Near infrared spectroscopy forage and feed testing network: a model of cooperation

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Introduction and network development

Commercial forage testing laboratories in the Midwest region have made tremendous changes in testing forage crops within the last twelve years. The adaptation of near infrared (NIR) spectroscopic technology has increased the volume of samples tested by ruminant livestock producers, especially dairymen, and gives a competitive economic advantage to users of this technology. Rapid changes in forage testing must continue to keep producers economically competitive in the future. Cooperative ventures between land grant universities and commercial forage testing laboratories is an approach to meeting the challenges of the future. This paper describes the formation of a consortium of forage testing laboratories to monitor instruments and develop and share equations for the purpose of increasing the accuracy and knowledge of NIR forage testing.

Dairy farmers in the Midwest utilize forage testing to reduce feed costs and maximize production. Many commercial forage testing laboratories within the Midwest use NIR reflectance spectroscopy to analyze forage samples for dairymen, commercial hay producers and other livestock owners. Farmers are concerned about accuracy of forage test results and repeatability of sample analyses from lab to lab. Gray and Hill[1] demonstrated forage test results on the same alfalfa samples sent to two commercial forage testing labs were different between reference methods and NIR reflectance analysis. In a prior study,[2] eight commercial forage testing laboratories cooperated in a project from September 1990 through May 1991 to examine the feasibility of matching spectra generated by model 4250 NIRS instruments together through master instruments at the University of Minnesota, University of Wisconsin and Pennsylvania State University. Diagnostic evaluation showed six of the eight instruments needed repair. After instrument repairs were completed, the study showed all instrument spectral output could be matched and a single calibration equation could be developed for all instruments. From that project the NIRS Consortium, the identified network within this paper, was established.

The group focus centered on education of forage test users (clients) as being essential and the NIRS Consortium needed to be a leader in education on sampling, testing and equipment. Universities and commercial businesses within the NIRS Consortium must work together to achieve education goals. The Mission Statement adopted by the group is as follows: The NIR Forage and Feed Testing Consortium is dedicated to increasing the accuracy and knowledge of NIR forage testing.

During the consortium's first year, the group expanded from the original eight to eighteen participating labs. The first objective was to standardize all instruments. The consortium consisted of seven different models of NIRSystems instruments (4250A, 4250B, 6250, 6350, 4500, 5000 and 6500) and seven different versions of Infrasoft International (ISI) NIRS software. Two lab visits were made to initial consortium members. Diagnostics were completed on instruments, indicating many instruments were in need of adjustment and/or repair. Once the instruments were determined to be in good working order, spectra were collected from sealed characterization and check sample sets. The instruments were then standardized to an ISI master instrument. The group developed standard operating procedures for instruments, evaluated analytical capabilities of calibration equations in regards to regionalism and standardized relative feed value (RFV) equation throughout the Midwest. In addition, three calibration equations were developed, using procedures of Shenk *et al.*[3] for use by consortium members to analyze sorghum silage, forage dry matter, and grain dry matter.

The consortium held the first of three training sessions to-date in May of 1993. The training was conducted by Tom Boyd of Perstorp Analytical and focused on diagnostics, linearization, equation evaluations, routine operations and calibration development. After the training session, it was obvious that the multiple versions of software and equipment was going to make our job more complicated and delay implementation of new product equations.

Most laboratories began to upgrade their instruments to the newer models. By mid 1995, virtually all of the participants will have the latest NIR equipment. Also, the consortium negotiated a volume purchase of ISI 3.0 software and N level equations at a reduced rate. All participators took advantage of the offer and as a group we have moved from seven different instruments to three and seven different versions of software to a single version used by the entire group.

Consortium training sessions in 1994 and 1995 included basic training for new operators and advanced training on calibration and equation development.

Accomplishments to-date are:

1. Upgraded instrument operator skills in diagnostics, routine analysis and monitoring calibrations.
2. Uniform instrument spectra output to obtain uniform test results between labs.
3. Evaluation of labs reference method accuracy showed consortium labs were more accurate than average of (NFTA) National Forage Test Association certified labs, Table 1.

In three years, the NIRS Consortium has expanded the area serviced from Wisconsin, Minnesota, Illinois, Iowa, Nebraska and South Dakota to include labs in Indiana and Kansas. Work has begun on a number of product equations, with some coming to completion in 1994 and new projects taking their place. Projects started in 1995 are bypass protein calibration on roasted soybeans, *in vitro* digestible NDF calibrations, *in situ* digestible dry matter calibrations, calibrations for new products and evaluation and updating hay, haylage and corn silage expandable equations.

Table 1. Comparison of NIRS Consortium and National Forage Testing Association (NFTA) chemistry values for lst quarter, 1994 certification sample.

Statistic	DM	CP	ADF	NDF
	Tests, % of dry weight			
Consortium mean	92.89	21.08	30.94	40.23
Consortium std	0.85	0.52	0.76	0.77
Number	9	9	9	9
NFTA mean	92.63	21.33	31.42	40.77
NFTA std	0.81	0.54	1.84	2.42
Number	89	93	93	86

Activity results

Evaluation of matched instruments

Similar instruments models had spectral output matched to a consortium master using ISI's 29 sample characterization sample set according to ISI procedures.[4] Each instrument was standardized during a lab visit by the Consortium Manager. Spectra were collected from 10 instruments and standardized to a single master instrument using 14 sealed hay test samples. Generation of tests results with the standardized spectra on 14 sealed hay samples using a common calibration equation demonstrated excellent comparisons between instruments (Figure 1).

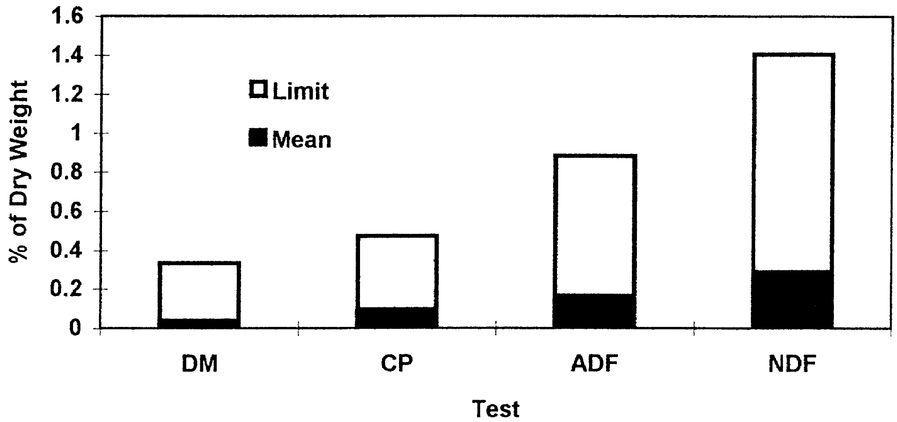

Figure 1. Mean standard errors for test values predicted using a common calibration equation across 14 sealed hay samples of 10 standardized Consortium NIRSystems Model 5000 instruments.

However, when the standardized spectra collected from 14 sealed hay samples were used to analyze hay samples with each lab's equation, variation between laboratories was unacceptable. Some labs use calibrations developed internally, others used ISI equations. Labs with the same level of ISI equations generated differing results, often due to internal lab equation "biasing". Therefore, the consortium decided to evaluate accuracy of their lab reference methods which were used to develop equations.

Reference method evaluation

After examining laboratory performance data from consortium labs submitted to NFTA, labs within the consortium were asked to analyze 12 hay and 12 corn silage samples to determine which

Table 2. Calibration statistics of original ISI expandable hay equation (base), inclusion of 60 consortium samples (base + 60) and a downsized library file plus 60 sampled (reduced base + 60).

	Expandable base equation			Base + 60			Reduced base + 60		
Statistics	CP	ADF	NDF	CP	ADF	NDF	CP	ADF	NDF
Number	1230	1230	550	1280	1279	605	631	629	315
Mean	17.03	35.02	54.42	17.04	35.12	53.79	18.21	35.73	50.94
SEC	0.92	1.66	2.63	0.79	1.51	2.45	0.74	1.38	2.31
RSQ	0.97	0.93	0.95	0.97	0.94	0.95	0.96	0.93	0.94
SECV	0.93	1.70	2.73	0.82	1.53	2.56	0.78	1.42	2.56
1 – *VR*	0.96	0.93	0.94	0.97	0.94	0.95	0.96	0.93	0.93

Table 3. Calibration statistics of original ISI expandable corn silage equation (base) and inclusion of 60 consortium corn silage samples (base + 60).

	Base			Reduced base + 60		
Statistic	CP	ADF	NDF	CP	ADF	NDF
	% of dry weight					
Number	378	368	292	436	435	349
Mean	9.33	26.82	49.35	9.28	27.14	49.03
SEC	0.45	1.04	2.24	0.41	1.15	2.19
RSQ	0.97	0.96	0.93	0.97	0.96	0.93
SECV	0.47	1.12	2.36	0.45	1.24	2.27
1 – *VR*	0.97	0.96	0.92	0.97	0.95	0.92

labs the consortium could use for its Reference Method labs. Three laboratories were selected from a group of 13 labs by eliminating the top and bottom 25% of labs based on values above and below the mean. The standard error between labs was %DM, 0.28 and 0.22; %CP, 0.26 and 0.29; %ADF, 0.69 and 0.62; and %NDF, 0.64 and 0.69; for hay and corn silage, respectively. Within the remaining six labs, three labs reproduced acceptable accuracy for all tests of hay and corn silage, respectively, with standard errors of %CP, 0.19 and 0.16; %ADF, 0.32 and 0.26; and %NDF, 0.75 and 0.50; for hay and corn silage, respectively, and both products, hay and corn silage.

Monitoring of ISI expandable equations

A goal of the consortium is to improve the accuracy of testing, especially by reducing the size of laboratory errors. As a step towards this goal, theconsortium is currently working with hay and corn silage. Sixty samples each of hay and corn silage were selected from random samplings (approximately 400–500 samples each of hay and corn silage) from 16 consortium laboratories within the midwestern United States. The sample populations were divided and analyzed in duplicate by the three designated reference laboratories for CP, ADF and NDF. Commercially available ISI expandable equations were monitored for bias and standard error of prediction corrected for bias [*SEP*(C)] hay, Table 2 and corn silage, Table 3. Data collected from the 60 hay

Table 4. Statistics for monitoring the ISI expandable hay equation (base) and a down-sized library file plus 60 consortium hay samples (reduced base + 60) with the 60 consortium hay samples.

	Base			Reduced base + 60		
Statistic	CP	ADF	NDF	CP	ADF	NDF
	% of dry weight					
SEP	1.73	2.83	4.20	1.07	2.46	3.25
Mean—Ref method	17.56	36.94	48.48	17.56	36.94	48.48
—NIR	18.73	35.73	51.10	17.76	36.38	49.28
Bias	–1.17[a]	1.21[a]	–2.62[a]	–0.20	0.56	–0.80
Bias limit	0.55	0.99	1.58	0.45	0.83	1.39
SEP(*C*)	1.29[a]	2.58[a]	3.30	1.06[a]	2.42[a]	3.18[a]
SEP(*C*) limit	1.20	2.15	3.42	0.97	1.80	3.01
Std deviation—RM	4.23	6.17	9.21	4.23	6.17	9.21
—NIR	3.80	4.75	8.00	14.09	5.55	9.22
Slope	1.06	1.20	1.08	41.00	1.02	0.94
R^2	0.91	0.85	0.88	0.94	0.85	0.88
Average *H*	1.17	1.17	1.17	1.30	1.30	1.30

[a]Indicates limit exceeded.

Table 5. Statistics for monitoring the ISI expandable corn silage calibration (base) and inclusion of 60 consortium corn silage samples (base + 60) with 60 consortium corn silage samples.

	Base			Reduced base + 60		
Statistic	CP	ADF	NDF	CP	ADF	NDF
	% of dry weight					
SEP	0.63	2.62	3.03	0.48	1.31	2.53
Mean—Ref method	9.30	28.88	47.72	9.30	28.88	47.72
—NIR	9.36	26.67	48.42	9.37	28.28	48.22
Bias	–0.06	2.21[a]	–0.70	–0.07	0.59	–0.50
Bias limit	0.27	0.63	1.35	0.25	0.69	1.31
SEP(*C*)	0.63[a]	1.41[a]	2.97[a]	0.48	1.17	2.50
SEP(*C*) limit	0.59	1.35	2.92	0.54	1.49	2.85
Std deviation—RM	2.92	5.05	7.88	2.92	5.05	7.88
—NIR	2.80	4.57	6.88	2.91	4.77	7.14
Slope	1.02	1.06	1.06	0.99	1.03	1.05
R^2	0.95	0.93	0.86	0.97	0.95	0.90
Average *H*	1.61	1.61	1.61	1.20	1.20	1.20

[a]Indicates limit exceeded.

samples showed bias limits had been exceeded for CP, ADF, and NDF, Table 4.[5] Crude protein and ADF also exceeded *SEP*(*C*) limits indicating the need for recalibration. However, expanding the base equation with the 60 samples reduced SEC of the expanded equation, Table 2, but did not change monitoring results. More samples are being collected. Down-sizing the base calibration library using the ISI MATCH program, then adding the 60 hay samples improved the monitoring of hay, Table 4. Data collected from the 60 corn silage samples showed bias limits had been exceeded for ADF, Table 5. Crude protein, ADF and NDF all exceeded *SEP*(*C*) limits indicating the need for recalibration. Performance of updated equations will be reported.

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Rock River Laboratory, Inc., Watertown, WI 53094;
Stearns DHIA Central Laboratory, Sauk Centre, MN 56378;
University of Wisconsin, Madison, WI 53706;
Commercial Testing Laboratory, Colfax, WI 54730;
Farmland Industries, Inc., Kansas City, KS 66109;
Growmark, Inc., Mendota, IL 61342;
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Midwest Alfalfa Co., Willmar, MN 56201;
Wisconsin DHI Cooperative, Bonduel, WI 54107;
Forage Genetics, West Salem, WI 54669.

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