Comparison of NIRS based methods to determine legume content of mixed swards

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ABSTRACT

NIRS calibrations were compared for their ability to predict red clover content of the same set of independent samples of mixed sward. All published calibrations used in this study with wide range of samples, proved their capability to determine the legume content in samples of very different origin without any loss of precision.

INTRODUCTION

Legume content of mixed swards is a key factor for feed quality and for N-balances in organic farming. Estimation of the legume content of mixed swards is difficult and laborious. Several authors have reported NIRS (Near Infrared Reflectance Spectroscopy) to be a promising tool for the determination of legume content in mixed sward samples (Petersen et al., 1987, Coleman et al., 1990, Wachendorf et al., 1999 and Locher et al., 2005a, b). Different calibration strategies with material from different kinds of mixed swards were used. The critical part of NIRS-calibrations is their successful validation with independent samples (Reeves, 2000). For a widespread use of a calibration it is necessary to compare it with different calibrations at the same set of independent samples to judge advantages and shortcomings of existing calibrations. Then it will be possible to profit from the advantages of NIRS (low cost, fast, simple) and to use calibration transfer for data exchange between labs even across different instrumentation (Reeves, 2000).

Therefore we compared the performance of four existing and two preliminary calibrations, in their ability to determine legume content in Finnish red clover-grass mixtures of known legume content. Additionally we demonstrate the performance of

MATERIALS AND METHODS

The test samples from Finland were collected at eight different fields under organic and conventional farming and at three cutting times in 2003. Ten samples were mixtures of known red clover content and 17 samples each were pure grass and pure red clover.

Sample origin, calibration procedure and NIRS-instruments were different at Kiel and Munich, but both applied PLS regression to develop calibrations based on the whole spectra measured. Wachendorf et al. (1999) used 282 natural red clover-grass mixtures, harvested at weekly intervals from differently fertilised plots, and measured them with a monochromator (FOSS). Reference values came from hand-sorted samples harvested nearby (calibration K). Locher et al. (2005a) developed three calibrations from hand-sorted multi-species legume-grass mixtures harvested at several organic farms in Bavaria, Germany, between 1999 and 2002. Calibration M1 was developed from 334 pure grass and pure legume samples collected at one farm, calibration M2 contained further 63 artificial mixtures of grasses and legumes from the same farm and for calibration M3 another 120 pure legume and pure grass samples from diverse origins were added to the data set. These samples were measured with an FT-NIR (Vector, BRUKER, Ettlingen, Germany).

At Munich one preliminary calibration was developed based on the 17 pure grass and pure clover Finnish samples (calibration F1) and at Kiel twelve Finnish samples (one pure grass, one pure red clover and the ten known mixtures) were used to develop another calibration (calibration F2). In both cases the preliminary calibration was developed using PLS regression as comprised by the software packages ISI (Kiel) and OPUS (Munich).

RESULTS

NIRS-predicted values of the ten known mixtures from Finland were in a close linear relationship to the true values in all calibrations. The prediction error (RMSEP: root mean square error of prediction) was well within the reported range of 5% (3.6-8.3 % legume content, Table 1) and there were only minor differences found between the published calibrations. Surprisingly the red clover specific calibration K was not superior to the other published calibrations, but it had the smallest bias. The preliminary calibration F1 showed the highest RMSEP and bias, while the values of calibration F2 were very good. However, in case of calibration F2, calibration and validation sets were not independent, because ten of the twelve samples used for calibration were the test-samples as well. In this case RMSEP comes close to an RMSECV (root mean square error of cross-validation). (Table 1)

Table 1. Comparison of the NIRS models to predict legume content of legume-grass samples. The model cross-validation errors are shown together with the prediction errors evolved from the determination of ten known mixtures from Finland.

Reference for calibration	name	RMSECV	SEP	bias	RMSEP	$SEP_{biascor}$
		---------- % legume content ----------				
Wachendorf et al. 1999	K		6.5	0.4	6.5	6.5
Locher et al. 2005a	M1	2.3	5.2	-2.0	5.6	5.2
Locher et al. 2005a	M2	2.5	5.0	1.6	5.3	5.0
Locher et al. 2005b	M3	3.7	4.4	-0.5	4.4	4.4
	---------- calibrations solely based on Finnish samples ----------					
	F1	2.5	4.8	-6.8	8.3	4.8
	F2		3.6	-0.3	3.6	3.2

DISCUSSION

The small prediction errors confirm the ability of all published calibrations (K, M1-M3) to determine legume content with an acceptable error. The calibration procedure seems to be of no importance for the prediction power of the calibrations, which confirms the findings of Locher et al. (2005a, b). But, the present results prove as well the necessity to determine the aim of a calibration: the more specific it is, as with calibration K, the prediction error may increase if quite different samples have to be predicted but it will be smaller with samples from the same population. The decrease in RMSEP from calibration M1 through M2 to M3 confirms this, because in the same sequence the variability included in the model increases.

Locher et al. (2005 b) did not find this effect. For the deviation from their expectation they discussed a too small deviation of their test samples from the calibration samples as one reason, which was confirmed by our results.

The preliminary calibrations highlighted the capability of NIRS to develop a good calibration even if there are only a limited number of samples. Obviously such calibrations will be much more prone to errors if new samples have to be predicted, because they do not include much variation.

CONCLUSION

All published calibrations used in this study proved their capability to determine legume content in samples of very different origin without any loss of precision. The average RMSEP of these calibrations (5%) was good compared to other errors, which may occur during sampling and sample preparation. Especially studies at field level may profit from these methods. But, one has to consider, that samples needed for other analyses (chemical composition of the partner in the mixture) still have to be separated / sorted by hand.

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