Sources of Error in Determination of Available Lysine in Cottonseed and Peanut Meals

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ABSTRACT

Experiments were made to identify the sources of error in the Rao procedure for available lysine in cottonseed and peanut meals and to estimate the magnitude of each. The partition of squares in analyses of variance of the data reported revealed that the major error can be attributed to sampling. The variances due to other possible sources of error, such as dinitrophenylation, hydrolysis, chromatography, etc., were not significantly different from the error variance, which (after the sampling error is accounted for) corresponds to confidence limits at the 5% level of probability of ±0.5% of the mean of the available lysine concentration.

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INTRODUCTION

Data for the available lysine content of oilseed meals have been found useful in feeding experiments with nonruminant animals. One of the analytical procedures for available lysine that finds frequent use is that described by Rao et al. (1). Apparently substantial variations in the determinations with oilseed meals are observed in practice with this method, and it therefore becomes worthwhile to identify the sources and measure the magnitude of errors encountered.

Sources of error encountered in the determinations include those attributable to sampling, dinitrophenylation of the oilseed meal, removal of unreacted dinitrofluorobenzene, hydrolysis of the dinitrophenylated meal, age of the hydrolyzate, chromatographic separation of 2,4-dinitrophenyl lysine from the other colored substances in the hydrolyzates and determination of the absorbance of the effluent from the column. Included also are the differences between chromatographic columns and various dilution errors. In addition, errors increase when the available lysine datum is expressed (as is conventional) as a function of the total nitrogen of the meal, since the nitrogen analysis is also subject to analytical errors.

EXPERIMENTAL PROCEDURES

Four oilseed meals were selected. One was derived from glandless cottonseed, one from glanded cottonseed and two from peanuts.

Each meal was ground to pass through a 40 mesh screen, and the screenings were thoroughly mixed. A 50 g sample was taken from each meal; the residual meal was mixed again and a second 50 g sample was taken. This procedure was repeated to produce a total of four 50 g samples from each of the cottonseed and peanut meals, making a grand total of 16.

TABLE I

Grams Available Lysine per 16 Grams Meal Nitrogen for Two Cottonseed and Two Peanut Meals

Meal	Replication, g 1	2
Cottonseed meal no. 1 (from glandless cottonseed)		
Subsample no. 1	4.34 (1)	4.34 (2)
2	4.20 (1)	4.20 (4)
3	4.15 (4)	4.12 (3)
4	4.11 (4)	3.95 (3)
Cottonseed meal no. 2 (from glanded cottonseed)		
Subsample no. 1	4.02 (1)	4.02 (2)
2	3.85 (1)	3.85 (2)
3	3.78 (3)	3.78 (4)
4	3.80 (1)	3.77 (2)
Peanut meal no. 1		
Subsample no. 1	3.34 (1)	3.35 (2)
2	3.23 (3)	3.23 (4)
3	3.08 (1)	3.20 (2)
4	3.21	3.26
Peanut meal no. 2		
Subsample no. 1	3.00 (2)	3.00 (3)
2	2.93 (1)	2.93 (2)
3	2.98 (2)	2.98 (3)
4	2.47 (2)	2.47 (3)

TABLE II

Per Cent Nitrogen in Air Dry Cottonseed and Peanut Meals

Meal	Replication, % 1	2
Cottonseed meal no. 1		
Subsample no. 1	9.113	9.116
2	8.719	8.726
3	8.625	8.644
4	8.720	8.727
Cottonseed meal no. 2		
Subsample no. 1	8.609	8.551
2	8.594	8.621
3	9.041	9.046
4	9.070	9.071
Peanut meal no. 1		
Subsample no. 1	7.826	7.801
2	7.754	7.756
3	7.756	7.761
4	7.778	7.772
Peanut meal no. 2		
Subsample no. 1	8.345	8.342
2	8.551	8.551
3	8.839	8.863
4	8.403	8.404

TABLE III

Partition of Squares–Available Lysine Data

Source of variance	Sum of squares	Degrees of freedom	Mean square
Total	9.2659	31	
Between meals	8.6439	3	
Within meals	0.6211	28	0.0222
Replication within meals			
Cottonseed no. 1	0.0045	1	
Cottonseed no. 2	0.0001	1	
Peanut no. 1	0.0041	1	
Peanut no. 2	0.0000	1	
Total replication within meals	0.0087	4	0.0022
Samples within meals			
Cottonseed no. 1	0.1009	3	
Cottonseed no. 2	0.0754	3	
Peanut no. 1	0.0423	3	
Peanut no. 2	0.3802	3	
Total samples within meals	0.5988	12	0.0499
Error	0.0139	12	0.0011

TABLE IV

Partition of Squares--Total Nitrogen Data

Source of variance	Sum of squares	Degrees of freedom	Mean square
Total	6.785640	31	
Between meals	5.763297	3	
Within meals	1.022344	28	0.036512
Replication within meals			
Cottonseed no. 1	0.000163	1	
Cottonseed no. 2	0.000078	1	
Peanut no. 1	0.000072	1	
Peanut no. 2	0.000061	1	
Total replication within meals	0.000374	4	0.000093
Samples within meals			
Cottonseed no. 1	0.276306	3	
Cottonseed no. 2	0.430686	3	
Peanut no. 1	0.004037	3	
Peanut no. 2	0.308113	3	
Total samples within meals	1.019142	12	0.084928
Error	0.002828	12	0.000253

A 50 g sample was spread on aluminum foil and mixed thoroughly by plowing and stirring with a spatula. The sample was *not* rolled by tilting a corner of the foil, because this operation tends to segregate hull fragments. The sample was then shaped into the form of a disc and marked off in quarters. Lots of ca. 100 mg were then taken randomly and successively from each quarter until a total of ca. 12 g were accumulated. This 12 g sample was then thoroughly mixed and quartered, as noted above. Ten milligram portions were collected randomly and successively from each quarter, until a total of ca. 500 mg (500.0-501.0) was accumulated. This subsample was weighed to four significant figures and was set aside for a total nitrogen determination. All of the residues from the 50 g sample were then recombined, mixed and sampled as described above for a second 500 mg subsample that was also set aside for a total nitrogen determination. Two additional 500 mg subsamples were selected in the same way for replicate available lysine determinations.

Four comparable 500 mg samples were taken from each of the 50 g samples of the remaining meals; a total of 64 subsamples was collected.

The dinitrophenylation procedure described by Rao et al. was followed meticulously, as was the preparation of the columns, the hydrolysis of the dinitrophenylated meals, etc. Fresh solvent mixtures of ethyl methyl ketone and aqueous HCl were prepared daily.

All of the volumetric glassware was carefully calibrated, and the appropriate correction was made when necessary.

The eluate containing the dinitrophenyl lysine was collected and made up to 25 ml, as directed by Rao et al., and the absorbance was determined with a Beckman Model B spectrophotometer where the slit width was set at 0.2 mm. The data were recorded to three significant figures.

Four columns were prepared for the analyses reported here and were used interchangeably in the course of the work.

Total nitrogen was determined by a micro-Kejhdahl procedure; 25 ml concentrated sulfuric acid was used for each 500 mg sample of meal. Mercuric oxide was used as the oxidation catalyst. The ammonia distilled from the digest was trapped in standard sulfuric acid and was determined by back titration with standard NaOH (carbonate-free), with methyl purple as the indicator. This indicator shows a strong color change at pH 5.5--the pH of the inflection point observed when ammonium hydroxide is titrated potentiometrically with sulfuric acid.

RESULTS AND DISCUSSION

Aliquots of 2 ml each of the dinitrophenylated meal hydrolyzates were used to produce the data reported in Table I. The column used in each instance is identified by the number in parentheses.

The available lysine data reported in Table I are expressed as grams of available lysine per 16 g meal nitrogen, where the appropriate average of replicate nitrogen analyses was used in the calculations. The nitrogen data are reported in Table II.

An analysis of variance of the available lysine data is shown in Table III, while an analysis of variance of the total nitrogen data is shown in Table IV.

The analysis in Table III reveals that the sampling error is the major error in the determination of available lysine in this series of determinations. It is obvious that with comparable materials meticulous care must be taken in sampling of the meals, to assure a representative sample if highly reproducible results are to be obtained.

The mean square due to replication in the assay for available lysine is of the same order of magnitude as the error mean square; apparently errors attributable to the manipulative operations, e.g., dinitrophenylation, hydrol-

TABLE V

Comparison of Columns 1 and 2

Source of variance	Sum of square	Degrees of freedom	Mean square
Total	3.0876	13	
Between columns	0.0007	1	0.0007
Between samples	3.0799	6	0.5133
Error	0.0070	6	0.0012

TABLE VI

Comparison of Columns 3 and 4

Source of variance	Sum of square	Degrees of freedom	Mean square
Total	0.9938	7	
Between columns	0.0045	1	0.0045
Between samples	0.9805	3	0.3268
Error	0.0088	3	0.0029

TABLE VII

Comparison of Charge on Column

Source of variance	Sum of square	Degrees of freedom	Mean square
Total	0.0051	7	
Between charges	0.0025	3	0.0008
Within charges	0.0026	4	0.0007

ysis, etc., were not significant.

The analyses shown in Table III indicate confidence limits for the available lysine data of ca. 0.5% and 0.7% of the available lysine concentration for the 5 and 1% levels of probability. Actually, an experimental error of ca. 0.5% is inherent in reading the absorbance with the Beckman Model B spectrophotometer; the third significant figure is obtained by estimation.

The analysis of variance of the total nitrogen data, as shown in Table IV, indicates that the major error in total nitrogen assay was also due to sampling. The data indicate confidence limits of ca. 0.1% of the total nitrogen.

The data in Table I permit an assessment of the column effect. Analyses of variance of the data obtained with columns 1 and 2 and with columns 3 and 4 are shown in Tables V and VI. It is evident that the mean square due to column effect is of the same order of magnitude as the error mean square, and it is concluded that the column effect is too small to be detected in these experiments.

The effect of varying the charge on the column from 1 to 5 ml, e.g., 1, 2, 3 and 5 ml, of hydrolyzate was also investigated. An analysis of the data for the ratio of each datum to that obtained with 2 ml is shown in Table VII. No differences in the assay for available lysine were observed that can be attributed to loading of the column, in the loading range studied.

The age of the hydrolyzates of the dinitrophenylated meals up to 24 days (the period of observation) had no measurable influence on the assay.

It is concluded that available lysine data may be reproduced to within ca. 0.5% of the available lysine content of the meals when sufficient care is taken in sampling the meal. Obviously the major source of error in this series of analysis is attributable to the sampling, when the sampling (the four 50 g samples from each meal) is typically that used for conventional assay.

REFERENCES

1. Rao, S.R., F.L. Carter and V.L. Frampton, Anal. Chem. 35:1927 (1963).

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