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Adaptation and Management of Forage Legumes – Strategies for Improved Reliability in Mixed Swards

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

Keywords: NIRS, legume content, red clover

INTRODUCTION

The legume content of mixed swards is a key factor determining feed quality and N-balances in organic farming but estimation of the legume content of mixed swards is difficult and laborious. Visual estimates are subjective and involve high errors and low repeatability, whilst botanical analyses by hand sorting are accurate but time consuming. Therefore a fast and accurate assessment of the legume content is needed.

Several authors have reported NIRS (Near Infrared Reflectance Spectroscopy) to be a promising tool for the determination of the legume content of 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 have been used. The critical part of NIRS-calibrations is their successful validation with independent samples (Reeves, 2000). For widespread use of a calibration it is necessary to compare it with different calibrations of the same set of independent samples in order to compare their advantages and shortcomings. 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 across different instrumentation (Reeves, 2000).

We compared the performance of four existing calibrations, developed at Kiel or Munic their ability to determine the legume content of Finnish red clover-grass mixtures of k legume content. Additionally, we demonstrate the performance of two prelim calibrations, which were based on the Finnish samples.

MATERIALS AND METHODS

The test samples from Finland were collected from eight different fields under organi conventional farming at three different dates in 2003. Ten samples were mixtures of k red clover content and 17 samples each were pure grass and pure red clover. Sample o calibration procedure and NIRS-instruments were different at Kiel and Munich, but applied PLS regression to develop calibrations based on the whole spectra meas Wachendorf *et al.* (1999) used 282 natural red clover-grass mixtures, harvested at w intervals from differently fertilised plots, and measured them with a monochromator (F Reference values came from hand-sorted samples harvested nearby (calibration K). Loc *al.* (2005a) developed three calibrations from hand-sorted multi-species legume- mixtures harvested at several organic farms in Bavaria, Germany, between 1999 and Calibration M1 was developed from 334 pure grass and pure legume samples collected a farm, calibration M2 contained 63 artificial mixtures of grasses and legumes more fro same farm and for calibration M3 another 120 pure legume and pure grass samples of di origins were added to the data set. These samples were measured with an FT-NIR (V BRUKER, Ettlingen, Germany). At Munich one preliminary calibration was developed t on the 17 pure grass and pure clover Finnish samples (calibration F1) and at Kiel tv Finnish samples (one pure grass, one pure red clover and the ten known mixtures) were to develop another calibration (calibration F2). In both cases the preliminary calibratio developed using PLS regression as comprised by the software packages ISI (Kiel) and C (Munich).

RESULTS

NIRS-predicted values of the ten known mixtures from Finland were in a close linear relationship with 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). However, the present results also emphasise the need to determine the aim of a calibration: the more specific it is (as with calibration K in this study) the more the prediction error will increase if quite different samples are to be predicted. The decrease in RMSEP from calibration M1 through M2 to M3 confirms this, since including more samples to the prediction set increases the variability included in the model. However, this result unexpectedly disagrees with the findings of Locher *et al.* (2005 b). The reason for discrepancy might be too small a deviation of their test samples from their calibr samples, which is what we found in the present study.

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

CONCLUSION

All published calibrations used in this study proved their ability to determine the leg content of samples of very different origin without any loss of precision. The average RM of these calibrations (5%) was good compared to other errors that may occur during samp and sample preparation. Field studies, in particular, may profit from these methods. How one has to consider that samples needed for other analyses (e.g. chemical compositio mixture components) still must be separated / sorted by hand.

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