Quality Evaluation of Distillers' Dried Grain by Near-Infrared Analysis[1]

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ABSTRACT

A by-product of fuel ethanol production from barley and marketed as distillers' dried grain (DDG) is fed to livestock either directly or as an ingredient in a formula feed. Samples of DDG were obtained from the Montana plants producing fuel ethanol. These samples were used to fabricate a set of calibration samples for use with near-infrared (NIR) analysis . From the fabricated calibration set, regression coefficients were obtained to provide NIR analysis of DDG for crude protein content, moisture content, and the percentages of crude fiber, acid detergent fiber (ADF), neutral detergent fiber (NDF), and amino acids. The correlations between standard laboratory values and NIR analysis values for the calibration samples were crude protein 0.966, crude fiber 0.964, ADF 0.911, and NDF 0.945. Amino acid correlations ranged from 0.993 for glutamic acid to 0.933 for methionine. Correlation coefficients between lab and NIR values on verification samples were protein 0.968, crude fiber 0.890, ADF 0.660, and NDF 0.627. Correlation coefficients between lab and NIR amino acid values on the verification samples range from 0.946 for alanine to 0.210 for proline. The standard error of difference between lab and NIR methods for the calibration samples was protein 1.10%, crude fiber 0.925%, ADF 1.28%, NDF 2.51%, and amino acids from 0.916 for glutamic to 0.011 for tyrosine.

Near-infrared (NIR) reflectance techniques have been applied to numerous products in addition to their use in the analysis of wheat for protein and moisture content and oleaginous crops for total lipid content. Successful determination of nutritional constituents in feed stuff and analysis of forage legumes and grasses by Shenk et al (1981) has opened a new area for NIR analysis in quality evaluation of agricultural products. Their work showed that NIR analytical results of forage quality parameters were highly correlated with lab results obtained by traditional methods. Marten et al (1983) demonstrated the applicability of analyzing annual forage quality by NIR reflectance. Their standard errors of calibration for NIR-predicted assays were equivalent to or lower than those commonly reported for the respective laboratory assays.

Predictions of amino acid content of barley and wheat using NIR reflectance (Williams et al 1984) suggest a new use for this analytical tool in the food and feed industry. These authors reported standard errors of prediction of less than 2.0 μmol/g of dry matter for three of four amino acids measured in wheat. Their low standard errors of prediction using NIR model 6350 show a high degree of accuracy for estimating up to 14 amino acids in barley.

Gill et al (1979) calibrated NIR equipment to read lysine content of barley grain. Their range of lysine values was 0.31–0.80%. The correlation coefficient between lab and NIR data was 0.98. The standard error of difference between the methods was 0.035 on verification samples and 0.030 on their calibration set. These researchers claim adequate accuracy for screening large barley populations.

Gill et al (1979) and Baker (1980) found NIR estimates of barley grain nitrogen content within the limits of accuracy and precision of the traditional Kjeldahl method. Between-lab error for Kjeldahl ranged from a low of 0.17 to a high of 0.40 on separate collaborative sample sets (Baker 1980). NIR between-lab error was 0.08 and 0.06 on these same two sets.

Obtaining results at the point of sale or manufacture allows the feed formulator an immediate evaluation of product quality parameters. Compared to traditional laboratory procedures, the NIR analysis gives results of feed analysis with precision, accuracy, and the expediency of immediate data presentation (Norris et al 1976).

One problem of data acquisition by NIR analysis is that of calibration of the equipment for a given application. NIR calibration requires a set of samples that has maximum variation for the parameters of interest. The variation in protein content of distillers' dried grains (DDG) from random samples taken over two years was inadequate for a good NIR calibration. To maximize the variation from routine production samples to achieve a good calibration, some manipulation of those samples was required. A major objective of this research was, therefore, to create a suitable calibration set for each of the parameters of interest, such as the amount of protein, moisture, crude fat, crude fiber, neutral detergent fiber (NDF), acid detergent fiber (ADF), and the various amino acids commonly found in cereals. Such calibrations may have applications for quality evaluations other than for the spent grains from fuel-ethanol-manufacturing facilities. The feed quality traits studied are widely used in the formula feed industry.

MATERIALS AND METHODS

Samples of DDG came from A. E. Montana (Amsterdam, MT) and Alcotec, Inc. (Ringling, MT). Both of these businesses produce fuel-grade ethanol from barley. Eleven samples of DDG were taken at weekly intervals, five from Ringling and six from Amsterdam. Each sample was milled into two fractions on a Buhler mill. Samples of DDG were milled without temper at a feed rate of 100 g/min. Following first-break rolls, the stock was sifted on a 40-mesh wire followed by an 88-mesh wire. Overs of the 40-mesh sieve went to second break. Throughs of the 88-mesh sieve were flour. Second- and third-break sieves were similar to first break, except a 44-mesh sieve replaced the 40-mesh sieve at third break. Overs of the 44-mesh sieve went to bran. Overs of each 88-mesh sieve on the break side went to first reduction. Each of the three reduction sifters was fitted with an 88-mesh sieve. Throughs of these were flour. Overs of the third reduction sifter were classified as shorts. The fine (F) fraction was a combination of the throughs of the 88-mesh sieves. The course (C) fraction was a mixture of bran and shorts. The F and C fractions from each sample were then blended in proportions of 30F:70C, 40F:60C, 50F:50C, 60F:40C, and 70F:30C, to create a range in their contents of dry matter, protein, ether extract, crude fiber, ADF, NDF, and amino acids. These mixtures were then ground through a 0.5-mm screen in the Udy cyclone mill. The 55 samples were analyzed by AOAC (1980) methods for dry matter, protein, ether extract, crude fiber, ADF as modified by Robertson and Van Soest (1977), NDF as modified by Roth et al (1981), and by a standard amino acid analytical procedure (AAA Laboratory, 6206 89th Ave. SE, Mercer Island, WA 98040).

A Technicon 400R infrared-reflectance analyzer was used for this project. The equipment was calibrated as recommended by the manufacturer. Log data were transferred to a CP-6 Honeywell computer and analyzed by a BMD P9R—all possible subsets regression (BMDP Statistical Software, Inc., Westwood Blvd., Ste. 202, Los Angeles, CA. Published 1964). This program regresses lab

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values for a given parameter on log readings from NIR. Regression coefficients from each wavelength are given as well as coefficients from all combinations of wavelengths in groups of two, three, and four through nine. The multiple correlation coefficient is given for each regression as is the F statistic for regression. Selection of wavelengths and regression coefficients for the optimum calibration depended on the magnitude of the multiple correlation coefficient. When data for all possible wavelength combinations had been compared, the data giving the largest R^2 and a highly significant F for regression with the smallest number of wavelengths was selected. Regression coefficients from those sets were used for subsequent NIR analysis.

Verification samples of DDG from the two fuel ethanol plants had been obtained for other purposes. Protein content had been previously determined on 14 samples, moisture content on 13, crude fiber on 10, NDF on eight, ADF on nine, and the amino acid profile on 10. None of these were included in the set of calibration samples, but all were analyzed by NIR reflectance as well as by the traditional lab methods.

Cultivar names of the barley used by the alcohol manufacturers were not known. The crop used in this work was grown near the processing plants during 1982 and 1983.

RESULTS

The best wavelength combination for a given parameter was selected by choosing the largest R^2 value from the fewest number of wavelengths as well as the magnitude of F for the regression. After the selection of wavelengths was made and the regression coefficients entered in the NIR analyzer, calibration samples were evaluated for the parameters under consideration. Any adjustments for bias were made in the intercept according to the equipment operators' manual to obtain best agreement between lab and NIR data. The selection of wavelengths and regression coefficients may be seen in Table I.

Protein Content

The wavelengths selected from the calibration data were 2,208, 2,190, and 2,100 nm. The R^2 value of 0.980 explains almost all of the variability between the Kjeldahl and the NIR log readings at the above wavelengths for the calibration samples. Protein content ranged from 14.0 to 36.1%, and the the 55 calibration samples were distributed over that range. After entering the regression coefficients in the NIR apparatus, the 55 calibration samples were analyzed. Protein data from that set are in Table II. A least squares regression analysis was done on the lab and NIR data. For this regression the F value was 735.7 and $R = 0.966$ (significant at the 0.01 level), $\beta = 0.984$ with its standard error of 0.036, and the standard error of difference between methods was 1.10. This standard error compares to 0.924 for the Kjeldahl method when the 55 calibration samples were analyzed in duplicate.

A verification set of 14 production samples was analyzed by Kjeldahl and NIR methods. Data from this comparison were analyzed by a least squares regression analysis; results are shown in Table III. The mean for Kjeldahl protein was 24.0, and that for NIR analysis was 24.6. The two methods correlated very highly, with $R = 0.968$ and F for regression of 59.0 (0.01 level), $\beta = 0.831$ with its standard error of 0.108, and a standard error of difference between the methods of 1.41.

Moisture Content

Moisture levels in DDG calibration samples were in the range of 3.6–7.5%. Regression coefficients were obtained for a moisture calibration (Table I), but this calibration failed to predict moisture values in verification samples. DDG are routinely dried quickly at a high temperature as the last step in their production. This high-temperature drying may affect the capability of NIR analysis to estimate moisture.

Ether Extract

The eight wavelengths selected are given in Table I along with their regression coefficients, but the $R^2 = 0.582$ value for the log values of wavelength readings versus lab ether extract values was deemed to be too low for a useful calibration. Ether extract estimates on calibration verification samples bore this out. These data are not reported here.

Crude Fiber

Four wavelengths (Table I) were selected for estimating this parameter. Those wavelengths along with their coefficients of regression are given in Table I. The $R = 0.963$ accounts for much of the variability between NIR log readings and the crude fiber data provided by the wet lab method. The highly significant F for regression shows the degree of association between the lab data and the NIR log values. The calibration samples read on NIR for crude fiber produced data comparable to the wet lab data. Statistical analysis of these two sets of crude fiber data used the same procedure used for the protein data. The correlation coefficient was 0.964 (0.01 level), $\beta = 1.10$ with its standard error of 0.041, the standard error of difference between lab and NIR was 0.925, and the F for regression was 704.0 (0.01 level). The means and range of crude fiber as measured by the respective methods are given in Table II.

The verification set contained 10 samples ranging from 14.6 to 24.6% crude fiber measured by the lab method. Statistical analysis of these samples assayed by both methods produced the data in

TABLE I
Filter Selection and Corresponding Regression Coefficients for Estimating Moisture Content (MO), Protein Content (CP), Ether Extract (EE), Crude Fiber (CF), Acid Detergent Fiber (ADF), and Neutral Detergent Fiber (NDF) in 55 Samples of Barley Distillers' Dried Grains

Filter Wavelength (nm)	Parameter					
	MO	CP	EE	CF	ADF	NDF
2,336	−55.03		−438.2			
2,348						1,651
2,310						
2,270			322.8	489.6	823.6	
2,208	713.6	−741.3				
2,190	−581.1	1,243				
2,139				−1,873	−785.4	−3,464
1,982			−243.7		−695.2	
1,818	−38.11		−584.2			2,899
2,100		−474.7	159.9	1,257		1,392
1,940	−13.79		136.7		380.1	
1,722			738.7		−675.8	−2,548
1,445			−88.64	97.89		
1,680					907.8	
Intercept	−6.79	7.778	6.206	19.30	18.23	62.88
R^2	0.821	.980	.582	0.963	0.914	0.943
F regression	82.77**[a]	1,109.0**	18.45**	681.1**	167.8**	358.4**

[a]** Significant at the 0.01 probability level.

TABLE II
Data from a Least Squares Regression Analysis of Reference Laboratory Methods and Near-Infrared (NIR) Analysis of 55 Calibration Samples of Distillers' Dried Grain

	Crude Protein	Crude Fiber	Acid Detergent Fiber	Neutral Detergent Fiber
NIR $\bar{x}$	24.3	18.0	26.7	54.2
NIR range	13.2–37.50	12.6–25.8	21.9–31.8	42.0–67.5
Lab $\bar{x}$	24.2	17.9	26.7	54.1
Lab range	14.0–36.1	12.1–26.7	20.7–33.9	38.5–71.1
Correlation coefficient	0.966**[a]	0.964**	0.911**	0.945**
β (slope of reg line)	0.984	1.10	1.09	1.08
SE β	0.036	0.041	0.069	0.052
SED	1.10	0.925	1.30	2.51
F reg	735.7**	704.0**	248.2**	438.9**

[a]** Significant at the 0.01 probability level.

Table III. The correlation coefficient of 0.890 (0.01 level) was highly significant with a $\beta = 1.20$ (SE $\beta = 0.212$). This regression line is greater than 1.00 but shows a positive significant relationship between the NIR analysis and lab method. The standard error of differences was 1.44, along with a highly significant F for regression of 31.9 (0.01 level).

ADF

After the NIR instrument had been calibrated, the set of 55 samples was read for ADF using that calibration. The simple correlation between lab and NIR data was 0.911 (0.01 level), with a $\beta = 1.09$ (SE $\beta = 0.69$), and a standard error of difference between the two methods of 1.28% (Table II). The F for regression was 248.2 (0.01 level) when these data were analyzed by a least squares procedure. The mean ADF was 26.7% for both lab and NIR. The range for NIR was 21.9–31.8% and 20.7–33.9% for the lab method.

A standard error of 0.944% was calculated from duplicate lab values for ADF. This compares to 1.28% as the standard error of differences between lab and NIR.

A verification set of nine samples was available (Table III) for ADF. NIR analysis of these samples produced a mean of 26.2% compared to the lab mean of 27.9%. The simple correlation was 0.67 (0.05 level) between the two methods with $\beta = 0.50$ (SE $\beta = 0.213$) and a standard error of the difference between the two methods of 2.05%. F for regression was 5.42 (0.05) when the data were analyzed by a least squares technique.

With only nine samples available for this calibration verification, the variability was larger than expected. This suggests that the calibration needs further modification or else some lab values were inaccurate. A larger number of verification samples may also have helped to reduce the magnitude of the standard error of difference. The wavelengths selected for this calibration along with the regression coefficients are given in Table I.

NDF

Five wavelengths and their regression coefficients for assaying NDF are given in Table I. The R value for the multiple regression relationship among wavelengths and the log values was 0.943. The

TABLE III
Results of Analysis of Verification Distillers' Dried Grain Samples by Lab and Near-Infrared (NIR) Analysis

	Protein		Crude Fiber		Neutral Detergent Fiber		Acid Detergent Fiber	
Sample ID	Kjeldahl (%)	NIR (%)	Lab (%)	NIR (%)	Lab (%)	NIR (%)	Lab (%)	NIR (%)
1109-5	25.0	27.9	19.2	17.4	65.4	66.3	28.6	24.2
1123-2	28.7	27.8	15.6	14.0	69.7	62.6	29.8	26.5
0105-1	30.4	30.5	15.9	14.8	70.0	61.6	28.7	27.6
0315-1	26.3	25.8	16.3	17.4	68.8	68.2	27.6	27.6
0825-1	26.8	27.9	15.7	16.5	...	...	...	...
1015-1	28.3	29.1	14.6	14.6	...	...	22.2	26.2
1101-1	28.0	27.7	15.5	16.4	66.1	60.3	26.5	25.0
0713-1	25.1	25.6	20.5	19.8	57.5	65.6	27.4	29.5
0810-1	25.0	26.0	20.5	20.2	64.2	65.0	27.6	30.1
1109-1	13.5	14.0	24.6	23.6	78.9	84.1	32.4	35.2
1118-1	17.9	16.2						
0301-3	19.1	20.3						
0315-11	18.5	21.1						
0609-1	22.9	24.7						
$\bar{x}$	24.0	24.6	17.8	17.1	66.5	67.6	27.9	26.2
Range	13.5–30.4	14.0–30.5	14.6–24.6	14.0–23.6	57.5–78.9	60.3–84.1	22.2–32.4	24.2–35.2
Correlation coefficient (lab vs. NIR)	0.968*[a]		0.89**		0.627		0.66*	
β	0.831		1.20		0.55		0.50	
SE (β)	0.108		0.212		0.281		0.213	
SE (dif)	1.41		1.44		4.75		2.05	
F reg	59.0**		31.9**		3.89		5.42*	

[a] *, ** Significant at the 0.05 and 0.01 probability levels, respectively.

TABLE IV
The Regression Coefficients that Correspond to Each Filter Wavelength for the Estimation of 16 Amino Acids in Barley Distillers' Dried Grains

		Filter Wavelength (nm)													
Amino Acid	Intercept	2,336	2,310	2,270	2,230	2,139	2,180	1,982	2,100	1,759	1,940	1,722	1,445	1,680	R^2	F Reg
Alanine	0.350						31.21	−9.47	−24.60				4.17		0.953	528.3**[a]
Arginine	0.075		12.85				32.22	−11.82	−40.33				8.59		0.963	545.0**
Aspartic acid	0.805						28.39		−26.82						0.931	717.1**
Glutamic acid	−1.671						270.00		−200.10		−41.55	−82.47	66.21		0.985	8.0**
Glycine	0.374		24.14	−45.56			43.19	−10.58	−11.52						0.916	186.1**
Histidine	0.591						12.78		−13.56				1.02		0.940	550.9**
Isoleucine	0.773						26.43	−1.01	25.02						0.977	1,477.0**
Leucine	1.121				−40.20		84.28		−42.91						0.958	809.1**
Lysine	−0.238	−68.42	56.97		24.91				−7.81				17.57	−22.66	0.971	576.9**
Methionine	0.413				−17.52	62.12			−45.40						0.870	237.2**
Phenylalanine	0.778						50.52	−6.37	−122.00						0.980	1,739.0**
Proline	−1.005				142.6		29.33	−43.34					29.60	−28.66	0.969	526.3**
Serine	0.777						26.69		−25.70						0.936	788.7**
Threonine	0.956			−16.76	−75.0		91.70		−24.23						0.952	695.3**
Tyrosine	0.846			−2.43			27.19								0.972	1,230.0**
Valine	−0.281						45.08		−38.26	75.03	−19.35		17.69	−75.52	0.916	1,887.7**

[a] ** Significant at the 0.01 probability level.

F for regression was 358.4 (0.01 level) as a result of a least squares regression analysis of the log data from the five wavelengths and the NDF lab values for the calibration set. After the regression coefficients (Table I) were entered in the NIR equipment, the 55 calibration samples were read. Data from the lab method and NIR readings were compared as noted above. The statistics from this analysis are in Table II. NDF means were 54.2% and 54.1% for NIR and lab methods, respectively. The correlation between the two methods was 0.945 (0.01 level) with $\beta = 1.08$ (SE $\beta = 0.052$) and the standard error of difference between the methods of 2.51%. F for regression was 438.9 (0.01 level).

Duplicate lab analysis afforded a calculation of a standard error of 2.27% for NDF. The value of 2.51% between methods compares favorably to that lab standard error.

Only eight samples were available for a verification of this calibration. These data are in Table III. Even though the means and ranges of NDF values were similar for the two methods, neither the correlation coefficient nor the F for regression were significant.

Amino Acid

Data from amino acid lab analysis of the 55 calibration samples were submitted to the computer along with log data from each of 19 wavelengths. Regression coefficients selected for each of the 16 amino acids are shown in Table IV. The least squares regression analysis produced R as low as 0.870 for methionine and as high as 0.985 for glutamic acid. F for regression was highly significant for each analysis. The magnitude of these values may be seen in Table IV.

TABLE V
Statistics for the Calibration of Near-Infrared (NIR) Equipment to Estimate Amino Acid Content of Distillers' Dried Grains

	Calibration Samples											
	Lab Range		NIR Range		Least Squares Regression Data				Correlation Coefficients[a]			
	Max	Min	Max	Min	β[b]	SE β[c]	SED[d]	F Reg	K vs. AA[e]	NP vs. NA[e]	NP vs. AA[e]	NA vs. AA[e]
Alanine	1.34	0.62	1.17	0.51	0.604	0.086	0.144	48.80**[f]	0.94	0.797	0.891	0.690
Arginine	1.87	0.84	1.67	0.71	1.04	0.073	0.133	205.5**	0.93	0.961	0.898	0.892
Aspartic acid	2.08	0.95	1.98	1.00	1.06	0.080	0.152	175.6**	0.94	0.979	0.929	0.876
Glutamic Acid	11.10	3.37	10.70	3.80	1.07	0.064	0.916	279.5**	0.98	0.979	0.803	0.917
Glycine	1.18	0.58	1.03	0.54	1.02	0.105	0.087	94.19**	0.85	0.867	0.900	0.800
Histidine	0.81	0.34	0.79	0.39	0.604	0.086	0.144	48.78**	0.94	0.970	0.931	0.883
Isoleucine	1.52	0.55	1.48	0.60	0.829	0.088	0.178	88.10**	0.98	0.852	0.914	0.915
Leucine	3.34	1.22	3.21	1.33	1.06	0.070	0.267	230.4**	0.96	0.980	0.915	0.902
Lysine	1.26	0.52	1.25	0.59	1.11	0.063	0.081	303.7**	0.94	0.941	0.915	0.923
Methionine	0.66	0.22	0.66	0.30	1.08	0.087	0.062	151.7**	0.91	0.880	0.889	0.861
Phenylalanine	2.44	0.79	2.29	0.72	1.09	0.060	0.193	330.3**	0.97	0.993	0.930	0.928
Proline	5.00	1.38	4.59	1.64	1.00	0.086	0.538	136.2**	0.96	0.976	0.906	0.849
Serine	1.83	0.72	1.68	0.77	1.09	0.075	0.136	207.3**	0.94	0.997	0.889	0.892
Threonine	1.41	0.59	1.38	0.69	1.08	0.075	0.099	207.5**	0.95	0.966	0.904	0.893
Tyrosine	1.47	0.50	1.42	0.56	1.10	0.064	0.011	298.1**	0.97	0.995	0.926	0.921
Valine	2.07	0.78	1.61	0.41	1.00	0.071	0.161	197.0**	0.89	0.432	0.855	0.888

[a] r Values >0.35 significant at 0.01 probability level.
[b] β = Slope of the regression line.
[c] SE β = standard error of β.
[d] SED = standard error of differences between NIR estimates and AAA laboratory values.
[e] Kjeldahl protein, K; AAA lab amino acids, AA; NIR protein, NP; NIR amino acids, NA.
[f] ** Significant at the 0.01 probability level.

TABLE VI
Statistics for the Verification Sample Set to Estimate Amino Acid Content of Distillers' Dried Grains by Use of Near-Infrared (NIR) Analysis

	Verification Samples								
	Lab Range		NIR Range		Least Squares Regression Data				
	Max	Min	Max	Min	R[a]	β[b]	SE β[c]	SED[d]	F reg
Alanine	1.29	0.78	1.29	0.74	0.946**[e]	0.905	0.110	0.49	68.4**[e]
Arginine	1.81	0.85	1.79	0.99	0.866**	1.02	0.208	0.133	24.0**
Aspartic Acid	1.98	1.02	1.88	1.25	0.840**	1.48	0.339	0.155	19.1**
Glutamic Acid	8.87	4.40	8.85	4.12	0.712*	0.727	0.254	0.859	8.2*
Glycine	1.29	0.54	1.14	0.60	0.894**	1.07	0.191	0.094	31.7**
Histidine	0.82	0.32	0.76	0.43	0.767**	1.19	0.352	0.090	11.4**
Isoleucine	1.32	0.77	1.32	0.74	0.881**	0.947	0.180	0.074	27.8**
Leucine	2.60	1.52	2.57	1.53	0.869**	1.11	0.223	0.171	24.7**
Lysine	0.89	0.57	1.03	0.44	0.343	0.211	0.204	0.109	1.07
Methionine	0.63	0.29	0.52	0.37	0.826**	1.81	0.436	0.053	17.2**
Phenylalanine	1.68	1.06	2.05	0.96	0.687*	0.351	0.131	0.126	7.15*
Proline	3.42	1.49	3.83	2.14	.210	0.196	0.322	0.503	0.37
Serine	1.84	0.80	1.60	0.95	0.836**	1.54	0.356	0.163	18.6**
Threonine	1.12	0.67	1.10	0.68	0.819**	0.882	0.218	0.075	16.3**
Tyrosine	1.16	0.56	1.23	0.58	0.691*	0.704	0.260	0.120	7.32*
Valine	1.61	0.99	1.97	0.83	0.450	0.365	0.256	0.183	2.03

[a] R = correlation coefficient.
[b] β = Slope of the regression line.
[c] SE β = standard error of β.
[d] SED = standard error of difference between NIR estimates and AAA lab values.
[e] *, *** Significant at the 0.05 and 0.01 probability levels, respectively.

All calibrations except for lysine and methionine have wavelength 2,180 in common, and all except for threonine have 2,100 in common. The lysine calibration uses wavelengths 2,310, 2,336, 2,230, 2,100, 1,445, and 1,680 but does not include a primary protein wavelength.

Analysis of the calibration samples by NIR produced the data shown in Table V along with lab values for 16 amino acids. Data from the two methods were compared by the same statistical procedure as noted above. In addition, simple correlations were determined between Kjeldahl protein content and each AAA laboratory amino acid value (AA), NIR protein (NP) content and AA, NP and NIR amino acid data (NA), and between NA and AA.

For the calibration samples, simple correlations between NA and AA ranged from 0.69 (0.01 level), for alanine to 0.928 (0.01 level), for phenylalanine. All of these correlations were highly significant statistically. The slope of the regression line for each amino acid was not different from 1.0, showing a good prediction of each amino acid by use of the NIR calibration selected. The significant F for regression for each amino acid bears this out.

When data for verification samples (Table VI) were compared as for the calibration samples, the correlations between methods for amino acids were all significant or highly significant except for lysine, proline, and valine. The slope of the regression line was significantly different from zero for all amino acid data except for lysine, proline, and valine. F for regression shows significance for all except these three amino acids.

DISCUSSION

NIR estimates of protein content in 55 fabricated calibration DDG samples compared favorably with Kjeldahl samples. The standard error of duplicate Kjeldahl analyses on these samples was 0.924%. This compares with the standard error of differences (SED) between Kjeldahl and NIR of 1.10%. The SED of 1.41% on the verification set is higher but acceptable. The correlation coefficient = 0.968, b = 0.831, and the F (reg) = 59.0 (0.01 level), all determined on the verification samples, support the acceptability of this calibration for NIR estimates of protein in DDG. The wavelengths used for NIR protein estimates correspond to those used for urea (2208), cellulose (2100), and protein (2190).

Crude fiber estimates in DDG samples by NIR are acceptable in view of the statistics in Tables II and III. The correlations between lab and NIR of 0.964 (calibration) and 0.890 (verification) show the close relationship between the methods. The SED between methods is higher in the verification set (1.44) than in the calibration set (0.925) but is still acceptable. These compare with a standard error for the lab method of 0.812% on duplicate analyses.

NDF values and statistical comparisons between lab and NIR methods were acceptable for the calibration samples. With only eight samples in the verification set, the correlation between methods along with the regression analysis data suggest that NIR predictions are less than adequate. Additional lab values were not available to use to improve the statistical analysis of this comparison.

ADF values of DDG from NIR show good agreement with lab analyses. The SED for the calibration set (1.28) and verification set (2.05) are similar. The standard error for duplicate lab analyses was 0.944. The NIR may be entirely acceptable in view of the time required for lab analysis.

Amino acid analyses by the traditional lab procedure are costly in money and time. The NIR data show that DDG samples in the calibration set gave reasonable values for each of the 16 amino acids in Table IV. The verification set showed reasonable values for all except lysine, proline, and valine. The SED and correlation data in Table VI suggest that NIR could be used for estimates of the 13 other amino acids listed.

Correlations between protein content of DDG and amino acid content of DDG were all positive and highly significant. These relationships are as expected. The correlations between the NIR-predicted amino acid values and the lab values show the degree of predictability of DDG amino acids by NIR, both in the calibration samples and in the verification set. This is, of course, exclusive of the three amino acids noted above. We have no explanation for NIR-predicted values for them among the samples in the verification set.

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