

Table I. Accuracy (% Recovery) and Precision of HMB Analyses of Supplemented Test Feeds Based on 15 Independent Analyses at Each Supplemental Level

N	HMB, %		recovered, %	CV, %
	theory	found \pm 1 SD		
15	0.05	0.048 \pm 0.002	96	4.2
15	0.10	0.096 \pm 0.004	96	4.2
15	0.20	0.195 \pm 0.006	98	3.1
15	0.40	0.397 \pm 0.013	99	3.3
			97 ^a	3.7 ^a

^a Average value.

the region of 9–11 min. These peaks make it difficult to quantify any dimer that might be present. In this case, the dimer peaks are seen as shoulders on the interfering peaks. Chromatogram 1C is characteristic of a feed sample, supplemented with 0.1% HMB, taken through the entire extraction and hydrolysis procedure. The potassium hydroxide added to the feed extract rapidly hydrolyzes any oligomers present to monomer. Total quantitation is achieved with all of HMB characterized as monomer in a region of the chromatogram free from interferences. Addition of phosphoric acid lowers the pH of the hydrolyzed sample to pH 4–5, a range allowing for good column separation.

Accuracy (percent recovery) and precision of the method were determined by analyzing laboratory-supplemented feeds for DL-HMB-FA at four concentration levels ranging from 0.05 to 0.40%. By the method of calibration described, HMB recoveries of 15 independent analyses at each supplemental level averaged 97% of theory (Table I). Precision was determined by calculating the coefficient of variation for each set of 15 analyses. This CV averaged 3.7%. These statistics are comparable to the GC and ITP methods for determining HMB in supplemented feeds and also to that reported for analysis of DL-methionine in feeds. Analyses of feed samples supplemented with radiolabeled DL-HMB-FA confirm an extraction efficiency of 98–100%.

In addition to these analyses, 54 commercial broiler, layer, and turkey feeds ranging in concentration from 0 to 0.6% DL-HMB-FA were analyzed by HPLC and also by the GC procedure (Day et al., 1983). The excellent agreement between the two methods is illustrated by a linear regression performed on the pairs of data ($R = 0.98$). Samples analyzed for DL-HMB-Ca correlate equally well. Under the conditions described in this paper, HMB levels as low as 0.008% can be determined (signal/noise 2).

In summary, the HPLC method for the analysis of DL-HMB-Ca and DL-HMB-FA has proven to be rapid and reliable, providing analyses in a time-efficient manner. The procedure yields accurate and precise results and exhibits excellent correlation with the GC procedure. Since sample preparation is simple and the HPLC instrumentation is accessible in many laboratories, the method could be an effective quality control technique for many feed operations worldwide.

Registry No. HMB, 583-91-5.

LITERATURE CITED

- Beck, A.; Schmidburn, H.; Spender, M.; Tanner, J. *Literature Digest for the Feedstuffs Industry*; Degussa AG: Federal Republic of Germany, 1978.
- Day, R. J.; Borowy, D. E.; Schisla, R. M. *J. Agric. Food Chem.* **1983**, *31*(4), 889–891.
- Feit, M. J.; Daun, R. J.; Hodel, H. H.; Zietlow, D. C. *Poultry Sci.* **1984**, *63*, 100 (abstract).
- Holten, C. H. *Lactic Acid—Properties and Chemistry of Lactic Acid and Derivatives*; Verlag Chemie: Weinheim, 1971.
- McCarthy, T. E.; Sullivan, M. X. *J. Biol. Chem.* **1941**, *141*, 871–876.
- Vinjamoori, D. V.; Schisla, R. M. *Poultry Sci.* **1984**, *63*, 199 (abstract).
- Vinjamoori, D. V.; Schisla, R. M. *J. Assoc. Off. Anal. Chem.* **1986**, *69*(3), 474–477.

Received for review August 4, 1986. Accepted April 17, 1987. The material included in this paper was presented in part at the Poultry Science Association Meeting, Aug 1985, Ames, IA.

Protein Value of Dry Bean Cultivars: Factors Interfering with Biological Utilization

Jose Fernando Durigan, Valdemiro Carlos Sgarbieri,* and Eduardo Antonio Bulisani

The protein content and protein nutritive value of cooked dry beans from 12 cultivars were studied. Methionine and cysteine were consistently the most limiting amino acids. Best values for methionine (0.96–1.99 g/100 g of crude protein) were obtained by BrCN reaction with bean flour followed by GLC of methyl thiocyanate formed and for cysteine (0.5–2.2 g/100 g of crude protein) by GLC of the amino acid derivatives. Methionine determined by BrCN/GLC correlated well ($r = 0.820^{**}$, $p = 0.01$) with bioavailable methionine (0.23–0.77 g/100 g of crude protein). PER (0.79–1.16) showed linear positive correlation ($r = 0.718^{**}$, $p = 0.01$) with total sulfur (120–270 mg/100 g of whole bean) and a weak negative correlation ($r = -0.511$, $p = 0.10$) with total tannin (470–570 mg/100 g of whole bean).

Dry beans (*Phaseolus vulgaris*, L.) have been used in human diets mainly as a source of protein and carbohy-

drate. They furnish about 28% of the protein and 12% of the calories in the Brazilian diet (Sgarbieri and Garruti, 1986).

The nutritive properties of the dry bean protein have been studied by a large number of investigators (Jaffé, 1950a; Bressani et al., 1961; Evans and Bandemer, 1967; Sgarbieri et al., 1982), among others. It has been shown that limiting sulfur amino acids and the presence of protein

Departamento de Planejamento Alimentar e Nutrição (DePAN), 13100 Campinas, SP, Brazil (J.F.D., V.C.S.), and Seção de Plantas Leguminosas, IAC, Campinas, SP, Brazil (E.A.B.).

Table I. Crude Protein, Total Digestible Carbohydrates, Total Lipids, and Mineral Contents of Beans from Various Cultivars (Dry Matter)

cultivar (local name)	crude protein, ^a %	digestible carbohydrate, starch %	total lipids, %	ash, %
Aroana	29.0 ± 1.0	57.4 ± 0.5	1.3 ± 0.1	4.1 ± 0.1
Cara Suja	28.2 ± 0.7	65.2 ± 1.0	1.6 ± 0.0	4.5 ± 0.2
Jalo	26.6 ± 0.6	68.6 ± 0.7	1.5 ± 0.1	4.0 ± 0.3
Goiano Precoce	26.8 ± 0.7	64.1 ± 1.0	1.3 ± 0.1	3.9 ± 0.1
Carioca	23.0 ± 0.7	69.7 ± 1.1	1.4 ± 0.1	3.7 ± 0.3
Piratã-1	27.4 ± 0.7	67.5 ± 1.0	1.3 ± 0.0	4.5 ± 0.0
Iguaçu	27.2 ± 0.3	60.7 ± 1.5	1.3 ± 0.0	4.8 ± 0.1
Rico 23	24.5 ± 0.6	60.7 ± 0.9	1.0 ± 0.1	5.1 ± 0.3
Aeté 1	26.2 ± 0.8	60.7 ± 0.8	1.1 ± 0.0	5.1 ± 0.1
Aeté 3	25.0 ± 0.0	65.2 ± 0.6	1.4 ± 0.1	4.3 ± 0.1
Rosinha G2	23.0 ± 0.4	61.9 ± 1.3	1.4 ± 0.1	4.3 ± 0.1
Roxinho	25.5 ± 0.6	61.9 ± 1.0	1.3 ± 0.0	5.0 ± 0.6

^a N × 6.25.

and nonprotein antinutritional substances are responsible for the low protein value of dry beans.

Among the toxic and antinutritional factors recognized as protein, the protease inhibitors and the lectins are the ones that have received more attention (Jaffé, 1950b; Jaffé and Vega Lette, 1968; Jaffé and Brucher, 1972; Pusztai and Palmer, 1977).

A comprehensive review of the physicochemical and nutritional properties of dry bean protein was published recently (Sgarbieri and Whitaker, 1982).

Other antinutritive factors that have received considerable attention by various investigators are the phenolic compounds (Elias et al., 1979; Martin-Tanguy et al., 1977; Bressani et al., 1980).

In this paper, the protein values of 12 dry bean cultivars are compared and some of the factors influencing protein utilization are discussed.

MATERIALS AND METHODS

Bean Cultivars. Local names and the colors of the cultivars were as follows. Light brown: (1) Aeté 3; (2) Goiano Precoce; (3) Jalo; (4) Carioca. Dark brown: (5) Aeté 1; (6) Piratã 1; (7) Cara Suja; (8) Aroana. Light pink: (9) Rosinha G2. Dark pink: (10) Roxinho. Black: (11) Rico 23; (12) Iguaçu. They were all cultivated in the same plot at the Agronomic Institute of Campinas, S.P., Brazil, under the same cultural practices to avoid as much as possible distinctive environmental factors.

Preparation of Samples for Chemical Determinations and Biological Assays. The raw beans with approximately 10% water content were ground in a hammer mill to pass a 70-mesh screen and used for the chemical determinations.

For the biological assays the beans were first soaked in water (1:4, w/v) at room temperature for 8 h, autoclaved (10 min, 121 °C), cooled, frozen and freeze-dried, and then ground to pass the 70-mesh screen.

Chemical and Physical Determinations. Moisture content and total lipid were determined according to the AOAC (1970). Total nitrogen and crude protein (% N × 6.25) were determined according to AOAC procedures (1975). Extraction and fractionation of the proteins were performed by the procedure of Sgarbieri et al. (1982). Nonprotein nitrogen was determined in the TCA-soluble fraction by the procedure of Becker et al. (1940). Amino acid composition was determined by ion-exchange chromatography in a Beckmann 119CL amino acid analyzer (Beckman Instruments, 1966) and by gas-liquid chromatography of the amino acid derivatives (Irrman, 1974) using a Varian 244055 gas chromatograph. Total methionine was also determined by the reaction of cyanogen bromide (BrCN) followed by quantification of the methyl thiocyanate formed in the reaction by gas-liquid chromatog-

Table II. Globulins, Albumins, and Globulin to Albumin Ratios in Beans from Various Cultivars

cultivar (local name)	globulins (% N × 6.25) ^a	albumins (% N × 6.25) ^a	globulin/albumin
Aroana	13.8 (52.9) ^b	3.5 (13.5) ^b	3.93
Cara Suja	12.8 (50.9)	2.9 (11.4)	4.46
Jalo	9.4 (39.5)	2.2 (9.2)	4.28
Goiano Precoce	11.3 (47.5)	3.2 (13.5)	3.52
Carioca	9.1 (44.3)	3.6 (17.4)	2.55
Piratã-1	9.7 (39.6)	4.0 (16.5)	2.41
Iguaçu	8.1 (33.4)	3.9 (15.9)	2.10
Rico 23	8.5 (38.8)	2.6 (12.0)	3.24
Aeté 1	9.9 (42.2)	4.5 (19.3)	2.20
Aeté 3	9.6 (43.0)	4.5 (20.1)	2.14
Rosinha G2	10.2 (49.6)	3.8 (18.5)	2.68
Roxinho	10.8 (46.2)	4.1 (17.6)	2.63

^a Results are given in grams/100 g of dry matter, after extraction with 0.5 M NaCl and exhaustive dialysis against distilled water.

^b Results in parentheses represent the percentage of total bean nitrogen contributed by the extracted globulins and albumins.

raphy (Apostolatos and Hoff, 1981). A methionine standard curve was constructed by reacting methionine with BrCN and plotting micrograms of methionine on the abscissa vs. the ratio of the areas of methyl thiocyanate to ethyl thiocyanate (this used as internal standard) on the ordinate. Elimination of interference by glutamyl-S-methylcysteine, naturally occurring in the seeds, was made by extraction of the samples with 70% ethanol as proposed by McIntosh and Ellinger (1976). Tryptophan was determined colorimetrically by the method of spies (1967). Total sulfur was determined by turbidimetry according to Tabatabai and Bremmer (1970). Total tannin was determined by the method of Joslyn (1970). For determination of total digestible carbohydrate the samples were prepared by the method of Kaneshiro et al. (1977) and the equivalents of glucose determined by the method of Dubois et al. (1956).

Preparation of Diets and Biological Assays. Protein values of the cultivars was determined by the NPR (net protein ratio) and by the ratio of body weight gain/protein ingested during a 10-day period (PER₁₀). The diets were prepared to contain 10% protein, all from beans, 8% corn oil, 4% mineral mix (Rogers and Harper, 1965), 2% vitamin mix (NBC, 1977/1978), and carbohydrate mix (corn starch to sucrose, 75:25) to complete 100%. The contents of lipid mineral and vitamin in the bean cultivars were disregarded in the diet formulation. Total carbohydrate of beans was counted with the added carbohydrate to make up for the total carbohydrate of the diets. Wistar rats weighting 40–50 g initial body weight were used, receiving water and diet ad libitum for 10 days. An identical group of six rats was maintained in a protein-free diet during the

Table III. Essential Amino Acid Composition^a of Beans from Various Cultivars Determined in the Amino Acid Analyzer (IEC) and in the Gas-Liquid Chromatograph (GLC)

cultivar (local name)	Lys		His		Thr		Met		CysH		Ile		Leu		Val		Phe		Tyr		Trp ^b	
	IEC	GLC	IEC	GLC	IEC	GLC	IEC	GLC	IEC	GLC	IEC	GLC	IEC	GLC	IEC	GLC	IEC	GLC	IEC	GLC	IEC	GLC
Aroana	6.3	8.0	3.2	...	5.2	4.4	1.0	0.2	0.4	1.6	3.8	3.0	8.2	8.9	4.9	4.6	5.7	5.8	3.3	3.6	1.0	1.1
Cara Suja	6.5	8.2	2.9	...	5.3	4.5	1.0	0.4	0.2	0.5	3.9	3.2	7.9	9.4	4.6	4.7	5.5	5.7	3.1	3.5	1.0	1.1
Jalo	6.4	7.4	3.1	...	5.0	4.0	1.1	0.7	0.3	1.6	4.4	2.9	7.5	8.4	5.5	4.5	5.7	5.4	3.2	3.3	1.0	1.0
Goiano Precoce	6.8	8.0	2.9	...	5.3	4.1	1.0	0.2	tr	1.7	4.8	2.9	7.8	8.8	5.2	4.7	5.6	5.5	3.1	3.3	1.0	1.1
Carioca	6.3	7.3	2.2	...	5.7	4.6	1.1	0.3	0.9	1.9	4.0	2.9	7.4	8.4	5.2	4.7	5.5	5.2	3.1	3.3	0.9	1.0
Piratã-1	7.0	8.3	2.9	...	5.1	4.6	1.1	0.4	0.7	1.8	3.9	3.2	7.5	8.5	5.2	5.0	5.4	5.9	2.9	3.5	1.7	1.8
Iguaçu	6.5	6.8	2.9	...	5.7	4.5	1.0	0.5	0.7	1.9	4.0	3.0	7.7	8.4	4.7	4.1	5.6	5.6	3.3	3.7	1.1	1.2
Rico 23	6.7	7.5	2.8	...	5.0	4.6	1.1	tr	0.9	2.0	4.4	3.1	8.0	8.8	4.9	5.1	5.6	5.6	3.3	3.5	1.1	1.1
Aeté 1	7.0	7.8	3.0	...	5.5	4.5	1.1	0.3	0.7	1.8	4.7	3.1	7.6	8.7	5.1	4.5	5.4	5.7	3.3	3.4	1.0	1.0
Aeté 3	7.0	7.5	3.3	...	5.3	4.0	1.0	tr	1.0	2.2	4.0	3.2	7.3	8.7	4.8	4.3	5.2	5.6	2.9	3.8	0.9	1.0
Rosinha G2	6.5	7.1	2.6	...	5.5	4.7	1.1	0.2	0.7	1.8	3.9	2.9	7.8	8.7	5.5	4.7	5.5	5.4	3.2	3.3	1.4	1.4
Roxinho	7.0	8.8	2.8	...	5.3	3.8	1.0	0.5	0.4	1.6	4.4	2.8	7.8	8.3	5.1	4.0	5.4	6.0	3.2	3.7	1.0	1.1

^a Results are given in grams of amino acid/100 g of amino acids recovered. Key: ...; destruction (unrecovered); tr, trace. ^b Method of Spies (1967).

Table IV. Methionine Contents^a and Bioavailable Methionine^b from Various Cultivars (g/100 g of Crude Protein)

cultivar (local name)	Met content	bioavailable Met
Aroana	1.60	0.54
Cara Suja	0.96	0.23
Jalo	1.30	0.48
Goiano Precoce	1.99	0.57
Carioca	1.30	0.35
Piratã-1	1.51	0.56
Iguaçu	1.96	0.77
Rico 23	1.67	0.43
Aeté 1	1.28	0.44
Aeté 3	1.74	0.70
Rosinha G2	1.54	0.59
Roxinho	1.35	0.49

^a Reaction with BrCN, determination of methyl thiocyanide formed by GLC. ^b Rat biological assay.

period. Apparent digestibility was calculated by the ratio of nitrogen absorbed to nitrogen ingested, expressed as percentage. The nitrogen absorbed was the difference between the intake and the excretion in the feces. Bioavailable methionine was determined by rat assays following the procedure of Sgarbieri et al. (1979). In each assay five groups of four rats were used. One group received autoclaved bean diet with no addition of methionine (basal diet); the other four groups received different and increasing levels of L-methionine. The growth response to added methionine was essentially linear. A plot of the growth responses on the ordinate vs. added methionine on the abscissa permitted, by extrapolation of the growth-response curve, the calculation of the bioavailable methionine originally present in the beans, which corresponds to the point where the extrapolated growth curve crosses the extended abscissa.

RESULTS AND DISCUSSION

The contents of crude protein, starch, total lipids, and minerals in dry beans from 12 cultivars are shown in Table I. Starch and protein are the main components of dry beans. For this reason they are considered a good source of dietary protein and energy. Protein contents, on a dry basis, varied from 23 to 29% and starch contents from 57.4 to 69.7%. Fat contents were quite low (1.0–1.6%) while mineral contents ranged from 3.9 to 5.1%.

Bean proteins are mainly globulins and albumins. Both groups of proteins are soluble in salt solutions and fractionated by exhaustive dialysis of a salt extract against distilled water. The globulins precipitate during dialysis while the albumins remain in water solution. Table II gives the amounts of globulin and albumin (grams/100 g of beans, dry basis) as well as the ratio of globulin to albumin from each cultivar. This ratio varied from 2.10 to 4.46. The numbers in parentheses (Table II) represent percentages of total bean nitrogen contributed by the extracted globulins and albumins. Unextracted nitrogen plus nitrogen losses during dialysis should represent the nitrogen unaccounted for in Table II.

Table III presents the essential amino acids, composition, and the cysteine and tyrosine contents of beans from all cultivars used in this study. Ion-exchange chromatography gave lower recoveries of lysine, cysteine, and leucine and higher recoveries of histidine, threonine, methionine, isoleucine, and valine as compared to gas-liquid chromatography. The recovery of cysteine was particularly good in the GLC determination, but that of methionine was very poor. We have no adequate explanation for the very low recovery of methionine in the GLC method. It could be either incomplete derivatization of methionine,

Table V. Nutritional Evaluation of Dry Bean Proteins from Various Cultivars^a

cultivar (local name)	protein consumption, ^b g	body wt gain, ^b g	PER ₁₀ ^c	NPR ^d	app digestibility (<i>D_A</i>), ^e %
Goiano Precoce	34.4	25.2	0.79	2.36	65.2
Jalo	40.0	32.5	0.81	2.19	64.4
Iguaçu	34.6	28.8	0.83	2.24	65.0
Rosinha G2	41.9	39.0	0.93	2.31	64.0
Piratã-1	38.4	37.4	0.97	2.27	71.7
Cara Suja	38.8	37.5	0.97	2.15	72.6
Aeté 3	38.7	37.5	0.97	2.42	59.3
Roxinho	43.4	44.9	1.03	2.23	64.9
Carioca	31.7	33.0	1.04	2.53	64.0
Rico 23	40.2	42.5	1.05	2.35	65.8
Aeté 1	42.8	46.5	1.09	2.40	61.3
Aroana	41.3	48.0	1.16	2.54	64.1

^a Beans were soaked in distilled water (8 h) and autoclaved 10 min, 121 °C. ^b Values for six rats during 10 days. ^c PER₁₀ = PER determined in a 10-day feeding period. ^d NPR = [weight gain group on 10% protein + weight loss group on protein-free diet]/protein intake group on 10% protein. ^e *D_A* (%) = [N intake - N in feces]/N intake × 100.

incomplete recovery from the chromatographic column, or both. Overall correlation for all amino acids in each bean cultivar determined by ion-exchange chromatography (IEC) and by GLC ranged from 0.903 for the cultivar Aroana to 0.958 for Goiano Precoce.

Determination of methionine was also done by GLC of methyl thiocyanate formed in the reaction of bean flour with BrCN. The results of these determinations are reported in Table IV and should be compared with bioavailable methionine determined by rat assays. From the three methods used for quantification of methionine the reaction with BrCN followed by GLC of methyl thiocyanate formed gave the highest values, except for the cultivar Cara Suja. It has been shown by McIntosh and Ellinger (1976) that legume seeds contain glutamyl-S-methylcysteine, which also reacts with BrCN, releasing methyl thiocyanate interfering with the quantification of methionine. According to these authors extraction of the beans with a 70% ethanol solution should remove all glutamyl-S-methylcysteine with elimination of interference. Assuming complete removal of the interfering factor the BrCN reaction-GLC procedure seems to be a good method for quantification of methionine in beans. Good linear positive correlation ($r = 0.820^{**}$, $p = 0.01$) was found between methionine contents encountered by BrCN reaction-GLC of methyl thiocyanate and the bioavailable methionine determined by rat assays ($y = 0.672 + 1.687x$). In this equation y is total methionine determined by the BrCN-GLC procedure and x the bioavailable methionine, both in grams/100 g of protein. This method seems to be promising in the estimation of total and available methionine in a large number of bean samples as, for example, in breeding programs.

Results of nutritional evaluation of bean protein from 12 cultivars are shown in Table V. Nutritional evaluation was performed in terms of body weight gain, the ratio of body weight gain to protein consumption (PER), net protein ratio (NPR), and apparent digestibility (*D_A* %). Body weight gain/protein consumption in the 10-day period (PER₁₀) varied from 0.79 to 1.16, NPR from 2.15 to 2.54, and apparent digestibility from 59.3 to 72.6%.

There was no apparent correlation between bioavailable methionine (Table IV) and either PER or NPR (Table V). We have no adequate explanation for this lack of correlation except that it suggests that methionine deficiency is not the only growth-limiting factor in cooked beans. Previous detailed study in one of the cultivars (Rosinha G2) led to the conclusion that at least two types of compounds are present in dry beans with antinutritional properties (Sgarbieri et al., 1982). One type is represented by heat-labile and toxic compounds that are inactivated by heat treatment. These are mainly lectins and digestive

Table VI. Contents of Sulfur, Tannin, and Nonprotein Nitrogen in Beans from Various Cultivars (Dry Basis) (mg/100 g)

cultivar (local name, color)	total sulfur	total tannin	non-protein nitrogen
Aroana (dark brown)	230 ± 17	791 ± 21	570
Cara Suja (dark brown)	190 ± 17	825 ± 11	560
Jalo (light brown)	120 ± 10	996 ± 52	520
Goiano Precoce (light brown)	180 ± 20	977 ± 23	630
Carioca (light brown)	250 ± 17	760 ± 51	470
Piratã-1 (dark brown)	180 ± 10	536 ± 8	750
Iguaçu (black)	160 ± 17	1016 ± 16	560
Rico 23 (black)	260 ± 10	846 ± 12	500
Aeté 1 (dark brown)	280 ± 20	877 ± 21	560
Aeté 3 (light brown)	270 ± 17	698 ± 8	570
Rosinha G2 (light pink)	250 ± 17	990 ± 73	530
Roxinho (dark pink)	240 ± 10	806 ± 8	530

enzyme inhibitors that were completely inactivated by autoclaving the soaked beans 10 min at 121 °C. The other type includes heat-stable compounds like the phenolics and probably others that have not been isolated and characterized.

Results from Evans and Bauer (1978) and Sgarbieri et al. (1982) showed that added methionine to cooked bean containing diets did not improve PER as expected. The same amount of methionine added to soybean or to isolated dry bean protein promoted much higher improvement in PER. This seems to indicate that whole beans contains heat-resistant growth-inhibiting substances that counteract the function of added methionine.

The digestibility of the bean proteins was generally low, around 60–65% except for two cultivars that were about 72%.

Table VI shows total sulfur, total tannins, and non-protein nitrogen contents for the 12 cultivars studied. A positive and statistically significant correlation ($r = 0.718^{**}$, $p = 0.01$) was found between the total sulfur and the ratio of body weight gain to protein consumed (PER₁₀) and weaker correlation ($r = 0.599^{**}$, $p = 0.05$) between total sulfur and NPR. A weak negative correlation ($r = -0.511$, $p = 0.10$) was found between tannin contents and PER.

Data obtained in our laboratory (Oliverira and Sgarbieri, 1986) showed that diets containing either raw or cooked beans stimulated a higher excretion of body (endogenous) nitrogen, when compared to a bean-free diet.

Evaluation of bean protein quality is a difficult task at present, due to various factors interfering with biological utilization of the proteins and their amino acids. The nature and the mode of action of some of these interfering factors are still not understood.

ACKNOWLEDGMENT

We express our gratitude to CNPq for the financial help made available for execution of the work reported here.

Registry No. Met, 63-68-3; S, 7704-34-9.

LITERATURE CITED

- AOAC *Official Methods of Analysis*, 11th ed.; Association of Official Analytical Chemists: Washington, DC, 1970.
- AOAC *Official Methods of Analysis*, 12th ed.; Association of Official Analytical Chemists: Washington, DC, 1975.
- Apostolatos, G.; Hoff, J. E. *Anal. Biochem.* **1981**, *112*, 126.
- Becker, H. C.; Milner, R. T.; Naget, R. H. *Cereal Chem.* **1940**, *17*, 447.
- Beckman Instruments *Procedures Manual* 120-PM-1; Spinco Division: Palo Alto, CA, 1966.
- Bressani, R.; Elias, L. G.; Navarete, D. A. *J. Food Sci.* **1961**, *26*, 525.
- Bressani, R.; Braham, J. E.; Elias, L. G. 5th Congreso Latinoamericano de Nutricion, Puebla, Mexico, 1980; p 44.
- Dubois, M.; Gilles, K. A.; Hamilton, J. K.; Rebers, P. A.; Smith, F. *Anal. Chem.* **1956**, *28*, 350.
- Elias, L. G.; Fernandez, D. G.; Bressani, R. *J. Food Sci.* **1979**, *44*, 524.
- Evans, R. J.; Bandemer, S. L. *J. Agric. Food Chem.* **1967**, *15*, 439.
- Evans, R. J.; Bauer, D. H. *J. Agric. Food Chem.* **1978**, *26*, 779.
- Irrman, M. A. K. *J. Chromatogr.* **1974**, *97*, 175.
- Jaffé, W. G. *Arch. Venez. Nutr.* **1950a**, *1*, 107.
- Jaffé, W. G. *Proc. Soc. Exp. Biol. Med.* **1950b**, *75*, 219.
- Jaffé, W. G.; Vega Lette, C. L. *J. Nutr.* **1968**, *94*, 203.
- Jaffé, W. G.; Brucher, O. *Arch. Latinoam. Nutr.* **1972**, *22*, 267.
- Joslyn, M. A. *Methods in Food Analysis: Physical, Chemical and Instrumental Methods of Analysis*, 2nd ed.; Academic: New York, 1970.
- Kanesiro, M. A. B.; Faleiros, R. R. S.; Bellingieri, P. A.; Albertini, P. E. G. *Ciética* **1977**, *5*, 276.
- Martin-Tanguy, J.; Guillaume, J.; Kossa, A. *J. Sci. Food Agric.* **1977**, *28*, 757.
- McIntosh, A.; Ellinger, G. M. *Proc. Nutr. Soc.* **1976**, *35*, 148A.
- NBC *Diet Catalog of the INC*; Nutritional Biochemicals Corp.: Cleveland, OH, 1977/1978.
- Oliveira, A. C.; Sgarbieri, V. C. *J. Nutr. Sci. Vitaminol.* **1986**, *32*, 425.
- Pusztai, A.; Palmer, R. *J. Sci. Food Agric.* **1977**, *28*, 620.
- Rogers, Q. R.; Harper, A. E. *J. Nutr.* **1965**, *87*, 267.
- Sgarbieri, V. C.; Whitaker, J. R. In *Advances in Food Research*; Chichester, C. O., Mrak, E. M., Schweigert, B. S., Eds.; Academic: New York, 1982; Vol. 28, p 93.
- Sgarbieri, V. C.; Garruti, R. S. *Can. Inst. Food Technol. J.* **1986**, *19*, 202.
- Sgarbieri, V. C.; Antunes, P. L.; Almeida, L. D. *J. Food Sci.* **1979**, *44*, 1306.
- Sgarbieri, V. C.; Antunes, P. L.; Junqueira, R. G. *Cienc. Tecnol. Aliment.* **1982**, *2*, 1.
- Spies, J. R. *Anal. Chem.* **1967**, *39*, 1412.
- Tabatabai, M. A.; Bremner, J. M. *Agron. J.* **1970**, *62*, 805.

Received for review September 18, 1986. Accepted April 1, 1987.

Hemicellulose Digestibility by Steers Fed Sun-Cured Hay and Drum-Dehydrated Alfalfa and Coastal Bermuda Grass

William R. Windham,* Henry E. Amos, and John J. Evans

Four ruminally and abomasally cannulated steers were used in a 4 × 4 Latin square design experiment to determine the digestibility of hemicellulose (HC) in coastal Bermuda grass (CBG) and alfalfa (Alf) and to assess the effect of analytical method on the determination and digestibility of HC. Forages: (a) CBG-hay (CBG-H); (b) CBG-dehydrated (CBG-D); (c) Alf-hay (Alf-H); (d) Alf-dehydrated (Alf-D). Hemicellulose was determined by three methods: (1) conventional (HC-C), as the difference between neutral detergent fiber (NDF) and acid detergent fiber (ADF) determined on separate aliquots; (2) sequential (HC-S) as the material solubilized by acid detergent treatment of NDF; (3) trifluoroacetic acid soluble (HC-TFA), as the material solubilized by TFA treatment of NDF. Xylose and arabinose were the major components of HC-TFA. Apparent digestibility (AD) of HC was higher ($P < 0.05$) in CBG than in Alf and appeared to be related to differences in lignin content and hemicellulosic monosaccharides. Xylose was less digested than arabinose in all forages. Across all forages, analytical methods ranked HC content and AD differently reflecting a method × diet interaction ($P < 0.002$). Results indicate that grass and legume HC differ in digestibility and that the choice of analytical methods may significantly affect the interpretation of HC degradation in the ruminant.

The plant cell wall has been envisioned to be composed of cellulose fibers embedded in an amorphous mixture of hemicellulose (HC), pectin, glycoprotein, and lignin. The HC fraction is a group of cell wall polysaccharides that comprise a large portion of the dry matter of many tropical and temperate grasses with lesser amounts in legumes. Hemicellulose polysaccharides have the ability to bind noncovalently through hydrogen bonding to cellulose and

to bind covalently to the pectin polysaccharide (Albersheim, 1976). The HC polysaccharides may therefore serve to interconnect the cellulose fibrils and the pectin polysaccharides of the cell wall. Considerable research has subsequently been focused on the carbohydrate composition and digestion of HC (Gaillard, 1962; Bailey and Ulyatt, 1970; Bailey et al., 1976; Daughtry et al., 1978; Bacon, 1979; Windham et al., 1983; Bittner and Street, 1983; Pitman and Moore, 1985). However, because of a wide variety of analytical protocols and nomenclature, results are difficult to compare. Often plant polysaccharides are classified according to the analytical procedure used to separate given components. These procedures generally fall into two categories: (a) those involving hydrolysis of the cell wall; (b) those involving extraction

Richard B. Russell Agricultural Research Center, Plant Structure and Composition Research Unit, USDA-ARS, Athens, Georgia 30613 (W.R.W., J.J.E.), and Department of Animal and Dairy Science, University of Georgia, Athens, Georgia 30602 (H.E.A.).