Interrelationships of the Protein and Amino Acid Contents of Inbred Lines of Corn[1]

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Average total protein (N × 6.25) and amino acids (automated ion exchange) in 114 samples of inbred corn grain indicated that their potential quality as a source of protein for monogastric animals would not be much better than ordinary hybrid corn, even though 25 of the lines had lysine concentrations greater than 0.45% (whole sample basis). Relationships among basic amino acid concentrations (percent of sample and percent of protein) were all positive and highly significant. Relationships among acidic and neutral amino acid concentrations (percent of sample and percent of protein) were all positive and most were significant. Knowledge of such relationships may be useful in estimating amino acid concentrations and relative proportions if other than automated ion exchange methods are employed for assay. Further study of some of the relationships reported in this paper may explain more dependent variables by including additional independent variables in the regression analyses.

The discovery that a single gene mutation can markedly affect the amino acid pattern of corn protein (Mertz *et al.*, 1964) has stimulated interest in the search for inbred lines of corn of high nutritional quality. The development of such lines of corn of high nutritional quality requires determination of total protein and amino acids in hundreds of samples. Even with the automated methods available today, analyzing the large number of necessary corn samples becomes impracticable. Thus, there is a need not only to evaluate the nutritional potential of inbred corn by protein and amino acid analysis, but also to search for a simpler method of screening large numbers of samples.

One approach to screening is to study the relationships among amino acid quantities in corn. Prior to development of automated ion exchange chromatography, Frey (1951) studied interrelationships among several amino acid quantities in corn protein by microbiological assay and concluded that the relationships among valine, leucine, and isoleucine were close enough so that analyses for all three amino acids were not necessary. The examination of the interrelationships among 17 amino acid quantities in inbred corn was feasible, since automated assay equipment was available for the present study.

MATERIALS AND METHODS

One-hundred fourteen samples of inbred corn containing approximately 12% moisture were obtained from Dr. Alfred Manwiler, South Carolina Agricultural Experiment Station, Florence, S.C. 29501.

Estimation of Crude Protein. All samples were ground in a Wiley mill to a 40-mesh particle size and nitrogen was determined in 0.25 g of the whole ground kernel by the micro-Kjeldahl method. Crude protein was estimated by multiplying the nitrogen concentration by 6.25.

Amino Acid Analysis. Amino acid analysis of the ground whole kernel was performed on a Phoenix K-8000 VG automatic amino acid analyzer employing the accelerated Stein and Moore system (Spackman *et al.*, 1958). Samples representing 100 mg of corn protein were weighed on an analytical balance to the nearest 0.1 mg and transferred quantitatively to 16 × 250 mm borosilicate glass test tubes which had been constricted at the neck by the use of heat. Five milliliters of concentrated HCl and 5 ml of distilled water were added, and the tubes were placed in an acetone-dry ice bath until the contents were frozen, then attached to a freeze-dryer manifold, and evacuated to approximately 50 to 100 μ of Hg. Prepurified nitrogen gas was flushed three times intermittently into the tubes. After the third flushing, the tubes were heat-sealed under vacuum, allowed to equilibrate to room temperature, and placed upright in a forced-air oven at 110° ± 1° C for 22 hr. After removal from the oven and cooling to room temperature, the tubes were broken open and the hydrolyzed material filtered through a fine sintered glass funnel. The hydrolyzates were evaporated to dryness under reduced pressure by means of a rotary evaporator and brought to a final volume of 10 ml with pH 2.2 sodium citrate buffer. All hydrolyzates were stored at −20° C until amino acid analyses were performed.

A 0.2 ml aliquot of the hydrolyzate was placed on the 60 × 0.9 cm column to elute the acidic and neutral amino acids and 0.1 ml aliquot was placed on the 10 × 0.6 cm column to elute the basic amino acids. Chromatograms from each hydrolyzate were calculated using the height-times-width method of integration for each peak, as suggested by Spackman *et al.* (1958). By this method, the micromoles of each amino acid were calculated. Final values for all amino acids were expressed on the basis of percent of the sample and percent of the sample protein. No corrections were applied to account for possible destruction of any of the amino acids.

Data Evaluation. Means and ranges of total protein

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Table I. Ranges and Means of Protein and Amino Acid Contents of 114 Inbred Lines of Corn[a]

Total Protein, %	Range 9.77–16.25		Mean[b] 12.90 ± 1.14	
Amino acid	% of whole sample	% of protein	% of whole sample	% of protein
Lysine	0.21–0.57	2.20– 5.24	0.39 ± 0.07	3.48 ± 0.58
Histidine	0.09–0.59	1.01– 5.62	0.36 ± 0.09	3.22 ± 0.76
Arginine	0.21–0.97	2.27– 8.14	0.56 ± 0.12	4.96 ± 0.97
Aspartic	0.51–1.67	4.89–14.52	0.82 ± 0.16	7.21 ± 1.17
Threonine	0.28–0.67	2.55– 5.85	0.45 ± 0.07	4.03 ± 0.54
Serine	0.06–0.85	0.58– 7.43	0.61 ± 0.11	5.37 ± 0.84
Glutamic	1.41–3.37	13.85–26.83	2.32 ± 0.36	20.48 ± 2.45
Proline	0.47–2.26	3.84–17.26	1.01 ± 0.19	8.87 ± 1.35
Glycine	0.31–0.85	2.81– 6.43	0.52 ± 0.08	4.67 ± 0.67
Alanine	0.25–1.46	2.15–13.70	0.95 ± 0.18	8.42 ± 1.48
Cystine	0.06–0.36	0.48– 3.46	0.13 ± 0.04	1.12 ± 0.33
Valine	0.09–0.97	0.96– 9.09	0.61 ± 0.11	5.41 ± 0.90
Methionine	0.07–0.26	0.61– 2.39	0.13 ± 0.03	1.14 ± 0.30
Isoleucine	0.13–0.55	1.10– 5.53	0.43 ± 0.07	3.81 ± 0.55
Leucine	0.38–2.22	3.48–18.79	1.49 ± 0.27	13.04 ± 2.03
Tyrosine	0.08–0.47	0.79– 4.22	0.26 ± 0.07	2.31 ± 0.57
Phenylalanine	0.34–0.95	3.01– 9.09	0.58 ± 0.10	5.09 ± 0.78

[a] Moisture-free basis. [b] Mean ± SD.

Table II. Relationships between Total Protein Content and Amino Acids in the Whole Sample and in the Protein of Inbred Corn

	Coefficient of Correlation[a] between Protein and Amino Acid		
Amino Acid	Amino Acid as % of Whole Sample	Amino Acid as % of Protein	Regression Equation[b]
Lysine	0.428	−0.037	$\hat{y} = 0.034 + 0.028X$
Histidine	0.311	−0.014	$\hat{y} = 0.041 + 0.025X$
Arginine	0.404	0.023	$\hat{y} = 0.044X - 0.003$
Aspartic	0.522	0.073	$\hat{y} = 0.074X - 0.120$
Threonine	0.428	−0.191	$\hat{y} = 0.118 + 0.025X$
Serine	0.538	0.041	$\hat{y} = 0.051X - 0.040$
Glutamic	0.635	0.058	$\hat{y} = 0.198X - 0.199$
Proline	0.496	0.004	$\hat{y} = 0.081X - 0.030$
Glycine	0.399	−0.183	$\hat{y} = 0.138 + 0.029X$
Alanine	0.362	−0.123	$\hat{y} = 0.182 + 0.058X$
Cystine	0.326	0.035	$\hat{y} = 0.011X - 0.014$
Valine	0.467	−0.010	$\hat{y} = 0.014 + 0.046X$
Methionine	0.249	−0.112	$\hat{y} = 0.028 + 0.008X$
Isoleucine	0.476	−0.068	$\hat{y} = 0.035 + 0.030X$
Leucine	0.527	0.036	$\hat{y} = 0.126X - 0.125$
Tyrosine	0.422	0.116	$\hat{y} = 0.027X - 0.069$
Phenylalanine	0.509	0.001	$\hat{y} = 0.045X - 0.007$

[a] Significant ($P < 0.01$) $r = 0.241$; significant ($P < 0.05$) $r = 0.185$.
[b] $\hat{y}$ = % amino acid in the whole sample; X = % protein.

content and amino acid concentrations were calculated for the 114 corn samples. Interrelationships among variables were studied through calculation of simple correlation coefficients and use of linear regression analyses (Steele and Torrie, 1960).

RESULTS AND DISCUSSION

Protein and Amino Acid Contents of Samples. On a moisture-free basis, total protein concentration of inbred lines ranged from 9.77 to 16.25%, with a mean of 12.90% (Table I). Compared with S.C.-236 hybrid corn, these inbreds averaged about 4% higher in total protein content.

Except for cystine, methionine, and tyrosine, the average percent of all amino acids was greater than that of S.C.-236 hybrid corn. Expressed as percent of protein, inbreds averaged lower in concentrations of proline, cystine, and methionine; about the same in arginine, glutamic acid, and phenylalanine; and higher in all other amino acids assayed than S.C.-236 hybrid corn.

Lysine percentages averaged about 1.7 times greater than in S.C.-236 hybrid corn, but about 0.1% less than that reported for opaque-2 corn (Cromwell *et al.*, 1967). However, 25 inbreds analyzed more than 0.45% lysine (dry-weight basis). Histidine content was similar to that found in opaque-2 corn but arginine averaged 0.2% less than in opaque-2. Tryptophan values were not obtained in these analyses.

Relationships between Total Protein Content and Amino Acids. Relationships between percent protein and percent amino acid in the whole sample (Table II) were positive, and correlation coefficients ranged from 0.249 (methionine) to 0.635 (glutamic acid). Thus, as protein content increased in the corn, statistically significant increases in concentrations of amino acids analyzed were detectable. Variations in total protein concentration were more highly associated with variation in glutamic acid content than with any other amino acid and 40.4% of the change in glutamic acid was attributed directly to a change in protein content. The relationship of percent protein to serine content (r = 0.538) indicated a slightly smaller rate of change as protein changed, and 29.0% of the variation in serine was attributed to protein variation.

Leucine and aspartic acid ranked next in relationship to total protein content with r values of 0.527 and 0.522, respectively. Variations in leucine and aspartic acid content attributable to variation in protein percentage were 27.8 and 27.2%, respectively.

Phenylalanine ranked fifth in its association with protein content, (r = 0.509), and 25.9% of the change in phenylalanine concentration could be explained by a change in percent protein. Ranking lower were proline, isoleucine, and valine, with r values of 0.496, 0.476, and 0.467, respectively, with similar proportions of variation resulting from changes in the protein content of the corn.

Amounts of lysine and threonine and identical degrees of relationship to total protein (r = 0.428), and only 18.3% of the variation in these two amino acids could be attributed to changes in the protein content. In contrast, Miller *et al.* (1950) showed that about 70% of the change in lysine content of single cross corns was attributed to changes in total protein concentration. The relationship of tyrosine to protein was similar to that of lysine and threonine (r = 0.422); 17.8% of its variation was due to changes in protein content.

Arginine, glycine, alanine, cystine, histidine, and methionine all ranked lower than other amino acids in relationship to total protein, with less than 17.0% of their change attributable to change in protein percentage. Miller *et al.* (1950) found a correlation coefficient of 0.83 between methionine and crude protein and between tryptophan and crude protein in single cross corns.

Most correlation coefficients between protein content and amino acid concentration were rather low, suggesting limited predictive value for amino acids when percent protein is employed as the independent variable.

A higher content of basic amino acids and lower content of leucine were reported to contribute markedly to the quality of opaque-2 corn (Mertz *et al.*, 1964). Leucine content in these inbred lines increased rapidly with increases in corn protein. Concentrations of basic amino acids increased at a much slower rate. Increased amounts of leucine in corn protein are indicative of increased zein (Frey *et al.*, 1949), and zein is practically devoid of lysine and tryptophan (Bressani and Mertz, 1958a). These findings suggest that the quality of protein in inbred lines, on the average, would not be much better than that reported for ordinary hybrid corn.

Table II also demonstrates a similar analysis of this same data when all amino acids were expressed as a percent of the protein in the corn. Significant relationships (except for threonine) were not detectable when amino acids were expressed as percentages of the protein. Nevertheless, the correlation coefficients revealed the amino acids that tended to change in concentration within the corn protein and in what direction as total protein percent increased. Concentrations of tyrosine, aspartic acid, glutamic acid, and serine increased slightly as the percent protein increased. Concentrations of isoleucine, methionine, alanine, glycine, and threonine decreased, though not significantly, as total percent increased. Amounts of leucine, cystine, arginine, proline, phenylalanine, valine, histidine, and lysine remained essentially unchanged as the percent protein increased.

The changes in concentration of certain amino acids, when expressed as per unit of protein, become important when related to variation in total protein in corn. For example, if lysine concentration is not increased in proportion to total protein, this amino acid will become more limiting for animal growth, since requirements for lysine are higher when dietary protein levels are higher (Bressani and Mertz, 1958b). Requirements for other essential amino acids are also based on total protein intake. Thus, data in Table II suggest that since amounts of other essential amino acids in corn protein (isoleucine, methionine, threonine, and valine) either decreased slightly or were unchanged as total protein increased, inbred corn of higher protein content could be of lower biological value.

Interrelationships among Basic Amino Acids. Correlation coefficients calculated for lysine, histidine, and arginine (Table III) indicated that an increase in concentration of any one basic amino acid was associated significantly with increases in concentrations of the other two, both in the whole corn sample and within the protein of the corn. The association between lysine and histidine or arginine was closer (r = 0.750) than that between arginine and histidine (r = 0.639) when these amino acids were expressed as percent of the whole

Table III. Correlation Coefficients Among Basic Amino Acid Concentrations in Inbred Corn

Amino acid	Lysine	Histidine	Arginine
Lysine		0.750[a]	0.750[a]
Histidine	0.709[b]		0.639[a]
Arginine	0.688[b]	0.560[b]	

[a] Correlation coefficients calculated from percent amino acid in the whole sample. Significant (P < 0.01), r = 0.241. [b] Correlation coefficients calculated from percent amino acid in the corn protein. Significant (P < 0.01), r = 0.241.

Table IV. Correlation Coefficients Among Acidic and Neutral Amino Acid Concentrations in Inbred Corn[a]

Amino Acid	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Cys	Val	Met	Iso	Leu	Tyr	Phe
Aspartic		0.496	0.428	0.623	0.380	0.399	0.368	0.207	0.458	0.319	0.497	0.512	0.386	0.520
Threonine	0.376		0.755	0.864	0.587	0.811	0.600	0.323	0.704	0.609	0.825	0.681	0.650	0.854
Serine	0.212	0.630		0.733	0.534	0.594	0.522	0.339	0.600	0.416	0.694	0.661	0.533	0.630
Glutamic	0.477	0.823	0.561		0.632	0.699	0.567	0.324	0.700	0.550	0.827	0.782	0.610	0.880
Proline	0.200	0.500	0.365	0.368		0.372	0.457	0.216	0.615	0.212	0.591	0.575	0.360	0.567
Glycine	0.262	0.784	0.433	0.476	0.243		0.512	0.438	0.623	0.612	0.720	0.570	0.596	0.688
Alanine	0.249	0.560	0.401	0.352	0.393	0.448		0.219	0.570	0.425	0.596	0.503	0.384	0.600
Cystine	0.035	0.182	0.192	0.159	0.067	0.316	0.080		0.213	0.219	0.295	0.228	0.335	0.270
Valine	0.305	0.621	0.443	0.357	0.502	0.527	0.486	0.051		0.413	0.693	0.622	0.501	0.663
Methionine	0.226	0.582	0.293	0.435	0.150	0.588	0.401	0.112	0.348		0.541	0.456	0.568	0.576
Isoleucine	0.353	0.795	0.558	0.595	0.497	0.664	0.453	0.115	0.614	0.500		0.872	0.547	0.803
Leucine	0.348	0.572	0.376	0.504	0.216	0.448	0.401	0.057	0.516	0.382	0.817		0.471	0.649
Tyrosine	0.239	0.538	0.379	0.359	0.216	0.448	0.269	0.215	0.371	0.480	0.438	0.283		0.636
Phenylalanine	0.376	0.797	0.437	0.670	0.443	0.602	0.523	0.098	0.549	0.525	0.745	0.468	0.535	

[a] Correlation coefficients above and to the right of the diagonal were calculated from percent amino acid in the whole sample. Correlation coefficients below and to the left of the diagonal were calculated from percent amino acid in the corn protein. Significant (P < 0.01), r = 0.241; significant (P < 0.05), r = 0.185.

sample. Similar, but slightly lower, correlation coefficients were found when the amino acids were expressed on the basis of percent of protein.

The high degree of covariation which existed among the basic amino acids suggests that analyses for all three would be unnecessary. This would be advantageous only if methods other than automated column chromatography were employed for analysis.

Interrelationships among Acidic and Neutral Amino Acids. Acidic and neutral amino acids were positively associated with each other when expressed as percent of the whole sample (Table IV). With the exception of cystine, almost all associations among amino acid concentrations were significantly greater than zero. Under the conditions which the acidic and neutral amino acids were eluted from the chromatographic column, "one-half-cystine" appeared as a broad, flat peak probably resulting from destruction during acid hydrolysis. As a result, it was difficult to obtain a half-height reading on the cystine curve using the H $\times$ W method of integration.

Within the group of 14 acidic and neutral amino acids, six are considered indispensable in the diet of the rat. Within the group of essentials, methionine was the only amino acid that did not show relatively high correlations with all the others. Nevertheless, except for associations with threonine, the size of most of the correlation coefficients indicated that limitations would exist if one were seeking a reliable parameter for prediction purposes.

When concentrations of acidic and neutral amino acids were expressed as percent of the corn protein (Table IV), correlation coefficients indicated that an increase in any one amino acid within the protein was accompanied by increases in all the others. With few exceptions, this group of correlation coefficients paralleled those found among amino acids expressed as percent of the whole sample. However, the associations were not as strong as those found in the whole sample, nor uniform enough with respect to any one amino acid to be of value in seeking reliable prediction parameters. Nevertheless, Table IV indicates the degree of change in the concentration of each acidic or neutral amino acid with respect to all others in the protein of corn.

Other interrelationships (those between the basic amino acids and acidic or neutral amino acids) are not shown here. These relationships will be discussed in a subsequent publication.

LITERATURE CITED

Bressani, R., Mertz, E. T., *Cereal Chem.* **35,** 227 (1958a).
Bressani, R., Mertz, E. T., *J. Nutr.* **65,** 481 (1958b).
Cromwell, G. L., Rogler, J. C., Featherston, W. R., Pickett, R. A., *Poultry Sci.* **46,** 705 (1967).
Frey, K. J., *Cereal Chem.* **28,** 123 (1951).
Frey, K. J., Brimhall, B., Sprague, G. F., *Agron. J.* **41,** 399 (1949).
Mertz, E. T., Bates, L. S., Nelson, O. E., *Science* **145,** 279 (1964).
Miller, R. C., Aurand, L. W., Flach, W. R., *Science* **112,** 57 (1950).
Spackman, D. H., Stein, W. H., Moore, S., *Anal. Chem.* **30,** 1190 (1958).
Steele, R. G. D., Torrie, J. H., "Principles and Procedures of Statistics," pp. 99–194, McGraw-Hill, New York, Toronto, and London, 1960.

Received for review October 17, 1969. Accepted March 12, 1970.