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The amino acid profiles of the whole plant and of four plant residues from temperate and tropical forages¹

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ABSTRACT: This study compared the amino acid (AA) profile of five residues (original forage, borate-phosphate buffer residue (BPR), neutral detergent fiber residue with (NDF+) and without (NDF-) sodium sulfite, and acid detergent fiber residue (ADF). Fourteen grasses and legumes from tropical and temperate regions were used in this study. The use of sodium sulfite did not affect the NDF concentration, but the NDF insoluble protein was lower ($P < 0.05$) in the NDF+ than in the NDF- (3.9 vs 4.5% DM, respectively). For all of the amino acids tested, the amino acid content, expressed as a percentage of CP, was lower in the ADF residue than in the original forage. There were no differences in the amino acid concentrations of the NDF- and NDF+ extracts ($P > 0.05$). Only in the case of methionine was there a difference in the amount of amino acid when the original forage was compared with the BPR (1.84 vs 1.45 % CP). When the AA profile of each residue was corrected for the AA content of the ADF, no difference was observed between the AA profile of

the original forage and of the BPR ($P > 0.05$). Similar to the result without correction for the amino acids in ADF, the AA profiles of the NDF+ and NDF- fractions were similar ($P > 0.05$). From this result, we infer that the sodium sulfite had similar effects on all AA in the NDF residue that we tested. There were differences in amino acid concentrations in the original forage and the NDF residues for several amino acids (Met, Cys, Lys, Thr, Arg, Ile, Leu, and Phe) ($P < 0.05$). When the amino acid values of the original forage and the BPR were used with animal data in the Cornell Net Carbohydrate and Protein System model, few differences in animal predicted performance were evident. These findings suggest that the AA profile of the original forage can be used to predict the AA profile of the undegraded intake protein instead of using the borate-phosphate buffer residue for amino acid analyses. This would simplify obtaining feed amino acid values for use in the Cornell Net Carbohydrate and Protein System.

Key Words: Amino Acids, Borates, Fiber, Models, Protein

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Introduction

Ruminants' protein requirements are met with microbial and undegraded intake protein (UIP) that escapes the rumen and is absorbed in the intestine (NRC, 2000). Endogenous protein supplies few AA under normal feeding conditions (O'Connor et al., 1993). Often,

UIP is used to supplement diets deficient in metabolizable protein (MP) (Volden, 1999) and provides much of the total protein.

The Cornell Net Carbohydrate and Protein System (CNCPS) divides feed into five protein fractions (A, B1, B2, B3, and C). Fractions A (NPN) and B1 (true protein) are soluble. The availability of fractions B1, B2, and B3 is based on their degradation and passage rates (Roe et al., 1990), most of which have been determined with in situ methods (England et al., 1997). Because soluble proteins are more rapidly degraded than those that are insoluble, soluble proteins are more likely to be utilized by the ruminal bacteria (Sniffen et al., 1992) and insoluble proteins have a greater probability of escaping the rumen (Pichard and Van Soest, 1977). Therefore, the AA profile of the insoluble protein is likely to be similar to the AA profile of the UIP fraction.

Soluble and insoluble proteins can be separated using borate-phosphate buffer (Krishnamoorthy et al., 1982) with insoluble true protein remaining in the borate-

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Table 1. Forages used in the analysis of the amino acid profile of different residues

Item	Name		Days of regrowth	Origin
	Common	Scientific		
Tropical				
Grass	Palisade grass	<i>Brachiaria brizantha</i> (A. Rich.) Stapf	36	Honduras
Grass	Star grass	<i>Cynodon nlemfuensis</i> Vanderyst	24	Honduras
Grass	Pangola grass	<i>Digitaria eriantha</i> Steud.	24	Honduras
Grass	Jaragua grass	<i>Hyparrhenia rufa</i> (Nees) Stapf	36	Honduras
Grass	Guinea grass	<i>Panicum maximum</i> Jacq.	24	Honduras
Legume	Stylo	<i>Stylosanthes guianenses</i> (Aubl.) Sw.	60	Brazil
Legume	Perennial soybean	<i>Neonotonia wightii</i> Lackey	55	Brazil
Legume	Forage peanut	<i>Arachis pintoi</i> Krap. & Greg.	60	Brazil
Temperate				
Grass	Reed canary grass	<i>Phalaris arundinacea</i> L.	—	New York
Grass	Orchard grass, early cut	<i>Dactylis glomerata</i> L.	—	New York
Grass	Timothy grass	<i>Phleum pratense</i> L.	—	New York
Legume	Alfalfa, early cut	<i>Medicago sativa</i> L.	—	New York
Legume	Alfalfa, late cut	<i>Medicago sativa</i> L.	—	New York
Legume	Red Clover	<i>Trifolium pratense</i> L.	—	New York

phosphate residue (**BPR**) after soaking. In the CNCPS, it is assumed that the AA profile of the BPR is similar to that of the UIP, although the AA profiles of various chemical residues (BPR, NDF, ADF) and the original forage have not been compared (Muscato et al., 1983).

This study was conducted to compare the AA profile of the original forage with those of the BPR, NDF, and ADF residues. Because sodium sulfite affects cell-wall proteins (Van Soest et al., 1991), the AA profiles of the NDF residue with (NDF+) and without (NDF-) sodium sulfite were compared.

Material and Methods

Forage Samples and Preparation

Grasses and legumes from tropical and temperate regions were chosen for this study to represent a wide range of forages commonly fed to animals (Table 1). Five tropical grasses were collected, frozen, and then freeze-dried in the Animal Science Department of the Escuela Agrícola Panamericana (Zamorano, Honduras). Three commonly used legumes were collected at the Instituto de Zootecnia (Nova Odessa, SP, Brazil), frozen, and freeze-dried in the Animal Science Department of the Escola Superior de Agricultura "Luiz de Queiroz," University of São Paulo (Piracicaba, SP, Brazil). In addition, three temperate grass hays and two temperate legume hays were provided by D. J. R. Cherney from the Department of Animal Science at Cornell University (Ithaca, NY).

Samples were ground in a Wiley Mill (Arthur H. Thomas, Philadelphia, PA) to pass a 1-mm screen and stored. Dry matter content was determined at 104°C in triplicate.

Preparation of Residues

Neutral Detergent Fiber Residue. The NDF fraction used for the AA analysis was prepared using a modifi-

cation of the method described by Van Soest et al. (1991). Approximately 15 g of lyophilized forage was processed to obtain enough NDF residue for the AA analysis. The relationship between air-dried sample and NDF solution was kept constant at 1 g of forage in 100 mL of neutral detergent solution. No α -amylase was used. The samples were placed in a 3-L beaker and brought to a boil. An Erlenmeyer flask (3 L) with a constant exchange of cold water, placed on top of the beaker containing the sample, was used for refluxing. After the sample boiled for 1 h, the residue was filtered under vacuum through a 37- μ m pore size nylon mesh cloth (Tetko, Briarcliff Manor, NY), rinsed with 2 L of boiling distilled water, and rinsed twice with acetone. The washed residue was dried in an oven at 50°C to constant weight, which was usually achieved by 48 h. The same procedure was used for the NDF residue with sodium sulfite (NDF+) except that 1 g of sodium sulfite was added for each gram of sample in the beaker (Van Soest et al., 1991).

Acid Detergent Fiber Residue. The ADF fraction was prepared as described by Van Soest et al. (1991). The procedure was identical to that used for the NDF residue preparation, except that approximately 20 g of air-dried forage was used. The nonsequential procedure was used. The ratio of 1 g of forage to 100 mL of acid detergent solution was used. The ADF solution was prepared from premixed ingredients acquired from Ankom Inc. (Fairport, NY).

Borate-Phosphate Buffer Residue. The BPR was obtained using the method described by Licitra et al. (1996). Approximately 10 g of air-dried sample was added to a beaker with buffer solution for 3 h at room temperature. The residue then was filtered under mild vacuum through a 40- μ m pore size nylon cloth, and rinsed with 500 mL of cold distilled water for each gram of forage sample. The washed residue was dried in an oven at 50°C to constant weight.

All residues were analyzed for essential amino acids (**EAA**: methionine, cysteine, lysine, threonine, arginine, isoleucine, leucine, valine, histidine, and phenylalanine) and nonessential amino acids (**NEAA**: glycine, serine, proline, alanine, aspartate, and glutamate) based on the AOAC method number 994.12 (Llames and Fontaine, 1994). The analysis of tryptophan was performed by HPLC after alkaline hydrolysis based on Fontaine et al. (1998). Methionine and cysteine were analyzed after oxidation according to AOAC method number 994.12 (Llames and Fontaine, 1994). The analyses of AA and protein were performed by Degussa Corp. (Allendale, NJ).

The ratio of true protein to crude protein was calculated by dividing the amount of AA-N by the total N (CP/6.25) of each sample. The amount of AA-N was calculated using the following contents of N (%) based on Lehninger et al. (1993): Met, 9.40; Cys, 11.57; Lys, 19.18; Thr, 11.76; Trp, 13.73; Arg, 32.18; Ile, 10.69; Leu, 10.69; Val, 11.97; His, 27.10; Phe, 8.48; Gly, 18.67; Ser, 13.33; Pro, 12.17; Ala, 15.73; Asp, 10.53; and Glu, 9.52. Methionine, valine, leucine, and isoleucine were classified as hydrophobic and the remaining EAA were hydrophilic. Cysteine was not included in this analysis.

Residue Calculations

All values were converted to a DM basis. Amino acids were expressed as a percentage of the CP of the respective residue for each forage. Because the protein content of ADF in forage is assumed to be unavailable to ruminants (Sniffen et al., 1992; Van Soest, 1994, chapters 16 and 18), the insoluble AA available was calculated as the AA of each residue minus the AA from the ADF residue, which was then divided by the CP of its respective residue (Eq. [1]) for each forage.

$$AAC_{P,ij} = \frac{AARes_{ij} \times Residue_j - ADFAA_i \times ADF}{CPRes_j \times Residue_j} \quad [1]$$

where $AAC_{P,ij}$ is the i^{th} amino acid content of the j^{th} residue expressed as a percentage of CP, $AARes_{ij}$ is the i^{th} amino acid content of the j^{th} residue expressed as a percentage of DM, $Residue_j$ is the j^{th} residue expressed as a percentage of DM, $ADFAA_i$ is the i^{th} amino acid content of the ADF (% DM), ADF is the acid detergent fiber (% DM), and $CPRes_j$ is the crude protein content of the j^{th} residue (% DM).

Statistical Analysis

The data were analyzed as a randomized complete block design (Kuehl, 2000) using the SAS package (SAS Inst. Inc., Cary, NC). Each AA was analyzed separately and the following statistical model was used:

$$Y_{ij} = \mu + \alpha_i + b_j + e_{ij}$$

where Y_{ij} = the concentration of amino acid measured, μ = overall mean, α_i = extraction method effect, b_j =

forage effect (as blocking factor), and e_{ij} = random experimental error.

Outliers were identified using the plot of studentized residue against the predicted (Y variable) and Cook's D influence statistic (SAS Inst. Inc., Cary, NC). Feeds with a studentized statistical residue outside the range -2.5 and 2.5 were considered to be outliers and removed from the database. The plot of the studentized residue against extraction methods and blocks was analyzed to test the assumption of identical variance (Kuehl, 2000). The PROC GLM was used to perform the analysis of variance, and the Tukey test was used for mean comparisons with significance assumed when $P = 0.05$ (SAS Inst. Inc.).

Results and Discussion

Table 2 summarizes the chemical composition of the forages analyzed. No difference was found between the NDF+ and NDF- ($P > 0.05$; 54.0 vs 54.5% in the DM, respectively), but the neutral detergent insoluble protein (**NDIP**) was lower in the NDF+ than in the NDF- ($P < 0.05$; 3.9 vs 4.5% of DM, respectively).

Addition of sodium sulfite to the NDF solution decreased NDIP from 3.86 to 1.88% of CP in NDF extracted with α -amylase (Hintz et al., 1996). Robertson and Van Soest (unpublished) observed that sodium sulfite and α -amylase alone or in combination decreased the NDIP value. An interaction between sodium sulfite and α -amylase in NDIP values was observed. The use of α -amylase enhanced the sodium sulfite effect, decreasing the NDIP value. Sodium sulfite is used to remove keratinaceous residues of animal origin (Van Soest et al., 1991) and to decrease within-sample variation (Hintz et al., 1996). It cleaves disulfide bonds, dissolving many protein cross-linkages (Van Soest et al., 1991), which reduces the NDIP value.

The soluble CP ranged from 8 to 49.4% of the CP, and the NDIP and ADIP varied from 13.3 to 80.4% and from 4.7 to 30.2% of the CP, respectively. Almost all of the protein bound in the cell wall of reed canary grass (*Phalaris arundinacea* L.) was unavailable (ADIP was 99% of NDIP), whereas only 17% of the NDIP of pangola grass (*Digitaria eriantha* Steud., formerly *D. decumbens*) was in the ADIP. The ADIP and NDIP comprised more of the NDF ($P < 0.05$) when sodium sulfite was used compared with the NDF- treatment (0.53 vs 0.43, respectively), confirming that sodium sulfite decreases the NDIP content.

Table 3 contains the AA profile of the residues studied. Reed canary grass (*Phalaris arundinacea* L.) and alfalfa early cut (*Medicago sativa* L.) were considered outliers and were not used in the statistical analysis.

The AA content of the ADF residue was lower than that of original forage, BPR, NDF-, and NDF+ residues (Table 3) when expressed as a percentage of CP of the respective residue. This suggests that most of the N in the ADF fraction of original forage or the residue is not true protein (Van Soest, 1994). In fact, on average only

Table 2. Chemical composition of the forage samples

Sample	NDF ⁻ , ^a %DM	NDF ⁺ , ^b %DM	ADF, %DM	CP, %DM	SolCP, ^c %CP	NDIP ⁻ , ^d %DM	NDIP ⁺ , ^e %DM	ADIP, ^f %DM
Tropical grasses								
<i>Brachiaria brizantha</i>	63.4	62.4	34.3	8.8	29.5	2.7	2.6	2.0
<i>Cynodon nlemfuensis</i>	72.7	71.8	37.1	15.8	8.0	7.4	7.8	3.8
<i>Digitaria eriantha</i>	64.7	65.1	37.1	15.9	47.2	5.6	6.4	1.0
<i>Hyparrhenia rufa</i>	73.0	71.3	45.4	5.1	12.1	4.1	3.3	1.3
<i>Panicum maximum</i>	70.5	70.0	42.6	7.8	27.3	3.6	4.8	1.4
Tropical legumes								
<i>Stylosanthes guianensis</i>	45.7	46.1	34.5	17.9	36.8	3.7	1.6	0.8
<i>Neonotonia wightii</i>	45.4	44.9	33.2	20.0	49.4	2.9	2.4	1.3
<i>Arachis pintoi</i>	41.2	42.8	26.6	20.1	24.3	5.4	3.3	1.5
Temperate grasses								
<i>Phalaris arundinacea</i>	67.8	64.8	36.6	15.3	35.3	4.7	3.8	4.6
<i>Dactylis glomerata</i>	60.4	56.5	29.2	25.8	40.3	9.1	7.3	3.0
<i>Phleum pratense</i>	54.5	54.3	28.2	17.0	46.0	4.8	4.9	2.3
Temperate legumes								
<i>Medicago sativa</i> , early	26.4	27.1	21.8	17.2	—	2.3	1.5	1.0
<i>Medicago sativa</i> , late	48.7	50.1	39.7	21.7	33.8	2.9	2.2	1.2
<i>Trifolium pratense</i>	28.6	29.0	22.0	24.0	20.4	3.8	2.6	1.3
Mean	54.5	54.0	33.5	16.6	31.6	4.5	3.9	1.9

^aNDF without sodium sulfite.

^bNDF with sodium sulfite.

^cSoluble crude protein is equivalent to fractions A + B1.

^dNeutral detergent insoluble protein from NDF without sodium sulfite.

^eNeutral detergent insoluble protein from NDF with sodium sulfite.

^fAcid detergent insoluble protein.

24.9% of the ADF protein was true protein, which differed ($P < 0.05$) from the original forage, BPR, NDF⁻, and NDF⁺ residues (59.3, 64.9, 64.1, and 59.9%, respectively). There was no difference between the concentration of amino acids in the original forage and in the BPR ($P > 0.05$; Table 3), except for Met, which was higher in the BPR than in the original forage (1.84 vs 1.45 %CP, respectively). The AA concentrations in the original forage and in the NDF residues were similar ($P > 0.05$; Table 3).

The amino acid profiles in the original feed and soluble fraction of several feedstuffs have been reported

previously (Crooker et al., 1978; MacGregor et al., 1978), but these authors did not correct the AA profile for the AA in the ADF. The potentially available AA was calculated by subtracting the ADF residue AA from the AA of other residues (Eq. 1). Table 4 lists the corrected amino acid contents of the residues after the unavailable AA (AA in the ADF) have been subtracted.

The ratio of AA to CP was not different between the original forage and BPR ($P > 0.05$; Table 4) across all AA analyzed, although some AA (Met, Trp, Ile, and His) had BPR values 25% greater than the original

Table 3. Comparison of amino acid concentration between original forage and four chemical residues without correction for the amino acid content of the ADF

Amino acid	Forage	Residue				SE
		BPR	NDF ⁻	NDF ⁺	ADF	
Methionine	1.45 ^b	1.84 ^a	1.71 ^{ab}	1.59 ^{ab}	0.68 ^c	0.14
Cysteine	0.95 ^a	0.78 ^{ab}	0.79 ^{ab}	0.81 ^{ab}	0.67 ^b	0.11
Lysine	4.21 ^a	4.57 ^a	4.94 ^a	4.53 ^a	1.87 ^b	0.36
Threonine	3.54 ^a	4.09 ^a	4.03 ^a	3.80 ^a	1.58 ^b	0.27
Tryptophan	1.32 ^a	1.69 ^a	1.29 ^a	1.46 ^a	0.49 ^b	0.23
Arginine	3.65 ^a	3.95 ^a	3.98 ^a	3.39 ^a	1.00 ^b	0.31
Isoleucine	3.28 ^a	3.90 ^a	3.79 ^a	3.48 ^a	1.60 ^b	0.25
Leucine	6.17 ^a	7.27 ^a	7.28 ^a	6.38 ^a	2.64 ^b	0.54
Valine	4.54 ^a	5.33 ^a	5.13 ^a	4.92 ^a	2.33 ^b	0.42
Histidine	1.46 ^a	2.00 ^a	1.79 ^a	2.02 ^a	0.67 ^b	0.28
Phenylalanine	3.87 ^a	4.47 ^a	4.47 ^a	3.93 ^a	1.72 ^b	0.37

^{a,b,c}Means with distinct subscripts in the same row differ ($P < 0.05$). Amino acid values are expressed as % in the CP of the respective residue. BPR = borate-phosphate residue, NDF⁻ = neutral detergent fiber without sodium sulfite, NDF⁺ = neutral detergent fiber with sodium sulfite, ADF = acid detergent fiber, and SE = standard error of the mean.

Table 4. Comparison of potentially available amino acid concentration between original forage and three chemical residues after the correction with the amino acid content of the ADF

Amino acid	Forage	Residue			SE
		BPR	NDF-	NDF+	
Methionine	1.60 ^b	2.14 ^{ab}	2.25 ^a	2.45 ^a	0.27
Cysteine	1.04 ^a	0.85 ^a	0.77 ^a	1.02 ^a	0.13
Lysine	4.77 ^c	5.38 ^{bc}	6.88 ^a	6.86 ^{ab}	0.66
Threonine	3.93 ^b	4.81 ^{ab}	5.42 ^a	6.15 ^a	0.61
Tryptophan	1.49 ^a	2.09 ^a	1.73 ^a	2.83 ^a	0.62
Arginine	4.16 ^b	4.69 ^{ab}	5.76 ^a	5.42 ^{ab}	0.63
Isoleucine	3.63 ^b	4.56 ^{ab}	5.07 ^a	5.33 ^a	0.50
Leucine	6.88 ^b	8.45 ^{ab}	9.96 ^a	9.57 ^a	1.02
Valine	5.05 ^b	6.29 ^{ab}	6.78 ^b	7.80 ^a	0.84
Histidine	1.64 ^b	2.49 ^{ab}	2.51 ^{ab}	3.88 ^a	0.71
Phenylalanine	4.32 ^b	5.14 ^{ab}	6.03 ^a	5.68 ^{ab}	0.67

^{a,b,c}Means with distinct subscripts in the same row differ ($P < 0.05$). The potentially available AA was calculated by subtracting the ADF residue AA from the AA of other residues. Amino acid values are expressed as a percentage in the CP of the respective residue. BPR = borate-phosphate residue, NDF- = neutral detergent fiber without sodium sulfite, NDF+ = neutral detergent fiber with sodium sulfite, ADF = acid detergent fiber, and SE = standard error of the mean.

forage values. This variation is likely to be due to the extraction methods used. A similar result was found when the data of Muscato et al. (1983) were reanalyzed. When the insoluble available AA (protein frac-

tions B2 and B3) and the original forage AA were expressed as the percentage of the CP of the respective residue, no difference was found in the AA profile of the original and BPR ($P > 0.05$). This result occurred

Table 5. Amino acid profile (%CP adjusted for AA in the ADF residue) in the original forages and in the borate-phosphate buffer residue for each forage analyzed

Item	Met	Cys	Lys	Thr	Trp	Arg	Ile	Leu	Val	His	Phe
AA in the original forage											
Palisade grass, <i>Brachiaria brizantha</i>	2.25	1.51	6.02	4.94	2.10	5.04	4.55	8.66	6.80	2.01	5.58
Star grass, <i>Cynodon nlemfuensis</i>	1.77	1.24	4.59	4.03	1.63	4.21	3.47	6.78	5.24	1.46	3.96
Pangola grass, <i>Digitaria eriantha</i>	1.33	0.89	3.43	3.27	1.38	3.12	2.80	5.11	4.43	1.23	3.18
Jaragua grass, <i>Hyparrhenia rufa</i>	2.45	1.21	7.10	5.94	2.96	5.89	5.11	10.76	7.54	2.28	6.44
Guinea grass, <i>Panicum maximum</i>	2.05	1.42	5.56	4.72	2.20	4.85	4.25	8.41	6.59	1.80	5.34
Stylo - <i>Stylosanthes guianensis</i>	1.43	0.96	4.81	3.48	0.29	4.38	3.54	6.55	4.32	1.64	4.01
Perennial soybean - <i>Neonotonia wightii</i>	1.45	1.00	4.86	3.43	1.30	4.16	3.45	6.41	4.29	1.64	4.05
Forage peanut - <i>Arachis pintoi</i>	1.29	0.84	4.78	3.39	0.17	4.01	3.33	6.18	4.18	1.68	3.95
Reed canary grass - <i>Phalaris arundinacea</i>	1.81	1.12	4.49	4.42	1.75	4.30	4.08	7.63	5.54	1.62	4.74
Orchard grass, early cut - <i>Dactylis glomerata</i>	1.49	0.84	3.83	3.71	1.46	3.77	3.41	6.25	4.46	1.41	4.03
Timothy grass - <i>Phleum pratense</i>	1.16	0.74	3.54	2.78	1.09	2.97	2.53	4.80	3.74	1.11	3.07
Alfalfa, early cut - <i>Medicago sativa</i>	1.31	1.11	4.75	3.89	1.32	4.03	3.69	6.37	4.73	1.72	4.21
Alfalfa, late cut - <i>Medicago sativa</i>	1.26	1.05	4.41	3.64	1.40	3.64	3.44	6.05	4.34	1.68	4.01
Red clover - <i>Trifolium pratense</i>	1.29	0.77	4.29	3.87	1.91	3.89	3.64	6.64	4.74	1.68	4.28
AA in the borate-phosphate buffer residue											
Palisade grass, <i>Brachiaria brizantha</i>	3.12	1.66	7.42	6.24	2.76	6.41	5.96	12.28	8.53	2.47	7.24
Star grass - <i>Cynodon nlemfuensis</i>	1.75	0.82	4.34	3.66	1.48	3.92	3.28	6.82	4.83	1.25	3.95
Pangola grass - <i>Digitaria eriantha</i>	2.12	0.73	5.33	4.66	1.91	4.66	4.56	8.96	5.80	1.86	5.23
Jaragua grass - <i>Hyparrhenia rufa</i>	2.61	0.92	6.21	5.78	2.63	5.82	5.53	11.60	6.84	2.25	6.86
Guinea grass - <i>Panicum maximum</i>	2.62	0.93	6.47	5.43	2.43	5.07	4.87	10.40	6.75	2.35	6.37
Stylo - <i>Stylosanthes guianensis</i>	1.64	0.45	4.88	3.83	1.23	4.68	4.03	7.44	4.85	1.75	4.47
Perennial soybean - <i>Neonotonia wightii</i>	1.89	0.62	5.61	3.99	1.05	4.38	4.07	8.16	5.33	1.85	5.31
Forage peanut - <i>Arachis pintoi</i>	1.63	0.60	4.94	3.92	0.58	4.60	3.97	7.28	5.00	1.81	4.57
Reed canary grass - <i>Phalaris arundinacea</i>	3.05	1.31	6.18	5.89	2.52	6.03	5.80	11.37	7.66	2.29	7.48
Orchard grass, early cut - <i>Dactylis glomerata</i>	2.42	0.95	5.08	5.12	2.15	5.24	4.61	8.98	6.07	1.97	5.93
Timothy grass - <i>Phleum pratense</i>	2.33	1.11	6.02	5.10	1.84	5.52	4.75	9.25	6.55	1.88	5.83
Alfalfa, early cut - <i>Medicago sativa</i>	1.75	0.91	5.60	4.18	1.42	4.54	4.13	7.82	5.16	1.78	5.11
Alfalfa, late cut - <i>Medicago sativa</i>	1.84	0.81	5.66	4.41	1.55	4.73	4.41	8.26	5.63	2.04	5.43
Red clover - <i>Trifolium pratense</i>	1.70	0.62	5.12	4.49	1.79	4.85	4.33	8.52	5.50	1.96	5.44

Table 6. Comparison of three amino acid profiles of six forages on animal performance predicted by the Cornell Net Carbohydrate and Protein System (CNCPS) model^a

Forage	CNCPS ^b	Original ^b	BPR ^b
Orchard grass hay (ME and MP allowable gain are 1.41 and 1.61 kg/d, respectively)			
AA allowable gain ^c , kg/d	1.49 (Lys)	1.58 (Lys)	1.70 (Lys)
First AA limiting ^c	Histidine (0 g/d)	Histidine (3 g/d)	Histidine (3 g/d)
Timothy grass hay (ME and MP allowable gain are 1.47 and 1.25 kg/d, respectively)			
AA allowable gain, kg/d	1.28 (Lys)	1.32 (Lys)	1.43 (Lys)
First AA limiting	Lysine (-3 g/d)	Leucine (-3 g/d)	Histidine (-1 g/d)
Alfalfa early hay (ME and MP allowable gain are 1.63 and 1.70 kg/d, respectively)			
AA allowable gain, kg/d	1.86 (Met)	1.72 (Lys)	1.81 (Lys)
First AA limiting	Methionine (0 g/d)	Histidine (0 g/d)	Histidine (0 g/d)
Guinea grass (ME and MP allowable gain are 1.23 and 0.75 kg/d, respectively)			
AA allowable gain, kg/d	0.77 (Lys)	0.84 (Lys)	0.86 (Lys)
First AA limiting	Lysine (-9 g/d)	Lysine (-7 g/d)	Lysine (-7 g/d)
Palisade grass (ME and MP allowable gain are 1.27 and 0.85 kg/d, respectively)			
AA allowable gain, kg/d	0.76 (Lys)	0.93 (Lys)	0.87 (Lys)
First AA limiting	Lysine (-10 g/d)	Lysine (-6 g/d)	Lysine (-8 g/d)
Perennial soybean (ME and MP allowable gain are 1.38 and 1.39 kg/d, respectively)			
AA allowable gain, kg/d	1.63 (Lys)	1.58 (Lys)	1.62 (Lys)
First AA limiting	Histidine (1 g/d)	Histidine (0 g/d)	Histidine (1 g/d)

^aThe simulation was based on a 200-kg Angus steer. The base DM diet had cracked corn grain (33%), polished rice grain (17%), and one of the evaluated forages (50%).

^bCNCPS is the AA profile as the feed library of CNCPS version 4.0, Original is the AA profile of the original forage (Table 5), and BPR is the AA profile of the borate-phosphate residue of the same forage (Table 5).

^cMP is metabolizable protein. AA allowable gain refers to the methionine or lysine allowable gain only. First AA limiting refers to the difference (g/d) of any AA supplied minus the net required AA to meet ME allowable gain.

because the different chemical extraction methods, except for the ADF, affected each of the tested amino acids similarly (Table 3).

The AA profile of the original forage may be used as the AA profile of the UIP, based on the following assumptions: 1) BPR has a higher probability of escaping ruminal fermentation (Pichard and Van Soest, 1977) than soluble proteins and 2) all amino acids in a given forage are degraded to the same relative extent in the rumen (MacGregor et al., 1978). The second assumption needs further validation because hydrophilic amino acids are more rapidly degraded than hydrophobic AA, such as Leu, Ile, and Val (Van Soest, 1994). Varvikko (1986) observed that Leu, Ile, and Val were more slowly degraded than other AA. We found that the ratio of hydrophobic to hydrophilic AA was similar ($P > 0.05$) among the original forage, BPR, NDF-, and NDF+ (0.86, 0.88, 0.87, and 0.85, respectively), but they were lower than the ADF residue ratio (1.04; $P < 0.05$), suggesting that the ADF method extracted more hydrophilic AA than hydrophobic. The same ratio for bacteria AA using Clark et al. (1992) values would be 0.82, very similar to the ratios found.

In analyzing the relationship between the degradation and escape of amino acids of canola meal, Boila and Ingalls (1995) found a strong linear relationship between effective degradability of N and amino acids in

the rumen. Their result indicated that AA had similar degradation rates relative to the feed N. In addition, the relationship between actual measurements (Boila and Ingalls, 1992) and predicted escape of AA using the effective degradability had an r^2 of 0.99 with no bias (Boila and Ingalls, 1995). On the other hand, the analysis of Von Keyserlingk et al. (1998) suggested that Lys, Met, Phe, Ile, Leu, and Thr of grass silage disappeared at different rates in the rumen, leading to a different profile between intake and escape protein.

Maiga et al. (1996) found no difference in the essential AA profile of five protein supplements between the original and residue degraded for 12 h in situ in the rumen. The escaping AA profile was similar to the original AA profile. Other studies have also determined that the AA profile of the UIP fraction is similar to the original dietary AA profile (Ganev et al., 1979; Varvikko et al., 1983), after correction for microbial contamination in the nylon bags. Likewise, King et al. (1990) fed corn silage to lactating cows and reported that the AA profile flowing into the duodenum closely resembled the dietary AA profile, suggesting that the bacterial AA did not alter the AA pattern contributed by escaped dietary protein. On the other hand, several studies reported different AA profiles between original feed and the fraction that escapes ruminal degradation (Rooke et al., 1984; Varvikko, 1986; Von Keyserlingk et al., 1996).

Erasmus et al. (1994) reported that the AA profile of UIP differed from the original AA profile but was similar to the absorbable AA profile in the intestine for several meal feeds. However, the difference in AA concentration was not consistent across feeds or AA (Erasmus et al., 1994).

Most of these studies used markers to correct UIP for ruminal microbial contamination. This correction is not accurate. Diaminopimelic acid (**DAPA**) has limitations because of its presence in the feedstuffs (Puchata et al., 1992), it is metabolized (Masson et al., 1991), and the bacterial cell wall proteins, where the DAPA resides, are not degraded to the same extent as protoplasmic proteins when bacterial cells turn over in the rumen (Broderick and Merchen, 1992). Uric acid, a urinary purine metabolite, has a positive relationship with microbial N flow, but the overall regression had an r^2 of 32% (Johnson et al., 1998). Estimates of duodenal flow of bacteria N using DAPA were higher than the results obtained with purines (Volden, 1999). Comprehensive discussions of estimation of ruminal microbial protein have been published (Broderick and Merchen, 1992; Obispo and Dehority, 1999). In addition to the problems in measuring ruminal microbial protein and UIP, there are few data on the amino acid content of microbial protein and there is wide variation in the data that exist (Clark et al., 1992).

The variation in the AA profile of the original forage and that of the escape protein is likely due to 1) microbial correction of the ruminally incubated bags and 2) the assumption that material that disappears from the ruminally incubated bags is completely absorbed.

For some AA, the profile was similar between the original material and the NDIP residue (Cys, Trp, Val, and His), and our evaluation indicated that the original forage AA profile did not differ from the AA profile of the borate-phosphate residue (Table 4). Based on the assumption that the degradation rate of each AA is similar (Boila and Ingalls, 1995; Maiga et al., 1996; Varvikko et al., 1983), the AA profile of the UIP fraction should be similar to that of the original forage.

A simulation using the CNCPS model (version 4.0) was performed to evaluate the effect of the AA profile of the original forage and the AA profile of the BPR on animal performance. Six forages from Table 1 were chosen, and their respective chemical composition (Table 2) and AA profile (Table 5) were entered in the model. The result is shown in Table 6. When only Lys and Met were considered, because they are the best-understood amino acids, Lys was the first-limiting AA for ADG for almost all simulations. Histidine was the first-limiting AA in legume forages. Tropical grasses had a consistently low concentration of lysine, whereas, in temperate grasses, histidine was the lowest. Except for timothy grass and alfalfa hay, the limiting AA was the same across the different AA profiles. No major difference ($P > 0.05$) was found in the AA-allowable ADG using the AA profile in the original forage compared with the BPR.

Implications

The use of sodium sulfite did not affect the neutral detergent fiber values but decreased the nitrogen that was insoluble in neutral detergent solution. Similarly, the use of sodium sulfite did not change the amino acid profile of a given residue when amino acid concentrations were expressed as percentages of the CP of the residue. The analyses of the borate-phosphate residue and the original forage indicated that there is no difference in the amino acid profiles of these two fractions after the correction for the amino acid in the acid detergent fiber fraction. This finding suggests that the amino acid profile of the original forage can be used as an indicator of the amino acid profile of the undegradable intake protein.

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