

EVALUATION OF NIRS-ESTIMATION FOR ENZYME-SOLUBLE ORGANIC MATTER (ESOM) IN GRASS SILAGES COMPARED TO THE CELLULOSE REFERENCE METHOD

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ABSTRACT

Enzyme-soluble organic matter (ESOM) of 167 Austrian grass silages was measured using the cellulase reference method, also predicted by near-infrared spectroscopy (NIRS) and a general linear model (GLM) based on chemical parameters. NIRS calibration showed much higher accuracy ($R^2 = 0.90$) than GLM ($R^2 = 0.57$). Results indicate that NIRS is a reliable and cost-effective method for estimating ESOM in grass silage.

Keywords: near-infrared spectroscopy, in vitro digestibility, cellulase-method, calibration

INTRODUCTION

In the German feeding recommendations for dairy cows (GfE 2023), the cellulase method developed by deBoever *et al.* (1986) is a recognized standard method in which the enzyme soluble organic matter (ESOM) is measured in order to calculate the digestibility of organic matter (OMD). According to Shenk and Westerhaus (1994), near-infrared spectroscopy (NIRS) provides a rapid, cost-effective analysis of nutrient composition with good estimation accuracy, which can be applied to the increasing demand for efficiency in livestock feeding. Using a subset of grass silage samples, deBoever *et al.* (1996) showed that NIRS equations based on partial least squares analysis (PLS) were better at explaining the variance in OMD than multiple linear regression using nutrient parameters. At AREC Raumberg-Gumpenstein, 167 grass silage samples from farms with extensive to intensive grassland management were analysed for ESOM in order to evaluate the results of estimation from NIRS and General Linear Model (GLM) in terms of variance and estimation error.

MATERIAL AND METHODS

The grass silage samples ([total] $n = 167$ from: [AREC 2017-2025] $n = 128$ and [Austrian silage project 2024 (ASP) described by Resch (2025)] $n = 39$) were oven-dried at 50°C for two days and then ground to 1 mm particle size. Dry matter content was determined using a BRABENDER apparatus (type MT-C) for 10 g of sample at 130 °C for 30 minutes. The enzymatic analysis-procedure is described in VDLUFA-Method 6.6.1 (VDLUFA, 2018). For the preparation of samples with cellulase (Onozuka R-10, 1 U/mg) and pepsin (≥ 2.0 FIP-U/mg), we use special glass frits (GTWA497) with a filter base of porosity 2 and GL32 screw cap. ESOM data presented below refer to dry matter. The NIRS measurements were performed using a Unity spectrometer (type SpectraStar™ XT) with a wavelength range of 880 to 2,600 nanometer (nm) and a resolution of 1 nm. The cuvette diameter was 86 mm. The chemometric evaluation of the spectroscopy data was performed using ZEISS SL Calibration Wizard software. For

NIRS calibration and GLM statistics, 41% of the total samples (AREC: $n = 48$; ASP: $n = 21$) with measured ESOM values were used and 59% for validation. NIRS calibration was performed in the same way as in deBoever *et al.* (1996) using PLS statistics. Linear analysis of regression factors was carried out by procedure GLM (Statgraphics Centurion XVII) at confidence level of 95%. The following parameters – contents based on dry matter – are required for GLM calibration and subsequent GLM ESOM-estimation: crude protein (CP), hemicellulose (aNDFom minus ADFom), cellulose (ADFom minus ADL), ADL, crude lipid (CL) and crude ash (CA).

RESULTS AND DISCUSSION

The ESOM contents of the 167 grass silages were normally distributed and ranged from 440 to 817 g/kg DM, with an average value of 609 g/kg DM. In contrast, the mean ESOM value of the calibration subset was 626 g/kg DM and that of the validation subset was 597 g/kg DM (Table 1). With GLM, we achieved an R^2 of 0.7 to 0.77, which is comparable to deBoever *et al.* (1996), who achieved an R^2 of 0.73, using the Weender parameters for prognosis of OMD. The mean value of the GLM estimation results was the same as the ESOM measurement in the calibration set, but there was a slight ESOM underestimation in the validation set. The mean estimation error for GLM was 21.2 and 32.5 g/kg DM, respectively. The difference in PLS calibration for the NIRS estimate was less about the mean ESOM value, but more about the mean estimation error.

GLM formula (1) total sample set ($R^2 = 0.70$); formula (2) calibration subset ($R^2 = 0.77$)

$$\text{ESOM} = 1021.04 + 0.147\text{CP} - 0.6634\text{Hemicellulose} - 0.6822\text{Cellulose} - 2.0108\text{ADL} + 1.8964\text{CL} - 1.1498\text{CA} \quad (1)$$

$$\text{ESOM} = 950.969 + 0.191\text{CP} - 0.7336\text{Hemicellulose} - 0.4785\text{Cellulose} - 2.1032\text{ADL} + 3.1286\text{CL} - 1.3052\text{CA} \quad (2)$$

Tab. 1: Descriptive statistics of grass silage samples for calibration and validation with regard to enzyme-soluble organic matter (ESOM) in g/kg DM, as well as statistics on ESOM estimation using NIRS or GLM

Source of variation	Descriptive Statistics – Wet Chemistry						NIRS – Estimation				GLM – Estimation			
	n	$\bar{x}$	med	SD	min	max	$\bar{x}$	$\bar{x}^R$	RSD	R^2	$\bar{x}$	$\bar{x}^R$	RSD	R^2
Total Sample Set	167	609	603	60.4	440	817	609	12.6	11.2	0.97	609	27.0	33.7	0.70
Calibration Subset	69	626	623	56.4	486	817	627	7.7	7.3	0.92	626	21.2	28.5	0.77
Validation Subset	98	297	587	60.7	440	769	595	16.1	12.1	0.90	592	32.5	24.0	0.57

n = number of samples, $\bar{x}$ = arithmetic average, med = median, SD = standard deviation, min = minimum, max = maximum, $\bar{x}^R$ = average of residuals (mean estimation error), RSD = standard deviation of residuals

In the calibration set, the NIRS estimation error for ESOM was 7.7 g/kg DM, which was three times lower than the GLM estimation error. For validation, the NIRS estimation error for unknown samples rose to 16.1 g/kg DM, but was

still half that of the GLM prognosis (Table 1). In the regressive XY plot – observed ESOM vs. predicted ESOM via NIRS – the explained variance (R^2) for NIRS validation was 0.90 and for GLM validation only 0.57.

CONCLUSION

We found that NIRS performed reasonably well in estimating ESOM for a very diverse set of grass silage samples from Austria. This confirmed similar results on NIRS reported by deBoever *et al.* (1996), Nousiainen *et al.* (2004), Gibaud (2007) and Spiekens *et al.* (2011). The accuracy of NIRS predictions significantly exceeds that of GLM predictions based on chemical parameter from nutrient analysis. Therefore, the cost-effective NIRS technology is recommended for ESOM in grass silage. This finding is of great importance for the implementation of the new feeding recommendations for dairy cows according to GfE (2023), because ESOM is a central parameter for the calculation of OMD and metabolisable energy (ME). For grass silage analysing laboratories, the use of NIRS with good calibrations can make a valuable contribution to reducing wet chemical ESOM analysis to a few reference measurements, that are needed to improve NIRS calibration.

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