Prediction of the D-value of grass silages with cellulase solubility and NIRS

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Introduction

The content of digestible organic matter in dry matter (D-value) is routinely predicted in Finland with near infrared reflectance spectroscopy (NIRS), which is calibrated with *in vitro* pepsin-cellulase solubility based D-values. The *in vivo* digestibility of regrowth grass silages is substantially lower at the same pepsin–cellulase solubility than that of primary growth grass silages (Nousiainen et al.[1]) This may cause over-prediction of pepsin–cellulase solubility based D-values of silages with NIR, especially those made of regrowth grass. The aim of this study was to compare different methods to predict D-value and to develop less biased prediction NIR equations.

Materials and methods

Silages

A total of 53 grass silage samples were obtained from the experimental station of MTT Agrifood Research Centre in Jokioinen, Finland (61°N). The swards were second year timothy meadow fescue leys. Twenty-five primary growth and 28 regrowth grass silages were harvested in years 1988–1991, 1994 and 1996–2000. The silages were the same as described by Nousiainen *et al*.[1,2]

The composition of silages

The content of digestible organic matter in dry matter (D-value) of grass silages was determined *in vivo* with wether sheep by total collection of faeces. Chemical composition and *in vivo* digestibility of samples has been reported earlier by Nousiainen *et al*.[1,2]

In vitro D-value was predicted with a pepsin–cellulase solubility method as described by Nousiainen *et al*.[2] A standard silage sample was included in every incubation batch to verify the activity of the enzyme.

Different equations were used to calculate the D-value from pepsin–cellulase solubility (OMS). Equation A[3] has been used in practice for several years for all types of grass and grass silage samples. To improve prediction accuracy, separate equations developed by Nousiainen and Huhtanen[4] were used for silages made from the primary growth (B) and regrowth (C) of grass.

Equation A: D-value = 0,93 x OM – 0,79 x OM_{ns}
Equation B: D-value = 160 + 0,818 × OMS – 1,09 × ash
Equation C: D-value = 120 + 0,85 × OMS – 1,16 × ash

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

NIR scanning

Silage samples were dried and ground through a 1 mm screen and scanned with a Foss NIRSystems model 6500 spectrophotometer over the wavelength range from 400 to 2498 nm. Spectra were trimmed to 1100–2498 nm using WinISI II 1.04a (Foss NIRSystems/Tecator, Infrasoft International) software. This gave a total of 700 data points for each spectrum. NIR calibrations were developed with WinISI II using PLS regression, math treatments 1,4,4,1 and standard normal variate and detrend (SNV&D) scatter correction.

Two different NIR calibrations (I and II) for D-value were compared. NIR calibration set I contained 294 silage samples selected from farm grass silage samples over a period of eight years. Reference D-values were based on pepsin–cellulase Equation A. NIR calibration II based on D-values calculated using specific pepsin–cellulase Equations B for primary growth and C for regrowth silage samples. The total number of samples in the calibration set II was 141 farm grass silages over an eight-year period.

Root mean square errors (*RMSE*) between different D-value prediction methods were compared. Proportions of mean bias, slope and random errors of the total *RMSE* were also calculated.

Results

Separate equations for primary growth (B) and regrowth (C) of grass improved the relationship between pepsin–cellulase solubility and *in vivo* D-values of silages compared to equation A (Figure 1). As shown in Table 1, prediction accuracy improved and proportions of slope and bias error of total *RMSE* decreased.

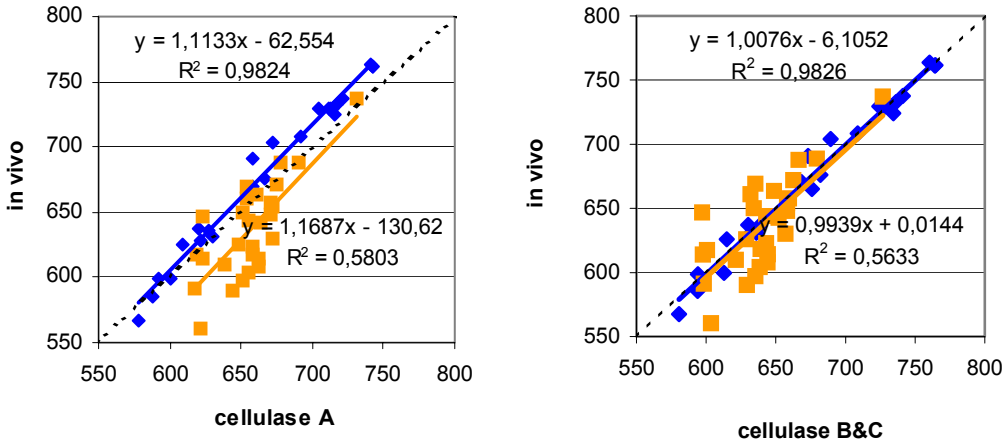

Figure 1. *In vivo* D-values (g kg–1 DM) of primary growth (♦) and regrowth (■) grass silages predicted with pepsin–cellulase Equation A and new equations B and C.

NIR calibration I produced more accurate predictions than calibration II (Figure 2), which might be due to the larger calibration set used in Calibration I. Despite of the smaller calibration set, calibration II decreased the proportion of bias and slope error between NIR and the cellulase method for regrowth silages, even though the absolute *RMSE* was not improved (Table 1).

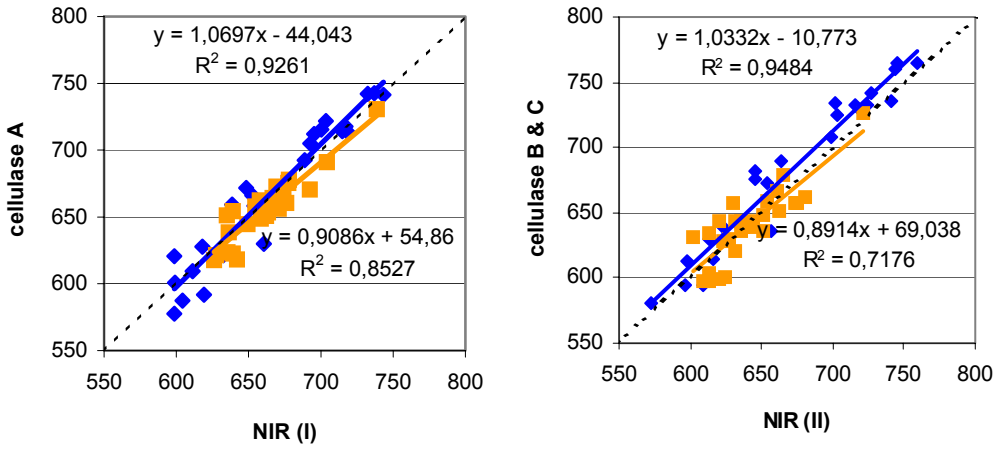

Figure 2. D-values (g kg^{-1} DM) of primary growth (♦) and regrowth (■) of grass silages calculated from pepsin–cellulase solubility compared to D-values predicted with NIR calibrations I and II.

The prediction accuracy of *in vivo* D-value was improved by NIR Calibration II in comparison to Calibration I (Figure 3). The total *RMSE* between NIR and *in vivo* D-values decreased and the proportions of bias and slope errors of the total *RMSE* were smaller with calibration II, especially for regrowth silages (Table 1).

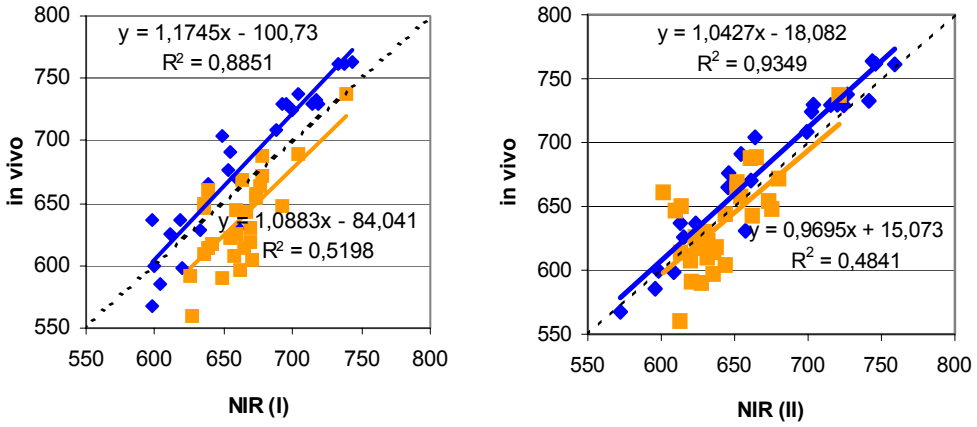

Figure 3. *In vivo* D-values (g kg^{-1} DM) of primary growth (♦) and regrowth (■) grass silages predicted with NIR calibrations I and II.

Table 1. D-value prediction statistics of the pepsin–cellulase method compared to the *in vivo* method, and NIR compared to the pepsin–cellulase method and the *in vivo* method.

Prediction	Method of							Proportion of *RMSE*		
method	comparison	cut[a]	n	Slope	Intercept	R^2	*RMSE*	Bias	Slope	Random
cellulase	*in vivo*									
A	*in vivo*	1	25	1,11	–62,6	0,982	16,7	0,632	0,135	0,233
A	*in vivo*	2	28	1,17	–130,6	0,580	31,5	0,403	0,017	0,580
A	*in vivo*	1&2	53	1,18	–122,8	0,800	25,4	0,029	0,082	0,889
cellulase	*in vivo*									
B	*in vivo*	1	25	1,01	–6,1	0,983	8,0	0,014	0,003	0,983
C	*in vivo*	2	28	0,99	0,1	0,563	24,6	0,024	0,000	0,976
B&C	*in vivo*	1&2	53	1,02	–13,4	0,882	18,6	0,018	0,002	0,980
NIR	cellulase									
I	A	1	25	1,07	–44,0	0,926	15,1	0,024	0,049	0,927
I	A	2	28	0,91	54,9	0,853	11,1	0,264	0,041	0,695
I	A	1&2	53	1,04	–27,4	0,901	13,0	0,022	0,012	0,966
NIR	cellulase									
II	B	1	25	1,03	–10,8	0,948	17,9	0,419	0,011	0,571
II	C	2	28	0,89	69,1	0,718	15,1	0,002	0,036	0,962
II	B&C	1&2	53	1,05	–26,9	0,904	16,3	0,098	0,018	0,884
NIR	*in vivo*									
I	*in vivo*	1	25	1,17	–100,7	0,885	27,0	0,330	0,097	0,573
I	*in vivo*	2	28	1,09	–84,0	0,520	36,5	0,499	0,004	0,497
I	*in vivo*	1&2	53	1,18	–126,0	0,670	32,1	0,038	0,044	0,919
NIR	*in vivo*									
II	*in vivo*	1	25	1,04	–18,1	0,935	18,8	0,317	0,016	0,667
II	*in vivo*	2	28	0,97	15,1	0,484	26,8	0,028	0,001	0,971
II	*in vivo*	1&2	53	1,08	–50,2	0,819	23,2	0,013	0,024	0,963

a) 1= primary growth, 2= regrowth

Conclusion

NIR calibration II seemed to perform quite satisfactorily although it was based on only half of the material as compared to calibration I. The total *RMSE* between *in vivo* and NIR D-values decreased and bias and slope errors were almost completely eliminated. The random error of regrowth samples might be decreased with a larger calibration set. The NIR calibration II should be redeveloped with a larger set of samples, to get more consistent results when applied to a wide range of farm grass silage samples.

References

1. J. Nousiainen, M. Rinne, M. Hellämäki and P. Huhtanen, *Anim. Feed Sci. Technol*. (submitted) (2003).
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3. I. Klemetti, M. Hellämäki and J. Nousiainen, *Ann. Zootech*. **44,** Suppl, 42 (1995).
4. J. Nousiainen, and P. Huhtanen, unpublished data (2003).