Comparison of the Amino Acid Composition and Connective Tissue Protein Contents of Selected Bovine Skeletal Muscles[1]

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The amino acid and collagen contents of selected bovine skeletal muscles were compared as potentially useful indices for evaluating their protein quality. In this chemical approach the content of collagen and collagen-like proteins of typical bovine skeletal muscles was determined from the amounts of 5-hydroxylysine found in their 96-h acid hydrolysates and the content of total connective tissue proteins from the amounts of 4-hydroproline present. Total collagen ranged from 1.9–3.6% in the longissimus dorsi of beef animals to 9.6% in the sternomandibularis muscle of cows. As the content of muscle collagen increased, the levels of lysine and other essential amino acids decreased compared to increased mean values found for the nonessential amino acids glycine, proline, and 4-hydroxyproline. The calculated protein efficiency ratios (PER), which for skeletal muscle proteins averaged 3.2, also varied with the amounts of collagen present.

Bovine skeletal muscle is the major tissue in beef carcasses. The myofibrillar and sarcoplasmic protein fractions constitute much of the intracellular proteins in muscle tissue, which account for about 85–95% of the total skeletal muscle proteins, and have long been recognized as an important source of high-quality protein in human nutrition. Although the intracellular skeletal muscle proteins have high contents of the essential amino acids, especially lysine, leucine, isoleucine, and the sulfur-containing amino acids (Pellet and Young, 1984), the extracellular matrix muscle proteins, composed principally of collagen and elastin (Hay, 1981; Carrino and Caplan, 1982; 1986; Sanes, 1986; Light et al., 1985), which are known to vary among bovine skeletal muscles (Bendall, 1967; McClain et al., 1971; Dransfield, 1977; Light and Champion, 1984), have low contents of the essential amino acids and are high in the nonessential amino acids such as glycine, proline, and 4-hydroxyproline [Pro(4-OH)]. Little quantitative information is available on the relative contribution of the intracellular and extracellular muscle proteins to the overall amino acid composition (and hence protein quality) of bovine skeletal muscles, nor have the effects of age, sex, or breed in amino acid composition of bovine skeletal muscles been carefully quantified. Accurate measurements of amino acid variations in bovine skeletal muscle tissues would be useful as a data base on muscle composition.

The official method for evaluating protein quality of meats in the United States (Bodwell, 1977; USDA, 1982; Bender, 1982) and Canada (Chapman et al., 1959) is the protein efficiency ratio (PER), as determined in rat bioassays (AOAC, 1984). The net protein ratio (NPR), relative NPR (RNPR), and available amino acid score (Alsmeyer et al., 1974; MacLaughlan et al., 1980; Bender, 1982; Happich et al., 1975; 1984; Sarvar, 1984) have also been proposed as alternative methods for assessing the nutritional value of meats. Although these bioassays can detect amino acid imbalances, processing damages, reduced amino acid availability, and decreased digestibility in the test diet, they do not provide any information about the identity of the limiting amino acid nor do they take into account information about the other amino acids in the test protein (Pellett and Young, 1984; Young and Pellett, 1984).

The improved Stubbs and More (1919) method (Pearson, 1975; Olsman and Slump, 1981; Ranken, 1984) used to evaluate the lean meat content of meats, which is defined as "lean muscle free of visible fat and connective tissue, that contains no more connective tissue than is naturally associated with the cut of meat used, and that neither the fat nor the connective tissue may exceed 10% by weight", is widely used in Britain and Australia for assessing the lean meat content of meats and poultry products. This procedure uses calculations of lean meat made on a fat-free basis (Pearson, 1970; Coomaraswamy, 1972) and are corrected by a proximate mean nitrogen fat-free factor appropriate for each meat-yielding species (Food Standards Committee, 1980). These procedures, however, do not distinguish between nitrogen derived from lean meat, i.e., myofibrillar and sarcoplasmic proteins, etc., and that derived from connective tissue proteins (Benedict, 1987).

To overcome the inherent limitations of these procedures, the Expert Work Group (FSIS, 1984) recommended the use of accurate amino acid composition data and connective tissue protein content of meat and poultry products as an alternative simple and practical method for assessing their protein quality. This approach for evaluating the protein quality of muscles, meats, and their products from their constituent amino acids was adopted because, first, a statistically significant correlation exists between PER values and the contents of several or all of the essential amino acids of a protein or protein mixture (Alsmeyer et al., 1974; Lee et al., 1978; Pellett and Young, 1984) and, second, the content of collagen in muscles or composite meats was highly negatively correlated (R = −0.99) to rat PER values reported by Lee et al. (1978) and Pellett and Young (1984). The connective tissue content of skeletal muscle is usually calculated from the amounts of Pro(4-OH) found in tissue hydrolysates (reviewed by Berg, 1982). Therefore, accurate analyses of the complete

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0021-8561/89/1437-1279$01.50/0

amino acid composition of muscles, meats, and their products, including analyses of those unique amino acids found in collagen and elastin would be helpful in relating amino acid composition of meat products to protein quality.

The purpose of the present study was to measure quantitatively the levels and variation of all amino acids, including 5-hydroxylysine [Lys(5-OH)] and Pro(4-OH), in typical bovine skeletal muscle tissues excised from young and adult animals with the analytical chromatographic methods developed previously (Zarkadas et al., 1986, 1987b). The amino acid composition and connective tissue protein content of the longissimus dorsi muscle, which has a relatively low connective tissue content, were measured in tissues from steers, heifers, cows, and bulls because this skeletal muscle has low connective tissue protein content is considered to be of high protein quality and were compared with the amino acid composition and connective tissue protein contents of the semitendinosus, semimembranosus, and external sternomandibularis skeletal muscles from mature Holstein–Friesian cows. The aim was to determine whether the levels of these amino acids and collagen content in bovine skeletal muscles could be used as an accurate measure of their protein nutritional quality.

MATERIALS AND METHODS

Chemicals and Resins. Types DC-6A (Lot No. 3280), DC-4A (Lot No. 750), and DC-5A (Lot No. 746) cation-exchange spherical resins, sized to 11.0 ± 1.0, 9.0 ± 1.0, and 6.0 ± 0.5 μm respectively, were purchased from Dionex Chemical Co., Sunnyvale, CA. The Type I standard amino acid calibration mixture was obtained from Beckman Instruments Inc., Palo Alto, CA. The unusual basic amino acid standards employed for the preparation of amino acid calibration standards were all supplied as chromatographically homogeneous compounds by Calbiochem-Behring Corp., La Jolla, CA, and included the following: diastereoisomer mixture of 5-hydroxyl-DL-lysine, N^6-methyl-L-lysine, N^6,N^6-dimethyl-L- and N^6,N^6,N^6-trimethyl-L-lysine bis(*p*-hydroxyazobenzenesulfonate) hydrate, N^τ-methyl-L-histidine, N^π-methyl-L-histidine hydrate, D-glucosamine hydrochloride, D-galactosamine hydrochlorite, and 4-hydroxyproline. These were prepared as described previously (Zarkadas, 1975, 1976; Zarkadas et al., 1986). Des and iDes were isolated by the preparative method described by Zarkadas (1979) using bovine ligamentum nuchae elastin purchased from Sigma Chemical Co., St. Louis, MO. All other chemicals and reagents were of the highest purity commercially available and were used without further purification.

Animals, Sampling, and Preparation of Muscle Tissues. To evaluate the overall protein quality of high-grade beef and low-grade cow's or bull's meat from knowledge of their connective tissue protein contents and amino acid composition data, the following four typical skeletal muscle tissues were selected for this investigation: longissimus dorsi, semimembranosus, semitendinosus, external sternomandibularis. Sections (10 cm thick) were taken from the right longissimus dorsi muscle at the 12th thoracic vertebrae of the right side of commercial grade bovine carcasses (Canadian Grade A1, steers and heifers; Grade C1, bulls and cows). Longissimus dorsi samples from three mature (8-year-old) Holstein–Friesian cows were obtained from Abattoir Soulange, Les Cedres, Quebec. The remaining samples were from crossbred bovine animals (11 steers, 4 heifers, 6 bulls), predominantly half- or three-fourths-blood Charolais, Simmental, or Chianina, ranging from 11 to 15 months of age, provided by Dr. L. E. Jeremiah and A. M. Martin of Research Station, Agriculture Canada, Lacombe, Alberta. For purposes of comparison, semimembranosus, semitendinosus, and external sternomandibularis muscles were all taken from three Holstein–Friesian cows. All muscle tissues (approximately 200 g each) were cleaned of adhering fat, cut into small cubes, ground, frozen (−170 °C), and lyophilized. The freeze-dried samples were then pulverized in a standard electrically driven end runner mill (coffee mill; Moulinex Canada Ltd., Weston, Ontario), passed through a No. 40 mesh sieve, and then stored at −20 °C in polypropylene bottles until needed. Proximate and elemental analyses in each of the cow's samples were carried out, and the results have been reported previously (Zarkadas et al., 1987a).

Procedure for Ethanol–HCl Extraction of Muscle Tissue. Skeletal muscle contains soluble amino acids and histidine dipeptides (Davey, 1960; Rangeley and Lawrie, 1977; Carnegie et al., 1984; Harris and Milne, 1987; Kohen et al., 1988) that must be removed completely with aqueous ethanol before amino acid analysis (Nguyen, 1987). Thus, bovine powders (10.0 g) were suspended in 200 mL of 0.1 M HCl in 75% ethanol (v/v) and then homogenized for 3 min in a VirTis Model 45 homogenizer (speed set at 30/100) as recommended by Rangeley and Lawrie (1976). The homogenates were centrifuged at 50000*g* (SS-34 Sorvall rotor) for 30 min at 2 °C, and the supernatants were removed. The pellet was extracted two more times with the same solvent, and the supernatants were combined. The pellets were then resuspended in 20 volumes of acetone and allowed to stand for 3 h inside a fume hood at room temperature. The suspension was again centrifuged, and the acetone extraction was repeated a further two times. The pellets from the final centrifugation were dried at 50 °C overnight and placed in a large vacuum desiccator to remove the last remnants of solvent. The extracted meat samples were finally ground to pass through a 40-mm screen and stored in plastic containers in a refrigerator at −20 °C.

Procedures for Amino Acid Analyses. Amino acid analyses were carried out on either a conventional (Beckman Spinco Model 120C) or an updated and fully automated amino acid analyzer (equivalent to Beckman Spinco Model 121 MB). The automated instrument was interfaced with a Varian Vista 402 (Varian, Walnut Creek, CA) chromatographic data reduction system (Zarkadas et al., 1986, 1987b) to enable both rapid quantitation of amino acids at the picomole range and accurate peak area analysis.

Complete amino acid analyses were carried out in each of the bovine muscle tissues, before and after the ethanol/acetone extractions. The dried, 40-mesh muscle tissue samples (0.1 g) were hydrolyzed in Pyrex test tubes (18 × 150 cm) under vacuum (<10 μmHg) with 15 mL of triply glass-distilled constant-boiling HCl (6.0 M) at 110 °C in duplicate for 24, 48, 72, and 96 h, respectively, with the usual precautions described previously (Zarkadas et al., 1987b, 1988). The data reported for serine, threonine, and tyrosine represent the average of values extrapolated to zero time of hydrolysis. The values for valine, isoleucine, leucine, and phenylalanine are averages of data from 48, 72, and 96 h of hydrolysis. All others are reported as the average values from 24, 48, 72, and 96 h of hydrolysis. The Pro(4OH) content of muscle tissues was determined separately from a concentrated hydrolysate (equivalent to 0.1 mg of protein/analysis) as described previously (Berg, 1982; Zarkadas et al., 1986), and recoveries were calculated relative to alanine.

Methionine and cyst(e)ine were determined separately (0.2-g samples) by the performic acid procedure by Moore (1963). Norleucine was added in the hydrolysates as an internal standard, and the recoveries of cyst(e)ine as cysteic acid and methionine as the dioxide were calculated in proportion to the yields obtained by the performic acid treatment of standard solutions of these amino acids and relative to alanine and leucine present in the sample. Tryptophan in muscle tissue samples (0.1 g) was determined separately after alkaline hydrolysis (Hugli and Moore, 1972) as described previously (Zarkadas et al., 1986, 1988).

Determination of the diastereoisomers of Lys(5-OH) and related compounds were carried out with concentrated hydrolysates (equivalent to 1–2 mg of protein) by the accelerated single-microcolumn (17.5 × 0.28 cm) system as described previously (Zarkadas et al., 1986) so that peaks adequate for these components would be obtained.

Protein Determination. Precise quantitation of the protein content in each muscle tissue hydrolysate was carried out by the method of Horstman (1979) as described previously (Nguyen et al., 1986; Zarkadas et al., 1988) with the expression

$$F = \sum_{i=1}^{18}(a_i b_i)/[1 - (a_{Trp} + a_{Cys})] \quad (1)$$

where F (μg) is the conversion factor used in the absence of tryptophan and cyst(e)ine, a_i is the mole fraction of an amino acid i per mole of total amino acid composition, and b_i is the weight (μg) of amino acid residue i. The protein concentration of each

Table I. Comparison of the Amino Acid Composition of Bovine Skeletal (Longissimus Dorsi) Muscle Tissue Excised from Young Steers, Heifers, and Bulls and Mature Cows (Grams of Amino Acid/Kilogram of Total Protein)

	11- to 15-month-old half- or three-fourths-blood crossbred Charolais, Simmental, or Chianina					signif level, age × sex × treatment (grade) interaction	
	Canada grade A1						
amino acid	steers (N = 11)[a]	heifers (N = 4)[a]	Canada grade C1 bulls (N = 6)[a]	8-year-old Holstein–Friesian Canada grade C1 cows (N = 3)[a]	wt mean ± SEM (N = 24)[a]	CV[a]	F[a]
aspartic acid	96.96 ± 0.15	92.96 ± 1.57	92.51 ± 0.52	90.92 ± 2.09	93.32 ± 0.49	2.47	0.89[ns]
threonine	44.66 ± 1.02[c]	52.94 ± 0.83[b]	45.71 ± 1.37[c]	42.38 ± 0.97[c]	46.59 ± 1.00	10.25	11.12**
serine	35.32 ± 0.78[c]	41.00 ± 1.25[b]	35.87 ± 0.69[c]	35.97 ± 0.45[c]	36.84 ± 0.71	9.20	9.58**
glutamic acid	151.72 ± 1.09[c]	157.89 ± 0.92[b]	156.64 ± 0.88[b]	152.77 ± 2.40[c]	155.24 ± 1.29	3.98	24.18**
proline	36.32 ± 0.53[c]	37.74 ± 0.38[c]	38.46 ± 0.51[c]	50.88 ± 7.94[b]	38.77 ± 1.14	14.04	18.47**
glycine	35.93 ± 0.57[c]	35.57 ± 0.44[b,c]	38.14 ± 0.67[b]	35.55 ± 0.80[b,c]	36.72 ± 0.42	5.47	5.35**
alanine	51.11 ± 0.59[c]	51.72 ± 0.55[b]	52.99 ± 0.34[b]	52.75 ± 0.28[b]	52.29 ± 0.47	4.28	8.67**
cysteine	10.04 ± 0.34	9.06 ± 0.42	9.87 ± 0.47	9.95 ± 0.02	9.93 ± 0.20	5.66	1.47[ns]
valine	59.75 ± 1.42	54.10 ± 1.03	53.96 ± 0.23	51.18 ± 0.08	56.93 ± 0.83	6.98	3.10[ns]
methionine	41.70 ± 2.02[b]	29.98 ± 1.85[c]	32.26 ± 0.89[c]	27.90 ± 1.03[c]	32.92 ± 1.21	34.04	8.03**
isoleucine	50.61 ± 0.46[d]	54.42 ± 1.09[b]	54.54 ± 0.41[b]	50.81 ± 0.56[c]	52.78 ± 0.64	5.82	23.09**
leucine	83.55 ± 0.71[c]	84.65 ± 1.24[b]	84.30 ± 0.19[b,c]	82.39 ± 0.63[b,c]	84.54 ± 0.54	3.07	6.07**
tyrosine	38.33 ± 0.84	37.40 ± 1.19	39.00 ± 1.18	39.40 ± 0.59	38.76 ± 0.58	7.19	0.95[ns]
phenylalanine	41.76 ± 0.37[c]	41.96 ± 0.44[b]	41.70 ± 0.16[b,c]	42.21 ± 0.45[b]	42.17 ± 0.27	3.05	5.84**
histidine	40.37 ± 0.58	39.24 ± 0.78	40.43 ± 0.75	39.30 ± 0.91	40.42 ± 0.39	4.61	0.51[ns]
lysine	93.74 ± 0.82	93.10 ± 0.86	92.99 ± 0.22	89.26 ± 3.69	93.80 ± 0.53	2.72	1.77[ns]
arginine	64.16 ± 0.41[c]	66.51 ± 0.83[b]	66.41 ± 0.24[b]	65.45 ± 0.11[b]	65.83 ± 0.53	3.84	24.75**
tryptophan	10.43 ± 0.02[d]	10.21 ± 0.07[d]	13.74 ± 0.32[b]	11.83 ± 0.37[c]	11.78 ± 0.57	13.72	44.03**
4-hydroxyproline	3.53 ± 0.57	2.04 ± 0.04	3.55 ± 0.03	2.23 ± 0.09	2.89 ± 0.28	27.84	6.27[ns]
5-hydroxylysine	0.395 ± 0.018[c]	0.233 ± 0.027[e]	0.575 ± 0.064[b]	0.329 ± 0.006[d]	0.391 ± 0.031		3.22*
ammonia	7.44 ± 0.21	6.83 ± 0.41	6.89 ± 0.41	10.09 ± 0.51	7.52 ± 0.25	16.08	10.93**
total AA nitrogen	173.6	171.4	171.4	173.9			
total protein,[b] g/kg dry matter	616.51	605.05	636.15	680.04			
WE,[b] μg/nmol	0.112125	0.111912	0.110051	0.111814			
F,[b] μg/nmol	0.118175	0.117889	0.116119	0.126133			

[a] Mean values ± standard error of measurements (SEM); N = replicates; $N \times 8$ = number of determinations. Significance: F, values from analysis of variance; **, $P < 0.01$; *, $P < 0.05$. Key: ns, not significant; CV, coefficient of variation. (b–e) Means, along a horizontal line with different superscripts, are significantly different (Duncan, 1955). [b] Calculated according to Horstmann (1979) using eq 1.

hydrolysate was then calculated by multiplying F by the total nanomoles (X_i) of amino acids found (Horstmann, 1979; Peterson, 1983) as follows:

$$P = F\sum_{i=1}^{18} X_i \tag{2}$$

Determination of Connective Tissue Proteins in Bovine Muscles. Based on the known amino acid compositions of purified, amorphous elastin (Foster, 1982) and skeletal muscle collagen isoforms (Miller and Gay, 1982; Light and Champion, 1984; Light et al., 1985), collagen and collagen-like proteins can be determined from the amounts of Lys(5-OH) present and the elastin content can be estimated by measuring the amounts of Des found. The following method was used to calculate collagen and elastin contents

$$P_j = C_i \frac{1000}{n_i'} \frac{\mathrm{WE(P}_j)}{M_r(i)} \tag{3}$$

where $\mathrm{WE(P}_j)$ is the weight equivalent of a specific muscle protein j, determined from eq 1 according to Horstman (1979) and Zarkadas et al. (1988), n_i' is the number of residues of a unique amino acid residue per 1000 amino acid residues, and $M_r(i)$ is the anhydrous molecular weight of the unique amino acid i. The following analytical conventions derived from eq 3 can therefore be used for calculating collagen and elastin as grams per kilogram of total protein:

$$\text{amt of collagen } (P_C) = \text{amt of Lys(5-OH)} \times 63.3 \tag{3a}$$

$$\text{amt of elastin } (P_E) = \text{amt of Des} \times 62.4 \tag{3b}$$

Similarly, the amount of total connective tissue proteins in these skeletal muscle tissues (in grams per kilogram of total protein) could also be calculated from the sum of collagen (P_c) and elastin (P_E) found in bovine skeletal muscle tissues as described previously (Zarkadas et al., 1988). The following analytical convention, derived from eq 3, can therefore be used for computing total connective tissue proteins (in grams per kilogram of total protein):

$$\text{amt of connective tissue } (P_{CT}) = \text{amt of Pro(4-OH)} \times 8.03 \tag{3c}$$

This value is in close agreement with that reported by Etherington and Sims (1981) and Etherington et al. (1984).

Statistical Analysis. Data processing and linear regression analysis of the results were carried out by a Fortran computer program developed for this purpose. Analysis of variance conducted on the amino acid data for a completely randomized block design (factorial) was carried out by the SAS (Statistical Analysis System) general linear model procedure (SAS, 1982). Differences among sample means were also tested for significance with Duncan's multiple-range test (Duncan, 1955).

RESULTS AND DISCUSSION

Results of the amino acid analyses carried out in this study, and levels of statistical significance obtained from analysis of variance, are summarized in Tables I–IV. Expression of the data as grams of amino acids per kilogram of anhydrous fat- and ash-free protein allows comparisons to be made between the present results and those given in food composition tables and permits calculations of percentage recovery of the amino acids by simple summation (Tristram and Smith, 1963; Eastoe, 1967; Tables I–IV).

The mean residue weight (WE, μg/nmol) and conversion factors F (μg/nmol) given in Tables I–IV can be used in all subsequent protein quantitations of the same protein or tissue by using previously described standard procedures as described by Horstmann (1979), Peterson (1983), Nguyen et al. (1986), and Zarkadas et al. (1988). The usual practice of subtracting the connective tissue content from

Table II. Amino Acid Composition of Longissimus Dorsi Skeletal Muscle from Young Steers, Heifers, and Bulls and Mature Cows after Solvent Extraction with 0.1 M HCl in 75% Ethyl Alcohol (Grams of Amino Acid/Kilogram of Total Muscle Protein)

amino acid (AA)	11- to 15-month-old half- or three-fourths-blood crossbred Charolais, Simmental, or Chianina: Canada grade A1 steers ($N = 3$)[a]	Canada grade A1 heifers ($N = 3$)[a]	Canada grade C1 bulls ($N = 3$)[a]	8-year-old Holstein-Friesian Canada grade C1 cows ($N = 3$)[a]	wt mean ± SEM[a] ($N = 12$)[a]	signif level, age × sex × treatment (grade) interaction: CV[a]	F[a]
aspartic acid	108.38 ± 1.36[b]	102.87 ± 0.57[c]	97.98 ± 0.79[d]	88.76 ± 1.11[e]	99.60 ± 2.51	7.14	56.61**
threonine	46.26 ± 1.78	44.70 ± 0.26	43.78 ± 0.45	42.02 ± 0.83	44.24 ± 0.61	3.92	2.07[ns]
serine	36.45 ± 2.25	38.05 ± 0.97	36.34 ± 0.24	35.36 ± 0.31	36.59 ± 0.57	4.44	0.68[ns]
glutamic acid	163.99 ± 0.80[b]	162.88 ± 0.99[b]	164.76 ± 0.97[b]	151.39 ± 0.12[c]	160.78 ± 1.73	3.04	41.82**
proline	39.67 ± 1.00[c]	34.20 ± 0.50[c]	36.56 ± 1.37[c]	55.45 ± 2.44[b]	41.44 ± 3.24	22.38	42.43**
glycine	31.87 ± 0.05	31.14 ± 0.01	32.62 ± 0.57	31.86 ± 0.12	31.90 ± 0.23	2.11	
alanine	52.33 ± 0.30	53.11 ± 0.05	52.89 ± 0.35	52.05 ± 0.43	52.65 ± 0.19	1.11	2.30[ns]
cysteine	8.02 ± 0.18[c]	7.94 ± 0.33[c]	10.03 ± 0.46[b]	9.99 ± 0.31[b]	9.01 ± 0.41	13.21	11.66*
valine	51.74 ± 0.58[c]	52.78 ± 0.05[b]	52.39 ± 0.15[b,c]	52.32 ± 0.32[b]	52.34 ± 0.24	1.41	3.93
methionine	28.35 ± 0.21[d]	30.10 ± 0.01[b]	29.49 ± 0.01[c]	28.88 ± 0.22[c]	29.24 ± 0.26	2.65	27.35**
isoleucine	49.51 ± 0.30[c]	52.22 ± 0.19[b]	51.54 ± 0.07[b]	51.49 ± 0.23[b]	51.24 ± 0.47	2.59	39.32**
leucine	82.54 ± 0.24[c]	86.89 ± 0.22[b]	87.09 ± 0.06[b]	84.82 ± 0.61[b]	85.44 ± 0.79	2.62	46.11**
tyrosine	38.68 ± 0.40[d]	40.41 ± 0.05[c]	40.27 ± 0.01[c]	42.19 ± 0.56[b]	40.44 ± 0.58	4.08	25.00**
phenylalanine	43.04 ± 0.41[c]	44.23 ± 0.09[b,c]	44.72 ± 0.21[b,c]	46.30 ± 1.33[b]	44.62 ± 0.61	3.88	5.50[ns]
histidine	28.23 ± 1.27	28.32 ± 0.24	30.26 ± 0.09	29.37 ± 0.77	29.07 ± 0.44	4.38	1.99[ns]
lysine	98.26 ± 1.74	95.56 ± 0.54	93.09 ± 4.40	99.22 ± 5.72	96.64 ± 1.73	5.08	0.66[ns]
arginine	66.24 ± 0.21	68.07 ± 1.16	69.61 ± 0.12	68.23 ± 1.55	68.26 ± 0.65	2.73	2.87[ns]
tryptophan	13.79 ± 0.39	14.66 ± 0.09	16.37 ± 1.32	16.08 ± 0.35	15.20 ± 0.52	9.76	3.69[ns]
4-hydroxyproline	2.49 ± 0.018[c]	2.44 ± 0.04[c]	2.70 ± 0.05[b]	2.74 ± 0.015[b]	2.59 ± 0.05	6.05	15.64*
5-hydroxylysine	0.372 ± 0.041[c,d]	0.307 ± 0.015[e]	0.542 ± 0.089[b]	0.322 ± 0.021[d,e]	0.386 ± 0.03		3.22*
ammonia	9.25 ± 0.79	8.03 ± 0.51	6.81 ± 0.71	10.25 ± 1.70	8.59 ± 0.64	21.54	1.88[ns]
total AA nitrogen	169.98	169.50	162.71	172.17			
total protein, g/kg dry matter	766.13	793.46	825.47	804.30			
WE,[b] μg/nmol	0.112539	0.112694	0.112762	0.112474			
F,[b] μg/nmol	0.11935	0.118805	0.119312	0.121988			

[a] Mean values ± standard error of measurements (SEM); N = replicates; $N \times 8$ = number of determinations. Significance: F, values from analysis of variance; **, $P < 0.01$; *, $P < 0.05$. Key: ns, not significant; CV, coefficient of variation. (b–e) Means, along a horizontal line with different superscripts, are significantly different (Duncan, 1955). [b] Calculated according to Horstmann (1979) using eq 1.

Table III. Comparison of the Amino Acid Composition of Selected Bovine Skeletal Muscles Excised from 8-Year-Old Holstein Friesian Cows (Grams of Amino Acids/Kilogram of Protein)

amino acid (AA)	semimembranosus[a]	semitendinosus[a]	external sternomandibularis[a]	wt mean ± SEM[a]	signif level between muscles (type): CV[a]	F[a]
aspartic acid	92.01 ± 0.33[b]	90.07 ± 0.15[c]	91.79 ± 0.31[b]	92.75 ± 0.10	0.49	33.63**
threonine	45.12 ± 0.96	45.63 ± 0.30	42.00 ± 0.71	44.89 ± 0.31	2.93	2.65[ns]
serine	42.45 ± 0.85[b,c]	41.06 ± 0.83[c]	44.53 ± 0.58[b]	43.51 ± 0.28	2.75	5.62*
glutamic acid	149.77 ± 0.53	149.33 ± 0.34	151.10 ± 1.43	152.47 ± 0.17	0.51	4.55[ns]
proline	36.46 ± 0.14[c]	40.10 ± 0.25[c]	45.75 ± 1.01[b]	41.44 ± 0.07	0.75	28.17**
glycine	39.47 ± 0.33[c]	44.81 ± 0.27[c]	52.14 ± 2.01[b]	47.94 ± 0.14	1.31	24.45**
alanine	53.01 ± 0.20[b]	56.16 ± 0.14[b,c]	56.34 ± 0.71[b]	56.05 ± 0.07	0.63	8.02*
cysteine	10.80 ± 0.71[b]	13.17 ± 0.17[b]	11.29 ± 0.01[b]	11.94 ± 0.12	4.69	1.07*
valine	51.78 ± 0.29[c]	54.40 ± 0.19[b]	47.66 ± 0.23[d]	52.07 ± 0.12	1.09	89.08**
methionine	27.73 ± 0.37	27.03 ± 0.20	25.90 ± 0.23	27.31 ± 0.12	1.94	4.47[ns]
isoleucine	48.57 ± 0.40[b]	48.36 ± 0.21[c]	43.54 ± 0.11[d]	47.55 ± 0.14	1.38	68.08**
leucine	81.71 ± 0.31[b]	81.59 ± 0.14[b]	76.23 ± 0.50[c]	81.09 ± 0.14	0.74	24.72**
tyrosine	41.34 ± 0.80[b]	34.45 ± 0.24[c]	38.73 ± 1.00[b]	38.81 ± 0.34	3.76	16.24**
phenylalanine	40.83 ± 0.24[b]	40.90 ± 0.11[b,c]	39.10 ± 0.29[c]	40.41 ± 0.12	1.31	7.67*
histidine	41.66 ± 0.50[b]	38.95 ± 0.31[b]	32.82 ± 1.00[c]	38.39 ± 0.05	0.67	15.86**
lysine	87.96 ± 0.45	87.08 ± 0.08	85.26 ± 0.50	88.15 ± 0.12	0.62	5.15[ns]
arginine	66.02 ± 0.30	66.70 ± 0.14	67.58 ± 0.05	67.84 ± 0.12	0.84	106.09**
tryptophan	17.70 ± 0.39[b]	17.21 ± 0.40[b]	14.45 ± 0.34[c]	16.70 ± 0.11	3.13	8.63*
4-hydroxyproline	4.62 ± 0.14[c]	5.94 ± 0.20[c]	16.02 ± 1.10[b]	9.31 ± 0.35	16.03	55.84**
5-hydroxylysine	0.589 ± 0.030[c]	0.637 ± 0.109[c]	1.420 ± 0.035[b]	0.899 ± 0.098	7.46	278.98**
ammonia	18.19 ± 1.79[b]	14.79 ± 0.83[b,c]	10.61 ± 0.42[c]	14.75 ± 0.50	14.63	5.55*
total AA N[b]	182.34	180.31	183.24			
total protein,[c] g/kg dry mass	751.37	738.68	747.57			
WE,[d] μg/nmol	0.111662	0.110457	0.109938			
F,[d] μg/nmol	0.115061	0.114674	0.114314			

[a] Mean values and standard error of measurements (SEM) for 3 replicates and 48 determinations. Significance: F, values; **, $P < 0.01$; *, $P < 0.05$. Key: ns, not significant; CV, coefficient of variation. (b–d) Means, along a horizontal column with different superscripts, are significantly different. [b] Calculated according to Heidelbaugh et al. (1975). [c] Protein mass determined according to Horstmann (1979) and dry mass as reported previously (Zarkadas et al., 1987a). [d] The WE and F constants calculated according to Horstmann (1979).

Table IV. Amino Acid Composition of Selected Holstein-Friesian Cows' Skeletal Muscles Extracted with 0.1 M HCl in 75% Ethyl Alcohol prior to Acid Hydrolysis (Grams of Amino Acids/Kilogram of Protein)

amino acid (AA)	semimembranosus[a]	semitendinosus[a]	external sternomandibularis[a]	wt mean ± SEM[a]	signif level between muscles (type) CV[a]	signif level between muscles (type) F[a]
aspartic acid	98.50 ± 0.07	95.52 ± 0.86	94.87 ± 0.40	96.24 ± 0.51	1.31	5.23[ns]
threonine	45.95 ± 0.67	44.88 ± 0.86	43.76 ± 0.33	44.84 ± 0.36	2.01	1.15[ns]
serine	36.08 ± 0.27[c]	37.56 ± 0.60[c]	39.29 ± 0.33[b]	37.63 ± 0.75	4.87	21.50*
glutamic acid	157.31 ± 0.30[b]	152.94 ± 0.37[c]	151.25 ± 0.19[c]	153.75 ± 0.71	1.12	36.94**
proline	37.31 ± 0.43[d]	41.22 ± 0.27[c]	46.06 ± 0.34[b]	41.53 ± 1.80	10.38	166.23**
glycine	39.81 ± 0.15[d]	46.29 ± 0.90[c]	53.50 ± 1.10[b]	46.55 ± 2.77	14.19	76.95**
alanine	53.71 ± 0.19[c]	57.69 ± 0.70[b]	56.02 ± 0.51[b]	55.78 ± 0.82	3.75	19.50*
cysteine	7.83 ± 0.00	8.29 ± 0.09	8.56 ± 0.04	8.22 ± 0.17	5.43	5.78[ns]
valine	55.26 ± 0.40[b]	56.35 ± 0.91[b]	51.19 ± 0.14[c]	54.22 ± 0.87	3.95	13.70*
methionine	29.04 ± 0.30[b]	27.75 ± 0.10[c]	26.91 ± 0.20[c]	27.87 ± 0.31	2.75	13.99*
isoleucine	50.95 ± 0.35[b]	50.41 ± 0.21[b]	46.04 ± 0.35[c]	49.09 ± 0.84	4.12	50.01**
leucine	86.05 ± 0.45[b]	84.54 ± 0.08[b]	80.25 ± 0.10[c]	83.55 ± 0.82	2.36	59.99**
tyrosine	38.16 ± 0.15[b]	35.93 ± 0.10[c]	34.44 ± 0.10[d]	36.15 ± 0.55	3.81	152.05**
phenylalanine	40.22 ± 0.40	40.60 ± 0.35	39.18 ± 0.10	38.57 ± 1.24	7.88	0.80[ns]
histidine	30.01 ± 0.02[b]	29.88 ± 0.10[b]	26.88 ± 0.05[c]	28.87 ± 0.55	4.83	547.38**
lysine	87.88 ± 0.33	84.76 ± 0.56	85.99 ± 0.50	86.16 ± 0.54	1.62	9.47[ns]
arginine	70.16 ± 0.37[c]	67.95 ± 0.23[d]	71.32 ± 0.18[b]	69.78 ± 0.80	2.82	60.58**
tryptophan	15.43 ± 0.09	14.29 ± 0.34	12.99 ± 0.34	14.22 ± 0.41	7.13	13.97*
4-hydroxyproline	4.09 ± 0.00[a]	6.63 ± 0.25[c]	15.19 ± 0.22[b]	9.60 ± 2.44	55.82	472.95**
5-hydroxylysine	0.484 ± 0.047[c]	0.528 ± 0.024[c]	1.528 ± 0.039[b]	0.847 ± 0.119	6.73	644.95**
isodesmosine	0.13 ± 0.00	0.47 ± 0.07	0.26 ± 0.02	0.33 ± 0.05	25.36	3.38[ns]
desmosine	trace	0.45 ± 0.04[a]	0.15 ± 0.08[b]	0.20 ± 0.05	17.97	14.69*
ammonia	15.15 ± 1.70	15.45 ± 0.16	14.37 ± 1.84	15.51 ± 0.58	9.14	0.11[ns]
total AA N[b]	177.16	177.46	181.94	179.61		
total protein,[c] g/kg dry mass	819.68	796.57	868.06			
WE,[d] μg/nmol	0.111508	0.110110	0.109139			
F,[d] μg/nmol	0.118249	0.117467	0.118020			

[a] Mean values and standard error of measurements (SEM) for 24 determinations. Significance: F, values; **, $P < 0.01$; *, $P < 0.05$. Key: ns, not significant; CV, coefficient of variation; DM, dry matter. (b–d) Means, along a horizontal column with different superscripts, are significantly different (Duncan, 1955). [b] Calculated according to Heidelbaugh et al. (1975). [c] Protein mass determined according to Horstmann (1979) and dry mass reported previously (Zarkadas et al., 1987a). [d] The WE and F constants calculated according to Horstmann (1979).

such complex protein mixtures or tissue hydrolysates (Olsman and Slump, 1981; Ranken, 1984) can be eliminated by using this method of calculation.

The amino acid composition (grams per kilogram of protein) of unextracted longissimus dorsi skeletal muscle from young heifers, steers, bulls, and mature cows was similar although female animals had lower Lys(5-OH) ($P < 0.05$) than bulls and steers, and longissimus dorsi from heifers had significantly higher ($P < 0.01$) threonine, serine, glutamic acid, alanine, isoleucine, leucine, phenylalanine, and arginine than longissimus dorsi from the other classes of animals (Table I). Age, sex, and age × sex interaction had significant effects on individual amino acids, but no other general patterns of differences were discernible. Longissimus dorsi from all groups of animals had high glutamic and aspartic acid contents, ranging from 245 to 257 g/kg of total protein (Table I). Hence, the acidic amino acids constitute approximately one in four of the total amino acids compared with one in five of the total amino acids constituted by the basic amino acids (Table I).

The results in Table I include the soluble amino acids and histidine dipeptides (carnosine, anserine) that occur in variable amounts in muscle tissues (Carnegie et al., 1984; Harris and Milne, 1987; Kohen et al., 1988) and can be compared with analyses on the same tissues after these amino acids and dipeptides had been extracted (cf. Tables I and II). Extraction removed 26.6% of total histidine in unextracted tissue but had little effect on the other amino acids, probably because the total free amino acids in longissimus dorsi constituted only 0.75% of the total amino acid content (expressed as protein) in this muscle.

The amino acid compositions of semitendinosus, semimembranosus, and external sternomandibularis muscles before and after extraction are presented in Tables III and IV. The relative proportions of amino acids are, on the whole, similar to those found in the longissimus dorsi muscle of bovine animals (Tables I and II), with characteristic differences in their contents of proline, glycine, Pro(4-OH), and Lys(5-OH) and with sporadic variations within the general profiles. Specifically, it should be noted that although animal to animal variability in amino acid content for individual muscles was very low (Tables III and IV), the external sternomandibularis muscle contained significantly higher levels of proline, glycine, Pro(4-OH), and Lys(5-OH), compared to the other three bovine muscles, indicating that muscle to muscle variation in connective tissue protein content may be the cause for much of the differences observed in the levels of these four amino acids. Muscle to muscle variation was also found to be highly significant for all but a few of the standard amino acids (Tables III and IV).

As in the case of the longissimus dorsi, a comparatively small proportion (1.29%) of the total amino acid nitrogen, expressed as protein content, can be extracted from either of these three muscle tissues with 0.1 M HCl in 75% ethanol prior to acid hydrolysis. As much as 24.9% histidine, 13.6% serine, and 31.2% cyst(e)ine were extracted from these bovine muscle tissues. While it is not known whether the high values found for free serine and cyst(e)ine represent the actual levels of the metabolic pools found in skeletal muscle, the results obtained on soluble histidine are in accord with those of Carnegie et al. (1984). These authors have reported values for histidine bound to β-

Table V. Evaluation of the Protein Quality of Selected Bovine Skeletal Muscles Based on Their Amino Acid Composition Data

skeletal muscle type and treatment	essential amino acids (EAA)					PER predicted by[d]		collagen content,[e] % total protein	elastin content,[e] % total protein	connective tissue content,[f] % total protein
	total EAA,[a] g/g N	EAA[b] index	chem[b] score	EAA_7,[c] % total protein	EAA_{10},[c] % total protein	eq 4 (PER_7)	eq 5 (PER_{10})			
Longissimus Dorsi										
steers										
untreated	2707.3	79	71	41.58	53.07	3.25	3.39	2.48	nd	2.84
extracted				39.97	50.80	3.12	3.06	2.33		2.00
heifers										
untreated	2779.3	79	70	41.12	52.72	3.31	3.18	1.46	nd	1.64
extracted				40.65	51.75	3.18	3.12	1.93		1.96
bulls										
untreated	2779.8	82	79	40.55	52.61	3.17	3.17	3.61	nd	2.85
extracted				40.21	51.83	3.14	3.12	3.40		2.17
cows										
untreated	2671.3	74	79	38.61	50.27	3.01	3.02	2.06	nd	1.79
extracted				40.51	51.86	3.17	3.12	2.02		2.20
Semimembranosus										
cows										
untreated	2634.5	80	79	38.37	50.37	2.99	3.03	3.70	nd	3.71
extracted				39.35	51.10	3.07	3.08	3.04	0.81	3.28
Semitendinosus										
cows										
untreated	2594.2	80	80	38.50	50.79	3.00	3.06	4.00	nd	4.77
extracted				38.93	50.14	3.04	3.02	3.31	2.81	5.82
External Sternomandibularis										
cows										
untreated	2474.5	76	81	35.97	47.45	2.80	2.85	8.91	nd	12.86
extracted				37.33	48.45	3.13	2.91	9.59	1.62	12.20

[a] Computed from reference protein standards (FAO/WHO, 1976). [b] Calculated according to Block and Mitchell (1946) and Oser (1951). [c] Calculated according to Lee et al. (1978). EAA_7: threonine, valine, methionine, isoleucine, leucine, phenylalanine, and lysine. EAA_{10}: EAA_7 plus histidine, arginine, and tryptophan. [d] PER were calculated according to Lee et al. (1978) from eq 4 ($PER = 0.08084(EAA_7) - 0.1094$) and eq 5 ($PER = 0.06320(EAA_{10}) - 0.1539$). [e] Total collagen was calculated from the amounts of Lys(5-OH) using eq 3a, while the elastin content was determined from the amount of Des found from eq 3b. nd = not determined. [f] The total connective tissue content of skeletal muscles was calculated from the amounts of Pro(4-OH) present with eq 3c.

alanine (carnosine) that ranged from 7.9 to 9.3 g/kg protein in mixed-beef muscles compared to 9.95 g of free histidine/kg of protein found in the present study (Tables III and IV). Carnosine was originally suggested to act as a buffer to neutralize lactic acid produced in skeletal muscle during glycolysis (Davey, 1960) but is now believed to serve as an endogenous antioxidant in muscle and brain (Kohen et al., 1988).

Comparison of the essential amino acid (EAA) patterns (milligrams per gram of dietary nitrogen) of the four bovine skeletal muscles examined in this study, as recommended by Block and Mitchell (1946), Oser (1951), and FAO/WHO (1965; 1973), indicate that bovine skeletal muscles contain significant amounts of all EAA required for human nutrition. Mean values for total essential amino acids ranged from 2474 mg/g of N in the external sternomandibularis muscle of cows compared to 2729 mg/g of N found in the longissimus dorsi muscle excised from either heifers or bulls. Similar results were obtained from the EAA indices and chemical scores.

Although these predictive tests are based on knowledge of the constituent amino acids of a protein or protein mixtures (Mitchell and Block, 1946), they fail to take into account differences in the digestibility, the quality of the various proteins present, and the availability of individual amino acids. Because of this, Lee et al. (1978), Pellett and Young (1984), and Young and Pellett (1984) have recommended that both the complete amino acid composition and the collagen content of skeletal muscle tissues be used as indices for assessing their protein quality. Lee et al. (1978) defined total EAA in two ways, consisting of either seven or ten amino acids, the seven (EAA_7) being threonine, valine, methionine, isoleucine, leucine, phenylalanine, and lysine, and the ten (EAA_{10}) being these seven plus histidine, arginine, and tryptophan.

Mean values for total EAA_7 and EAA_{10} ranged, respectively, from 38.5–41.5% to 50.1–53.1% in the selected bovine muscle tissues evaluated (Table V). These results are consistent with those listed in the paper by Pellett and Young (1984) for beef muscles. Because this scoring procedure is by definition limited to the essential amino acids, Lee et al. (1978) developed equations (eq 4 and 5, listed in Table V) for predicting the protein efficiency ratios (PER) of bovine muscle proteins from amino acid data. In using the prediction, eq 4 (EAA_7) and eq 5 (EAA_{10}) both show mean PER values close to a value of 3.2 for skeletal muscle proteins, depending upon the amount of connective tissue proteins present.

The results summarized in Table V also show that the amount of collagen and collagen-like (Anglister et al., 1976; Porter and Reid, 1978) proteins in the longissimus dorsi muscle of young heifers (11 to 15 months old), estimated from the amount of Lys(5-OH) found in their acid hydrolysates (Zarkadas, 1981) averages 1.93% and in the adult (8-year-old) cow's muscle averages 2.02% of the total muscle protein. Young steers, by contrast, contained 2.3–2.4% collagen compared to 3.4–3.6% found in the longissimus dorsi muscle of young bulls. These results are in good agreement with the collagen content of 2.3% and 2.76% found in the longissimus dorsi muscle of 18- to 24-month-old Hereford or Hereford × Friesian steers by Bendall (1967) and Dransfield (1977), respectively. The mean total connective tissue protein values for the bovine longissimus dorsi muscle calculated from the amounts of

Pro(4-OH) found ranged from 1.64 to 2.20% in female and from 2.0 to 2.85% in male animals, which are lower than the values found from the Lys(5-OH) content of this muscle. These results indicate that the bovine longissimus dorsi skeletal muscle contained lower amounts of Pro(4-OH) than other vertebrate connective tissues and that this unique amino acid shows larger variation in level than Lys(5-OH).

Mean values for total collagen of the other three bovine muscles ranged from 3.04% in semimembranosus to 3.31% in semitendinosus compared to 9.6% found in the external sternomandibularis muscle of mature Holstein-Friesian cows. The semitendinosus muscle in cows contained higher levels of total connective tissue proteins (4.8–5.8%) compared to that found in the semimembranosus (3.3–3.7%). These results are similar to the values presented by Bendall (1967), McClain et al. (1971), and Dransfield (1977) for the corresponding muscles from young steers and heifers. Differences in collagen and total connective tissue protein content among different muscles may be due to the anatomical arrangement of these proteins in each level of muscle organization (i.e., epimysium, perimysium, and endomysium), which obviously influence it and appear to be under genetic control (Bailey et al., 1979; Borgand Caulfield, 1980; Kovanen, 1984; Light and Champion, 1984; Light et al., 1985; Cheah, 1985).

Mean values of the elastin content of these three bovine muscles, as estimated from their Des content after extraction, were 0.81% for semimembranosus, 2.8% for semitendinosus, and 1.6% for external sternomandibularis. Light et al. (1985) reported that the extracellular matrix of the bovine semitendinosus muscle contained a higher proportion of elastin and only 54% collagen in the perimycial septa (Rowe, 1987). These data, however, are slightly higher than those reported by Bendall (1967) for the same bovine skeletal muscles. The content of the total connective tissue proteins, estimated from the sum of collagen and elastin reported in Table V, averaged 3.9% in semimembranosus, 6.8% in semitendinosus, and 11.2% in the external sternomandibularis muscle.

The results presented show that as the content of total connective tissue proteins in different skeletal muscle increased, the concentration of glycine, proline, and Pro(4-OH) of muscle also increased, while the levels of lysine and other essential amino acids decreased. These results indicate that amino acid composition and connective tissue protein contents of different skeletal muscles can be used as potentially useful indices for evaluating their protein quality, as recommended by the Expert Work Group (FSIS, 1984), Pellett and Young (1984), and Young and Pellett (1984).

ACKNOWLEDGMENT

We are indebted to Dr. L. E. Jeremiah and A. M. Martin of Agriculture Canada, Research Station, Lacombe, Alberta, for providing some of the meat samples used in this study. This investigation was part of an M.Sc. Thesis (Q.N.), McGill University (1987), Montreal, Quebec, and was supported by Agriculture Canada (DSS Contract No. 0SU81-00523) and by Operating Grants (No. 2012 and 84038). We also acknowledge J. P. Emery and D. Tutte for skillful technical assistance and Roberta Boyle for typing this manuscript.

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Received for review July 25, 1988. Accepted April 11, 1989.