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Protein Hydrolysis of Animal Feeds for Amino Acid Content

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An investigation was carried out to determine the suitability of the screw cap tube method of acid hydrolysis with nitrogen flushing for routine amino acid analysis of animal feeds. Amino acid values determined by the screw cap tube method were compared to those from three other methods, including the conventional reflux method under nitrogen for soybean meal, wheat, meat and bone meal, and casein. The screw cap tube method was shown to be suitable for routine analysis of animal feeds, since similar results to the reflux method were obtained. Methionine was unstable during acid hydrolysis without prior oxidation, for all methods, particularly in soybean and wheat samples, but not in casein. Therefore for routine analysis of animal feeds methionine should be preoxidized to methionine sulfone before acid hydrolysis. Similar values were obtained for methionine and cystine plus cysteine in animal feeds using two different preoxidation procedures.

INTRODUCTION

Conditions for acid hydrolysis of proteins have been investigated by many workers (Mason et al., 1980; Phillips, 1983; Lucas and Sotelo, 1982; Savoy et al., 1975; Roach and Gehrke, 1970; Mondino and Bongiovanni, 1970; Kohler and Palter, 1967; Finlayson, 1965).

The most common methods employed are (a) open reflux under an atmosphere of nitrogen, (b) hydrolysis in evacuated sealed tubes, and (c) hydrolysis in screw cap tubes in an atmosphere of nitrogen. Each method has disadvantages. Hydrolysis under reflux, by comparison with other procedures, is constrained by space and equipment allowing only a limited number of hydrolyses to be performed in each batch. Although hydrolysis using evacuated sealed tubes is most widely used, the method is time consuming and operator skill is required for flame sealing and evacuation. Methods based on screw cap tube hydrolysis are simple and rapid and therefore suitable for routine analysis. However this method has the disadvantage that oxygen is not excluded from the screw cap tube and sensitive amino acids may be oxidized.

The purpose of this study was to compare the screw cap tube method of hydrolysis with three other methods including the classic technique of refluxing. Since the screw cap tube procedure is less time consuming than other methods its use would offer considerable advantage in routine amino acid analysis of animal feeds.

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EXPERIMENTAL SECTION

Materials. Samples of soybean meal, meat and bone meal, wheat, and casein were ground to pass a 500-μm mesh screen. Each of the four samples was analyzed in duplicate for total amino acids by using four different hydrolysis procedures. In addition cystine plus cysteine and methionine were determined after oxidation as cysteic acid and methionine sulfone, respectively, by three different procedures.

Methods. Screw Cap Tube Hydrolysis (SC). Acid hydrolysis was conducted according to a modified method of Roach and Gehrke (1970). Samples of 200 mg were hydrolyzed in 50-mL screw cap culture tubes with 47 mL of 6 N HCl containing thioglycollic acid (0.01 mmol/mL). The contents were thoroughly wetted and mixed on a vortex mixer until all of the sample was finely distributed in the acid. After mixing, the air space above the solution was flushed with oxygen free nitrogen for 10 s and the teflon-lined screw cap quickly screwed onto the tube. The solutions were hydrolyzed at 110 °C for 24 h in an air draft oven.

Screw Cap Tube Hydrolysis—Nitrogen Purged (SC_N). Hydrolysis was performed as described above for screw cap tubes except that instead of nitrogen flushing,

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Table I. Comparison of Acid Hydrolysis Methods Average Deviation Relative to the Reflux Method Expressed as a Percent of the Reflux Method Value

	soybean meal			wheat			meat and bone meal			casein		
	SC[a]	SC_N[b]	OR[c]	SC	SC_N	OR	SC	SC_N	OR	SC	SC_N	OR
Asp	−4.1	−2.5	−6.6	−10.2	−6.3	−9.5	−2.6	−3.4	−8.2	+0.8	+1.6	−0.9
Thr	0	+2.2	+1.7	−2.6	−1.3	−1.3	+0.9	+0.3	−0.6	+5.7	+5.3	+2.3
Ser	+1.0	+3.7	−3.7	0	+1.8	−0.7	+1.8	+2.1	−7.2	+5.7	+7.1	0
Glu	−1.1	−0.5	−1.1	−3.9	−3.2	+0.7	−1.7	−1.7	−2.5	+1.3	+1.8	+0.9
Gly	−2.3	+0.9	+3.2	−4.4	−1.9	+0.9	−1.4	−0.7	+2.8	+1.6	+3.6	+3.1
Ala	0	−1.6	−4.6	−0.8	−2.3	−0.8	−0.4	−2.3	−7.8	+0.6	+1.3	+1.0
Val	−7.7	−6.0	+0.6	−1.7	0	+0.2	−2.9	−3.1	+1.4	−1.3	−1.9	+0.9
Iso	−9.0	−4.2	+3.0	−6.9	−3.6	0	−5.8	−6.1	−0.1	−2.7	−2.8	+3.8
Leu	−1.9	+0.5	0	−1.2	+0.5	+0.5	−1.7	−2.7	−1.0	+1.8	+1.3	+2.1
Tyr	−0.3	+1.1	−8.6	−1.3	−1.3	−49.3	−1.3	+0.9	−82.6	+3.8	+3.4	−40.2
Phe	+1.2	+0.8	−3.1	−1.7	−2.4	−7.6	+2.0	+1.7	−12.3	+2.7	+2.0	+1.2
His	−1.5	−1.5	+0.7	−3.6	−1.8	+3.6	−2.4	−4.9	+1.8	0	+0.7	+1.3
Lys	−1.4	−1.9	+0.3	−2.9	−2.9	0	−1.5	−1.9	+0.8	+1.0	+1.1	+0.2
Amm	−9.4	+1.0	+1.1	−9.9	−12.3	−3.2	−15.2	−18.1	−1.5	+12.6	−4.5	−4.5
Arg	−1.4	−1.4	−2.3	−0.8	−0.8	0	−1.3	−1.3	−0.8	+1.3	+0.8	+2.4
prot rec[d]	−2.6	−0.5	−2.1	−3.3	−3.3	+0.2	−2.2	−2.7	−2.4	+2.8	+0.8	−0.3

[a] Screw cap tube. [b] Screw cap tube with nitrogen purging. [c] Preoxidation followed by reflux under nitrogen. [d] Protein recovery. Recovery of amino acid nitrogen as a percentage of total nitrogen. Recovery values of 96.9, 93.4, 94.1, and 96.6 were obtained for soybean meal, wheat, meat and bone meal, and casein, respectively, by using the reflux method.

nitrogen was bubbled through the hydrolysate at 100 mL/min with a pasteur pipette with the tapered end near the base of the tube. After 5 min the pasteur pipette was rapidly disconnected from the nitrogen line and the cap screwed tightly onto the tube with the pipette inside.

Reflux Hydrolysis (R). Reflux under nitrogen was carried out on electric mantles with 400 mg of sample plus 94 mL of 6 N HCl containing thioglycollic acid (0.01 mmol/mL), for 24 h, according to the method of Mondino and Bongiovanni (1970).

Oxidation–Reflux Hydrolysis (OR). Hydrolysis was conducted as described in method 8 of Bech-Andersen et al. (1980) except for the following modifications. A sample weight of 200 mg was oxidized with 25 mL of performic acid containing 50 mg of phenol. After oxidation 2 g of sodium metabisulfite was added to remove excess performic acid.

Sulfur-Containing Amino Acids. Cystine plus cysteine and methionine were determined by the following procedures: (a) The oxidation reflux method of Bech-Andersen et al (1980) described above. (b) The oxidation procedure described by Moore (1963) with 200 mg of sample, 25 mL of performic acid, and 3 mL of 48% HBr. Acid hydrolysis was performed by using the screw cap tube procedure described previously without thioglycollic acid. (c) The oxidation procedure described by Jamalian and Pellett (1968) using 200 mg of sample, 25 mL of performic acid, but no HBr. After oxidation the solution was evaporated at 100 °C on a water bath and the residue hydrolyzed with the modified screw cap procedure of Roach and Gehrke (1970) described previously but omitting the thioglycollic acid.

Amino Acid Analysis. After hydrolysis, the sample hydrolysates were diluted and filtered through a 540 Whatman filter paper, and a 5-mL aliquot taken for evaporation on a Buchi rotary evaporator capable of holding five 100-mL flasks on a spider attachment. The residue was dissolved in pH 2.2 sodium citrate buffer and stored at 4 °C. Before analysis the hydrolysate was again filtered through a disposable membrane filter (0.45 μm, Gelman Acrodisc), attached to a Luer syringe.

Amino acid chromatography was performed with a Kontron Liquimat 3 Automatic Amino Acid Analyzer on a column 28 cm long and 4 mm internal diameter, filled with Durrum DC 6A resin. The sodium citrate elution buffers were prepared as follows: (a) pH 3.30, 0.2 N (Na^+), and 1% propanol; (b) pH 4.25, 0.2 N (Na^+); (c) pH 6.40, 1.1 N (Na^+), and 5% propanol.

Cystine plus cysteine and methionine were measured in a separate run as cysteic acid and methionine sulfone, respectively, with the first elution buffer described above, and the column temperature set at 54 °C.

Statistical Analysis. Differences between methods for amino acid content were examined by analysis of variance using Duncan's new multiple range test (Duncan, 1957).

Table II. Statistical Analysis Showing Significant Differences between Methods for Amino Acid Content of Animal Feeds[a]

amino acids	methods					
	SC[b]	SC_N[c]	R[d]	OR[e]	OJ[f]	OM[g]
Asp	a	a	b	c		
Thr	a	a	a	a		
Ser	a,[b] a[c]	a[b]	a,[c] a[d]	a[d]		
Glu	a	a	a	a		
Gly	a[b]	a,[b] a[c]	a,[b] a[c]	a[c]		
Ala	a	a	a	a		
Cys				a	b	a
Val	a	a	b	b		
Meth	a	a	b	d	c	d
Iso	a	a	b	b		
Leu	a	a	a	a		
Tyr	a	a	a	b		
Phe	a	a	a	b		
His	a[b]	a[b]	a,[b] a[c]	a[c]		
Lys	a	a	a	a		
Arg	a	a	a	a		
pro rec[h]	a	a	b	a		

[a] Within rows methods that do not have the same letters and footnotes are significantly different ($P < 0.01$). [b] Screw cap tube. [c] Screw cap tube with nitrogen purging. [d] Reflux. [e] Oxidation reflux method of Bech-Andersen (1980). [f] Oxidation method of Jamalian and Pellet (1968). [g] Oxidation method of Moore (1963). [h] Protein recovery. Recovery of amino acid nitrogen as a percentage of total nitrogen.

RESULTS AND DISCUSSION

Table I shows a comparison of results of the acid hydrolysis methods calculated as a percentage deviation relative to the reflux hydrolysis method of Mondino and Bongiovanni (1970). The reflux procedure was used as the standard reference method since it is recognized as an accurate method (Williams, 1982).

The statistical analysis of the results is shown in Table II.

As shown in Tables I and II the reflux method recovered significantly more nitrogen as amino acid nitrogen than the screw cap tube method. Reflux hydrolysis has an advantage over other procedures because during hydrolysis the solution is continually agitated and therefore higher recoveries of amino acids can be expected. This observation is supported by Otterburn and Sinclair (1973) who found that constant agitation during acid hydrolysis of chicken muscle was necessary when using evacuated sealed tubes for hydrolysis. Bech-Andersen et al. (1979) also obtained significantly lower values for the evacuated sealed tube method compared to the reflux method. However in contrast Cavins et al. (1972) obtained similar results for soybean meal by using both methods. Experience gained in our laboratory with the screw cap method of hydrolysis has indicated that optimal recoveries are obtained only after thorough wetting and mixing of the sample before acid hydrolysis.

Tyrosine is susceptible to reaction with chlorine during acid hydrolysis, particularly under oxidizing conditions (Mason et al., 1979). The results illustrated in Tables I and II show that the reducing agent thioglycollic acid prevented the formation of significant amounts of tyrosine derivatives during screw cap tube hydrolysis.

Although the amounts of most amino acids determined by the screw cap method were less than by the reflux method (Table I) the deviations were small, except that there were significant differences ($P < 0.01$) with aspartic acid, valine, and isoleucine. Since both valine and isoleucine are difficult to liberate from peptide linkages during acid hydrolysis, it can be expected, as discussed previously, that the reflux method would have a higher recovery for these amino acids. It is interesting to note that significantly lower values for valine and isoleucine were obtained with the evacuated sealed tube method compared to those with the reflux method by Bech-Andersen et al. (1979). To compensate for the incomplete liberation of valine and isoleucine, a recovery factor which is experimentally determined after extrapolation to 72-h hydrolysis is used in the calculation to determine the concentration of both these amino acids in feeds (Blackburn, 1978). After this correction is made it can be expected that both methods of analysis would produce similar results for valine and isoleucine.

In general the results listed in Table I show that using the screw cap tube method for routine analysis of animal feeds, results very similar to those of the reflux method were obtained.

As shown in Tables I and II purging the screw cap tube with nitrogen for 5 min before hydrolysis had little affect on the recovery of amino acids.

Significantly large losses ($P < 0.01$) were observed for tyrosine and phenylalanine and less significant losses in the case of aspartic acid in the oxidation reflux method of Bech-Andersen et al. (1980) (see Tables I and II). The destruction of phenylalanine and part of the tyrosine was most likely due to the low ratio of phenol scavenger to performic acid in the oxidation mixture.

Comparison of results for methods for cystine plus cysteine and methionine methods are shown in Table III. The deviations have been expressed as a percentage relative to the value obtained by the oxidation–reflux method of Bech-Andersen et al. (1980).

Results for methionine analysis confirm previous findings of other workers that methionine is unstable during acid hydrolysis, particularly in the presence of carbohydrates (Jennings, 1969; Mason et al., 1979; Sarwar et al., 1983). Significantly greater destruction of methionine occurred in the screw cap tube hydrolysis method than in the reflux method (see Table III) even though a reducing agent thioglycollic acid was present during hydrolysis. Purging with nitrogen before acid hydrolysis in the screw cap tube did not prevent destruction of methionine (Table II). For the casein sample, which had a crude protein content of 96% on a dry basis, all methods of methionine analysis were in agreement (Table II), whereas for soybean meal and wheat and to a much lesser extent meat and bone meal large differences between methods were obtained for methionine (Table II). This indicates, that in the absence of constituents such as carbohydrates, fats, mineral salts, and other naturally occurring materials, methionine is stable during acid hydrolysis. These results emphasise the fact that the usefulness of any hydrolysis procedure is related directly to the material being analyzed and particularly the amounts of non-protein components in the product.

Table III. Comparison of Cystine Plus Cysteine and Methionine Methods[a]

feed	anal method	cystine plus cysteine	methionine
soybean	SC[b]		−36.0
soybean	SC_N[c]		−36.0
soybean	R[d]		−18.6
soybean	O-J[e]	−0.6	−0.6
soybean	O-M[f]	0	−0.6
wheat	SC		−24.0
wheat	SC_N		−20.3
wheat	R		−17.2
wheat	O-J	−8.8	−12.0
wheat	O-M	−0.4	−1.0
meat and bone	SC		−10.2
meat and bone	SC_N		−9.5
meat and bone	R		−2.0
meat and bone	O-J	−2.5	−5.4
meat and bone	O-M	+0.8	−0.7
casein	SC		−2.0
casein	SN_N		−1.0
casein	R		−2.3
casein	O-J	+2.5	−2.3
casein	O-M	+5.0	−1.7

[a] The average deviation relative to the oxidation–reflux method of Bech-Andersen et al. (1980) expressed as a percentage of the oxidation reflux method. [b] Screw cap tube. [c] Screw cap tube with nitrogen purging. [d] Reflux. [e] Oxidation method of Jamalian and Pellet (1968). [f] Oxidation method of Moore (1963).

As expected, the modified method of Jamalian and Pellett (1968) produced significantly lower values for half cystine and methionine compared to the methods of Moore (1963) and Bech-Andersen et al (1979) (Table III). However, except for wheat, the agreement for the other meals was surprisingly good (Table II) considering the high potential for reactivity during evaporation of performic acid.

As shown in Table III no significant difference was obtained between the method of Moore (1963) and that of Bech-Andersen et al. (1980) for cystine plus cysteine and methionine.

Our results confirm those of Kohler and Palter (1967) who found that the evacuated sealed tube method of acid hydrolysis was of no advantage over N_2 flushing for cysteic acid and methionine sulfone. In conclusion these results show that when using the screw cap tube hydrolysis method, the recovery of essential amino acids is satisfactory for routine feedstuff analysis. In addition this study confirms that the level of methionine in animal feeds must be analyzed by an alternative procedure to the conventional acid hydrolysis method without prior oxidation.

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Comparison of Laboratory Methods for the Prediction of in Vitro Dry Matter Digestibility in Three Maturing Grasses

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Maturing reed canary grass, Russian wild rye, and smooth bromegrass were freeze-dried and ground. Samples were analyzed gravimetrically for detergent fiber components and cell wall constituents by using spectrophotometric and gas-liquid chromatographic assays. The relationship between cell wall composition and in vitro dry matter digestibility (IVDMD) was investigated by using regression methods. Acid detergent fiber and acid detergent lignin were the best single parameters for predicting IVDMD. However, multiple linear regression equations utilizing the monomeric constituents of plant cell wall polysaccharides, lignin and silica, provided the best estimates of IVDMD. The arabinose:xylose ratio and galactose content may reflect the importance of hemicellulosic polymer branching on the digestibility of forages.

Digestibility is one of the major factors determining the feeding values of forages. Since cell contents are considered readily available, dry matter digestibility becomes largely a function of cell wall digestibility (Van Soest, 1975). Presently, almost all descriptions of forage composition use gravimetric methods that fractionate cell walls on the basis of their solubility in a particular solvent system (Goering and Van Soest, 1970). These methods fail to accurately estimate digestibility over a variety of conditions (Oh et al., 1966; Barton et al., 1976). Inglett and Falkenhag (1979) reported that plant cell walls may be quantified by their monosaccaride constituents. Separation of plant cell walls into their constituent monosaccarides may allow for more accurate predictions of digestibility, as well as lead to a better understanding of the plant related factors that influence digestibility.

The purpose of this study was to compare two chemical schemes of analysis as predictors of in vitro dry matter digestibility (IVDMD) in three maturing grass species. The detergent fiber system of analysis and the monomeric constituents of plant cell wall polysaccarides were utilized.

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MATERIALS AND METHODS

Reed canary grass (*Phalaris arundinacea* L.) leaf blades were collected at six stages of maturity from April 22 to June 4 from the Utah State University dairy farm. Maturities ranged from early leaf to milk stage. Smooth bromegrass (*Bromis inermis* L.) whole plants were harvested at six stages of maturity from April 16 to June 16. Stages of maturity ranged from early leaf to dough stage. Russian wild rye (*Elymus juceus* Fisch.) plants were collected at five stages of maturity between April 24 and June 16 from a neighboring USDA plant breeding test plot. The first four Russian wild rye samples were harvested between the immature and dough stages, while the fifth was harvested as immature regrowth on June 16, ten days after the plot had been mowed to a 15-cm stubble height. Upon collection, samples were immediately frozen on dry ice, freeze-dried, and ground in a Wiley mill equipped with a 1-mm screen. Half of each ground sample was reground through a cyclone mill equipped with a 0.5-mm screen.

Coarsely ground plant samples were analyzed gravimetrically for neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) as outlined by Goering and Van Soest (1970), except that asbestos was not used in the ADL determinations. Acid insoluble ash (AIA) was determined by the method of Fonnesbeck (1976).

Approximately 5 g of finely ground plant material was refluxed for 1 h in 150 mL of 80% aqueous ethanol, while being stirred continuously with a magnetic stirring bar.

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