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Near-Infrared Reflectance Spectroscopy (NIRS) Enables the Fast and Accurate Prediction of Essential Amino Acid Contents. 2. Results for Wheat, Barley, Corn, Triticale, Wheat Bran/Middlings, Rice Bran, and Sorghum

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Further NIRS calibrations were developed for the accurate and fast prediction of the total contents of methionine, cystine, lysine, threonine, tryptophan, and other essential amino acids, protein, and moisture in the most important cereals and brans or middlings for animal feed production. More than 1100 samples of global origin collected over five years were analyzed for amino acids following the Official Methods of the United States and European Union. Detailed data and graphics are given to characterize the obtained calibration equations. NIRS was validated with 98 independent samples for wheat and 78 samples for corn and compared to amino acid predictions using linear crude protein regression equations. With a few exceptions, validation showed that 70−98% of the amino acid variance in the samples could be explained using NIRS. Especially for lysine and methionine, the most limiting amino acids for farm animals, NIRS can predict contents in cereals much better than crude protein regressions. Through low cost and high speed of analysis NIRS enables the amino acid analysis of many samples in order to improve the accuracy of feed formulation and obtain better quality and lower production costs.

KEYWORDS: NIRS calibration; NIRS validation; amino acids; crude protein; crude protein regression; equation, feed ingredient evaluation; methionine; cystine; lysine; threonine; tryptophan

INTRODUCTION

An accurate adjustment of the amino acid contents in compound feed is crucial, because a lack of methionine, lysine, threonine, and other essential amino acids can limit the growth of farm animals. Wheat, corn, barley, and other cereals contribute not only energy in the feed but also up to 70% of the total protein in the ration, especially in swine nutrition. Thus, it is important to use accurate figures for amino acid contents in cereals and not to check only protein carriers such as soybean meal.

Wet chemical amino acid analysis is quite complicated and labor intensive and needs a minimum of 3 days of processing time. NIRS combined with chemometric calibration algorithms has been used for more than 30 years in feed analysis, mainly to determine moisture, crude protein, and other crude nutrients. In 1978 Rubenthaler and Bruinsma (*1*) first developed successfully a NIRS calibration for the lysine content in wheat and barley. In the following years, some further publications about NIRS prediction of amino acids in feedstuffs followed. The results of Szalánczy and Fülopp (*2*), Letellier and Cuq (*3*), Van Kempen and Bodin (*4*), Jaikaran et al. (*5*), Gill et al. (*6*), William et al. (*7*), Szalánczy (*8*), Workman (*9*), Dyer and Feng (*10*), and Rhône-Poulenc (*11*), dealing with amino acid calibrations for the feedstuffs reported herein, will be compared with our data later.

Already in 1997 Dyer and Feng (*12*) assessed that besides proximate analysis NIRS can also predict energy contents and amino acids accurately and that this technique will improve feed formulation and quality management in the feed industry tremendously. During the past years, in fact, NIRS has become a major tool for feedstuff evaluation, including amino acids.

For many years, our laboratory has been doing a worldwide analytical service for the feed industry. Thus, >1100 cereal samples have been analyzed chromatographically for their amino acid composition during the past few years. This is an optimum basis to develop robust NIRS calibrations for amino acids. Recently, we (*13*) published the results of the NIRS amino acid calibrations of protein-rich feedstuffs developed by the Degussa laboratory. In this second paper we will continue and describe our progress in the development of amino acid calibrations for cereals including brans or middlings. We will illustrate that NIRS calibrations of good accuracy can be obtained for cereal products. Amino acid predictions based on linear regression to the crude protein, as described in the amino acid composition tables of Degussa (*16*), will also be compared to NIRS

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predictions. Finally, two validations with 98 independent wheat samples and 78 independent corn samples will illustrate the agreement between NIRS predictions and the results of reference analysis.

MATERIALS AND METHODS

Samples were ground, using a 0.5 mm sieve, analyzed by wet chemical methods, and scanned by NIRS. The ground samples were filled in airtight 50 mL polyethylene bottles and stored in a freezer to enable the repetition of chemical analysis and NIRS measurement for subsequent NIRS calibration work. All samples were of feedgrade quality.

Wheat (*Triticum aestivum durum* and other varieties), barley (*Hordeum vulgare*), corn (*Zea mays*), and triticale (*Triticale hexaploide* and other varieties) were usually received as total grains. Wheat brans and middlings or pollards, the byproducts from wheat flour production, were compared on the basis of their NIR spectra and amino acid composition and showed strong overlapping. Thus, we formed a common calibration population. Rice bran or similar products such as polishings, including fat-extracted (deoiled) samples, were also combined to one calibration population. Sorghum or milo (*Sorghum caffrorum* or *bicolor* and other varieties) is a variable cereal. It was differentiated from the similar millet grains through its methionine (Met) content relative to crude protein (CP). Only samples up to 2.1% Met/CP were used for the calibration; millet has >2.8% Met/CP and is seldom used as feedstuff.

Chemical and Chromatographic Methods. The nitrogen content of the samples was determined by the Dumas method according to Official Method 990.03 of the AOAC International (*14*). Crude protein was obtained using the conversion factor of 6.25. Dry matter (moisture) was determined by drying samples in a ventilated oven for 4 h at 103 °C weighing them back. All amino acids except tryptophan were analyzed with a procedure that meets the requirements of the official European method of amino acid analysis in feed (*16*) and of Official Method 994.12 of the AOAC International (*14*). A sample amount containing ~10 mg of nitrogen was weighed, and 5 mL of performic acid was added to oxidize methionine to methionine sulfone and cystine to cysteic acid during 16 h in an ice bath. According to the above-mentioned official methods this step does not interfere with the determination of the other amino acids, which were used for our NIRS calibrations. After the performic acid had been destroyed with sodium metabisulfite, 25 mL of 6 mol/L hydrochloric acid was added, and the protein was hydrolyzed for 24 h at 110 °C in a closed 50 mL glass bottle with a screw cap. Then norleucine was added as internal standard, and the hydrolysate was diluted with buffer and adjusted to a pH of 2.20. The amino acids were separated on a cation exchanger resin and were postcolumn reacted with ninhydrin following a photometric detection at 570 nm. For the detailed wet chemical procedure see Degussa AG (*16*, *17a*) and Llames and Fontaine (*18*).

The tryptophan content of the samples was analyzed after alkaline hydrolysis with barium hydroxide in an autoclave. The hydrolysate was adjusted to a pH of 3.0, filtered, diluted with 30% methanol, and injected into a C-18 reversed phase HPLC column for separation. A very specific fluorescence detection was applied using an excitation wavelength of 280 nm and an emission wavelength of 356 nm. The procedure conforms to the official European method for tryptophan (*19*), the development and performance of which were reported by Fontaine et al. (*20*). Details of the analytical procedure were also published by Degussa AG (*16*, *17b*).

Because of laboratory capacity reasons single analysis of amino acids was performed, but *t* outliers of the NIRS calibration statistics were checked with a second analysis (see below).

NIR Spectroscopy: Instrumentation. A NIRSystems Composite Monochromator 5000 with spinning sample module and reflectance detector with autogain function was employed. WinISI II routine and calibration software for PC (Foss NIRSystems Inc., Silver Spring, MD) were used.

NIRS: Sample Measurement. All samples that were used for chemical analysis were also scanned by NIRS. Two ring cups were filled with the finely ground material (<0.5 mm sieve) and scanned between 1100 and 2500 nm in 2 nm steps. The reflectance at each wavelength was expressed as $\log(1/R)$ using a ceramic plate as reference [see Shenk and Westerhaus (*21*)]. The root-mean-square error test (RMS) of the WinISI software was used to check for eventual spectral differences caused by errors in sample cup filling or sample inhomogeneity. RMS is a very similar calculation to the standard error of differences, but calculated for each wavelength pair over all wavelengths of the compared spectra.

NIRS: Calibration Development. A minimum of 50 samples were collected before a first version of the calibration equation was developed. Updates were prepared regularly with approximately one year intervals. A table containing the laboratory codes and the results of reference analyses for dry matter (DM), crude protein (CP), methionine (Met), cystine (Cys), the sum of methionine and cystine (Met +Cys), lysine (Lys), threonine (Thr), tryptophan (Trp), arginine (Arg), isoleucine (Ile), leucine (Leu), and valine (Val) was prepared and imported to WinISI to establish the calibration CAL file. Other (nonessential) amino acid contents were not calibrated, although available, because they are not commonly used for the feed formulation. All reference data in the calibration set and all spectra were compared and checked carefully to detect and eliminate untypical samples before the calibration, which were wrongly classified in our data system because of poor information from the customer having sent the respective feedstuff sample. Criterion was the typical amino acid profile of the feedstuff [see Degussa (*16*)] and the general spectral shape, which differs from feedstuff to feedstuff visually.

Different calibration algorithms on spectra or derivatives were tried [see Shenk and Westerhaus (*21*), Martens and Naes (*22*), and Kramer (*23*)]. The following procedure gave the best results: Spectra were first treated with the WinISI scatter correction program "SNV and detrend" as recommended for samples with <15% moisture to reduce differences in the spectra poulation that are caused by particle size effects only and not by changes in the constituents. The spectra were then smoothed over four data points (8 nm), and the first and second derivatives of the calibration spectra were calculated using a gap of four data points. The modified partial least-squares regression (MPLS) algorithm was applied, which transforms the data points in the spectra population to terms not only based on the most important differences in the spectra but also taking the reference data into consideration. A limit of 12 terms was set to avoid MPLS regressions on "spectral noise". In the case of 200−300 samples in the population, up to 16 terms were allowed, although the software usually stopped much below this limit using the cross-validation results as criterion. MPLS was run both with the first- and second-derivatives of the spectra, and the obtained statistical performance data were used to decide for each parameter in each calibration separately which derivative gave optimal results. Additionally, care was taken that the fractions of explained variance of cross validation, 1-VR, agree well with RSQ of calibration for all variables and that the standard errors SECV and SEC are also similar and low. Normally, the data of cross-validation are somewhat worse than those of the calibration statistics, but a very big gap is a hint for "overfitting calibrations".

The results of the calibration calculation were checked by observing the *t* ouliers with $t > 2.5$. In the case of *t* outliers, the samples were taken from the freezer and analyzed again by chemical analysis. The new analytical results were used in the following way: If the deviation to the first analytical result was reasonable compared to the precision of the related reference method, that is, a relative difference <1% for DM, <3% for CP, <10% for methionine or cystine, and <6% for all other amino acids, the average result was used for the second calibration run. If the deviation was higher and the second laboratory value was closer to the NIRS prediction, the first laboratory result was removed as an outlier. Except for tryptophan, which could not be analyzed in all samples, the contents of amino acids, crude protein, and dry matter in all samples were used to obtain the calibration equation.

In the case of calibration updates, care was taken to add spectral deviating samples such as global *H* outliers or especially samples containing low or high crude protein levels compared to the present calibration population. The basis for optimal correlation was found to be a broad distribution of, for example, the crude protein contents in the calibration population after the partial removal of too many similar, very common sample qualities laying in its center. In addition to the

Table 1. NIRS Calibration Statistics of Wheat (Number of Samples, $n = 213$; Trp, $n = 124$): Linear Regression of Amino Acid Contents Relative to Crude Protein for the Same Sample Population

	content (%) of variables in the sample population[a]				NIRS performance data				linear regression of amino acids to CP		
					calibration		cross-validation				
variable	mean	CV	min	max	SEC	RSQ	SECV	1-VR	intercept	slope	RSQ_{CP}
dry matter	89.1	1.76	83.7	93.3	0.293	0.97	0.318	0.96			
crude protein	12.5	14.2	7.88	17.6	0.209	0.99	0.218	0.99			
methionine	0.19	14.0	0.13	0.28	0.008	0.91	0.009	0.90	0.014	0.0141	0.88
cystine	0.28	13.3	0.18	0.40	0.011	0.92	0.012	0.91	0.038	0.0194	0.86
Met + Cys	0.47	13.4	0.30	0.68	0.017	0.93	0.018	0.92	0.052	0.0335	0.89
lysine	0.34	10.6	0.23	0.45	0.011	0.91	0.015	0.84	0.135	0.0166	0.65
threonine	0.36	13.0	0.23	0.49	0.009	0.97	0.010	0.96	0.043	0.0249	0.92
tryptophan	0.15	12.0	0.10	0.20	0.007	0.84	0.008	0.81	0.053	0.0077	0.77
arginine	0.60	13.2	0.38	0.81	0.025	0.90	0.027	0.89	0.089	0.0408	0.84
isoleucine	0.42	14.9	0.25	0.60	0.010	0.97	0.012	0.96	–0.012	0.0341	0.96
leucine	0.82	14.1	0.52	1.14	0.017	0.98	0.018	0.98	0.014	0.0644	0.98
valine	0.53	13.9	0.33	0.74	0.018	0.94	0.019	0.94	0.029	0.0397	0.93

[a] The given contents of variables in the calibration population are the wet chemical analyses. CV is the respective coefficient of variation (relative standard deviation) of individual dry matter, crude protein, or amino acid contents in the samples.

use of reference data for this selection, also the three-dimensional score plots of the principal component analysis (PCA) and the WinISI algorithms CENTER and SELECT based on that were used to detect samples with very similar spectra in the calibration population and to remove a part of them, observing the effect on calibration results.

RESULTS AND DISCUSSION

Statistics for NIRS Calibration. Tables 1−7 summarize the performance parameters obtained for the calibration equations. Additionally, a linear regression between amino acid contents and crude protein for the same sample populations was calculated (slope, intercept, and RSQ_{CP}).

The calibration statistics for wheat, obtained from the reference results of 213 samples, of which 124 were also analyzed for tryptophan contents, are shown in **Table 1**. With the exceptions of lysine and tryptophan, the RSQ of calibration and 1-VR of cross-validation are between 0.89 and 0.98 for all amino acids. For crude protein, a level of 0.99 was reached. The amino acid composition of the protein is very stable for wheat as a plant material, because the CVs of the protein and of all amino acid contents are similar, between 13.0 and 14.9%, and high crude protein contents correlate with high amino acid levels. Lower CVs were observed for lysine (10.6%) and tryptophan (12.0%), which may explain the somewhat lower correlation achieved. As a consequence, the RSQ_{CP} of the linear regression of amino acids to crude protein is mostly high for the sample population and equal to or slightly below the results of the NIRS calibration (RSQ). Indeed, for lysine NIRS explains much more of the variance (0.91) than the CP regression with a poor correlation of 0.65. As mentioned above, Rubenthaler and Bruinsma (*1*) were the first to report, in 1978, about a NIR amino acid prediction. They calibrated the ratio Lys/crude protein for several small wheat populations and obtained coefficients of correlation, *r*, between 0.85 and 0.98, which is equivalent to RSQ values of 0.72−0.96. Also, validation results with an RSQ of 0.86 are reported. They concluded that NIR predicts amino acids independent of crude protein. Szalánczy and Fülopp (*2*) also reported a successful NIR calibration for methionine and lysine in wheat and achieved with 40 samples an SEC of 0.0078 and an RSQ of 0.88 for methionine and an SEC of 0.016 and an RSQ of 0.84 for lysine, the first similar to and the latter somewhat worse than our data. Letellier and Cuq (*3*) published in 1991 a NIR estimation of in vitro available lysine in wheat flour. Using nine samples treated with different heating times at 140 °C in an autoclave to introduce Maillard reactions at the ϵ-amino group, they achieved good correlation of two different in vitro methods to NIR absorbances having tried different filter combinations. Van Kempen and Bodin (*4*) compared in 1998 the NIRS estimation of digestible lysine, methionine, and threonine, based on a calibration with 23 wheat samples with the crude protein regression equation. They achieved for lysine RSQs of 0.55 for NIRS and 0.41 for CP regression; for methionine no important difference (0.84−0.77) was found, and for threonine even better prediction by CP regression (0.69−0.85) was achieved. They concluded that both methods are equivalent for wheat. Our data, based on a much higher sample population and the more accurate laboratory values of total amino acid contents, show always a slightly better NIRS performance, and for lysine indeed a much better estimation, than by the CP regression. Jaikaran et al. (*5*) developed a wheat amino acid calibration by selecting 100 representative samples out of a population of 736 Canadian samples, said to represent the full range of chemical characteristics. They calibrated whole grain wheat as well as ground samples and allowed the elimination of *t* outliers (see above). For whole grain/ground samples the following SECV data were obtained: crude protein (0.428/0.283), methionine (0.014/0.012), lysine (0.027/0.024), threonine (0.026/0.024), and tryptophan (0.012/0.013). These observed standard errors are by far higher than in our calibration, probably caused by the less precise precolumn derivatization technique used for the determination of amino acids. Nevertheless, it is interesting that the prediction of amino acids in whole grain wheat was nearly as accurate as in ground and homogenized samples.

The calibration statistics for barley, obtained from the reference results of 185 samples, are shown in **Table 2**. With the exception of cystine, the RSQ of calibration and 1-VR of cross-validation are between 0.84 and 0.97 for all amino acids. For crude protein, an excellent level of 0.97 was reached. Also for barley the amino acid composition of the protein is quite stable, because the CVs of the protein and of all amino acid contents are between 9.8 and 13.3% and high crude protein contents correlate with high amino acid levels. As a consequence, also the RSQ_{CP} of the linear regression of amino acids to crude protein is mostly good for this sample population, but slightly below the results of the NIRS calibration (RSQ) and clearly lower for methionine, cystine, Met + Cys, and lysine, the most important amino acids for feed formulation. Several publications deal with NIR amino acid analysis in barley. First Rubenthaler and Bruinsma (*1*) achieved good correlation to the laboratory assay for the ratio lysine/crude protein and obtained

Table 2. NIRS Calibration Statistics of Barley (Number of Samples, $n = 185$; Trp, $n = 109$): Linear Regression of Amino Acid Contents Relative to Crude Protein for the Same Sample Population

	content (%) of variables in the sample population[a]				NIRS performance data				linear regression of amino acids to CP		
					calibration		cross-validation				
variable	mean	CV	min	max	SEC	RSQ	SECV	1-VR	intercept	slope	RSQ_{CP}
dry matter	89.9	2.34	83.0	94.6	0.296	0.98	0.347	0.97			
crude protein	11.7	12.1	8.35	15.6	0.216	0.98	0.240	0.97			
methionine	0.19	12.1	0.14	0.26	0.009	0.85	0.009	0.84	0.023	0.0140	0.77
cystine	0.25	10.5	0.19	0.34	0.009	0.88	0.012	0.79	0.064	0.0163	0.76
Met + Cys	0.44	10.8	0.34	0.60	0.015	0.91	0.017	0.87	0.087	0.0302	0.82
lysine	0.40	9.8	0.31	0.51	0.012	0.90	0.015	0.86	0.121	0.0241	0.76
threonine	0.39	10.8	0.28	0.51	0.011	0.94	0.012	0.92	0.059	0.0278	0.91
tryptophan	0.14	10.8	0.12	0.19	0.004	0.92	0.006	0.87	0.024	0.0102	0.89
arginine	0.57	11.4	0.42	0.76	0.019	0.92	0.021	0.90	0.077	0.0419	0.86
isoleucine	0.40	13.2	0.28	0.54	0.010	0.97	0.011	0.95	–0.021	0.0355	0.93
leucine	0.79	12.0	0.55	1.04	0.016	0.97	0.019	0.96	0.023	0.0651	0.96
valine	0.56	11.8	0.41	0.73	0.011	0.97	0.013	0.96	0.033	0.0447	0.94

[a] The given contents of variables in the calibration population are the wet chemical analyses. CV is the respective coefficient of variation (relative standard deviation) of individual dry matter, crude protein, or amino acid contents in the samples.

Table 3. NIRS Calibration Statistics of Corn (Number of Samples, $n = 258$; Trp, $n = 156$): Linear Regression of Amino Acid Contents Relative to Crude Protein for the Same Sample Population

	content (%) of variables in the sample population[a]				NIRS performance data				linear regression of amino acids to CP		
					calibration		cross-validation				
variable	mean	CV	min	max	SEC	RSQ	SECV	1-VR	intercept	slope	RSQ_{CP}
dry matter	89.4	1.80	84.8	94.5	0.459	0.92	0.465	0.92			
crude protein	8.77	12.2	6.06	12.8	0.144	0.98	0.154	0.98			
methionine	0.18	13.4	0.13	0.27	0.011	0.78	0.013	0.72	0.035	0.0167	0.54
cystine	0.20	10.9	0.15	0.29	0.008	0.86	0.009	0.81	0.044	0.0174	0.76
Met + Cys	0.38	11.5	0.28	0.54	0.016	0.86	0.018	0.82	0.079	0.0342	0.71
lysine	0.26	10.5	0.19	0.34	0.012	0.82	0.013	0.77	0.100	0.0181	0.52
threonine	0.31	11.3	0.23	0.43	0.007	0.96	0.008	0.95	0.030	0.0319	0.95
tryptophan	0.07	10.5	0.05	0.09	0.003	0.82	0.003	0.78	0.023	0.0049	0.67
arginine	0.41	11.1	0.32	0.54	0.014	0.90	0.016	0.87	0.097	0.0359	0.71
isoleucine	0.29	12.9	0.20	0.42	0.008	0.96	0.009	0.95	–0.005	0.0342	0.93
leucine	1.06	15.8	0.67	1.67	0.034	0.96	0.036	0.95	–0.254	0.1494	0.93
valine	0.41	11.5	0.30	0.54	0.010	0.95	0.012	0.94	0.042	0.0415	0.91

[a] The given contents of variables in the calibration population are the wet chemical analyses. CV is the respective coefficient of variation (relative standard deviation) of individual dry matter, crude protein, or amino acid contents in the samples.

for 21 samples of different varieties an RSQ of 0.92. One year later Gill et al. (*6*) succeeded also with a lysine prediction in barley based on 40 finely ground calibration samples and validated with 69 independent samples. They achieved an excellent RSQ of 0.96 or 0.92, respectively, and prediction errors SEC of 0.030% or SEP of 0.035%, thus a better correlation but 2 times higher errors than our data. Also, calibrations of specific genotypes were established, which did not improve the prediction errors. The calibration of the ratio Lys/CP gave good correlations (RSQ of 0.72 or 0.62), showing again that NIR can predict lysine independent of crude protein. Williams et al. (*7*) used 48 barley samples to calibrate with a NIR filter instrument all amino acids except cystine and tryptophan (not available due to analytical problems) and obtained for each amino acid the best correlations for individual up to four filter wavelengths. They observed that for protein calibrations wavelengths other than those used for most amino acids were used, showing the independence of these calibrations. With 15 independent samples they obtained for methionine, lysine, and threonine RSQ validation results of 0.83, 0.90, and 0.96, respectively. Converting their SEP results from micromoles per gram to grams per 100 g, standard errors of prediction of 0.014, 0.044, and 0.026 were reported. In our laboratory using NIR spectra and the powerful MPLS algorithm, far smaller prediction errors SECV are achieved for barley. Those authors also compared NIR to CP regression to predict amino acids and stated NIR performed much better. Szalánczy (*8*) reported calibration data for methionine and lysine in barley based on ~30 samples. She reported RSQ values of 0.92 and 0.94 and SECs of 0.009 and 0.017, respectively.

The calibration statistics for corn, obtained from the reference results of 258 samples, are shown in **Table 3**. This ingredient, with an average of only 8.77% for crude protein, is problematic in NIRS amino acid calibrations because the spectral response is the lowest of all cereals. As a result, the obtained RSQ and 1-VR values lay somewhat lower, between 0.72 and 0.96 for amino acids, than for other grains, but for crude protein the obtained correlation is 0.98. The standard errors are low enough to enable NIR predictions for amino acids in corn. When calculated relative to the mean amino acid contents of the population, SECV/mean values of 2.5−7.1% are observed. Again, the amino acid composition of the protein is quite stable, the CVs of the protein and of all amino acid contents are between 10.5 and 13.4%, and high crude protein contents correlate with high amino acid levels. For leucine a higher CV of 15.8% is observed in corn. The RSQ_{CP} of the linear regression of amino acids to crude protein is fairly good for this sample population; nevertheless, for methionine, cystine, Met + Cys, lysine, and tryptophan, the NIRS calibrations show clearly higher RSQ than the CP regression data. First, Szalánczy (*8*) published results for the NIR estimation of methionine and lysine in corn, achieving SECs of 0.009 and 0.030 and correlation

Table 4. NIRS Calibration Statistics of Triticale (Number of Samples, $n = 122$; Trp, $n = 104$): Linear Regression of Amino Acid Contents Relative to Crude Protein for the Same Sample Population

	content (%) of variables in the sample population[a]				NIRS performance data				linear regression of amino acids to CP		
					calibration		cross-validation				
variable	mean	CV	min	max	SEC	RSQ	SECV	1-VR	intercept	slope	RSQ_{CP}
dry matter	88.6	1.66	83.9	92.1	0.339	0.95	0.344	0.95			
crude protein	11.5	13.0	8.49	14.5	0.188	0.98	0.235	0.98			
methionine	0.19	12.0	0.14	0.22	0.006	0.92	0.008	0.88	0.024	0.0141	0.89
cystine	0.26	12.2	0.19	0.34	0.009	0.92	0.013	0.83	0.043	0.0192	0.81
Met + Cys	0.45	11.8	0.33	0.56	0.018	0.89	0.019	0.87	0.067	0.0334	0.88
lysine	0.37	11.1	0.28	0.46	0.009	0.95	0.014	0.89	0.090	0.0245	0.79
threonine	0.35	12.2	0.26	0.44	0.009	0.96	0.011	0.94	0.031	0.0277	0.94
tryptophan	0.12	9.80	0.10	0.16	0.004	0.91	0.005	0.81	0.040	0.0071	0.76
arginine	0.57	14.3	0.39	0.73	0.018	0.95	0.024	0.91	–0.013	0.0508	0.87
isoleucine	0.38	14.1	0.27	0.48	0.008	0.98	0.012	0.95	–0.019	0.0348	0.94
leucine	0.74	13.0	0.53	0.93	0.014	0.98	0.016	0.97	0.010	0.0634	0.98
valine	0.51	13.1	0.36	0.65	0.013	0.96	0.015	0.95	0.009	0.0432	0.95

[a] The given contents of variables in the calibration population are the wet chemical analyses. CV is the respective coefficient of variation (relative standard deviation) of individual dry matter, crude protein, or amino acid contents in the samples.

RSQs of 0.95 and 0.79, respectively, based on only 27 samples. Workman (*9*) presented calibration data based on 111 calibration samples, which were selected to avoid spectral similarities from 400 corn samples. Using equipment and a calibration algorithm similar to those used in our laboratory, he achieved RSQ values between 0.62 and 0.89 for 12 amino acids. The SECVs obtained were 0.02−0.14. A validation with 30 independent samples with RSQs from 0.23 to 0.58 gave very low correlations for five amino acids and standard errors SEP of 0.01 (Cys and Trp) or 0.02 (Met, Lys, and Thr). Showing also data for soybean meal, he nevertheless concluded that NIRS has a sound potential as a method for rapid amino acid measurement in major feed ingredients. Dyer and Feng (*10*, *12*) have used NIR amino acid calibrations for screening purposes in the development of genetically altered grains. On the basis of ~150 corn samples they reported the following statistical data: methionine, RSQ = 0.78, SECV = 0.012; cystine, SECV = 0.013; lysine, RSQ = 0.93, SECV = 0.017; threonine, SECV = 0.013. Thus, despite good correlation the standard errors are mostly above those obtained in our laboratory based on very accurate reference analysis. A brochure of the amino acid producer Rhône-Poulenc (*11*) contains calibration data for total and digestible amino acids in corn, which were not published elsewhere. No number of samples or fractions of explained variance are given. The following SECV data are listed: crude protein, 0.33; methionine, 0.01; cystine, 0.02; Met + Cys, 0.03; lysine, 0.02; threonine, 0.02; tryptophan, 0.01; arginine, 0.03; isoleucine, 0.03; leucine, 0.08; and valine, 0.03. Except for methionine these standard errors are about or more than double our results. All of these published results were based on ground corn samples.

The calibration statistics for triticale, obtained from the reference results of 122 samples, are shown in **Table 4**. The obtained fractions of explained variance RSQ and 1-VR lay between 0.81 and 0.97 for amino acids and 0.98 for crude protein. The standard errors SEC and SECV correspond very well to each other and are small. With 11−14% the CV of the amino acid contents in the sample population is similar to that of crude protein, except for tryptophan. It is therefore observed that the values RSQ_{CP} for amino acids obtained by the linear CP regressions are very similar to those of the NIRS calibration, with the exception of lysine and tryptophan, which can be measured more accurately by NIRS. Wheat, triticale, and rye are genetically close to each other. Calibration trials have shown that for these cereals also a combined NIRS equation with good performance data can be obtained. We intend to thoroughly validate its performance relative to the three dedicated calibrations. For triticale and the following feedstuffs no publications of NIR amino acid analysis are available.

The calibration statistics for wheat bran and middlings, obtained from the reference results of 109 samples, are shown in **Table 5**. This side product of wheat flour production is an important feed ingredient in many countries. The protein content is higher than in whole wheat grain, but due to the high fiber content, the content in animal feed has to be limited. In this processed plant product the variation CV of the amino acid contents does not follow the protein variation of only 7.8%. The lysine contents show a doubly large variation, and also methionine, threonine, tryptophan, arginine, and valine do not follow the crude protein contents closely. As a result, the obtained RSQ and 1-VR values for amino acids, 0.90−0.98 and 0.83−0.97, respectively, are very good, but the CP regressions are clearly inferior to NIRS. For tryptophan the CP regression fails totally with an RSQ_{CP} of 0.08. The amino acid prediction in wheat bran requires more information about the sample than just the crude protein content. In the NIR spectra there are absorbance bands related to starch and fiber contents in the samples available, which can be used for correlations to the amino acid contents. The standard errors SEC and SECV agree well and are low, compared to the means of the variables. The relative standard error SECV/mean of amino acid is only 3% for methionine and lysine and 2.1% for threonine. Thus, NIRS is a real advantage for the accurate prediction of amino acid contents in wheat bran and similar products.

The calibration statistics for rice bran, obtained from the reference results of 90 samples, are shown in **Table 6**. This feedstuff, mainly used in Asia, is more variable in the crude protein contents (CV = 11.9%) than the wheat bran products, but the amino acid composition is more stable and follows the CP contents. At 0.93−0.99 and 0.88−0.98, the RSQ and 1-VR values, respectively, obtained for amino acids and crude protein are excellent. The SEC and SECV figures correspond well with each other and are both small compared to the mean of the variable. Here also the CP regression results are good with the exception of methionine and lysine, for which NIRS predictions show a much higher fraction of explained variance. Thus, highly informative and accurate predictions were obtained by NIRS.

The calibration statistics for sorghum or milo, obtained from the reference results of 167 samples, are shown in **Table 7**. This feedstuff has many varieties around the world, and here we observed the highest CV of the crude protein content (16.9%) of all cereal calibrations reported herein. The mean protein

Table 5. NIRS Calibration Statistics of Wheat Bran plus Wheat Middlings (Number of Samples, $n = 109$; Trp, $n = 61$): Linear Regression of Amino Acid Contents Relative to Crude Protein for the Same Sample Population

	content (%) of variables in the sample population[a]				NIRS performance data				linear regression of amino acids to CP		
					calibration		cross-validation				
variable	mean	CV	min	max	SEC	RSQ	SECV	1-VR	intercept	slope	RSQ_{CP}
dry matter	89.9	1.38	87.2	92.8	0.149	0.99	0.196	0.98			
crude protein	16.3	7.82	13.1	19.3	0.217	0.97	0.252	0.96			
methionine	0.24	9.84	0.19	0.30	0.006	0.93	0.007	0.91	–0.018	0.0159	0.73
cystine	0.34	7.97	0.27	0.42	0.009	0.90	0.011	0.83	0.076	0.0161	0.58
Met + Cys	0.58	7.49	0.47	0.69	0.011	0.93	0.013	0.91	0.063	0.0318	0.87
lysine	0.64	15.2	0.44	0.85	0.015	0.98	0.019	0.96	–0.319	0.0587	0.60
threonine	0.51	9.50	0.39	0.64	0.009	0.96	0.011	0.95	–0.057	0.0350	0.84
tryptophan	0.26	12.1	0.20	0.31	0.007	0.95	0.010	0.89	0.145	0.0069	0.08
arginine	1.07	14.5	0.72	1.35	0.020	0.98	0.026	0.96	–0.371	0.0886	0.66
isoleucine	0.50	7.35	0.40	0.60	0.011	0.94	0.012	0.92	0.059	0.0270	0.68
leucine	0.98	7.34	0.77	1.15	0.013	0.97	0.015	0.97	0.129	0.0522	0.72
valine	0.73	9.07	0.59	0.90	0.013	0.96	0.017	0.93	–0.011	0.0457	0.85

[a] The given contents of variables in the calibration population are the wet chemical analyses. CV is the respective coefficient of variation (relative standard deviation) of individual dry matter, crude protein, or amino acid contents in the samples.

Table 6. NIRS Calibration Statistics of Rice Bran (Number of Samples, $n = 90$; Trp, $n = 52$): Linear Regression of Amino Acid Contents Relative to Crude Protein for the Same Sample Population

	content (%) of variables in the sample population[a]				NIRS performance data				linear regression of amino acids to CP		
					calibration		cross-validation				
variable	mean	CV	min	max	SEC	RSQ	SECV	1-VR	intercept	slope	RSQ_{CP}
dry matter	91.5	2.09	87.7	96.1	0.394	0.96	0.460	0.94			
crude protein	14.4	11.9	10.1	18.3	0.203	0.99	0.267	0.98			
methionine	0.28	13.2	0.19	0.36	0.010	0.93	0.012	0.90	0.009	0.0191	0.76
cystine	0.30	10.7	0.22	0.37	0.009	0.93	0.011	0.88	0.054	0.0172	0.82
Met + Cys	0.59	11.5	0.40	0.72	0.016	0.95	0.020	0.91	0.066	0.0361	0.84
lysine	0.64	15.3	0.36	0.87	0.024	0.94	0.031	0.90	–0.042	0.0474	0.68
threonine	0.53	12.7	0.34	0.67	0.011	0.97	0.014	0.95	–0.028	0.0387	0.96
tryptophan	0.19	13.8	0.11	0.22	0.006	0.95	0.008	0.90	0.002	0.0124	0.95
arginine	1.10	12.0	0.68	1.38	0.029	0.95	0.039	0.91	0.063	0.0720	0.87
isoleucine	0.50	12.5	0.34	0.64	0.009	0.98	0.012	0.96	–0.011	0.0356	0.94
leucine	1.00	11.4	0.67	1.24	0.012	0.99	0.018	0.98	0.064	0.0651	0.95
valine	0.77	11.5	0.53	0.94	0.015	0.97	0.018	0.96	0.042	0.0504	0.95

[a] The given contents of variables in the calibration population are the wet chemical analyses. CV is the respective coefficient of variation (relative standard deviation) of individual dry matter, crude protein, or amino acid contents in the samples.

Table 7. NIRS Calibration Statistics of Sorghum (Number of Samples, $n = 167$; Trp, $n = 110$): Linear Regression of Amino Acid Contents Relative to Crude Protein for the Same Sample Population

	content (%) of variables in the sample population[a]				NIRS performance data				linear regression of amino acids to CP		
					calibration		cross-validation				
variable	mean	CV	min	max	SEC	RSQ	SECV	1-VR	intercept	slope	RSQ_{CP}
dry matter	89.0	1.38	85.9	93.7	0.223	0.97	0.257	0.96			
crude protein	9.77	16.9	5.71	14.6	0.174	0.99	0.214	0.98			
methionine	0.17	13.8	0.12	0.23	0.009	0.86	0.009	0.84	0.051	0.0117	0.71
cystine	0.18	12.7	0.13	0.25	0.008	0.87	0.009	0.85	0.062	0.0120	0.76
Met + Cys	0.35	12.6	0.24	0.48	0.014	0.89	0.015	0.88	0.113	0.0237	0.81
lysine	0.22	17.4	0.16	0.40	0.010	0.93	0.012	0.90	0.086	0.0137	0.35
threonine	0.32	14.2	0.20	0.46	0.007	0.98	0.008	0.97	0.058	0.0264	0.94
tryptophan	0.11	15.9	0.08	0.17	0.003	0.98	0.003	0.97	0.007	0.0104	0.93
arginine	0.38	18.1	0.25	0.71	0.017	0.94	0.019	0.93	0.059	0.0327	0.62
isoleucine	0.38	17.9	0.21	0.60	0.010	0.98	0.012	0.97	–0.015	0.0407	0.96
leucine	1.26	19.5	0.67	2.05	0.038	0.98	0.046	0.97	–0.099	0.1395	0.87
valine	0.48	16.4	0.28	0.73	0.013	0.97	0.014	0.97	0.020	0.0472	0.97

[a] The given contents of variables in the calibration population are the wet chemical analyses. CV is the respective coefficient of variation (relative standard deviation) of individual dry matter, crude protein, or amino acid contents in the samples.

content is, at 9.77%, low, only a bit higher than in the corn samples. Indeed, the obtained RSQ and 1-VR values for amino acids, 0.86−0.98 and 0.84−0.97, respectively, are very high. The variation of the amino acid contents is, with a CV of 12.7−19.5%, high for a cereal population, especially for lysine, arginine, isoleucine, and leucine (CV = 17.4−19.5%), which exceeds the total crude protein variation. This situation helps to establish good NIRS calibration equations, but it reduces the accuracy of predictions with CP regression. Here the RSQ_{CP} values of methionine, cystine, leucine, and especially lysine (0.35) and arginine (0.62) are clearly inferior to those obtained by NIRS, but this is not the case for threonine, isoleucine, and valine. The standard errors SEC and SECV are again very small, and when divided through the mean of the amino acid, relative

Table 8. NIRS Validation Statistics for Independent Samples of Wheat (Number of Samples, $n = 98$; Trp, $n = 56$)

	content (%) as analyzed with the reference method			content (%) as analyzed with the NIRS calibration			NIRS performance data of independent validation			
variable	mean	min	max	mean	min	max	SEP	RSQ_{val}	slope	SEP_{rel}
crude protein	12.6	9.00	17.9	12.7	8.88	18.6	0.290	0.98	1.00	2.29
methionine	0.19	0.13	0.28	0.19	0.14	0.27	0.009	0.89	0.91	4.64
cystine	0.28	0.22	0.39	0.28	0.21	0.41	0.015	0.86	0.96	5.36
Met + Cys	0.48	0.35	0.66	0.47	0.34	0.68	0.021	0.90	0.97	4.38
lysine	0.34	0.25	0.44	0.34	0.25	0.45	0.015	0.86	0.87	4.41
threonine	0.36	0.26	0.49	0.36	0.26	0.49	0.011	0.95	0.96	3.06
tryptophan	0.15	0.11	0.20	0.15	0.11	0.20	0.010	0.71	0.81	6.67
arginine	0.60	0.43	0.81	0.60	0.44	0.83	0.033	0.84	0.92	5.50
isoleucine	0.42	0.29	0.60	0.42	0.29	0.61	0.015	0.95	0.99	3.57
leucine	0.83	0.60	1.20	0.83	0.58	1.19	0.025	0.96	0.99	3.01
valine	0.53	0.39	0.73	0.53	0.37	0.76	0.021	0.92	1.00	3.96

SECVs of only 2.5−5.6% are found at this very low content levels. Also, for sorghum highly informative and accurate predictions can be obtained by NIRS.

Validation for Wheat. Ninety-eight independent samples of wheat were selected to check the NIRS calibration equations independently. A tool of the WinISI software, the global *H* value was used to eliminate samples that were not reflected in the calibration population, by using 3.0 for the maximum limit. This is also necessary when using NIRS equations in daily work. The statistics of this validation are summarized in **Table 8**. The mean, minimum, and maximum contents of the variables analyzed with the reference method and with NIRS agreed very well with each other. We conclude that the variation of amino acid contents in the samples is well depicted by the NIRS predictions. The standard error of prediction (SEP) and the fraction of explained variation (RSQ_{val}) were also in good agreement with the related parameters SECV and 1-VR of the cross-validation statistics. It is a normal finding that the standard errors obtained by validation are slightly higher and the RSQ values slightly lower than those parameters of the cross-validation. For methionine and lysine the standard errors SECV and SEP agree, and for Met + Cys and threonine the SEP is only relatively 16.7 and 10% higher than the SECV. In our opinion this shows that the cross-validation statistics give a very realistic estimate of the real performance of the developed NIRS calibrations. Additionally, the slope between laboratory values (*x*-axis) and NIRS predictions (*y*-axis) is given. For the wheat validation it is close to the ideal value of 1 for all variables, with the exceptions of methionine (0.91), lysine (0.87), and tryptophan (0.81). As an indication of the typical relative deviations between laboratory and NIRS, the parameter SEP_{rel} was calculated using the mean of the respective variable. For wheat, these relative deviations SEP_{rel} range only between 3 and 5.5%, with the exception of 6.7% for tryptophan, for which only 56 results were available. Results of collaborative trials (*15*, *18*, *19*) for chromatographic amino acid analysis show that such deviations were also observed for reference results of one sample in different laboratories.

In **Figure 1** the individual data for the wheat validation samples are plotted for methionine, lysine, threonine, and leucine. The scattering of data points around the ideal curve with the slope = 1 (dotted line) is larger for methionine and lysine than for threonine and especially leucine, and this agrees with the accuracy parameters SEP_{rel} in **Table 8**.

As a validation of the CP regression equations, we predicted the amino acids based on the analyzed reference crude protein content and calculated the individual differences from the laboratory value. In **Figure 3**, the mean of these differences, averaging the absolute values, is compared with the respective mean of the differences for the NIRS amino acid prediction. The obtained average accuracy is quite similar. For lysine this mean difference is ~3.5% for NIRS, but at 5% is clearly higher for the CP regression data. This had to be expected taking the RSQ_{CP} figures of **Table 1** into account, where only for lysine did the RSQ data of the prediction techniques differ greatly. If crude protein and amino acids are highly correlated as in wheat, it cannot be expected that NIRS predictions perform much better than the linear CP regression equations.

Validation for Corn. Seventy-eight independent samples of corn were selected to check the NIRS calibration equations independently. As for wheat, only samples with a global *H* value below 3 were used, which are covered by the sample population of the NIRS calibration. The validation statistics are summarized in **Table 9**. The mean contents of the variables analyzed with the reference method and with NIRS agreed well with each other. For corn, having the lowest amino acid values of all cereals, the minimum and maximum values of the validation samples differ a little more, with a tendancy of somewhat higher NIRS results. Generally the variation of amino acid contents in the corn samples is well depicted by the NIRS predictions. The standard errors of prediction SEP were in good agreement with the related parameters SECV, sometimes being below, more often above, the cross-validation result. Relative to the mean of the amino acid in the validation samples the standard error SEP is between 3.8−5.9%. The fraction of explained variation RSQ_{val} is, at 0.55−0.92 for amino acids, always below the 1-VR of the cross-validation statistics. Again, the slope between laboratory values (*x*-axis) and NIRS predictions (*y*-axis) is given. Only for crude protein, threonine, arginine, and leucine is the slope above 0.90. The validation of the other amino acids gave slopes of 0.63−0.8. One of the reasons is that the variation of the amino acid contents in the used corn validation samples was much lower than in the wheat validation population, and this results in poorer statistical performance data.

In **Figure 2** the individual data for the corn validation samples are plotted for methionine, lysine, threonine, and leucine. The scattering of data points around the ideal curve with the slope =1 (dotted line) is again larger for methionine and lysine than for threonine and especially leucine and generally higher than for the wheat samples. The data show that corn can be estimated by NIRS, but because of the low protein and amino acid contents, we are close to the application limits of the NIRS technique.

Also here the CP regression equations were used for the validation samples based on the analyzed reference crude protein content, and we calculated the invidual differences from the laboratory value. In **Figure 4**, the mean of these differences, averaging the absolute values, is compared with the respective

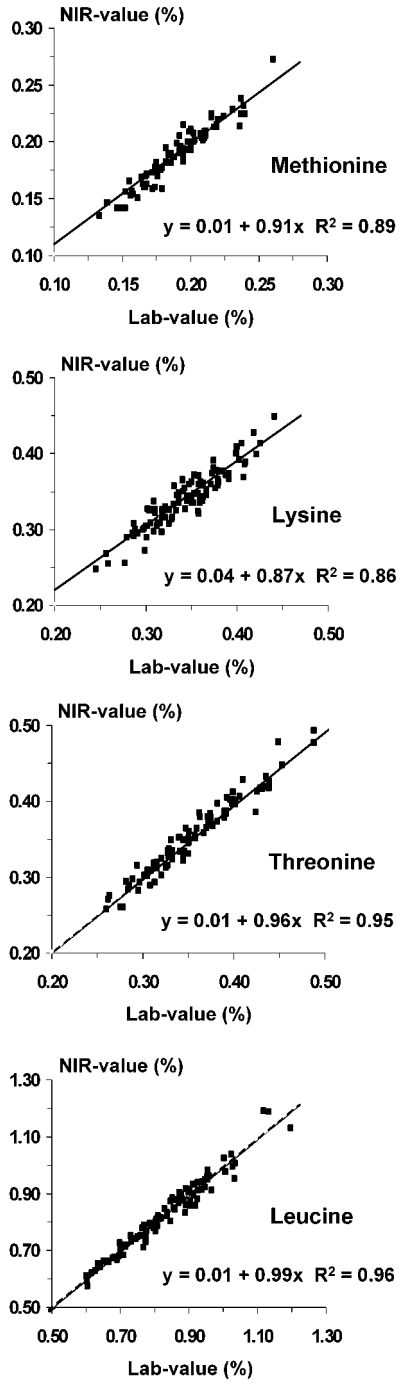

Figure 1. Validation of the NIRS amino acid predictions for wheat: methionine, lysine, threonine, and leucine contents compared to reference analysis (98 samples).

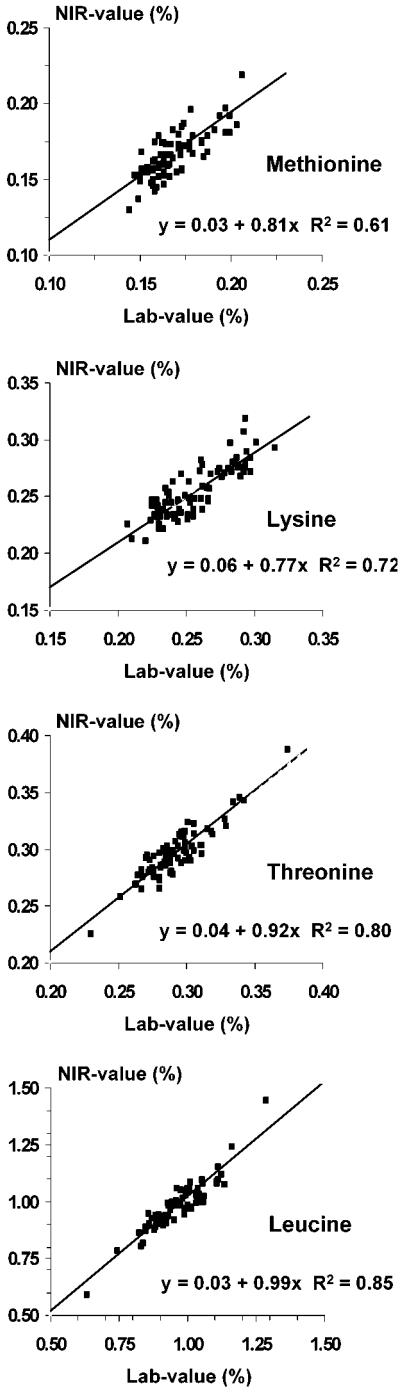

Figure 2. Validation of the NIRS amino acid predictions for corn: methionine, lysine, threonine, and leucine contents compared to reference analysis (78 samples).

mean of the differences for the NIRS amino acid prediction. With mean differences of ~4% relatively, the NIRS predictions prove to be accurate. The obtained average data for both prediction techniques were quite similar for some amino acids, but for methionine, lysine, isoleucine, and leucine NIRS is more accurate than the CP regression data. This had to be expected for methionine and lysine, taking the RSQ_{CP} figures of **Table 3** into account, but is surprising for leucine and isoleucine. In the NIR spectra besides protein absorbance also information about starch, fiber, fat, and other contents in the samples is contained and can be used in the calibration equation; this often results in a better accuracy of the NIRS predition compared to a CP regression equation.

Reproducibility of Reference Analysis. It is a general rule in chemometrics that to obtain excellent NIRS calibrations the standard deviation of the variable in the calibration population should be 20 times the standard error of the laboratory reference method. Especially in cereals, the CVs of the amino acids range only between 10 and 15%; thus, the reference method should have an analytical reproducibility CV of only 0.5−0.8%. Therefore, we have checked the precision for our reference analysis in the following way: A soybean meal and a barley sample were analyzed monthly for crude protein and amino acids over a one year period. The reproducibility of the analysis (CV) for the different variables was calculated on the basis of the 12 results per sample. The results are shown in **Figure 4**. For crude

Table 9. NIRS Validation Statistics for Independent Samples of Corn (Number of Samples, $n = 78$; Trp, $n = 14$)

	content (%) as analyzed with the reference method			content (%) as analyzed with the NIRS calibration			NIRS performance data of independent validation			
variable	mean	min	max	mean	min	max	SEP	RSQ_{val}	slope	SEP_{rel}
crude protein	8.38	6.18	11.0	8.31	6.03	11.2	0.165	0.95	0.92	1.97
methionine	0.17	0.14	0.21	0.17	0.13	0.22	0.010	0.61	0.81	5.94
cystine	0.19	0.17	0.22	0.19	0.16	0.23	0.008	0.55	0.66	4.30
Met + Cys	0.35	0.31	0.43	0.35	0.30	0.45	0.016	0.65	0.80	4.51
lysine	0.26	0.21	0.32	0.25	0.21	0.32	0.013	0.72	0.77	5.08
threonine	0.29	0.23	0.37	0.30	0.23	0.39	0.011	0.80	0.92	3.77
tryptophan	0.064	0.057	0.084	0.065	0.057	0.079	0.003	0.86	0.63	4.68
arginine	0.40	0.32	0.49	0.40	0.34	0.50	0.019	0.72	0.97	4.74
isoleucine	0.27	0.17	0.37	0.27	0.20	0.38	0.013	0.80	0.77	4.82
leucine	0.96	0.63	1.29	0.98	0.59	1.44	0.045	0.85	0.99	4.68
valine	0.39	0.27	0.47	0.38	0.30	0.50	0.018	0.92	0.71	4.65

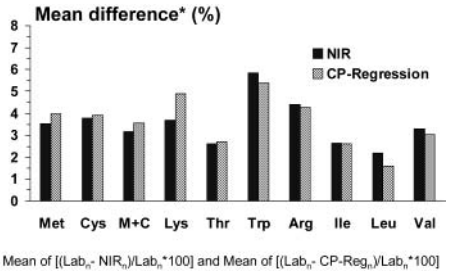

Figure 3. Mean difference of amino acid contents predicted by NIRS and by the linear CP regression equations as compared to the reference analysis for 98 independent samples of wheat.

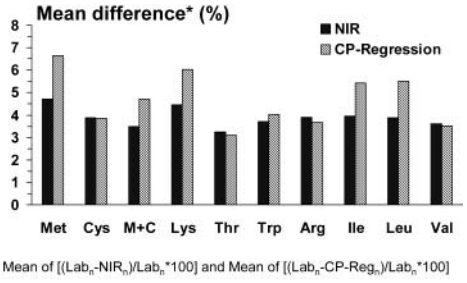

Figure 4. Mean difference of amino acid contents predicted by NIRS and by the linear CP regression equations as compared to the reference analysis for 78 independent samples of corn.

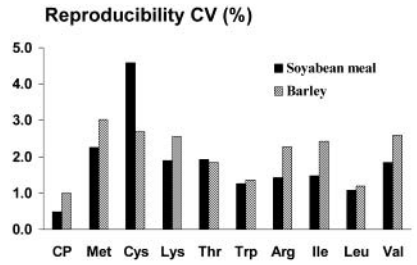

Figure 5. Reproducibility of chromatographic amino acid analysis and crude protein determination in the laboratory. Each sample was analyzed 12 times at 1 month intervals.

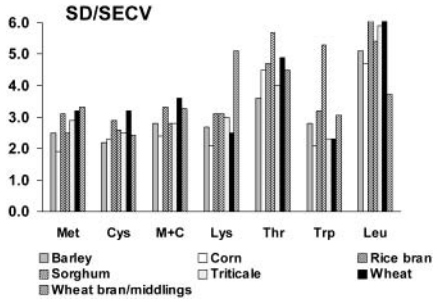

Figure 6. Ratio of the SD of the amino acid contents in the calibration samples to the standard error SECV of NIRS.

protein, the reproducibility (CV) of analysis was between 0.5 and 1% and therefore precise enough for excellent NIRS calibrations, but for most amino acids 2−3% analytical variations were observed (for cystine up to 4.5%). Reasons for this are (a) the sample preparation oxidation/hydrolysis is a complicated procedure and (b) chromatograpic determinations have additional errors, such as the injection onto the column, the postcolumn reaction with ninhydrin, and problems in peak area integration of the chromatograms. The observed reproducibility in our laboratory is excellent for amino acid analysis, and the quality of our sample preparation and chromatography is continuously monitored by participation in international collaborative trials (e.g., AAFCO ring tests). Most of the calibration samples were only analyzed once (see above), and thus the higher analytical errors for amino acids affect the possible accuracy of NIRS calibrations. There is no alternative way for analysis, and therefore the precision of the results could only be improved by repeated analyses. Following the statistical rules the analytical error of each amino acid content could be halved by using the average of four analyses for NIRS calibration. However, due to serious laboratory capacity problems, replicate assays for the large amounts of calibration samples could not be performed.

SD/SECV, a Measure for Meaningfulness of NIRS Predictions. A small number for the standard error SECV alone does not clearly reflect the usefulness of a NIRS calibration for the feedstuff evaluation. The ratio of the standard deviation (SD) of the amino acid in the calibration population to the SECV is a better measure of the information given. If the SD/SECV ratio is high, NIRS predictions enable a given amount of samples to be divided into some subgroups of low, medium, and high contents of the amino acid. Especially for the essential amino acids, this can improve the supplementation rate with crystalline amino acids and yield cost savings. In **Figure 5**, the ratio SD/SECV is shown for all cereal calibrations and the most important amino acids. If this ratio exceeds a value of 3, the calibration equation is very meaningful to predict the amino acid content, whereas in cases of values below 2, the applicability is limited. For methionine in rice bran, wheat, and wheat bran and for lysine in rice bran, sorghum, triticale, and wheat bran the SD/SECV exceeds 3. This is also the case for threonine in all cereal calibrations and for tryptophan in rice bran and sorghum. The values for the sulfur amino acids, lysine, and tryptophan lay for the other cereals between 2 and 3 with the exception of corn, for which mostly SD/SECVs of around 2 were found.

The best results were obtained for leucine, the amino acid with the best reproducibility in the chromatographic assay. The results show that our NIRS calibration equations are mostly able to give very meaningful predictions of the amino acid contents in cereal samples.

Applications. For the past three years, our laboratory has used NIRS as a customer service for amino acid analysis of feedstuff, and currently we have analyzed >10000 samples from all over the world. The advantage for our customers is not only the short processing time but also the huge series of ingredients that can be analyzed and evaluated in summary statistics. This enables a screening of the quality and variation of different sources of feedstuff suppliers for quality improvement and optimum feed formulation. Additionally, we are able to transfer our calibrations to other Foss NIR spectrometers of customers or internal laboratories (hosts), and our amino acid calibrations directly used in the quality laboratories of feed producers give the highest advantage. Four international collaborative studies showed that also transferred equations predict amino acids with good accuracy (unpublished results).

In summary, we conclude that the developed NIRS calibrations enable meaningful, fast, and accurate predictions of essential amino acids in cereals and are recognized as useful by our customers. The calibrations contain high and growing numbers of samples of global origin; consequently, they are very robust and applicable for samples from anywhere. It is especially due to these calibrations and their continuous updating and enlarging that makes our NIRS service unique worldwide.

ABBREVIATIONS USED

NIRS, near-infrared reflectance spectroscopy; SEC, standard error of calibration; RSQ, fraction of explained variance for the calibration samples (square of correlation coefficient *r*); SECV, standard error of cross-validation; 1-VR, fraction of explained variance for cross-validation (square of correlation coefficient *r*); CV, coefficient of variation (relative standard deviation); RSQ_{CP}, fraction of explained variance for linear crude protein regression (square of correlation coefficient *r*); SEP, standard error of prediction for independent validation samples; SEP_{rel}, SEP/(mean of lab values) × 100 (%); slope, slope of regression line between lab values (*x*-axis) and NIRS values (*y*-axis); RSQ_{val}, fraction of explained variance for independat validation samples (square of correlation coefficient *r*); SD, standard deviation of the variable in the sample population.

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