An international ring trial to assess differences between laboratories in forage analysis

D.J. Undersander,[a] I.A. Crowe,[b] P. Dardenne,[c] P.C. Flinn,[d] C. Paul,[e] P. Berzaghi,[f] N.P. Martin,[g] N.B. Büchmann[b]

[a] *University of Wisconsin, 1575 Linden Drive, Madison, WI 53706 USA*

[b] *FOSS Tecator AB, Box 70, SE-263 21, Höganäs, Sweden*

[c] *Centre de Recherches Agronomiques de Gembloux, 24 Chaussée de Namur, 5030 Gembloux, Belgium*

[d] *Department of Primary Industries, Pastoral and Veterinary Institute, Private Bag 105, Hamilton, Victoria, Australia 3300*

[e] *Federal Agricultural Research Centre, Bundesallee 50, D-38116 Braunschweig, Germany*

[f] *University of Padua, Department of Animal Science, Strada Romea 16, 35020 Legarno, Italy*

[g] *USDA-ARS, Dairy Forage Research Center, 1925 Linden Drive West, Madison, WI 53706-1108, USA*

Introduction

The quality of laboratory reference values has always been a major factor affecting NIR calibration accuracy. It is of particular relevance in the case of forage analysis, where several of the common quality measurements are "derived" properties rather than specific constituents, and slight differences in methodology can produce different values for a given property. This issue is even more important where a central laboratory conducts reference analysis, derives calibrations and transfers them to other instruments in a network or where spectral databases and reference values from instruments of different laboratories are combined to produce "global" calibrations.

Materials and methods

Seventy-four samples consisting of whole plant maize silage and fresh cut forage and grass/legume hay, silage and freshly cut forage from Europe, North America and Australia collected during 2001. All samples were oven dried at 55 C to approximately 5% moisture. Samples were ground through a grinder with a 1 mm screen, then subdivided using a sample splitter to produce 8 sets of samples each approximately 30 g in weight. All subsamples were scanned by NIR and any set showing variability among subsample spectra was recombined and the splitting and scanning process was repeated until uniformity was achieved. The set included 10 blind duplicate samples. Participating laboratories were asked to run wet chemical analyses for dry matter, protein, acid detergent fiber (ADF), and neutral detergent fiber (NDF) in duplicate on all samples.

Copyright 2004 NIR Publications. All rights reserved.

Near Infrared Spectroscopy: Proceedings of the 11th International Conference.
www.nirpublications.com

Results

The results presented (table 1) illustrate that the "real" error in laboratories (no prior knowledge of duplicate sample identity) is larger than would be perceived from an analysis of replicate samples.

All laboratories produced highly satisfactory results for Protein and NDF (tables 2 and 3) and ADF and Ash (results not shown). Results for Dry Matter were poor at sites where samples had been delayed in transit. Differences seen between laboratories were clearly systematic, with both slope and bias differences being evident. Random differences between laboratories occurred only with dry matter for the reasons mentioned previously.

The high quality of results, combined with the large sample number involved, produced one unexpected result: differences between laboratories (either slope or bias) were usually significant at a 1% level, even although slope differences from 1.0 or differences from 0.0 for bias were small.

Recommendations

- Allow sufficient time for sample collection (up to 15 months).
- For safe transit, place samples in medium grade airtight polythene bags that are heat-sealed or have integral airtight zip tops.
- Each individual sample should be placed inside another zip top polythene bag with wax seal attached. The purpose of the wax seal is to identify if a particular bag has been opened.
- All the samples should be placed within a single large plastic bag and shipped in a stout cardboard box.
- Expect difficulties when shipping biological samples across international borders. Obtain correct phytosanitary documentation, expect Customs difficulties.
- Include blind duplicates and run all analyses in duplicate

Table 1 Mean Standard Deviation of Analysis of Blind Duplicates

Laboratory	DM	Protein	ADF	NDF	Ash
1	0.39	0.22	0.36	0.30	0.24
2	0.20	0.18	0.47	0.31	0.27
3	1.14	0.20	0.57	1.14	0.19
4	0.79	0.19	0.88	0.55	0.25
5	0.36	0.06	0.37	0.52	0.14
6	0.60	0.30	0.53	1.24	0.38

Copyright 2004 NIR Publications. All rights reserved.

Table 2 Comparison of Protein Determination Among Laboratories

	Laboratory						
Comparison	1	2	3	4	5	6	7
Slope Sig Diff from 1.0 at 0.01	TRUE	TRUE	FALSE	TRUE	TRUE	TRUE	TRUE
Bias Sig diff at 0.01	TRUE	TRUE	FALSE	TRUE	TRUE	TRUE	TRUE
Correlation	1.00	1.00	1.00	1.00	1.00	1.00	1.00
RMSED	0.32	0.28	0.20	0.30	0.49	0.58	1.03
SED	0.23	0.21	0.19	0.22	0.28	0.53	0.52
Mean	12.63	12.04	12.85	13.07	12.45	12.60	12.05
Bias	0.23	-0.03	-0.05	-0.21	0.40	0.25	0.90
Slope	1.03	0.98	1.00	0.99	1.03	0.95	1.08
Intercept	-0.13	0.15	0.00	-0.03	0.02	0.87	-0.04
Minimum	2.30	2.26	2.29	2.33	2.16	1.65	2.15
Maximum	29.35	30.80	30.06	30.20	29.21	30.60	28.06

Table 3 Comparison of Neutral Detergent Fiber Among Laboratories

	Laboratory						
Comparison	1	2	3	4	5	6	7
Slope Sig Diff from 1.0 at 0.01	TRUE	TRUE	TRUE	FALSE	TRUE	FALSE	FALSE
Bias Sig diff at 0.01	FALSE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE
Correlation	1.00	1.00	1.00	0.99	0.99	0.99	0.99
RMSED	1.10	1.07	1.75	2.44	1.54	1.82	6.96
SED	1.07	1.19	1.14	1.21	1.40	1.75	2.11
Mean	48.36	46.53	49.44	50.23	47.44	48.64	43.70
Bias	-0.25	1.58	-1.33	-2.12	0.67	-0.54	6.64
Slope	0.96	1.05	0.96	1.01	0.96	1.00	1.08
Intercept	1.50	-0.88	0.49	-2.59	2.36	0.57	3.30
Minimum	23.81	24.46	29.19	28.80	23.31	27.65	18.43
Maximum	79.90	76.24	81.57	81.13	79.43	79.65	71.40

Copyright 2004 NIR Publications. All rights reserved.