Assessing the Myofibrillar and Connective Tissue Protein Contents and Protein Quality of Beef Tripe†

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The myofibrillar actin content of adult bovine tripe has been determined from the amounts of N^{τ}-methylhistidine found in acid hydrolysate. Collagen and elastin have also been determined from the amounts of 5-hydroxylysine and isodesmosine present, respectively. The beef tripe contained 13.36% actin and 9.59% myosin, corresponding to 37.9 and 27.1% of the myofibrillar protein (35.3% of the total protein). Beef tripe contained high levels of collagen (20.1%) and elastin (1.0%). Beef tripe provides adequate amounts of all of the essential amino acids (EAA_9) ranging from 40.3 to 41.9%, compared to FAO/WHO reference EAA_9 pattern value of 33.9% for a 2−5-year-old child, and it is limited only in tryptophan, reflecting the amount of connective tissue proteins present. Evaluation of protein quality of smooth muscles can be based on a knowledge of their digestibility and FAO/WHO amino acid scoring pattern and of their myofibrillar and connective tissue protein contents.

Keywords: *Smooth muscle; beef tripe; myofibrillar; myosin; actin; collagen; elastin; assessment; protein quality; amino acids; composition*

INTRODUCTION

Tripe refers to the bovine ruminant stomach, particularly the rumen (first stomach) and the reticulum (second stomach), which are the parts of the ruminant stomach most widely used in processed meat products. The third stomach, the omasum, is difficult to clean and it is not used for human food (Ockerman and Hansen, 1988). This tissue was chosen as a typical example of a visceral smooth muscle because it is composed of smooth muscle cells and collagenous tissue fibers and is relatively free from other nonmuscle cell types.

The muscular coat of the rumen consists of two layers of smooth muscle fibers: the external fiber layer, which is longitudinal; and the thicker internal layer of fibers, which are largely circular in direction. The muscle fibers of the reticulum, by contrast, consist of two layers of smooth muscle arranged in a network of honeycomb-like folds (Spooncer, 1988; Rust, 1988). This matrix, which is composed primarily of collagen and elastin fibers, plays an important role in determining the protein quality of this bovine smooth muscle.

Available evidence has also indicated that differences in the amount, isotype, and anatomical arrangement of the extracellular matrix protein components of smooth muscle, i.e., collagen, elastin, proteoglycans, and glycoproteins such as fibronectin, laminin or tenascin, and nitogen/entactin (Hay, 1981; Cheah, 1985; Hynes, 1985; Sanes, 1986; Martin and Timpl, 1987; Walsh et al., 1987; Timpl, 1989; van der Rest and Garrone, 1991; Kucharz, 1992) could be related to the function of different smooth muscle cells (Blumenfeld et al., 1983). Collagen and elastin are the key structural components of the mammalian visceral and arterial walls, particularly in the bovine ruminant stomach and reticulum. In concert with other extracellular matrix proteins, these two proteins provide the structural integrity and resiliency required of this specialized smooth muscle tissue.

Although the sliding filament mechanism of smooth muscle is similar to that of striated muscles (Small and Sobieszek, 1980; Somlyo et al., 1983), numerous studies have shown that these muscle types are very different with respect to their molecular weight and assembly of myosin and their ratios of myosin and actin (Somlyo et al., 1973). Vertebrate smooth muscle cells express two different heavy myosin chain isoforms with molecular masses of 204 and 200 kDa, two pairs of light chains 14−27 kDa (Eddinger and Murphy, 1988; Kawamoto and Adelstein, 1991; Babij et al., 1991; Kelley et al., 1992, 1993), and four different isoactins (M_r = 41 782 Da) (Vanderkerckove and Weber, 1979; Fatigati and Murphy, 1984; Katoh and Morita, 1993), each representing a different gene product.

Several researchers have determined the contractile protein content of different smooth muscles by quantitative electron microscopy and electrophoresis (Cohen and Murphy, 1978; Small and Sobieszek, 1980; Murakami and Uchida, 1985). Their results showed that the myosin and actin ratios varied from about 1:8 to 1:16 in chicken gizzard to 1:15 in vascular smooth muscle (Somlyo et al., 1973), compared to skeletal muscle with a ratio of 1:6 (Murakami and Uchida, 1985). Such wide variations reported among various smooth muscle tissues might be partly attributed to the extreme lability of myosin filaments in smooth muscle. These results pointed to the need for an accurate measurement of the amino acid profiles of smooth muscle.

Until recently the official method for assessing the protein quality and nutritive value of meat products and their ingredients in Canada (Chapman et al., 1959) and the United States (AOAC, 1984) has been the protein efficiency ratio (PER) method of Osborn et al. (1919), which measures the ability of a protein to support growth in rapidly growing rats (Campbell, 1963). These bioassay methods, however, tend to underestimate the protein quality of meats because rats have higher

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requirements for the sulfur-containing and other essential amino acids than humans. To overcome the inherent limitations of these bioassay procedures, the USDA-sponsored Expert Work Group of the Food Safety and Inspection Services (Expert Work Group, FSIS, 1984) and the FAO/WHO/UNU Expert Consultation Group (FAO/WHO/UNU, 1985; FAO/WHO, 1991) have recommended that an amino acid score, based on an accurate amino acid composition, corrected for digestibility of proteins, should be the preferred method for assessing protein quality of plant and animal proteins.

Quantitation of N^{τ}-methylhistidine [His(τ-Me)] has been proposed as an index for determining the principal myofibrillar proteins myosin and actin in muscle tissues (Zarkadas et al., 1988a−c; Zarkadas, 1992). Studies have shown that actin contains 1 mol of His(τ-Me) (Elzinga et al., 1973; Vandekerckhove and Weber, 1978, 1979; Elzinga and Collins, 1977) and myosin isolated from adult fast-twitch white skeletal muscle contains 1 mol of His(τ-Me) at position 754 in the amino acid sequence of each of its two heavy chains (MHO) (Maita et al., 1987a, 1991). Recent studies have indicated that His(τ-Me)is absent from chicken gizzard myosin (Nakayama et al., 1994). This modified residue has been substituted for proline in the myosin from the chicken gizzard (Onishi et al., 1986; Maita et al., 1987b).

The contents of collagen and collagen-like proteins can be determined from the amounts of 5-hydroxylysine [Lys(5-OH)] present, and the elastin content from the amounts of desmosine (Des) or isodesmosine (iDes) found in acid hydrolysates (Zarkadas et al., 1988c, 1993). Muscle collagens have a calculated average Lys-(5-OH) content of 10.0 residues per 1000 total amino acid residues (Light and Champion, 1984; Light et al., 1985), while muscle elastin contains 3.0 residues of Des per 1000 residues (Foster, 1982).

The present study was designed to quantitatively establish the levels and variation of all amino acids including the methylated basic amino acids, Lys(5-OH), Pro(4-OH), Des, iDes, and related compounds in the adult bovine tripe. The aims of the present study were, first, to provide statistically and experimentally sound total protein and amino acid data on beef tripe; second, to show that the ptotein quality of a typical smooth muscle, i.e., tripe, can be assessed from its digestibility and the FAO/WHO (1991) amino acid scoring pattern; and third, to show that the myofibrillar actin, collagen and collagen-like proteins, and elastin in tripe can be determined from the amounts of His(τ-Me), Lys(5-OH), and iDes present, respectively.

MATERIALS AND METHODS

Materials. Types DC-6A (lot 3280), DC-4A (lot 750), and DC-5A (lot 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 amino acid standards were obtained as follows: N^6-lysinoalanine [N^G-(DL-2−amino-2-carboxy-ethyl)-L-lysine] from Miles Analytical Laboratories, Inc., Elkart, IN; the diastereoisomer mixture of 5-hydroxy-DL-lysine, N^6-methyl-L-lysine, N^6-dimethyl-L- and N^6-trimethyl-L-bis(*p*-hydroxyazobenzene-*p*′-sulfonate)·H_2O, N^{τ}-methyl-D-histidine, N^{π}-methyl-L-histidine hydrate, D-glucosamine monohydrochloride, D-galactosamine monohydrochloride, and 4-hydroxyproline from Calbiochem-Behring Corp., La Jolla, CA; DL-ornithine (5-aminonorvaline) from Schwarz/Mann, Orangeburg, NY; norleucine and L-2-amino-3-guanidinopropionic acid from Pierce Chemical Co., Rockford, IL; and 3-nitro-L-tyrosine from Aldrich Chemical Co., Milwaukee, WI. The standard amino acid calibration mixture was purchased from Beckman Instruments, Inc., Palo Alto, CA. Bovine *Ligamentum nuchea* elastin was purchased from Sigma Chemical Co., St. Louis, MO, and desmosine and isodesmosine were prepared as described previously (Zarkadas, 1979). All reagents and buffers were made with high-purity laboratory water as described previously by Zarkadas et al. (1988c). All other chemicals and reagents were of the highest purity commercially available and were used without further purification.

Experimental Procedures. *Sampling and Preparation of Smooth Muscle Tissue.* All four compartments of the ruminant stomach are composed mainly of smooth muscle and connective tissue. The selected tripe specimens were from the bovine rumen and reticulum, which are the most widely used parts of the ruminant stomach for meat processing. The samples were excised from three randomly selected commercial carcasses from mature (8-year-old) Holstein-Friesian cows (Canada Grade C1) obtained from Abattoir Soulange, Les Cedres, PQ. The rumen and reticulum samples were initially freed of the stomach contents by cold-water flush followed by a hot-water wash (50−55 °C) at the abattoir and then washed with distilled water prior to sampling. The tripe specimens used for amino acid analysis were excised primarily from the dorsal and ventral sacs of the rumen and reticulum. The specimens were cut into small cubes, ground in an electrically driven end runner mill (Moulinex Canada Ltd., Weston, ON) while frozen (−173 °C), and lyophilized. They were then pulverized to pass through a 40 mesh screen and stored at −20 °C until needed.

Extraction Procedure for the Tripe. Tripe samples used for amino acid analysis before and after extraction were excised from the inner walls of the rumen and reticulum, which are largely tendinous. To remove all traces of soluble histidine dipeptides, including balenine, a β-alanyl-L-N^{τ}-methylhistidine, known to be present in certain muscle tissues (Carnegie et al., 1984; Harris and Milne, 1987), samples (10 g) of the pulverized tripe were extracted with a mixture of methanol/chloroform/water essentially as described by Bligh and Dyer (1959) and Zarkadas et al. (1988c). Since the moisture of the lyophilized samples was low, these were adjusted to a final moisture of 80 ± 1.0%. The volumes of chloroform/methanol/water before and after dilution were kept at the specified levels of 1/2/0.8 and 2/2/1.8, respectively. The delipidated muscle tissue in the methanolic layer was recoverd by filtration, and the extraction procedure was repeated two more times. The muscle tissue residue was dried overnight at room temperature, ground and sieved to pass through a 40 mesh screen, and stored at −20 °C until needed for analysis.

Procedures for Amino Acid Analyses. Amino acid analyses were carried out on either a conventional (Beckman Model 120C) or a fully automated amino acid analyzer (Beckman Model 121MB) equipped with a Varian Vista 402 chromatographic data reduction system (Varian Instruments Group, Walnut Creek, CA) as described previously (Zarkadas et al., 1986, 1987a, 1990).

Complete amino acid analyses were carried out on each of the three bovine tripe samples (100 mg), before and after extraction, according to standard chromatographic procedures described previously (Zarkadas et al., 1986, 1987a). Each of the three replicates was divided into two subsamples, i.e., A and B, which were then hydrolyzed under vacuum (below 10 μmHg) with 10 mL of triple glass-distilled constant boiling HCl (6.0 M; 20.5% v/v) at 110.0 ± 0.5 °C in duplicate for 24, 48, 72, and 96 h, respectively, following the precautions described previously (Zarkadas et al., 1987a, 1988a−c).

Methionine and cyst(e)ine were determined separately (100 mg samples) according to the performic acid procedure of Moore (1963). Norleucine was added to the hydrolysate as an internal standard. Recoveries of cyst(e)ine as cysteic acid and methionine as methionine *S,S*-dioxide were calculated in proportion to the yields obtained by the performic acid treatment of standard solutions of these amino acids. The data were then normalized relative to alanine, valine, leucine, and isoleucine present in the sample and represent the average of 24 determinations.

Tryptophan in bovine tripe samples (100 mg) was also determined separately (equivalent to 0.1 mg of protein/analysis) after alkaline hydrolysis (Hugli and Moore, 1972) on a single column (21 × 0.6 cm) packed with Dionex type DC-

Table 1. Amino Acid (AA) Composition of Beef Tripe (Grams of Amino Acids per Kilogram of Protein) before and after Solvent Extraction with a Mixture of Methanol/Chloroform/Water According to the Bligh and Dyer (1959) Method

	tripe						
	untreated		extracted with methanol/ chloroform/water		significance levels (between treatments)		USDA Handbook 8-13 (U.S. Department of Agriculture, 1986)
AA	mean ± SEM[a]	CV	mean ± SEM	CV	CV	F^a	
aspartic acid	83.76 ± 1.11	3.11	85.00 ± 1.19	3.34	1.18	0.04[ns]	85.1
threonine	38.33 ± 0.93	5.68	44.95 ± 1.76	9.24	3.17	2.81[ns]	38.9
serine	39.33 ± 1.45	8.67	50.47 ± 1.21	5.69	6.66	15.16[ns]	50.5
glutamic acid	138.11 ± 2.62	4.47	137.40 ± 2.07	3.57	1.91	0.07[ns]	146.8
proline	65.09 ± 3.76	13.56	62.90 ± 2.79	10.52	5.81	0.13[ns]	87.5
glycine	80.38 ± 3.19	9.32	77.10 ± 6.19	19.04	4.17	0.33[ns]	9.9
alanine	60.75 ± 0.41	1.63	59.19 ± 1.32	5.30	1.76	1.59[ns]	69.8
cysteine	6.76 ± 0.10	3.65	11.74 ± 0.38	7.78	4.61	63.67*	12.7
valine	51.97 ± 2.28	10.32	48.89 ± 1.38	6.67	7.17	11.24[ns]	46.3
methionine	25.46 ± 0.39	3.64	28.06 ± 1.48	12.46	2.02	0.78[ns]	23.8
isoleucine	42.41 ± 0.51	2.80	43.08 ± 1.27	7.01	1.69	0.02[ns]	44.4
leucine	73.50 ± 1.38	4.41	75.43 ± 2.47	7.77	2.07	0.10[ns]	71.5
tyrosine	35.18 ± 0.73	4.90	39.69 ± 1.46	8.73	3.78	3.39[ns]	29.9
phenylalanine	36.69 ± 1.17	7.53	36.99 ± 0.76	4.85	2.33	0.00[ns]	35.5
histidine	26.10 ± 1.61	14.46	22.19 ± 0.69	7.42	11.50	54.82*	27.4
lysine	70.74 ± 3.19	9.69	64.35 ± 2.30	10.03	2.73	0.21[ns]	78.8
arginine	76.78 ± 1.21	3.72	76.71 ± 0.40	1.24	4.50	0.03[ns]	75.1
tryptophan	5.99 ± 0.36	14.15	6.20 ± 0.58	22.25	4.49	0.03[ns]	8.6
4-hydroxyproline	37.59 ± 0.77	4.78	25.44 ± 3.23	30.10	5.64	7.30[ns]	
5-hydroxylysine	3.43 ± 0.21	14.28	3.18 ± 0.57	40.84	6.85	0.08[ns]	
isodesmosine	0.204 ± 0.022	18.81					
desmosine	0.159 ± 0.024	34.12	0.159 ± 0.04	53.84	13.63	0.01[ns]	
N^6-methyllysine	0.078 ± 0.01	27.77					
N^6-dimethyllysine	0.555 ± 0.069	26.23					
N^6-trimethyllysine	0.340 ± 0.008	43.97	0.291 ± 0.054	41.26	29.26	11.21[ns]	
N^τ-methylhistidine	0.416 ± 0.045	22.50	0.484 ± 0.012	5.35	2.36	0.37[ns]	
ammonia	12.27 ± 1.28	24.52	8.64 ± 1.57	43.14	31.18	3.84[ns]	
total AA nitrogen (N)							
g of AAN/16 g of N[b]	90.19		92.59				
g of AAN/kg of protein	177.39		161.07				
g of AAN/kg dry weight	158.39		161.07				
total protein[c]							
g/kg of dry weight	901.95		946.77				
WE, mg/nmol	0.105696		0.10569				
CF, mg/nmol	0.106753		0.107465				
CF′, mg/nmol	0.119552		0.118466				

[a] Mean values and standard error of measurements (SEM) for three replications. Significance: F values; *, $P < 0.05$; CV, coefficient of variation; ns, not significant. [b] Total amino acid nitrogen was determined according to the method of Heidelbaugh et al. (1975). [c] Protein mass and WE, CF, and CF′ constants were determined according to the method of Horstmann (1979).

6A resin as described previously (Zarkadas et al., 1986), using 3-nitrotyrosine as the internal standard. The data presented in Table 1 represent the average of 24 determinations.

4-Hydroxyproline [Pro(4-OH)] was determined separately from a concentrated 24 h hydrolysate (equivalent to 0.1 mg of protein/analysis) using a single column (21 × 0.6 cm) packed with Dionex type DC-6A resin (Zarkadas et al., 1986). Recoveries of Pro(4-OH) were calculated relative to alanine, isoleucine, and leucine. The Pro(4-OH) data represent the average values of 24 determinations.

The determination of the methylated basic amino acids, the diastereoisomers of Lys(5-OH), Des, and related compounds were carried out with concentrated 96 h hydrolysates (equivalent to 100−250 μg of protein per analysis) by use of analytical chromatographic methods developed to quantitate these unique amino acids (Zarkadas et al., 1986, 1987a) so that peaks adequate for these components (100−250 pmol) could be obtained. Recoveries of these unique amino acids were calculated on the basis of total protein found in each 96 h hydrolysate determined according to the procedure described by Horstmann (1979).

Determination of Total Protein. The content of total protein in each of the smooth muscle replicate samples was determined according to two methods: first, by the conventional Kjeldahl method (AOAC, 1984) as reported earlier (Zarkadas et al., 1987b), and, second, by the summation of the amino acid residues of which each sample or acid hydrolysate is composed by the procedure of Horstmann (1979) as described previously (Zarkadas et al., 1988a−c). According to this method a mean residue weight (WE, in micrograms per nanomole) is calculated for the 18 standard amino acid residues plus Pro(4-OH) and Lys(5-OH) constituting the proteins in the bovine tripe using the expression

$$\mathrm{WE} = \sum_{i=1}^{20} (a_i b_i) \quad (1)$$

where a_i is the mole fraction of a specific amino acid i found in the analyzed aliquot and b_i is the molecular weight of the amino acid residue i. A conversion factor CF (in micrograms per nanomole) was used for determining the protein mass in each hydrolysate in the absence of tryptophan, methionine, and cyst(e)ine. Similarly, CF′, which is the apparent average residue molecular weight in micrograms per nanomole, was also used to calculate protein concentration in the absence of tryptophan, methionine, cyst(e)ine, Pro(4-OH), and Lys(5-OH) and can be calculated as

$$\mathrm{CF}' = \sum_{i=1}^{15} (a_i b_i) / [1 - (a_{\mathrm{Trp}} + a_{\mathrm{Met}} + a_{\mathrm{Cys}} + a_{\mathrm{Pro(4\text{-}OH)}} + a_{\mathrm{Lys(5\text{-}OH)}})] \quad (2)$$

These factors, WE, CF, and CF′, can be used in all subsequent quantitations of a given sample. The protein concentration P (in micrograms) of each hydrolysate was calculated by multiplying CF or CF′ by the total nanomoles (χ_i) of amino acids found (Horstmann, 1979; Peterson, 1983) as follows:

$$\mathrm{P} = \mathrm{CF}' \sum_{i=1}^{15} \chi_i \quad (3)$$

Determination of Connective Tissue Proteins in the Bovine Tripe. An attempt was also made to relate the amounts of the unusual protein-bound amino acids, which occur exclusively in vertebrate connective tissue proteins, i.e. collagen and elastin, to the contents of these extracellular smooth muscle

proteins in the bovine tripe as described previously (Zarkadas et al., 1988a,b, 1995). A method for calculating the amount of a specific protein *j* in smooth muscles has been described previously (Zarkadas et al., 1988c) and is

$$P_{j=1} = C_i \frac{[1000]}{n_i} \frac{\mathrm{WE}_{P_j}}{M_{\mathrm{r}(i)}} \quad (4a)$$

where WE_{P_i} is the weight equivalent of a specific connective tissue protein *j*, determined from eq 1 according to the method of Horstmann (1979), n_i is the number of residues of a unique amino acid per 1000 amino acid residues, and $M_{r(i)}$ is the anhydrous molecular weight of the unique amino acid *i*.

In this chemical approach the distribution of elastic fibers (elastin) in this mammalian smooth muscle tissue could be calculated from the amounts of iDes found in the acid hydrolysates of this tissue. The total collagen and collagen-like proteins of the tripe could also be determined from the amounts of the aLys(5-OH) diastereoisomer present in their acid hydrolysates. Amorphous elastin (Foster, 1982), which was used as standard for comparison, contains 3 residues of Des/1000 total amino acids and has a mean residue weight (WE) of 85.06. The anhydrous molecular weight of iDes is 454.54. Similarly, a mean for the diastereoisomers of Lys(5-OH) content of $n_i' = 10.0$ residues/1000 total amino acid residues in smooth muscle collagen could be computed from the relative distribution of collagen types and their respective Lys(5-OH) contents as described previously for skeletal muscle (Light and Champion, 1984; Light et al., 1985; Zarkadas et al., 1988a,b) and by Blumenfeld et al. (1983) for smooth muscle tissues. The average residue weight (WE) for collagen is 91.1, and each of the diastereoisomers of Lys(5-OH) has an anhydrous M_r of 145.18.

The following analytical conventions derived from eq 4a can therefore be used for calculating collagen as grams per kilogram of total protein (Zarkadas et al., 1988a,b)

$$\text{amt of collagen } [P_c] = [\text{amt of Lys(5-OH)}] \times 63.3 \quad (4b)$$

and for computing total elastin

$$\text{amt of elastin } [P_E] = (\text{amt of iDes}) \times 62.4 \quad (4c)$$

The method used for calculating the amount of total connective tissue proteins in the bovine tripe (in grams per kilogram of total protein) is based on the known Pro(4-OH) and amino acid composition of purified collagen [$n = 105.8$] [see Zarkadas et al. (1988c); Miller and Gay (1987); Light (1987) and amorphous elastin ($n_i = 22$) (Foster, 1982) and the anhydrous molecular weight of Pro(4-OH) ($M_{r(i)} = 113.12$)]. The following analytical convention described previously (Berg, 1982; Zarkadas et al., 1988c) can therefore be used for computing total connective tissue proteins (in grams per kilogram of total protein):

$$\text{amt of connective tissue } [P_{CT}] = [\text{amt of Pro(4-OH)}] \times 8.03 \quad (4d)$$

Determination of the Myofibrillar Proteins. Since sequence studies (Elzinga et al., 1973; Vandekerchkhove and Weber, 1979; Vandekerchkhove et al., 1986; Fatigati and Murphy, 1984; Maita et al., 1987a) have shown that 1 mol of actin (A) contains 1 mol of His(τ-Me) and His(τ-Me) is absent from smooth muscle myosin (M) (Onishi et al., 1986; Maita et al., 1987b; Nakayama et al., 1994), the quantitation of protein-bound His(τ-Me) can be used as an index for determining actin in smooth muscle tissues. Considerable care must be taken, however, to extract all soluble histidine dipeptides, especially balenine (Carnegie et al., 1984; Harris and Milne, 1987), prior to acid hydrolysis of smooth muscle tissues.

The relative amount of actin [M_r(A)= 41 782; Elzinga et al., 1973] in beef tripe can be calculated from the total concentration, C_T in grams per kilogram of total protein (see eq 4a), present in the acid hydrolysates by the equation

$$P_A = \frac{M_r(A)}{n_i + M_{r(i)}} C_T \quad (5a)$$

or

$$P_A = \frac{41782}{1 + 151.2} C_T \quad (5b)$$

where P_A is the amount of actin in smooth muscles (grams per kilogram of total protein), $M_{r(A)}$ is the molecular weight of actin (41 782 Da), n_i=1 is the number of unique amino acid residues *i* per mole of actin, and $M_{(i)}$ is the anhydrous molecular weight of His(τ-Me), which is 151.2.

Therefore, the following equation can be used to calculate actin as grams per kilogram of total protein:

$$\text{amt of } P_A \text{ (g/kg)} = [\text{His}(\tau\text{-Me}); \text{(g/kg)}] \times 276 \quad (5c)$$

The myosin content (grams per kilogram of total protein) in bovine tripe can be calculated relative to actin as

$$P_M = P_A \frac{M_{r(M)}}{M_{r(A)}} \frac{1}{16} \quad (6a)$$

where P_M and P_A are the concentrations of tripe myosin and actin (grams per kilogram of total protein), respectively, and $M_{r(M)}$ and $M_{r(A)}$ are the respective molecular weights of cardiac myosin and actin.

Substituting the molar ratio of actin to myosin A/M = 16 reported by Murakami and Uchida (1985) and the molecular weight of myosin [$M_{r(M)} = 480\,000$; Babij et al., 1991; Kelley et al., 1992, 1993] in eq 6, the sum of actin and myosin in bovine smooth muscles can then be calculated as

$$P_M = P_A \frac{480000}{41782} \frac{1}{16} = 0.718 P_A \quad (6b)$$

Yates and Greaser (1983) have shown that the sum of actin and myosin in myofibrils accounts for 65% of the total myofibrillar protein by weight. Thus, the total myofibrillar protein in grams per kilogram of total protein in porcine skeletal muscle can also be calculated as

$$\text{amt of myofibrillar protein} = \frac{\sum_{j=1}^{2} (P_A + P_M)}{0.65} C_T \quad (6c)$$

Statistical Analysis. Data processing and statistical analysis of the results were carried out by a FORTRAN computer program developed for this purpose. Analysis of variance conducted on the amino acid for bovine tripe for completely randomized block design (factorial) was carried out according to the Statistical Analysis System (SAS, 1991) by the general linear model procedure.

RESULTS AND DISCUSSION

To further study the use of the amino acid composition data and/or myofibrillar and connective tissue contents as potentially useful indices for assessing the protein quality of a typical vertebrate smooth muscle tissue, accurate and detailed amino acid determinations were carried out on the ruminant stomach (beef tripe) of adult cows.

Samples were excised primarily from the dorsal and ventral sacs of the rumen, and also from the inner wall of the reticulum, and were thoroughly mixed prior to amino acid analysis. Protein determinations in each acid hydrolysate of the bovine tripe, before and after extraction (Bligh and Dyer, 1959), were carried out according to the method of Horstmann (1979) as described previously (Zarkadas et al., 1988b,c), and the

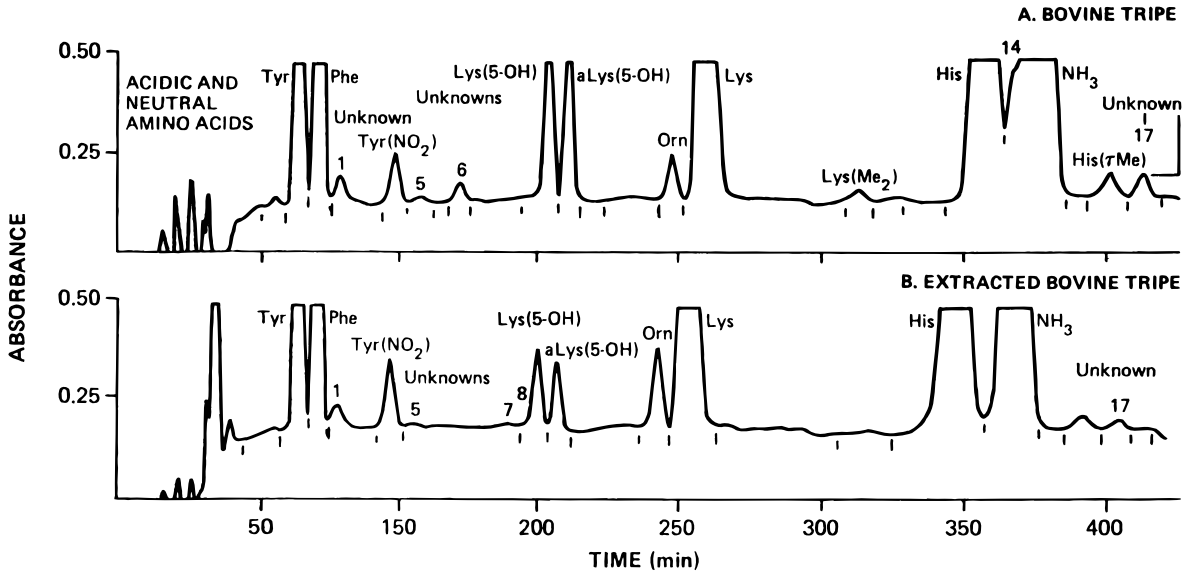

Figure 1. Typical chromatographic elution profile of the methylated basic amino acids in bovine tripe on an analytical 50 × 0.28 cm microcolumn of Dionex DC 4A resin: (A) separation of an untreated 96 h acid hydrolysate of bovine tripe sample; (B) elution profile of an extracted bovine tripe 96 h hydrolysate sample. Tyr(NO_2), 3-nitrotyrosine; Lys(5-OH), 5-hydroxylysine; aLys(5-OH), *allo*-5-hydroxylysine; Orn, ornithine; Lys(Me_2), N^6-dimethyllysine; His(τ-Me), N^{τ}-methylhistidine.

results are summarized in Table 1. The mean residue weight (WE, micrograms per nanomole) and conversion factors CF and CF′ (micrograms per nanomole) given in Table 1 can be used in all subsequent protein quantitations of this tissue following standard procedures (Horstmann, 1979).

The overall amino acid compositions of the adult bovine tripe before and after extraction, and levels of statistical significance obtained from analysis of variance as presented in Table 1, represent the average values of three replicates and duplicate determinations obtained from duplicate 24, 48, 72, and 96 h hydrolysates. Glutamic acid was the most abundant amino acid in the tripe and accounts for almost 1.5 residues in 10 and aspartic acid approximately 1 in 12, giving a total acidic amino acid frequency of about 23.1%. The frequency of total basic amino acids, including arginine, lysine, Lys(5-OH), and histidine, accounted for almost 17.2% of the total, which is below that of the acidic amino acids. The high glycine and arginine contents and lower levels of lysine reflect the amount of connective tissue proteins present. These data correspond closely to those reported by Skurray and Perryman (1980) and Anderson (1988) for raw and processed tripe and in the USDA Handbook 8-13 (U.S. Department of Agriculture, 1986) for raw beef tripe. The differences noted in the levels of glycine, lysine, and those amino acids undergoing destruction on acid hydrolysis were not statistically significant.

The cyst(e)ine and histidine contents between untreated and extracted beef tripe, however, varied significantly ($P < 0.05$). The results of Table 1 show that about 15% of the total histidine found in this smooth muscle tissue was extracted with the methanol/chloroform mixture of Bligh and Dyer (1959), and probably most of it was present as the soluble dipeptide carnosine (Carnegie et al., 1984). It should also be noted that a sizable proportion (32.8%) of the total Pro(4-OH) in this tissue was present in the soluble pool. Quantitative amino acid analysis of the soluble extracts indicated that the total free amino acids and soluble peptides pool was very small (0.75%).

The values obtained for protein-bound His(τ-Me) in both the untreated and extracted specimens of the bovine tripe do not differ significantly and show high reproducibility and a low coefficient of variation. Previous studies have shown that one complication often encountered in the analysis of protein-bound His(τ-Me) in skeletal muscle tissues (Zakadas et al., 1988b,c) is the presence of variable amounts of soluble dipeptides, including carnosine, anserine, and balenine, which on acid hydrolysis yield β-alanine, histidine, N^{π}-methylhistidine [His(π-Me], and His(τ-Me) (Carnegie et al., 1984; Harris and Milne, 1987).

The chromatograms illustrated in Figure 1 are typical of the separations obtained of tissue hydrolysates (100 μL equivalent to 100−250 μg of protein per analysis) before and after extraction according to the present method (Zarkadas et al., 1987a). This method enabled the complete separation of His(τ-Me) and the diastereoisomers of Lys(5-OH) along with six as yet unidentified ninhydrin-positive compounds, designated 1, 5, 6, 7, 8, and 17 to indicate their relative elution times from the microcolumn.

One of the methylated compounds, His(π-Me), was absent from this smooth muscle tissue. This was notable since His(π-Me) is usually present in skeletal muscle tissues as a component of the soluble histidine dipeptide anserine (β-alanyl-L-N^{π}-methylhistidine; Carnegie et al., 1984) and elutes from the microcolumn between the ammonia and His(τ-Me) peaks (Figure 1). These results indicate that further studies are required to delineate the significance of the absence of His(π-Me) and the presence of a considerable amount of carnosine in this smooth muscle tissue.

As shown in Figure 1, one unknown peak, no. 8, appears to partially coelute with one of the diastereoisomers of Lys(5-OH), even after epimerization at 110 °C for 96 h (Zarkadas, 1975), thus interfering with its quantitation (Figure 1B). For this reason, the determination of the aLys(5-OH) diastereoisomer is now routinely used for quantitating this basic amino acid which is unique to collagen. An accurate determination of the levels of iDes, Des, and the diastereoisomers of Lys(5-OH) in acid hydrolysates (96 h) of the tripe was also carried out by a rapid analytical procedure using a 17 × 0.28 cm microcolumn of Dionex DC-5A resin (Zarkadas et al., 1986). Their typical chromatographic

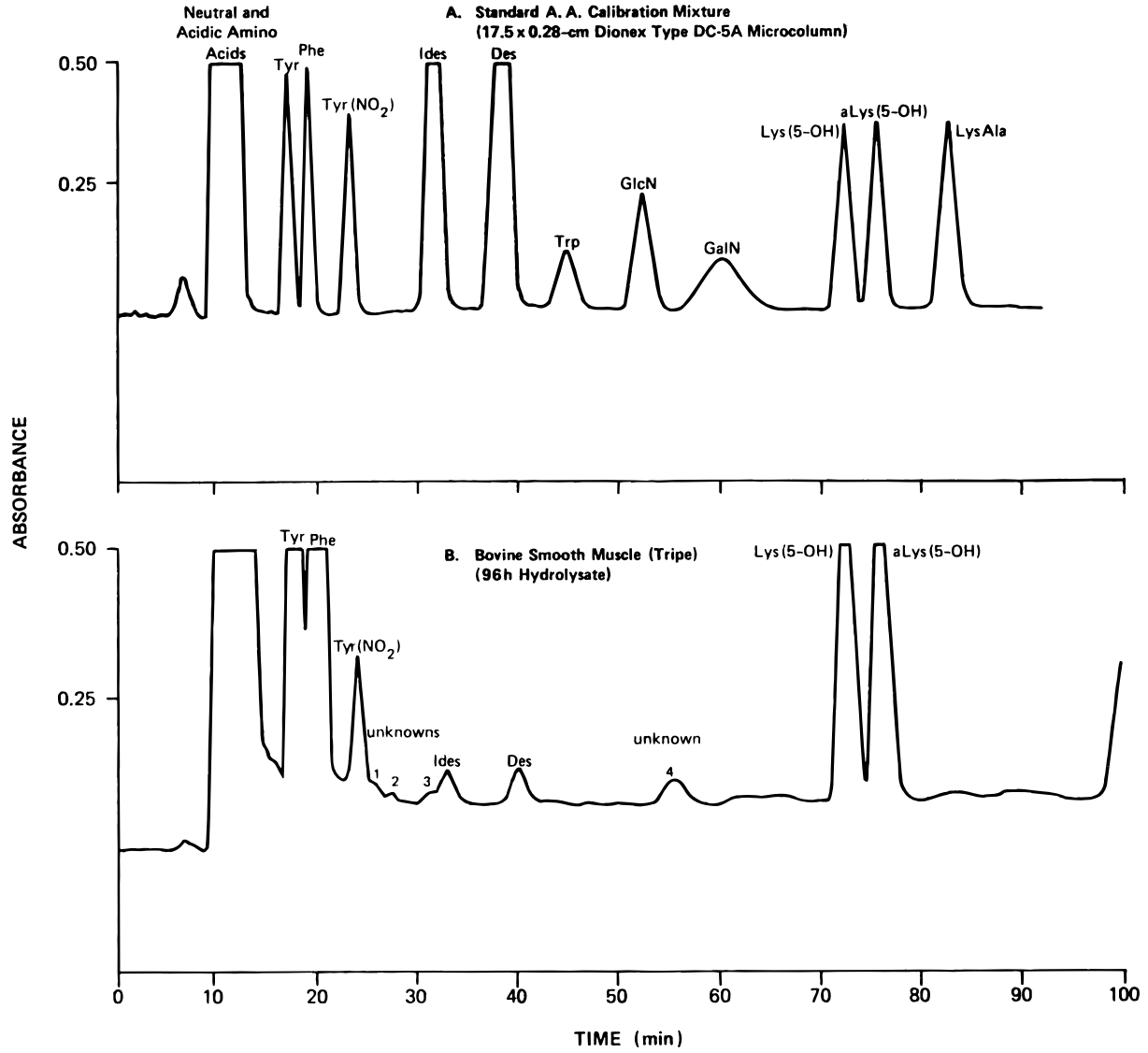

Figure 2. Chromatographic separation of 11 basic amino acids (0.5 nmol each) on an analytical 17.5 × 0.28 cm microcolumn of Dionex DC-5A resin: (A) separation of a synthetic basic amino acid calibration mixture; (B) analysis of a 96 h acid hydrolysate of bovine tripe. The curves show absorbance at 570 nm. Tyr(NO_2), nitrotyrosine; iDes, isodesmosine; Des, desmosine; GlcN, glucosamine, GalN, galactosamine; Lys(5-OH), 5-hydroxylysine; aLys(5-OH), *allo*-5-hydroxylysine; LysAla, lysinoalanine.

separations, along with four as yet unidentified ninhydrin-positive peaks, designated 1−4, are illustrated in Figure 2. This chromatographic procedure has an advantage over other methods in that complete separation of the elastin cross-links and the diastereoisomers of Lys(5-OH) is possible in a single analysis in less than 1.25 h. Thus, the data presented in Table 1 indicate that the tripe is a highly tendinous smooth muscle tissue containing high levels of collagen and elastin but lower amounts of myofibrillar proteins.

From the protein-bound His(τ-Me), Lys(5-OH), iDes, and Pro(4-OH) contents (Table 1) of beef tripe from adult cows, it has been possible to determine the myofibrillar and connective tissue protein contents of this smooth muscle tissue (Zarkadas et al., 1988b,c, 1993). The results, expressed as grams of protein per kilogram of total protein, are summarized in Table 2. This direct chemical approach is based on the determination of the myofibrillar actin and collagen contents of smooth muscle (tripe) from the amounts of His(τ-Me) and aLys-(5-OH) found in their acid hydrolysates, respectively. Elastin can also be determined from the amounts of Des using eq 4c. Total connective tissue proteins, which include collagen and elastin, are determined from the amounts of Pro(4-OH) present, multiplied by 8.03 (eq 4d).

Actin in beef tripe accounts for an estimated 13.36% of total smooth muscle protein or about 37.91% of the myofibrillar proteins (Table 2). The value obtained for beef tripe myosin (eq 6b) accounts for 9.59% of the total muscle protein, which corresponds to 27.1% of myofibrillar proteins (35.3%). These results were based on the molar ratio of myosin to actin 1:16 reported by Murakami and Uchida (1985), compared to cardiac and skeletal muscle tissues with ratios of 1:4 and 1:6, respectively. Previous studies have shown that the myofibrillar proteins of bovine, porcine, avian, and rabbit skeletal muscles represent 57.75% of the total muscle mass (Yates and Greaser, 1983; Khalili and Zarkadas, 1988; Zarkadas et al., 1988b,c). The myofibrils contained 11.5% actin and 23.0% myosin, corresponding to 21.1 and 44.0% of the myofibrillar proteins (Table 2).

Although the spindle-shaped smooth muscle cells contract by a sliding filament mechanism similar to that existing in striated muscles (Small and Sobieszek, 1980; Somlyo et al., 1983), the striking difference among the

Table 2. Myofibrillar and Connective Tissue Protein Contents (Grams per Kilogram of Total Smooth Muscle Protein) of Beef Tripe

	smooth muscle	skeletal muscle of total muscle protein			
			psoas major (rabbit)		
muscle protein	tripe[a] (extracted)	bovine diaphragm, Zarkadas et al. (1988c)	Yates and Greaser (1983)	Hanson and Huxley (1957)	Szent-Gyorgyi et al. (1955)
i. myofibrillar[b]					
actin	133.58	110.4	126.9	120.0	
myosin	95.91	229.4	248.2	340.0	250−300
actomyosin	229.49	339.8	375.2	460.0	
total	353.07	522.6	577.1	620.0	
other soluble proteins	435.72	430.0		340.0	41.60
ii. connective tissue[c]	204.28	25.9			
collagen[d]	201.29	26.2			
elastin[d]	9.92	1.3			
total[e]	211.21	27.5			

[a] Extracted according to Bligh and Dyer (1959). [b] Calculated from His(τ-Me) data (Table 1) using eqs 4b, 4c, 4d. These calculations are based on the molar ratio of myosin to actin 1:16 reported by Murakami and Uchida (1985). [c] Calculated from Pro(4-OH) data (Table 1) using eq 4d. [d] Calculated from Lys(5-OH) and Des data (Table 1) using eqs 4b and 4c, respectively. [e] Collagen plus elastin.

three types of muscles was in their myosin to actin molar ratios (Murakami and Uchida, 1985). The reason for such variation is unclear, but Murphy et al. (1974) and Cohen and Murphy (1978) have suggested that the low myosin to actin ratios might be associated with differences in smooth muscle function and the higher force developed by the various smooth muscle tissues during contraction, which can be equal to or greater than that produced in striated muscle. In addition, most of the functional diversity among smooth muscle tissues appears to be due to the levels of the contractile proteins within their minisarcomeres, the state of assembly of the myosin and actin filaments, and the manner of insertion of the latter on cytoplasmic and plasma membrane-bound dense bodies (Small and Sobieszek, 1980; Somlyo et al., 1983).

Myofibrillar protein variants have also been observed among smooth muscles (Buckingham, 1985). Many of the actin and myosin variants present in smooth muscles (Fatigati and Murphy, 1984; Eddinger and Murphy, 1988; Kawamoto and Adelstein, 1991) are expressed as structurally distinct, tissue and developmental stage specific isoforms encoded by either different genes (Mahdavi et al., 1986; Pollard and Cooper, 1986; Taylor, 1993; Cheney et al., 1993; Rayment et al., 1993; Pollard et al., 1994) or from the same gene by alternative splicing. Smooth muscle cells express two different heavy chains of estimated M_r 200 000 and 204 000 (Kawamoto and Adelstein, 1991; Kelley et al., 1992, 1993) and four different isoactins (Vandekerckhove and Weber, 1978). These include two variants specific to smooth muscle (α-SM and γ-SM) plus appreciable amounts of the two cytoplasmic actins found in virtually all eukaryotic cells (β-NM and γ-NM).

Beef tripe has high levels of collagen and elastin compared with skeletal muscle. Mean values for total collagen in beef tripe from mature animals averaged 20.12% of the total protein compared to 1.0% of elastin found in this smooth muscle tissue, corresponding to 21.12% of the total smooth muscle proteins. Total connective tissue proteins are also determined from the amounts of Pro(4-OH) present multiplied by 8.03 (eq 4d), and the results are included in Table 2. Although the data reported for the total connective tissue protein content of beef tripe in Table 2 (21.12%) are in reasonably good agreement with the 15.9% collagen value reported by Rust (1988) for bovine tripe, they differ considerably from those reported by Bendall (1967), Dransfield (1977), and Light et al. (1985) for the distribution of collagen (average 4.35%; spread 1.22−15.1%) and elastin (average 0.38%; spread 0.05−2.8%) in 34 bovine and 3 porcine skeletal muscles investigated. The results have an important bearing on the question of the protein quality of beef tripe. Recent studies have shown that collagen plays a key role in contributing to the overall textural quality of meat (Stanton and Light, 1990; McCormick, 1994; Nishimura et al., 1995). Laser-Reuterward et al. (1985) have shown that over 90.0% of the protein present in pig skin or bovine tendon was digestible by the rat, regardless of the heat treatment during processing or the age of the animal from which these tissues were taken. Thus, an amino acid score, based on an accurate amino acid composition and myofibrillar and connective tissue protein contents, corrected for digestibility of beef tripe proteins (Carpenter, 1984), should be the preferred method for assessing protein quality in beef tripe.

The essential amino acid (EAA) profile of bovine tripe before and after extraction ranged from 2614 to 2755 mg of EAA/g of nitrogen (Table 2). The data indicated that the adult bovine tripe contains a good complement of most of the amino acids considered essential in human nutrition, comparable to either whole egg (3215 mg of EAA/g of nitrogen) or cow's milk protein (3200 mg of EAA/g of nitrogen) (FAO/WHO, 1965). Similar results were obtained from the EAA indices of tripe calculated from its amino acid composition (Table 1) according to the methods of Block and Mitchell (1946) and Oser (1951). The calculated protein efficiency ratio (PER) of beef tripe, computed from the amino acid data of Table 1, using the prediction equation (eq 7) developed by Lee et al. (1978), show a mean PER value close to 2.50. These results show that the calculated mean PER values for beef tripe, which for other animal proteins, i.e., whole egg, milk, beef, etc., average 3.2, also varied with the amount of collagen present as a percentage of total muscle proteins. These results are consistent with those reported by Lee et al. (1978), Young and Pellett (1984), and Pellett and Young (1984) for meats containing up to 14.0% collagen and collagen-like proteins. Collagen has a unique amino acid composition, which is considerably lower than the FAO/WHO (1991) reference pattern and that of egg, milk, or the striated muscle tissues, which are considered to be excellent sources of high-quality proteins. A characteristic of collagen, compared to the proteins of muscle fibers, is its high concentration of glycine, proline, and Pro(4-OH). In addition, the level of total sulfur amino acids in collagen is quite low, and tryptophan is almost completely absent in both collagen and elastin.

Although these scoring procedures are based on a knowledge of the essential amino acid composition of various muscles and meats and are in part a function of the limiting amino acid in the test protein source

Table 3. Essential Amino Acid (EAA) Scores of Beef Tripe and Other Animal Proteins and EAA Requirements of Preschool Child

EAA	EAA[a] requirements for preschool child (2−5 years old)	EAA: bovine tripe, untreated	EAA: bovine tripe, extracted	EAA: other animal products, egg[a]	EAA: other animal products, beef[b]
	Milligrams of Amino Acid per Gram of Total Protein[c]				
histidine	19	26	22	22	34
isoleucine	28	42	43	54	48
leucine	66	73	75	86	81
lysine	58	71	64	70	89
methionine + cysteine	25	32	39	57	40
phenylalanine + tyrosine	63	63	76	93	80
threonine	34	38	45	47	46
tryptophan	11	6	6	17	12
valine	35	52	49	66	50
% total protein					
EAA_9[c]	33.9	40.3	41.9	51.2	50.4
EAA_{10}[d] including arginine		47.9	49.7		
total EAA,[e] mg/g of N		2615	2755		
PER_{10}[e] predicted by eq 8		2.52	2.49		
	Percent Protein Digestibility in Man				
		90	90	95	98
	Percent Amino Acid Score				
		54.5	54.5	100	100
	Protein Digestibility Corrected Amino Acid Score				
		50	50	95	98
limiting EAA		Trp	Trp		

[a] Data from FAO/WHO/UNU (1985) and FAO/WHO (1991). [b] Data taken from Bodwell (1987). [c] Calculation of protein ratings was carried out by comparison of the amino acid composition of bovine tripe with that of the reference pattern established by FAO/WHO/UNU (1985) for preschool child (2−5 years old). EAA_9: histidine, isoleucine, leucine, lysine, methionine plus cysteine, phenylalanine plus tyrosine, threonine, tryptophan, and valine. [d] Calculated according to Lee et al. (1978); EAA_{10}: EAA_9 plus arginine. [e] Computed from reference protein standards (FAO/WHO, 1965). [f] PER_{10} were calculated according to Lee et al. (1978) from eq 7 [$PER_{10} = 0.06320(EAA_{10}) - 0.1539$].

(Pellett and Young, 1984, 1990), they fail to take into account differences in the digestibility, the quality of the various proteins present, and the availability of individual amino acids.

The essential amino acid profiles and protein ratings of the beef tripe before and after extraction are compared with those of the reference pattern (FAO/WHO/UNU, 1985) for a 2−5-year-old child and with two high-quality animal proteins such as hen's whole egg and bovine skeletal muscle tissue, and the results are shown in Table 3. The egg and skeletal muscle have true protein digestibilities of 95 and 98%, respectively (FAO/WHO, 1991; Bodwell, 1987). Mean values for corrected amino acid scores ranged from 95% in hen's whole egg to 50% in beef tripe. Beef tripe provides adequate amounts of all of the essential amino acids ranging from 40.3 to 41.9%, which is considerably higher than the 33.9% reference pattern value given by FAO/WHO (1991), and it is limiting only in tryptophan, reflecting the amount of connective tissue proteins present. Unlike animal bioassays, which require several trials for the identification of the actual limiting amino acid, the actual advantage of using the FAO/WHO (1991) protein digestibility-corrected amino acid scoring procedure is that it can readily identify the limiting amino acid(s) in a protein source of a diet.

The results of the present study have provided statistically and experimentally sound amino acid data on bovine tripe and have shown that the protein quality of different smooth muscle tissues can be assessed from their digestibility and FAO/WHO (1991) amino acid scoring patterns. This study has also shown that the myofibrillar actin, collagen, and collagen-like proteins in smooth muscle can be determined from the amounts of His(τ-Me) and Lys(5-OH) present, respectively. Elastin in bovine tripe can be determined from the amounts of isodesmosine present.

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