procedures.

Results

Across all 39 muscles, variation was evident in all analyzed physical and chemical properties (Tables 1 - 4). Objective color (L^* , a^* , and b^*) values represent a color scale. The higher the L^* (ranging from 0 = black to 100 = white), the lighter the muscle. As a^* (ranging from negative 60 = green to positive 60 = red) increases, the muscle becomes more red. As b^* (ranging from negative 60 = blue to positive 60 = yellow) increases, the muscle becomes more yellow. The overall means and standard deviations observed for L^* , a^* , and b^* were 41.06 ± 4.55 , 29.57 ± 4.05 , and 22.78 ± 4.32 , respectively. The measurement of objective color can be correlated to other chemical properties such as pH and total heme-iron, which together can be related to the shelf-life of a specific muscle and the ultimate color of a processed product.

(Continued on next page)

Table 1. Properties of chuck muscles.

Muscle	Emulsion capacity (mL oil/2.5g)		Expressible Moisture (%)		L* - value		a* - value		b* - value	
	Mean	(s.d.)	Mean	(s.d.)	Mean	(s.d.)	Mean	(s.d.)	Mean	(s.d.)
Biceps brachii	169.71	(14.7)	37.52	(4.22)	38.56	(3.56)	28.65	(3.75)	21.85	(4.32)
Brachiocephalicus omo-transversarius	172.13	(18.1)	37.57	(5.95)	42.66	(2.70)	28.79	(3.27)	21.61	(3.34)
Brachialis	176.61	(20.6)	37.62	(5.41)	38.87	(4.03)	28.08	(3.74)	20.41	(4.28)
Cutaneous omo-brachialis	179.37	(27.3)	34.85	(8.17)	52.08	(5.63)	19.51	(4.54)	14.68	(4.45)
Complexus	175.10	(17.1)	36.34	(5.31)	40.58	(2.47)	31.10	(3.00)	23.69	(3.56)
Deltoides	173.07	(15.1)	38.56	(4.62)	43.80	(3.68)	27.47	(3.33)	20.71	(3.39)
Deep pectoral	175.53	(20.6)	37.46	(4.57)	41.31	(3.08)	29.79	(3.23)	22.56	(3.52)
Dorsalis oblique	175.04	(19.3)	37.59	(4.52)	43.35	(2.64)	30.09	(3.34)	22.79	(3.72)
Infraspinatus	171.45	(15.0)	37.63	(4.70)	38.85	(2.60)	31.25	(3.08)	24.82	(3.64)
Intertransversales	174.26	(14.9)	35.84	(5.24)	39.30	(4.04)	29.98	(3.17)	22.82	(3.71)
Latissimus dorsi	175.02	(19.6)	37.82	(5.15)	41.51	(3.50)	29.07	(4.03)	22.05	(4.35)
Longissimus capitus et Atlantis	175.15	(14.9)	37.19	(5.31)	39.71	(3.83)	29.90	(4.17)	22.63	(4.57)
Longissimus costarum	174.15	(18.3)	34.85	(5.90)	40.01	(4.31)	27.08	(3.77)	19.90	(3.84)
Longissimus dorsi	173.70	(16.8)	37.76	(4.27)	40.55	(3.03)	31.13	(3.46)	23.98	(4.00)
Levatores costarum	174.86	(19.7)	36.94	(5.28)	39.33	(3.87)	29.14	(3.62)	22.34	(3.88)
Multifidus/Spinalis dorsi	168.49	(17.0)	36.38	(5.47)	38.08	(3.34)	30.60	(3.87)	23.20	(4.94)
Rhomboideus	173.67	(16.2)	36.42	(4.77)	41.35	(3.04)	28.43	(3.48)	20.99	(3.90)
Scalenus dorsalis	170.82	(19.7)	37.51	(4.73)	44.61	(3.67)	29.55	(3.72)	21.74	(3.92)
Serratus ventralis	177.25	(16.4)	37.20	(5.72)	39.64	(2.98)	31.42	(2.87)	24.61	(3.49)
Splenius	179.35	(19.6)	36.34	(6.53)	40.49	(2.88)	29.40	(3.78)	21.98	(4.19)
Superficial pectoral	172.12	(15.9)	35.92	(5.52)	44.09	(3.75)	28.10	(3.06)	20.78	(3.07)
Subscapularis	173.06	(14.4)	37.85	(4.64)	38.65	(4.09)	30.30	(2.86)	23.45	(3.46)
Supraspinatus	178.80	(17.9)	38.73	(4.95)	40.82	(3.35)	30.92	(3.03)	23.83	(3.51)
Teres major	178.89	(24.1)	37.12	(4.83)	41.48	(3.75)	29.98	(3.74)	23.02	(4.21)
Tensor fascia antibrachii	173.10	(17.8)	37.92	(4.73)	42.47	(3.17)	28.03	(3.86)	20.40	(4.11)
Trapezius	175.24	(25.0)	35.64	(5.07)	44.89	(4.69)	25.65	(4.64)	18.44	(5.41)
Triceps brachii	169.64	(19.7)	36.41	(20.20)	39.47	(2.80)	31.50	(3.62)	24.78	(4.38)

Table 2. Properties of chuck muscles.

Muscle	Total Collagen (mg/g)		Heme – Iron (ppm)		pH		Fat (mg/g)		Moisture (mg/g)		Ash (mg/g)	
	Mean	(s.d.)	Mean	(s.d.)	Mean	(s.d.)	Mean	(s.d.)	Mean	(s.d.)	Mean	(s.d.)
Biceps brachii	13.14	(7.32)	24.70	(2.88)	5.79	(0.33)	6.79	(2.17)	72.74	(1.66)	1.07	(0.19)
Brachiocephalicus omo-transversarius	11.28	(6.48)	16.86	(3.12)	5.75	(0.31)	6.40	(2.14)	73.10	(1.47)	1.14	(0.18)
Brachialis	11.81	(4.36)	23.84	(2.85)	5.76	(0.30)	4.04	(1.23)	75.41	(1.19)	1.43	(0.22)
Cutaneous omo-brachialis	10.72	(5.68)	15.20	(6.47)	5.81	(0.32)	14.03	(4.19)	64.88	(6.70)	1.04	(0.19)
Complexus	12.59	(4.68)	22.14	(2.13)	5.76	(0.33)	8.37	(1.88)	72.02	(1.48)	1.48	(0.08)
Deltoides	13.57	(8.13)	16.72	(2.09)	5.77	(0.33)	6.45	(1.75)	73.32	(1.71)	1.23	(0.17)
Deep pectoral	10.56	(4.67)	19.90	(3.25)	5.73	(0.32)	5.49	(1.93)	72.66	(1.32)	1.41	(0.23)
Dorsalis oblique	10.13	(3.84)	18.35	(2.18)	5.88	(0.37)	9.07	(2.32)	71.91	(1.89)	1.12	(0.18)
Infraspinatus	8.72	(3.33)	23.55	(2.87)	5.78	(0.32)	9.18	(2.54)	70.81	(2.05)	1.08	(0.11)
Intertransversales	13.82	(5.55)	23.39	(2.92)	5.77	(0.34)	8.56	(2.45)	71.86	(1.96)	1.03	(0.14)
Latissimus dorsi	12.53	(7.67)	18.37	(2.94)	5.74	(0.32)	5.99	(1.51)	72.34	(1.41)	1.23	(0.16)
Longissimus capitus et Atlantis	11.87	(7.62)	21.44	(3.83)	5.79	(0.32)	6.49	(1.92)	73.23	(1.37)	1.07	(0.12)
Longissimus costarum	13.39	(8.19)	23.00	(3.40)	5.86	(0.33)	10.06	(3.48)	69.65	(2.75)	1.08	(0.20)
Longissimus dorsi	14.49	(9.07)	22.02	(4.48)	5.71	(0.27)	7.74	(1.95)	70.52	(1.58)	1.20	(0.14)
Levatores costarum	8.87	(4.40)	21.77	(1.96)	5.86	(0.32)	9.86	(2.45)	70.39	(1.95)	1.09	(0.17)
Multifidus/Spinalis dorsi	16.20	(11.71)	24.93	(2.45)	5.80	(0.41)	14.22	(2.67)	68.04	(2.11)	1.01	(0.21)
Rhomboideus	12.27	(5.09)	20.69	(3.45)	5.82	(0.32)	6.35	(1.93)	72.08	(1.43)	1.38	(0.15)
Scalenus dorsalis	10.06	(4.26)	15.75	(1.99)	5.75	(0.34)	9.11	(3.11)	71.22	(2.53)	0.98	(0.18)
Serratus ventralis	8.78	(3.82)	24.33	(3.13)	5.81	(0.28)	12.21	(3.05)	68.77	(2.43)	1.02	(0.11)
Splenius	11.16	(9.16)	19.37	(3.18)	5.71	(0.29)	4.35	(1.44)	74.43	(1.39)	1.33	(0.24)
Superficial pectoral	8.21	(4.90)	20.15	(4.97)	5.77	(0.27)	10.66	(2.90)	69.79	(2.29)	1.10	(0.09)
Subscapularis	10.64	(6.11)	20.55	(2.39)	5.85	(0.33)	4.60	(1.26)	73.21	(1.19)	1.31	(0.20)
Supraspinatus	17.77	(11.00)	21.47	(3.25)	5.82	(0.32)	4.95	(1.08)	74.29	(0.95)	1.37	(0.15)
Teres major	11.33	(3.74)	19.94	(2.33)	5.72	(0.34)	5.25	(1.29)	73.54	(1.11)	1.23	(0.31)
Tensor fascia antibrachii	9.95	(5.36)	14.74	(2.91)	5.79	(0.37)	4.57	(1.36)	74.08	(1.16)	1.24	(0.15)
Trapezius	8.85	(4.90)	16.03	(3.19)	5.82	(0.33)	8.65	(1.91)	71.62	(2.06)	0.93	(0.11)
Triceps brachii	9.97	(3.84)	21.53	(2.43)	5.78	(0.38)	5.65	(1.55)	73.23	(1.27)	1.44	(0.17)

Table 3. Properties of round muscles.

Muscles	Emulsion capacity (mL oil/2.5g)		Expressible Moisture (%)		L* - value		a* - value		b* - value	
	Mean	(s.d.)	Mean	(s.d.)	Mean	(s.d.)	Mean	(s.d.)	Mean	(s.d.)
Adductor	172.73	(17.9)	38.89	(4.41)	42.32	(4.08)	31.09	(3.71)	25.47	(3.00)
Biceps femoris	171.33	(16.5)	38.27	(6.09)	41.38	(2.78)	32.14	(2.61)	26.55	(2.62)
Gluteus medius	176.76	(21.2)	37.78	(4.72)	44.53	(3.55)	27.74	(4.42)	22.47	(3.14)
Gracilis	175.33	(18.8)	38.52	(4.99)	36.15	(2.92)	30.89	(3.05)	23.48	(3.54)
Pectineus	175.51	(18.8)	38.24	(5.20)	42.10	(4.54)	31.96	(2.14)	25.44	(2.51)
Rectus femoris	174.12	(19.5)	38.12	(4.89)	41.08	(3.01)	30.29	(3.32)	25.16	(2.38)
Sartorius	171.75	(20.0)	39.33	(4.66)	40.79	(3.07)	29.10	(3.13)	21.39	(3.15)
Semimembranosus	178.62	(23.7)	38.68	(4.48)	39.44	(2.96)	32.56	(2.53)	27.00	(2.57)
Semitendinosus	175.29	(17.1)	37.87	(4.11)	44.39	(3.11)	30.06	(2.23)	24.27	(2.11)
Vastus intermedius	173.25	(20.4)	37.84	(5.09)	35.22	(2.99)	30.16	(1.81)	23.32	(2.27)
Vastus lateralis	172.84	(16.1)	39.06	(4.24)	39.45	(2.75)	31.95	(2.35)	25.65	(2.70)
Vastus medialis	169.19	(12.8)	38.50	(4.23)	35.38	(3.53)	31.03	(2.65)	24.26	(3.51)

Table 4. Properties of round muscles.

Muscles	Total Collagen (mg/g)		Heme – Iron (ppm)		pH		Fat (mg/g)		Moisture (mg/g)		Ash (mg/g)	
	Mean	(s.d.)	Mean	(s.d.)	Mean	(s.d.)	Mean	(s.d.)	Mean	(s.d.)	Mean	(s.d.)
Adductor	12.31	(11.89)	22.57	(2.70)	5.76	(0.30)	4.57	(1.21)	72.86	(0.87)	1.49	(0.23)
Biceps femoris	12.36	(8.32)	22.43	(3.48)	1.69	(0.30)	6.86	(1.65)	71.61	(1.29)	1.29	(0.17)
Gluteus medius	11.80	(5.77)	19.76	(2.57)	5.76	(0.34)	5.94	(1.69)	71.44	(1.51)	1.40	(0.16)
Gracilis	15.20	(7.92)	24.31	(4.22)	5.76	(0.31)	3.93	(1.24)	74.78	(1.06)	1.51	(0.17)
Pectineus	12.97	(6.71)	21.28	(2.64)	5.84	(0.30)	3.16	(0.83)	74.45	(0.83)	1.56	(0.18)
Rectus femoris	11.06	(4.23)	19.60	(3.10)	5.72	(0.32)	5.11	(1.79)	73.33	(1.22)	1.50	(0.17)
Sartorius	10.49	(3.39)	19.40	(2.87)	5.75	(0.30)	3.14	(1.29)	74.69	(1.11)	1.54	(0.27)
Semimembranosus	10.40	(4.91)	21.22	(3.29)	5.74	(0.31)	4.36	(1.24)	72.79	(0.78)	1.75	(0.26)
Semitendinosus	11.56	(6.20)	14.65	(2.16)	5.72	(0.30)	4.08	(0.90)	73.27	(0.77)	1.53	(0.15)
Vastus intermedius	9.89	(3.46)	27.27	(2.92)	5.87	(0.40)	8.43	(2.56)	72.91	(1.77)	0.98	(0.11)
Vastus lateralis	12.71	(5.39)	20.29	(3.18)	5.77	(0.30)	4.44	(1.15)	73.54	(0.97)	1.53	(0.26)
Vastus medialis	14.92	(8.75)	25.45	(3.58)	5.78	(0.28)	4.35	(1.27)	75.02	(1.14)	1.47	(0.33)

The mean and standard deviation for expressible moisture was observed to be $37.50 \pm 5.15\%$. Expressible moisture (along with pH) can reveal a good understanding of protein functionality. Knowledge of the amount of moisture lost due to centrifugation allows product developers to use technologies to minimize the loss of moisture (loss of yield and palatability).

The mean and standard deviation of pH was observed to be 5.78 ± 0.32 . Muscle pH as previously mentioned reveals a better understanding of protein functionality. As muscle pH increases, expressible moisture decreases. However, higher pH meat appears to be darker in color (lower L* values) and also tends to have a shorter shelf-life.

The mean and standard deviation for emulsion capacity were observed to be 174.2 ± 18.8 mL oil bound/2.5 g of lean tissue. This property of muscle can also characterize a specific muscle, as higher amounts of oil bound in a protein system can be related to the amount of salt-soluble protein (major binding protein)

within that system. Such information can allow for increased yield and therefore increased profit in the production of sausage-type products.

The mean and standard deviation of total collagen was 11.69 ± 6.54 mg/g of lean tissue. This property of muscle can be related to the tenderness and texture of meat.

The mean and standard deviation for total heme-iron was 20.78 ± 4.43 ppm. Total heme-iron can reveal information about a muscle's physical appearance (appearance to the eye), which is a major factor in consumer acceptance. The concentration of these color pigments is also an important determinant of processed meat color.

Fat, moisture and ash had mean percentages and standard deviations of 6.86 ± 3.45 , 72.28 ± 2.83 , and 1.26 ± 0.28 , respectively.

To envision the variation between these 39 muscles, each muscle was categorized for each trait into three groups — desirable (white), intermediate (gray), or undesirable (black). These charts (Tables 5 and 6) show

specific physical and chemical properties (fat, pH, expressible moisture, emulsion capacity, total heme-iron, and total collagen) which provide a quick, overall picture of a particular muscles' characteristics. This can be useful in selection of candidate muscles for value-added products.

Through investigation of the effects of quality grade, yield grade and weight on the physical and chemical properties, quality grade was the effect that was most frequently significant ($P < .05$). Across all physical and chemical properties, 2 to 31, 1 to 9, and 0 to 8 muscles out of 39 showed an effect due to quality grade, yield grade, and weight, respectively. For muscles with a significant quality grade effect, moisture (19 of 23 muscles) and ash (6 of 15 muscles) decreased while fat (26 of 31 muscles) and pH (7 of 16 muscles) increased with an increase in quality grade. It was also observed that properties showing an increase with an increase in quality grade were fat (26 out of 31 muscles) and pH (7 out of 16 muscles).

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Table 5. Classification of beef chuck muscles by trait.

	Fat	pH	Expressible Moisture	Emulsion Capacity	Heme-Iron	Total Collagen
Biceps brachii						
Brachiocephalicus omot.						
Brachialis						
Cutaneous omo brachialis						
Complexus						
Deep pectoral						
Deltoideus						
Dorsalis oblique						
Infraspinatus						
Intertransversales						
Latissimus dorsi						
Longissimus cap. et Atlantis						
Longissimus costarum						
Longissimus dorsi						
Levatores costarum						
Multifidus and spinalis dorsi						
Rhomboideus						
Scalenus dorsalis						
Serratus ventralis						
Splenius						
Superficial pectoral						
Subscapularis						
Supraspinatus						
Tensor fascia antibroachii						
Teres major						
Trapezius						
Triceps brachii						

The white cells represent fat <5%, pH >5.8, WHC (expressible moisture) <36%, bind >175 mL, heme-iron >25 ppm, collagen <01 mg/g, while the dark gray cells represent fat >10%, pH <5.7, WHC >38%, bind <170 mL, heme-iron <20 ppm, collagen >15 mg/g. The values represented by the light gray cells are intermediate.

Table 6. Classification of beef round muscles by trait.

	Fat	pH	Expressible Moisture	Emulsion Capacity	Heme-Iron	Total Collagen
Adductor						
Biceps femoris						
Gluteus medius						
Gracilis						
Pectineus						
Rectus femoris						
Sartorius						
Semimembranosus						
Semitendinosus						
Vastus intermedius						
Vastus lateralis						
Vastus medialis						

The white cells represent fat <5%, pH >5.8, WHC (expressible moisture) <36%, bind >175 mL, heme-iron >25 ppm, collagen <01 mg/g, while the dark gray cells represent fat >10%, pH <5.7, WHC >38%, bind <170 mL, heme-iron <20 ppm, collagen >15 mg/g. The values represented by the light gray cells are intermediate.

Significant ($P < .05$) yield grade effects were seldom linear, reflecting inconsistent trends as yield grade increased or decreased.

Moisture (4 out of 5 muscles), L* value (7 out of 7 muscles), a* value (8 out of 8 muscles), b* value (6 out of 6 muscles), and expressible moisture (5 out of 6 muscles) increased with an increase in weight of carcass. How-

ever, pH (4 out of 4 muscles), fat (4 out of 5 muscles), and emulsion capacity (5 out of 5 muscles) decreased with an increase in weight of carcass. Total collagen showed no effect across all 39 muscles due to weight.

These data indicate a vast amount of variation in physical and chemical properties among muscles of the beef chuck and round. Knowledge of these

properties now allows individual muscles to be identified and utilized for production of value-added products.

¹Drew D. Von Seggern, graduate student. Chris Calkins, Professor, Animal Science, Lincoln. This project was funded by beef producers through their \$1/head checkoff and produced for the Cattlemen's Beef Board and State Beef Councils.

Fiber Type Composition of the Beef Chuck and Round

Kevin Kirchofer
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There is wide variability in fiber types of beef chuck muscles. This would be expected to create different processing characteristics which influence optimal muscle use in value-added products.

Summary

The fiber type composition of 38 muscles of the beef chuck and round was studied to facilitate optimal muscle use in value-added products. Select grade chucks and rounds (n=4 each) were used. Muscles containing greater than 40% β -red fiber numbers were classified as red; greater than 40% α -white were classified as white. All others were classified as intermediate. Nine of 12 round muscles were white, while chuck muscles were equally dispersed between red (10 of 26), intermediate (9 of 26), and white (7 of 26), indicating variation among muscles of the chuck, which may create differences in processing characteristics.

Introduction

There is a relationship between ultimate meat quality and muscle fiber type composition. Muscles with increased α white (α W) fibers have

more connective tissue, less intramuscular fat, and are less tender than muscles with more β -red (β R) fibers. Not only do individual muscles differ in fiber type composition, but muscle fiber types within a specific muscle may be affected by breed, sex, time on feed and maturity.

Muscle fiber types have been reported for many of the larger muscles of the beef carcass. Little attention has been given to the smaller muscles that comprise the chuck and the round. With many of these muscles going to further processing, there is a need for a fiber type profile of these muscles. The objective of this study was to characterize the histochemical muscle fiber type of 23 muscles of the beef round and 26 muscles of the beef chuck to help in the application of muscles into value-added products through the use of further processing.

Procedure

Select-grade chucks and rounds (n=4 each) were chosen representing two weight ranges (550-650 lbs, and 800-900 lbs) and two yield grades (yield grade 1 and 3). Twelve muscles of the beef round and 26 muscles of the beef chuck were fabricated and sampled. Muscle samples were frozen in liquid nitrogen within nine days post mortem and subsequently stored at -112°F until histochemical analysis was performed.

One cubic centimeter of frozen tissue was mounted on a cryostat chuck

in such a manner to set muscle fibers perpendicular to the cutting blade. The mounted cubes were allowed to equilibrate to -4.0°F before being sliced to a thickness of 12 μ m on a cryostat. The slices were mounted on slides and allowed to equilibrate to room temperature before being stained.

Muscle sections were stained according to a simultaneous staining technique, which included a stain for succinic dehydrogenase activity and a stain for acid-active adenosine triphosphatase activity after acid incubation. Cover slips were permanently mounted over the stained tissue to enable fiber classification.

Fibers were classified on the basis of stain reactions: β -red fibers stained dark brown, α -red fibers were clear in the middle and surrounded by a blue ring, and α -white fibers were clear. Fiber numbers were calculated by examining a minimum of 500 muscle fibers from muscle bundles containing at least 50 fibers per bundle. Muscle fiber percentage was calculated by counting the total number of each fiber type, dividing by the total number of fibers counted, and multiplying the quotient by 100:

$$\text{Fiber Number (\%)} = \frac{\text{Fiber Number (\beta-red, } \alpha\text{-red, or } \alpha\text{-white)}}{\text{Total Fiber Number}} \times 100.$$

Muscles were classified as red, intermediate, or white on the basis of the average muscle fiber number (%). Muscles were classified as red if they

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