Effect of Dietary Restriction during Gestation on Amino Acid Composition and Myofibrillar and Collagen Contents of Skeletal Muscle in Gilts Mated at Puberty†

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Sixteen Yorkshire gilts, mated at puberty, were used to study the effect of severe dietary restriction on the skeletal muscle amino acid composition, and its myofibrillar and connective tissue protein contents, and to determine their potential use as indices for evaluating carcass quality. The proposed chemical approach is based on the direct determination of the myofibrillar myosin and actin contents of skeletal muscle from the amounts of N^{τ}-methylhistidine found in their 96-h acid hydrolysates. Collagen and collagen-like proteins can be determined from the amounts of 5-hydroxylysine present. The semimembranosus muscle contained 10.9–12.4% actin and 22.8–23.9% myosin, corresponding to 21.0 and 44.0% of the myofibrillar proteins (51.9–54.3% of the total muscle proteins). The total skeletal muscle collagen ranged from 4.8 to 5.9% in the semimembranosus of once-mated gilts compared to 3.7–4.2% in the unmated controls. In all cases the increased muscle collagen appeared to be associated with changes during gestation, and meat from gilts that had farrowed tended to be less tender than that from controls.

INTRODUCTION

The effects on carcass quality of adequate and severely restricted nutrient intakes on female pigs (gilts) and other animals during gestation have been studied by various workers (Elliot and Lodge, 1978; Elliot et al., 1980, 1982; Baker and Speer, 1983; Moore and Brasel, 1984; Campbell et al., 1984; Pelletier et al., 1987). Faster weight gain in animals that are well nourished during gestation, compared to slower gaining animals, tends to be accompanied by increased fat deposition in relation to protein (Ontko, 1986; Thonney and Ross, 1987). Maternal undernutrition during the last 15–30 days of gestation produces a lower fat carcass of acceptable market weight (below 100 kg) in once-mated female gilts slaughtered soon after the birth of their first and only litter (Friend et al., 1979; Elliot and Lodge, 1978; Elliot et al., 1980, 1982; Campbell et al., 1984). Meat from such gilts tended to have more flavor but was consistently less tender than meat from unmated controls of the same age and breed (Elliot et al., 1982). Little is known, however, about the effects of dietary restriction during gestation on the levels and variation of amino acids, intracellular proteins, and collagen contents of skeletal muscle in such gilts.

The aims of this study were to ascertain (a) whether the amino acid composition and myofibrillar and connective tissue protein contents of skeletal muscles could be correlated with carcass quality and (b) if perceived differences in tenderness (Elliot et al., 1982) of the semimembranosus muscle tissue could be explained by differences in amino acid composition and myofibrillar and collagen content as determined by the procedures developed by Zarkadas (1979) and Zarkadas et al. (1986, 1987b, 1988a–c, 1990).

MATERIALS AND METHODS

Materials. Types DC-4A (Lot 750) and DC-5A (Lot 746) cation-exchange spherical resins, sized to 9.0 ± 0.5 and 6.0 ± 0.5 μm respectively, were purchased from Dionex Chemical Co., Sunnyvale, CA. Since this supplier has discontinued these products, alternative sources of spherical resins could be Interaction Chemicals Inc., Mountain View, CA, or Beckman Instruments, Inc., Palo Alto, CA. The unusual amino acid standards were obtained as follows: N^6-lysinoalanine [N^{α}-(DL-2-amino-2-carboxyethyl)-L-lysine] from Miles Analytical Laboratories, Inc., Elkhart, IN; the diastereoisomer mixture of 5-hydroxy-DL-lysine, N^6-methyl-L-lysine, N^6-dimethyl-L- and N^6-trimethyl-L-lysinebis(*p*-hydroxyazobenzene-*p*′-sulfonate)·H_2O, N^{τ}-methyl-L-histidine, N^{π}-methyl-L-histidine hydrate, ω-*N*-methyl-L-, ω-*N*-ω-*N*-dimethyl-L-, and ω-*N*,ω′-*N*-dimethyl-L-argininebis(*p*-hydroxyazobenzene-*p*′-sulfonate), D-glucosamine monohydrochloride, D-galactosamine monohydrochlorite, 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 nuchae* elastin was purchased from Sigma Chemical Co., St. Louis, MO, and Des and iDes were prepared as described previously (Zarkadas, 1979). All reagents and buffers were made with high-purity laboratory water (Zarkadas et al., 1987b) using activated carbon beds, mixed ion exchangers, glass distillation, and further deionization steps. All other chemicals and reagents were of the highest purity commercially available and were used without further purification.

Experimental Procedures. *Animals and Experimental Treatment.* The 16 female Yorkshire pigs (gilts) used in these experiments were 100 days of age and were selected randomly from 48 gilts raised at Agriculture Canada's Animal Research Centre, Ottawa, as described previously (Elliot, 1982). The

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0021-8561/92/1440-0098$03.00/0

Table I. Formulation and Composition of Experimental Diets

	diet	
	H	L
Ingredients, g/kg		
ground barley	850	840
soybean meal (475 g of crude protein/kg)	120	120
salt	5	5
limestone	10	10
dicalcium phosphate (160 g of Ca, 200 g of P/kg)	10	10
trace mineral premix[a]	2.5	7.5
vitamin premix[b]	2.5	7.5
Composition (Calculated on Air-Dry Basis)[c]		
digestible energy, MJ/kg	12.91	12.78
crude protein, g/kg (N × 6.25)	156	155
calcium, g/kg	6.1	6.1
phosphorus, g/kg	5.7	5.7

[a] Trace mineral premix contained, per kilogram of premix, 1.2 g of copper, 16.0 g of iron, 4.0 g of manganese, 10.0 g of zinc, and 20 mg of selenium. [b] Vitamin premix contained, per kilogram of premix, 28.7 g of β-carotene, 2.75 mg of vitamin D_3, 3.624 g of D-α-tocopherol, 800 mg of vitamin K (menadione), 600 mg of thiamin, 1.6 g of riboflavin, 8.0 g of niacin, 6.6 g of pantothenic acid, 5.6 g of vitamin B_{12}, 100 g of choline chloride, and 50 mg of biotin. [c] United States–Canadian Tables of Feed Composition (National Research Council, 1969).

selected gilts, which were of similar initial weight, were penned in four groups of four littermates each, were allowed fresh water ad libitum, and were maintained at appropriate environmental conditions for optimum performance (American Feed Manufacturers Association Nutrition Council, 1970). Before the study period all animals were allowed to consume a fortified barley–soybean meal (diet H; Table I) ad libitum, until breeding. From 120 days old all animals were checked daily for estrus (Elliot, 1982) and, after estrus, were mated and then transferred to individual pens (2.3 × 4.3 m) until farrowing. It should be noted that the gastation period for swine is 113 days. The eight mated gilts were given 1.36 kg/day of a typical high-concentrate diet (diet H) until day 85 of gestation, at which time these animals were randomly divided into two dietary treatment groups: a high-intake group (HIG), which continued to receive 1.36 kg/day of diet H; and a low-intake group (LIG), which was restricted to 0.45 kg/day of diet L (Table I). The LIG animals therefore received 33.1% of the total caloric intake of the HIG group. Body weights of both groups (HIG and LIG) were measured at mating, again at the time of imposition of the experimental diets, and daily thereafter to monitor daily weight gain.

The eight unmated gilts were divided into two control groups. One group of four gilts was fed ad libitum until slaughter at 105 kg live weight and served as initial heavy market weight controls (IC). The other four unmated gilts were designated final controls (FC) and were maintained on an intake of 1.36 kg/day of diet H. Each of the four FC animals that served as final controls was slaughtered at the same time as its experimental littermate, namely at 72 h after farrowing. Ages and final weights were reported for all control animals.

Nutrient Composition of the Diets. Minimum dietary nutrient requirements for sows during pregnancy have been established (National Research Council, 1979; Agricultural Research Council, 1981) from experiments with breeds and crosses used in commercial pork production (Baker and Speer, 1983; Miller and Kornegay, 1983; Pond, 1986). Details of the dietary formulations and composition for the high (HIG) and low (LIG) food intake groups used on this study are given in Table I. Diet L, however, was enriched in vitamins and mineral nutrients so that the animals receiving 0.45 kg of diet L daily (treatment LIG) would receive the same amount of vitamins and trace mineral nutrients (Miller and Kornegay, 1983) as those fed 1.36 kg of diet H (treatments HIG, IC, and FC). The digestible energy, crude protein, and calcium and phosphorus contents of the two diets are presented in Table I. The metabolic requirement of pigs for protein is related to the amounts of essential (EAA) and nonessential (NEAA) amino acids provided and not to protein per se. Baker and Speer (1983) and Pond (1986) have recently reviewed the research on which currently recommended levels of dietary amino acids are based. Thus, the total protein and overall amino acid compositions of the two diets are presented in Table II. The values represent the average of three replicates and duplicate determinations obtained from duplicate 24-, 48-, 72-, and 96-h hydrolysates as described previously (Zarkadas et al., 1988a). Since both diets consisted of similar mixtures of ground barley and soybean, their overall amino acid composition as presented in Table II appeared to be very similar.

Table II. Amino Acid Composition[a] of Experimental Diets (Grams of Amino Acid/Kilogram of Total Protein)

	exptl diets	
amino acid (AA)	H	L
aspartic acid	83.67 ± 1.69	82.71 ± 1.44
threonine	28.99 ± 0.58	33.91 ± 0.15
serine	29.21 ± 0.77	42.67 ± 0.09
glutamic acid	237.09 ± 1.99	228.74 ± 1.50
proline	96.95 ± 2.35	91.09 ± 1.37
glycine	37.75 ± 0.18	36.57 ± 0.29
alanine	38.90 ± 0.28	37.91 ± 0.27
cysteine	29.56 ± 1.02	28.63 ± 1.31
valine	54.60 ± 0.26	53.05 ± 0.61
methionine	27.37 ± 1.20	31.07 ± 1.12
isoleucine	44.05 ± 0.34	43.38 ± 0.16
leucine	77.76 ± 1.32	76.32 ± 1.04
tyrosine	15.54 ± 0.98	17.88 ± 0.23
phenylalanine	53.53 ± 0.52	52.30 ± 0.46
histidine	27.36 ± 0.11	26.77 ± 0.13
lysine	50.47 ± 0.45	49.62 ± 0.35
arginine	52.38 ± 0.61	53.21 ± 0.23
tryptophan	14.84 ± 0.06	14.75 ± 0.07
ammonia	38.65 ± 0.87	27.18 ± 0.40
total AA N	182.30	173.03
total protein,[b] g/kg dry matter	146.25	144.60
WE,[b] μg/nmol	0.112949	0.112688
F,[b] μg/nmol	0.113873	0.113703

[a] Mean values ± standard error of measurements (SEM); N, number of replicates; $N \times 9$ = number of determinations. [b] Calculated according to the method of Horstmann (1979) using eqs 1 and 2.

Experimental Material and Tissue Preparation. Food was removed 24 h before slaughter. Following slaughter, the carcasses were chilled to 5 °C for a minimum of 30 h, at which time the two roasts, namely the loin (first five ribs) and ham roasts, were obtained from the left side of the carcasses for sensory evaluation and chemical analyses. The rump portion of the ham was separated from the whole ham by cutting through the leg parallel with, and 4 in. posterior to, the initial cut (between the third and fourth sacral vertebrae). These cuts were then wrapped and frozen (−20 °C) until tested.

The four frozen ham roasts from each treatment were allowed to thaw in a refrigerator for approximately 48 h. The semimembranosus muscle from the rump portion of the ham was excised from each sample; the muscle tissues (approximately 100 g each) were cleaned of adhering fat and connective tissue, cut into small cubes, minced in a precooled (4 °C) meat grinder, frozen (−173 °C), and lyophilized. Moisture determination of each sample was carried out before and after lyophilization. The freeze-dried samples were then pulverized in a standard electrically driven end runner mill (coffee mill; Moulinex Canada Ltd., Weston, ON) and then stored at −20 °C in polypropylene bottles until needed.

Sensory Evaluation. On each day of testing four ham roasts from each treatment were evaluated. The frozen roasts were thawed for approximately 10 h before being cooked to an internal temperature of 76 °C in ovens set at 163 °C. Appropriate weights and times were recorded to calculate cooking rates and weight losses. Cooked slices (3 mm thick) from the semimembranosus muscle were evaluated, by 10 experienced judges, for flavor, tenderness, and juiciness, according to the method of descriptive analysis with scaling described previously (Larmond, 1977). The left and right anchor points on flavor evaluation were "lacking pork flavor" and "intense pork flavor", respectively. Corresponding anchor points for tenderness and juiciness were "very tough" to "very tender" and "dry" to "juicy", respectively. The four

samples evaluated each day were presented simultaneously to the judges to encourage comparison among samples.

Shear Value Determinations. Ten cores (16 mm long × 10 mm in diameter) were removed from each muscle parallel to the grain of the meat for shear value determination. The peak force required to shear the cores was recorded using the modified Warner-Bratzler shearing device (Voicey and Hansen, 1967).

Proximate and Elemental Composition. Standard methods from AOAC (1980) were followed for the determination of moisture (Sections 7.003 and 24.002) and total ash (Sections 24.009 and 31.012). Petroleum ether extractable lipids were determined according to the Crampton (1956) method (Sections 10.132 and 24.005). Preparation of quadruplicate samples for elemental analyses was carried out on a perchloric acid fume hood according to the wet digestion procedure using a mixture of concentrated nitric (15 mL) and perchloric (8 mL) acids in a 100-mL Kjeldahl flask as described by Parks and Dunn (1963). Phosphorus determinations were carried out according to the molybdovanadate method of Parks and Dunn (1963). Similarly, zinc, iron, potassium, magnesium, and sodium were determined separately according to the official lanthanum oxide method (Section 2.109; AOAC, 1980) using a fully automated atomic absorption spectrophotometer (Varian Model AA-975) equipped with a programmable sample changer (Varian Model 55) and printer plotter (Hewlett-Packard Model HP 82905A). Chlorine (Cl) was determined separately according to a modification of the Volhard method (Caldwell and Mayer, 1955), using an automatic chloride titrator (Amino-Cotlove American Instrument Co., Silver Spring, MD) equipped with a silver generator electrode and a silver anode as described previously (Zarkadas et al., 1987a). The total nitrogen content of muscle tissues was determined according to the official Kjeldahl method (Section 2.057) using the automated Technicon II system (Technicon Instruments Co., Tarrytown, NY) to analyze the digests (Section 24.028; AOAC, 1980).

Tissue Extraction Procedure. The lyophilized samples (10 g) were suspended in 200 mL of a mixture of 75% ethyl alcohol in 0.1 M HCl (Rangeley and Lawrie, 1977) and homogenized for 3 min in a VirTis Model 45 (VirTis, Gardiner, NY) homogenizer (speed set at 30/100), the homogenates were centrifuged at 50000*g* (SS-34 Sorvall rotor) for 30 min at 2 °C, and the supernatants were removed and dried under vacuum (Buchi, Rotovapor R) at 45 °C. The pellet was suspended in the same solvent, 75% ethyl alcohol/0.1 M HCl mixture, and the extraction procedure was repeated twice. The final pellets were suspended in 20 volumes of acetone, and the suspension was again centrifuged as before. The delipidated pellets from the final centrifugation were dried at 50 °C overnight, placed under vacuum to remove the last remnants of solvents, ground to pass through a 40-mm screen, and stored at −20 °C until needed.

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). The instruments were equipped with a module control (Autolab Spectra-Physics GmbH, Darmstadt, West Germany) and a companion Autolab system AA for computing peak concentrations (Zarkadas, 1979).

Complete amino acid analyses were carried out on each of the porcine semimembranosus samples before and after extraction. Duplicate samples of lyophilized powders (0.1 g) were hydrolyzed in Pyrex test tubes (18 × 150 mm) under vacuum (below 10 mm of mercury) with 10 mL of triple 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 (Hunt, 1985; Zarkadas et al., 1987b, 1988a). The 4-hydroxyproline [Pro(4-OH)] 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) according to the performic acid procedure of 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 methionine *S,S'*-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. Separate samples of 0.1 g were used for determination of tryptophan (Hugli and Moore, 1972) according to an improved chromatographic procedure using 3-nitrotyrosine as an internal standard (Zarkadas et al., 1986).

Determinations of the methylated basic amino acids, the diastereoisomers of Lys(5-OH), and related compounds were carried out with concentrated 96-h hydrolysates (equivalent to 100–300 μg of protein/analysis) in the single-microcolumn (50 × 0.28 cm) system packed with either Beckman Type AA-10 (Zarkadas, 1979) or Dionex DC-4A resins (Zarkadas et al., 1987a) so that peaks adequate for these components (100–250 pmol) could be obtained.

Determination of Total Protein Mass in Skeletal Muscle. Recoveries of all unique basic, 5-hydroxylysine, and other amino acids were based on the protein content of each muscle tissue hydrolysate determined according to the method of Horstmann (1979), as described previously (Zarkadas et al., 1988a) using the expression

$$\mathrm{WE} = \sum_{i=1}^{21} (a_i b_i) \tag{1}$$

where WE is the mean residue weight in micrograms per nanomole, a_i is the mole fraction of an amino acid i found in the analyzed aliquot, and b_i is the molecular weight of amino acid residue i. A conversion factor (F), which is the apparent average residue molecular weight (micrograms per mole), increased in proportion to the missing tryptophan, and cyst(e)ine values have been calculated as described by Zarkadas et al. (1988a). The protein concentration (P in micrograms) of each hydrolysate was then calculated by multiplying F(μg/nmol) by the total nanomoles (χ_i) of amino acids found (Horstmann, 1979; Peterson, 1983; Stoscheck, 1990) as follows:

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

(*a*) *Determination of Connective Tissue Proteins.* On the basis of the known amino acid compositions of purified skeletal muscle collagen isoforms (Miller and Gay, 1982, 1988; Light et al., 1985), the collagen and collagen-like protein contents of skeletal muscles can be determined from the amounts of Lys(5-OH) present as described previously (Zarkadas et al., 1988a,b). Collagen content was calculated according to the equation

$$P_j = C_i \frac{[1000]}{n'_i} \frac{\mathrm{WE}_{P_j}}{M_{r_i}} \tag{3}$$

where WE_{P_j} is the weight equivalent of a specific muscle protein j, determined from eq 1 according to the methods of Horstman (1979) and Zarkadas et al. (1988a,b), 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 as grams per kilogram of total protein:

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

Similarly, the amount of total connective tissue proteins in this skeletal muscle tissue (in grams per kilogram of total protein) could also be calculated from the sum of collagen (P_C) and elastin found in porcine skeletal muscle tissues as described previously (Zarkadas et al., 1988b). 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):

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

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

(*b*) *Determination of the Myofibrillar Proteins Myosin and Actin.* Since sequence studies (Elzinga et al., 1973; Maita et al., 1987) have shown that 1 mol of actin (A) contains 1 mol of His(τ-Me) and that 1 mol of myosin (M) contains 2 mol of His(τ-Me), the quantitation of protein-bound His(τ-Me) can be used as an index for determining these two principal myofibrillar

Table III. Mean Mineral Content, Proximate Composition, and Sensory Evaluation of Porcine Skeletal (Semimembranosus) Muscle Tissue Excised from Yorkshire Gilts Mated at Puberty and Given High and Low Food Allowances from Day 85 of Gestation

	dietary treatment, mean ± SEM (N = 4)[a]					significant[b] levels between treatments	
	HIG	LIG	control, unmated	control, market wt	weighted	SE	F
carcass data[c]							
food intake, kg/day	1.36	0.45	1.36	ad libitum			
age at slaughter, days	300.0	299.0	302.0	197.0	274.5	12.74	**
warm carcass wt, kg	87.5	76.7	93.4	83.9	85.4	7.26	**
nutrient, g/100 g, dry wt basis							
moisture	73.55 ± 0.22	73.64 ± 0.33	74.88 ± 0.73	73.76 ± 0.5	73.96	2.56	ns
total nitrogen	14.21 ± 0.15	14.68 ± 0.23	14.36 ± 0.34	13.63 ± 0.4	14.07	1.51	ns
total lipid	13.13 ± 1.46	10.34 ± 1.94	18.02 ± 5.7	18.60 ± 4.6	15.02	1.96	ns
total ash	4.29 ± 0.76	4.44 ± 0.08	4.58 ± 0.07	4.33 ± 0.1	4.41	0.53	ns
minerals, mg/100 g, dry wt, fat-free basis							
calcium	15.0 ± 1.0	16.0 ± 2.0	14.0 ± 1.0	15.0 ± 0.0	15.0	1.0	ns
phosphorous	771.0 ± 10.0	780.0 ± 16.0	809.0 ± 24.0	788.0 ± 14.0	787.0	1.0	ns
magnesium	82.0 ± 2.0	82.0 ± 1.0	84.0 ± 3.0	82.0 ± 3.0	82.0	9.0	ns
chlorine	177.0 ± 7.0	236.0 ± 24.0	209.0 ± 13.0	190.0 ± 6.0	203.0	9.0	ns
potassium	1440.0 ± 48.0	1390.0 ± 23.0	1440.0 ± 48.0	1376.0 ± 44.0	1394.0	22.0	ns
iron	3.76 ± 0.12	4.52 ± 0.17	4.36 ± 0.2	3.47 ± 0.1	4.01	0.14	**
zinc	8.77 ± 0.51	8.48 ± 0.67	9.88 ± 0.5	7.66 ± 0.5	8.70	0.33	ns
sodium	169.0 ± 4.00	200.0 ± 9.0	190.0 ± 13.0	187.0 ± 3.0	186.0	5.0	ns
sensory evaluation[d]							
flavor	7.2	8.0	8.0	7.9		0.3	ns
tenderness	4.5	6.2	6.3	7.4		0.32	*
juiciness	5.2	5.6	4.5	5.5		0.22	*
shear force, kg	2.443	2.567	2.247	1.983		0.168	*

[a] Mean values and standard error of measurements (SEM). [b] Significance: F, values from analysis of variance between treatments; **, $P < 0.01$; ns, not significant; N, number of replicates, $N \times 4$ = number of determinations; SE, standard error difference between means. [c] Data quoted from Elliot et al. (1982). [d] Determined according to the method of Larmond (1977) as described previously (Elliot et al., 1982).

proteins in muscle tissues (Zarkadas et al., 1988a,b). The amounts of myosin (P_M) and actin (P_A) in porcine muscles (grams per kilogram of total protein) can thus be calculated from the total amount (C_T) of His(τ-Me) and the molar ratio of actin and myosin per kilogram of total protein by the following equation:

$$\sum_{j=1}^{2}[P_A + P_M] = \left[\frac{A/M}{A/M+2}\frac{41782}{151.2} + \frac{1}{A/M+2}\frac{521000}{151.2}\right]C_T \quad (4)$$

When the molar ratio of actin to myosin reported by Murakami and Uchida (1985) ($A:M$ = 6) is substituted in eq 4, the total amount of actin and myosin in the semimembranosus muscle can be calculated as

$$\sum_{j=1}^{2}[P_A + P_M] = [207 + 431]C_T \quad (4a)$$

$$\sum_{j=1}^{2}[P_A + P_M] = 638C_T \quad (4a')$$

Since the sum of myosin and actin in the myofibril accounts for 65% of the total myofibrillar protein by weight (Yates and Greaser, 1983), the total myofibrillar protein in grams per kilogram of total protein in this porcine muscle can also be calculated as

$$\text{amt myofibrillar protein} = \sum_{j=1}^{2}\frac{[P_A + P_M]}{0.65}C_T = 981C_T \quad (4b)$$

Statistical Analysis. Data processing and linear regression analysis of the results were carried out by a FORTRAN computer program developed for this purpose, as described previously (Nguyen et al., 1986). Analysis of variance conducted on the amino acid data for a completely randomized block design with two factors (e.g., mated and unmated gilts) and two nutritional levels each was carried out according to the general linear model procedure (SAS, 1982).

RESULTS AND DISCUSSION

To study the effect of severe or moderate dietary restriction on gilts during late pregnancy, accurate chemical and detailed amino acid determinations of the porcine semimembranosus muscle were carried out. This muscle was chosen as a typical example of a porcine skeletal muscle tissue. The results obtained on the average mineral content and proximate components of this muscle, excised from once-mated and unmated gilts fed a restricted diet, are summarized in Table III. The overall composition of this muscle is 22–24% protein, 2.7–4.5% lipid, 2–3% other organic and inorganic constituents, and between 70 and 73% water. The concentrations of Kjeldahl nitrogen and total ash among these muscles were similar (Table III). Thus, protein represents 87,5% of the dry weight of this skeletal muscle. Values for total lipid ranged from a low of 10.3 g/100 g DWB (dry weight basis) in the food-restricted animals to a high of 18.0–18.6 g/100 g DWB obtained for the unmated controls of the same age. Many of the lipid components of skeletal muscle have been summarized by Waku (1977). Thus, animals that were given 1.36 kg daily (HIG) deposited fat during late pregnancy in amounts above that seen in pregnant food-restricted gilts (LIG) given 0.45 kg/day but in amounts well below that seen in the nonpregnant control animals (IC and FC). Therefore, there appeared to be preferential fat deposition during pregnancy, which occurred even when food intake was moderately restricted, at the expense of fetal growth. Such alterations in body composition had been observed previously with rats that were food restricted throughout pregnancy, lactation, and postweaning recovery (Moore and Brasel, 1984). Pekas (1985) has shown that feeding growing pigs 120% of ad libitum intake, via a gastric fistula, results in an increase in both muscle and fat deposition: fat increase of 32%; lean mass increase of 8%; and bone mass did not change. Similar developmental changes in response to overfeeding have been reported in

Table IV. Comparison of the Amino Acid Composition (Grams of Amino Acid per Kilogram of Total Protein)[a] of Porcine Skeletal (Semimembranosus) Muscle Tissue Excised from Yorkshire Gilts Mated at Puberty and Given High or Low Food Allowances from Day 85 of Gestation

	dietary treatment					significant levels between treatments	
amino acid (AA)	HIG, 1.36 kg of food/day	LIG, 0.45 kg of food/day	control, unmated, 1.36 kg of food/day	control market wt, ad libitum	weighted mean	SE	*F*
aspartic acid	90.79 ± 1.0	91.02 ± 0.95	91.66 ± 0.6	92.69 ± 0.67	41.35	0.67	ns
threonine	43.21 ± 1.06	43.23 ± 0.28	44.31 ± 0.21	43.46 ± 0.21	43.42	1.12	ns
serine	36.83 ± 1.57	37.67 ± 0.87	38.55 ± 0.29	38.66 ± 0.35	37.97	1.92	ns
glutamic acid	149.75 ± 0.53	149.57 ± 1.35	148.57 ± 0.81	148.57 ± 0.52	148.58	1.58	ns
proline	39.77 ± 0.72	41.24 ± 0.39	40.47 ± 0.56	40.26 ± 0.51	40.44	1.16	ns
glycine	41.27 ± 0.34	43.63 ± 0.91	41.95 ± 0.71	40.85 ± 0.62	41.85	1.63	ns
alanine	57.02 ± 0.27	57.26 ± 0.07	57.40 ± 0.37	56.19 ± 0.15	56.85	0.62	*
cysteine	11.21 ± 0.26	10.98 ± 0.19	10.87 ± 0.09	10.95 ± 0.10	11.07	0.28	ns
valine	52.08 ± 0.49	51.97 ± 0.20	52.02 ± 0.12	51.99 ± 0.22	51.81	0.57	ns
methionine	36.80 ± 0.61	36.70 ± 0.38	35.21 ± 0.46	36.69 ± 0.59	36.72	1.13	ns
isoleucine	50.24 ± 0.10	48.88 ± 0.14	49.05 ± 0.37	50.38 ± 0.47	49.63	0.88	*
leucine	82.09 ± 0.21	81.98 ± 0.30	81.36 ± 0.21	81.58 ± 0.25	81.30	0.55	ns
tyrosine	36.61 ± 1.48	37.04 ± 0.53	38.38 ± 0.23	38.91 ± 0.38	37.64	1.81	ns
phenylalanine	40.99 ± 0.09	41.08 ± 0.11	41.13 ± 0.07	41.14 ± 0.12	41.12	0.23	ns
histidine	43.55 ± 0.41	42.79 ± 0.70	43.38 ± 0.70	41.96 ± 0.18	43.04	1.17	ns
lysine	91.77 ± 2.28	89.96 ± 0.61	90.06 ± 0.27	90.64 ± 0.49	90.68	2.05	ns
arginine	65.35 ± 0.85	65.87 ± 0.17	65.76 ± 0.17	65.42 ± 0.19	65.44	0.87	ns
tryptophan	12.08	11.35	11.44	12.38	12.01	0.41	
4-hydroxyproline	4.72 ± 0.34	6.05 ± 0.74	4.78 ± 0.38	4.37 ± 0.27	5.02	1.07	ns
5-hydroxylysine	1.92 ± 0.04	2.49 ± 0.14	2.25 ± 0.17	1.91 ± 0.04	2.16	0.26	ns
N^6-methyllysine	0.27 ± 0.02	0.30 ± 0.03	0.35 ± 0.04	0.52 ± 0.07	0.36	0.02	ns
N^6,N^6-dimethyllysine	0.90 ± 0.15	0.79 ± 0.14	0.69 ± 0.11	0.99 ± 0.07	0.84	0.06	ns
N^6,N^6,N^6-trimethyllysine	1.50 ± 0.24	1.56 ± 0.07	1.56 ± 0.14	1.83 ± 0.15	1.68	0.06	ns
N^π-methylhistidine	2.65 ± 0.29	3.36 ± 0.19	2.83 ± 0.22	2.34 ± 0.20	2.83	0.14	ns
N^τ-methylhistidine	4.82 ± 0.09	5.91 ± 0.19	5.54 ± 0.25	4.13 ± 0.15	5.17	0.19	**
unknown peak 17	1.71 ± 0.07	1.18 ± 0.08	0.91 ± 0.24	1.75 ± 0.29	1.33	0.11	ns
ammonia	8.10 ± 0.78	7.34 ± 0.24	7.33 ± 0.28	7.23 ± 0.07			
total AA N, g/kg DM	179.49	180.77	181.69	179.82			
total protein,[b] g of protein/kg of dry wt	796.86 ± 3.9	795.77 ± 12.3	801.53 ± 15.0	785.96 ± 7.58			
WE,[b] μg/mmol	0.110765	0.110403	0.110640	0.110903			

[a] Mean values ± standard error of measurements (SEM); $N = 4$ (number of replicates); $N \times 8$ = number of determinations. Significance: *F*, values from analysis of variance: **, $P < 0.01$; *, $P < 0.05$; ns, not significant. SE, standard error of difference between means. [b] Calculated according to the method of Horstmann (1979) using eq 2.

studies of force-fed chicks and female Sprague-Dawley rats (Drewry et al., 1988). Chickens given 170% of ad libitum intake, via gastric intubation, had elevated gains of carcass protein and fat.

The mineral profiles (milligrams per 100 g DWFFB, dry weight, fat-free basis) of the semimembranosus muscle excised from once-mated or unmated gilts as presented in Table III (DWFFB) appeared to be similar. Because the high or low food allowances for gilts were adjusted to equalize intakes of trace minerals, as described by Elliot et al. (1982), there was no significant difference in the mineral composition between treatments, except for iron (Fe). The variation noted for Fe between treatments was statistically significant ($P < 0.01$), with the semimembranosus muscle of the control market weight gilts being consistently lower in total Fe than either the mated or unmated animals. Mean Fe (DWFFB) values for this important micronutrient ranged from 3.47 mg/100 g in control market weight gilts to 4.35 mg/100 g in semimembranosus for gilts under severe levels of food restriction. These values for Fe are much lower than those reported for porcine muscles by Zarkadas et al. (1987a), which ranged from 5.78 to 6.80 mg/100 g DWFFB in semimembranosus for boars and sows, respectively. Wagner et al. (1976) reported that the Fe content in porcine muscle was 1.72 mg/100 g WWB. Most of this storage iron is usually present in functional form in a variety of heme compounds (myoglobin, cytochromes), enzymes with iron–sulfur complexes, and other iron-dependent enzymes.

Sensory evaluation of the semimembranosus muscle of ham roasts from both mated and unmated gilts revealed no differences in flavor (Table III) among treatments (Elliot et al., 1982). On the other hand, the ham roasts from once-mated gilts tended to be more juicy than those that were excised from unmated market weight controls, but the results (Table III) were inconsistent and the differences small. The final controls (FI) were less juicy than either the initial controls (IC) or treatment HIG. In all cases ham roasts from gilts that had farrowed tended to be significantly ($P < 0.05$) less tender than those from maiden control gilts, possibly due to pregnancy or to differences in the myofibrillar and collagen contents in these muscles. The shear value results followed tenderness data quite closely.

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 IV and V. Protein determinations were carried out in each acid hydrolysate as described previously (Zarkadas et al., 1988a,b), and the results are summarized in Table IV. This method of calculating the protein mass in muscle tissues is based upon knowledge of the amino acid composition of the protein mixture and yields accurate estimates of the amount of protein present as determined by eqs 1 and 2. The mean residue weight (WE, micrograms per nanomole) given in Table IV is in accord with the values reported previously for Yorkshire pigs (Zarkadas et al., 1987b). The amino acid compositions (grams per kilogram of protein) of unextracted semimembranosus skeletal muscle from once-mated or unmated young gilts were similar. Dietary treatment had significant effects on a few individual amino acids, but no other general patterns of differences were discernible. The apparent differences noted in the levels of aspartic acid, proline, glycine, histidine, and Pro(4-

Table V. Amino Acid Composition[a] of Extracted Porcine Skeletal Muscle (Semimembranosus) Excised from Yorkshire Gilts Mated at Puberty and Given High or Low Food Allowances from Day 85 of Gestation (Grams of Amino Acid per Kilogram of Total Protein)

	dietary treatment			
amino acid	HIG, 1.36 kg of food/day	LIG, 0.45 kg of food/day	control, unmated, 1.36 kg of food/day	control, market wt, ad libitum
aspartic acid	94.46 ± 0.22	96.36 ± 0.38	94.67 ± 0.23	94.17 ± 1.52
threonine	43.46 ± 0.12	38.52 ± 0.12	43.53 ± 0.40	43.95 ± 0.32
serine	35.15 ± 0.16	35.75 ± 0.17	34.94 ± 0.41	37.68 ± 0.36
glutamic acid	150.20 ± 0.90	152.20 ± 0.34	151.14 ± 0.66	151.80 ± 1.29
proline	38.76 ± 0.29	39.01 ± 1.07	40.13 ± 1.41	38.78 ± 0.90
glycine	42.31 ± 0.46	41.94 ± 0.23	41.94 ± 0.35	41.15 ± 1.55
alanine	52.58 ± 0.57	52.82 ± 0.41	52.52 ± 0.44	53.46 ± 1.12
cysteine	11.54 ± 0.26	11.89 ± 0.19	11.20 ± 0.09	11.15 ± 0.10
valine	54.86 ± 0.43	54.09 ± 0.42	55.36 ± 0.33	55.12 ± 1.36
methionine	36.18 ± 0.97	33.38 ± 0.82	35.79 ± 0.66	38.37 ± 1.60
isoleucine	59.50 ± 0.16	61.67 ± 0.41	59.01 ± 0.25	56.86 ± 2.83
leucine	86.44 ± 0.44	87.31 ± 0.19	88.00 ± 0.26	86.58 ± 2.13
tyrosine	39.37 ± 0.07	38.10 ± 0.07	38.68 ± 0.04	38.79 ± 0.11
phenylalanine	42.25 ± 0.33	43.38 ± 1.56	42.14 ± 0.18	42.56 ± 0.80
histidine	31.45 ± 0.41	33.18 ± 0.17	32.41 ± 0.16	32.72 ± 0.68
lysine	93.5 ± 0.32	93.86 ± 0.64	92.21 ± 0.30	92.60 ± 1.24
arginine	66.65 ± 0.42	66.46 ± 0.51	66.57 ± 0.29	65.87 ± 0.48
tryptophan	12.44	10.98	11.44	12.60
4-hydroxyproline	6.14 ± 0.30	6.69 ± 0.20	5.89 ± 0.21	3.49 ± 0.21
5-hydroxylysine	0.94 ± 0.03	0.76 ± 0.03	0.66 ± 0.04	0.58 ± 0.06
N^r-methyl histidine	0.529 ± 0.03	0.549 ± 0.01	0.544 ± 0.03	0.554 ± 0.05

[a] Mean values and standard error of measurements (SEM).

Table VI. Evaluation of the Protein Quality of Porcine Semimembranosus Muscle Excised from Yorkshire Gilts Mated at Puberty and Given High or Low Food Allowances from Day 85 of Gestation

	dietary treatment			
	HIG	LIG	control, unmated	control, market wt
(i) essential amino acids (EAA)				
total EAA,[a] mg/g of nitrogen	2696.2	2654.1	2662.7	2704.5
EAA index[b]	79.9	78.9	78.6	79.9
chemical score[b]	79.8	80.1	80.1	79.2
limiting amino acids[b]	Phe, Val	Phe, Val	Phe, Val	Phe, Val
EAA_7,[c] of total protein	39.73	39.38	39.33	39.54
EAA_{10},[c] of total protein	51.82	51.38	51.39	51.51
(ii) protein efficiency ratio (PER)[d] predicted by				
eq 5 PER_7	3.102	3.074	3.074	3.086
eq 6 PER_{10}	3.121	3.094	3.094	3.102
(iii) myofibrillar proteins,[e] g/kg of total proteins				
actin	109.50	123.64	112.61	114.68
myosin	227.99	236.62	234.46	238.77
actomyosin	337.50	360.26	347.07	353.45
total myofibrillar	518.95	538.57	533.66	543.47
(iv) connective tissue proteins[f]	49.30	53.72	47.29	28.02
collagen and collagen-like proteins[g]	59.50	48.11	41.78	36.71

[a] Computed from reference protein standards FAO/WHO (1965). [b] Calculated according to the methods of Block and Mitchel (1946) and Oser (1951). [c] Calculated according to the method of Lee et al. (1978). EAA_7: threonine, valine, methionine, isoleucine, leucine, phenylalanine, and lysine. EAA_{10}; EAA_7 plus histidine, arginine, and tryptophan. [d] PERs were calculated according to the method of Lee et al. (1978) from eq 5 ($PER_7 = 0.08084(EAA_7) - 0.1094$) and eq 6 ($PER_{10} = 0.06320(EAA_{10}) - 0.1539$). [e] Calculated from eqs 4, 4a, 4a′ and 4b using His(τ-Me) data (Table V). [f] Calculated from Pro(4-OH) data (Table V) by eq 3a. [g] Calculated from Lys(5-OH) data (Table V) by eq 3b.

OH) were not statistically significant. There was, however, significant variation ($P < 0.05$) in the content of alanine and isoleucine between dietary treatments. Extraction of the soluble amino acids and histidine dipeptides (carnosine, anserine, and balenine) that occur in variable amounts in most muscle tissues (Harris and Milne, 1987; Kohen et al., 1988) removed 25.7–29.9% of total histidine in unextracted tissue, constituting only 0.75–1.0% of the total amino acid content in this muscle tissue.

When comparisons of the essential amino acid (EAA) profiles of semimembranosus skeletal muscles from mated and unmated gilts (Table VI) are made as recommended by Lee et al. (1978), Pellet and Young (1984), and Young and Pellet (1984), the results indicated that porcine muscle appears to be a good source of all amino acids commonly found in proteins with the exception of phenylalanine and valine and possibly isoleucine and tryptophan. Mean values for total EAA_7 and EAA_{10} ranged, respectively, from 39.3–39.7 to 51.3–51.8% in porcine semimembranosus muscles evaluated (Table VI). These results are consistent with those listed by Pellett and Young (1984) for skeletal muscles. Because this scoring procedure is by definition limited to the essential amino acids, Lee et al. (1978) developed equations (eqs 5 and 6 listed in Tables II and IV) for predicting the protein efficiency ratios (PERs) of bovine muscle proteins from amino acid data (Expert Work Group, 1984). In using these predictive equations, the results summarized in Table VI show mean PER values close to a value of 3.1 for the semimembranosus skeletal muscle proteins and that the PER values depend upon the amount of connective tissue proteins present in skeletal muscles.

The unique basic amino acid contents of this muscle tissue were also determined in the 96-h hydrolysates. The

weighted mean values obtained for the protein-bound His-(τ-Me) and Pro(4-OH) and Lys(5-OH) contents of this porcine skeletal muscle are also presented in Table V. Although dietary restriction of gilts during gestation had no significant effect on the unique basic amino acid content of the semimembranosus skeletal muscle evaluated, values for both His(τ-Me) and Lys(5-OH) varied between once-mated and unmated gilts fed restricted diets compared to those of the control animals. Values for His(τ-Me) ranged from 0.529 to 0.549 g/kg of protein in gilts mated at puberty to 0.544 to 0.554 g/kg of protein in control animals. By contrast, the Lys(5-OH) content ranged from 0.73 to 0.91 g/kg in gilts fed restricted diets compared to that found in control animals which was low and ranged from 0.57 to 0.64 g/kg of protein. These data corresponded closely to the Pro(4-OH) values reported in Table V, with the semimembranosus muscle from food-restricted animals being much higher in total Pro(4-OH) (5.94–6.39 g/kg of protein) compared with the lower values (3.43–5.72 g/kg of protein) found in the control animals. The variations noted between levels of His(τ-Me) and Lys(5-OH), within the same muscle of young gilts, may reflect variations in both the developmental stage of the semimembranosus muscle tissues of the young pigs and the anatomical location of the connective tissue proteins in the extracellular matrix.

Myofibrillar and Connective Tissue Protein Components of Porcine Muscles. From the protein-bound His(τ-Me), Pro(4-OH), and Lys(5-OH) contents (Table V) of porcine skeletal (semimembranosus) muscles from young once-mated and unmated gilts under dietary restriction, it has been possible to determine their myofibrillar and connective tissue protein contents as described previously (Zarkadas et al., 1988a,b), and the results are summarized in Table VI. Actin in the porcine semimembranosus muscle accounts for an estimated 11.0–11.5% of total muscle protein or about 21.35% of the myofibrillar proteins. The values obtained for myosin ranged from 22.8 to 23.9% of the total muscle protein in the semimembranosus muscle of gilts, corresponding to an average value of 43.9% of myofibrillar proteins.

Although the relative amounts of actin and myosin per unit of tissue among the porcine semimembranosus muscles investigated remain constant, the small differences noted in myofibrillar protein content of semimembranosus from the average figure of 57.71% reported by Yates and Greaser (1983) may reflect variations in the levels of fast, slow, or mixed fast/slow classes of myosin heavy-chain isoforms present in this skeletal muscle. Recent evidence has indicated that porcine G-actin from the semimembranosus muscle contained 0.97 mol of His(τ-Me)/mol of protein (Zarkadas et al., 1988b). The lower protein-bound His(τ-Me) content of the porcine semimembranosus muscle can be attributed to differences in the distribution of myosin heavy-chain isoforms in this skeletal muscle tissue. Staron and Pette (1987a,b) have shown that a maximum of 54–60 myosin isoforms can coexist in just two rabbit skeletal muscles (soleus and tibialis anterior). It has also been shown that all three adult forms of myosin in skeletal muscle, corresponding to two adult fast myosin heavy chains (fast oxidative IIA and fast glycolytic IIB) and one slow myosin heavy chain (slow oxidative, type I), are tissue-specific and developmentally regulated, with more than one myosin heavy-chain gene expressed in each muscle and developmental stage (Lowey, 1986; Gauthier, 1986; Mahdavi et al., 1986).

In the present study, an attempt was also made to relate the amounts of the unusual protein-bound amino acids, which occur exclusively in collagen and collagen-like proteins, i.e., the C1q protein component of the complement and eel acetylcholinesterase (Anglister et al., 1976; Porter and Reid, 1978), to the contents of connective tissue in the semimembranosus muscle of once-mated gilts under dietary restriction. In this chemical approach the content of total collagen and collagen-like proteins in this porcine skeletal muscle was determined from the amounts of Lys-(5-OH) found in their acid hydrolysates (Table V) using eq 3a and the content of total connective tissue proteins from the amounts of Pro(4-OH) present using eq 3b (Berg, 1982). The mean values for total collagen ranged from 3.67% in the semimembranosus of unmated initial control (IC) gilts to 4.18% in final control (FC) animals and from 4.81 to 5.95% in the semimembranosus muscle isolated from the low- and high-intake gilts, respectively. These results are in reasonably good agreement with those reported by Bendall (1967), Dransfield (1977), Light et al. (1985), and Zarkadas et al. (1988b) for the distribution of collagen (average 4.35%; range 1.22–15.1%) in 34 bovine and 3 porcine skeletal muscles investigated. The sensory evaluation data of Elliot et al. (1982) have shown that ham roasts from maiden gilts were significantly more tender than those from gilts that had farrowed. Further studies will be required to establish whether the small differences noted in collagen content (Table VI) between maiden and bred gilts, which do affect tenderness to a degree (Dransfield, 1977; Davey, 1984), may actually reflect variations in the proportion of heat stable to heat labile cross-linking amino acids in intramuscular collagen. Studies from Bailey's laboratory, however, have indicated that it is not only the quantity of collagen but also the amount of cross-linking, the nature, and the solubility properties of the collagen present in a muscle that play a role in determining meat toughness and texture (Bailey, 1984; Light et al., 1985). Recent studies by Mills et al. (1989) have shown that thermal stability of collagen declined while muscle collagen solubility increased during the first 24 h after slaughter. In addition, the physical characteristics of extracellular matrix connective tissue proteins changed. Thus, the early post-mortem degradation of collagen appeared to affect beef tenderness. Strange and Whiting (1990) have reported that restructured beef products are more acceptable when the raw materials are free of tendons and contain only limited amounts of epimysial collagen.

The results presented in Table VI also demonstrate that the content of collagen is higher in the semimembranosus muscle from once-mated gilts under either moderate or severe dietary restriction than that of the control animals. This could be associated with the increased demand on the muscle during pregnancy to support the increased weight of the foetus rather than the effect of feed restriction on collagen development. This suggestion is supported by recent studies of Kovanen et al. (1984), who have shown that differences in muscle collagen content appear to be related to the level of individual muscle fibers or even the level of perimysium and endomysium within a given fiber type (Bailey, 1984).

It thus became apparent that, in addition to myofibrillar and connective tissue proteins of the extracellular matrix, a large quantity of muscle protein is found in the intracellular muscle protein fraction of porcine muscles which ranged from 41.98 to 42.2% of the total muscle proteins. These results are considerably higher than those found (28–34%) by Hanson and Huxley (1957) as soluble proteins (sarcoplasmic) washed out of the glycerol-extracted muscle after it had been broken up into fibrils, but they are in accord with the 41.6% figure quoted by

Szent-Gyorgi et al. (1955), using two different extraction procedures, and with those reported by Zarkadas et al. (1988b) for porcine muscles. Thus, in addition to sarcoplasmic proteins (28.0%), a large quantity (14.0%) of intracellular skeletal muscle proteins exists, which may originate from organelles, the cytoskeleton and Z-bands, and other membrane structures.

From the foregoing results, it becomes evident that a potentially useful means for evaluating the protein quality of different muscles or meats might be based on a knowledge of their amino acid composition and myofibrillar and connective tissue protein contents, as recommended by the Expert Work Group (1984), Pellett and Young (1984), Young and Pellett (1984), and Zarkadas et al. (1988b, 1990).

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Received for review August 14, 1991. Accepted September 11, 1991.