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Predicting feeding value of forage maize hybrids harvested at different maturities and sites

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ABSTRACT. The objective of this study was to evaluate two near-infrared reflectance spectroscopy (NIRS) applications and different laboratory methods for determining indigestible neutral detergent fibre (iNDF) and organic matter digestibility (OMD) of forage maize hybrids harvested at high latitudes. Different *in situ* and *in vitro* methods have been used in calibrations of NIRS for routine determinations of forage feed value in the Nordic countries. Swedish and Finnish NIRS calibrations of iNDF generated biased estimates of low precision of iNDF determined *in situ*. All methods used to predict OMD in forage maize systematically underestimated the observed OMD calculated from the *in situ* determined value of iNDF. Digestibility was determined with least precision based on the enzymatic *in vitro* procedure. Values of OMD based on the *in vitro* incubation in buffered rumen fluid displayed the smallest error among the laboratory methods. The NIRS calibration developed with the forage maize samples of this study, suggested that NIRS has a great potential to predict iNDF and OMD of forage maize accurately and precisely. It was concluded that NIRS predictions of OMD can be a promising tool for evaluating performance of forage maize hybrids at high latitudes if NIRS calibrations of iNDF including forage maize samples are available.

Introduction

Forage maize (*Zea mays* L.) cultivation has been increasing in Northern Europe in recent years as a result of the development of early maturing hybrids and improved agronomic practices. Land used for cultivation of forage maize has increased almost ten-fold in Sweden since the crop was introduced in 1980's (Jordbruksverket, 2010). Yield and nutrient composition varies with hybrid, stage of maturity at harvest, and climatic conditions across locations used for forage maize cultivation in Sweden (Swensson et al., 2008). An accurate prediction of feed value is important for correct ranking of the performance of hybrids in plant breeding programmes. Digestibility is the most important trait of forages in feed value determinations (Huhtanen et al., 2006). Digestibility of organic matter (OM) in forage maize is positively related to high concentrations of starch and sugar, which are almost completely digestible in ruminants (Bal et al., 1997; Sutton et al., 2000). However, forage maize harvested at a more immature growth stage has less starch, but higher digestibility of neutral detergent fibre

(NDF) resulting in a possible small overall effect of increasing plant maturity on OM digestibility (OMD) (Givens and Deaville, 2001).

There are different analytical methods applied for the predictions of OMD in forages in the Nordic countries (Huhtanen et al., 2006; Åkerlind et al., 2011). Different *in vitro* methods related to *in vivo* OMD have been used for calibration of near infrared reflectance spectroscopy (NIRS) for routine determinations of forage OMD. Values reported to farmers are based on the country-specific reference laboratory method, which is affected by interactions between forage type and method (Krizsan et al., 2012). The reference laboratory method for forage maize in Denmark is an enzymatic 2-step *in vitro* procedure based on a mixture of enzymes degrading starch and fibre (Weisbjerg and Hvelplund, 1993). In Sweden, OMD in forage maize is predicted by a single 96-h *in vitro* incubation based on rumen fluid (Lindgren, 1979). Predictions of forage OMD performed almost equally well based on the 96-h *in vitro* incubation method as when predicted from indigestible NDF (iNDF) determined from a 288-h ruminal *in situ* incubation (Krizsan et al., 2012). It has been shown, however, that *in situ* was a more uniform method and would improve overall predictions of forage *in vivo* OMD at commercial laboratories in Nordic countries (Huhtanen et al., 2006; Krizsan et al., 2012). The performance of these analytical methods as predictors of OMD has not been evaluated for forage maize grown at higher latitudes before. The objective of this study was to evaluate the potential of NIRS calibrations of iNDF for feed value determination of forage maize, and to evaluate different laboratory methods used for predictions of *in vivo* OMD of forage maize hybrids harvested at different sites and stages of maturity at high latitudes.

Material and methods

Maize samples and chemical analysis

The plant material used to assess the different laboratory methods consisted of 72 samples of three forage maize hybrids harvested at four occasions with increasing maturity at three different geographical sites in Sweden. The hybrids were of different maturity indices as classified according to the Food and Agricultural Organization of the United Nations (FAO): Avenir with FAO 180 (Syngenta Seeds, Basel, Switzerland) and Isberi and Burli with FAO 190 and 210, respectively (Caussade Semences, Caussade, France). Samples were collected in an agronomic study of forage maize conducted by Mussadiq et al. (2012) during the 2009 growing season from Kristianstad (N 56°; E 14°), Skara (N 58°; E 13°) and Västerås (N 59°; E 16°). Growing conditions, cultivation, agronomic performance and nutritional qualities of the material have been described in detail by Mussadiq et al. (2012). Briefly, a randomized complete block design with two replicated blocks at each site was used. Plants were harvested on four occasions and in two replicates, which resulted in 24 samples at each site. After drying, plant samples were ground through a 2.0-mm screen for *in situ* incubations, and a 1.0-mm screen for *in vitro* incubations and chemical analysis using a Retsch SM 2000 Mill (Haan, Germany). Descriptive statistics of chemical composition across hybrids, sites and harvests of plant material are presented in Table 1.

Table 1. Descriptive statistics for the measured and predicted parameters (g · kg^{-1} dry matter unless otherwise stated) of all maize hybrid samples collected at all sites (n = 72)

Parameters[1]	Mean	SD[2]	Minimum	Maximum
Dry matter, g · kg^{-1}	242	58.2	150	433
Organic matter	952	8.1	929	972
Crude protein	80	18.5	44	123
Neutral detergent fibre	490	61.7	340	594
Starch	151	90.8	8	359
Water soluble carbohydrates	102	56.1	13	230
EFOS, g · kg^{-1}	653	42.2	535	750
VOS, g · kg^{-1}	777	26.7	703	844
$iNDF_{IN\ SITU}$	113	21.6	65	165
$iNDF_{NIRS-S}$	105	18.8	70	158
$iNDF_{NIRS-F}$	142	20.5	85	196
$iNDF_{NIRS-M72}$	113	20.8	64	171
$OMD_{IN\ SITU}$, g · kg^{-1}	693	29.7	621	757
OMD_{EFOS}, g · kg^{-1}	679	30.8	593	749
OMD_{VOS}, g · kg^{-1}	679	24.0	612	739
OMD_{NIRS-F}, g · kg^{-1}	678	30.2	594	736

[1] EFOS – enzyme digestible organic matter; VOS – rumen fluid organic matter solubility; $iNDF_{IN\ SITU}$ – indigestible neutral detergent fibre (iNDF) determined from a 288-h ruminal *in situ* incubation in dairy cows; $iNDF_{NIRS-S}$ – from the Swedish near infrared reflectance spectroscopy (NIRS) calibration; $iNDF_{NIRS-F}$ – from the Finnish NIRS calibration; $iNDF_{NIRS-M72}$ – from the NIRS calibration developed in this study; $OMD_{IN\ SITU}$ – organic matter digestibility (OMD) calculated based on the iNDF determined by the 288-h ruminal *in situ* incubation (Huhtanen et al., 2013); OMD_{EFOS} – predicted from EFOS (Weisbjerg and Hvelplund, 1993); OMD_{VOS} – predicted from VOS (Lindgren, 1979); OMD_{NIRS-F} – predicted based on the Finnish NIRS calibration; [2] SD – standard deviation

In situ and *in vitro* methods

Concentrations of iNDF in forage maize samples were determined following a 288-h *in situ* incubation (Huhtanen et al., 1994) using three ruminally cannulated lactating Swedish Red cows. The cows were fed a diet consisting of 600 and 400 g · kg^{-1} diet dry matter (DM) of grass silage

and concentrate (Vida 175; Lantmännen Lantbruk AB, Stockholm, Sweden), respectively, to meet production requirements. Samples of 2 g were weighed into pre-weighed polyester bags with pore size of 12 µm and a pore area equal to 6% of the total surface (Saatifil PES 12/6; Saatitech S.P.A., Veniano, Como, Italy). The sample size to surface area ratio was 10 mg · cm^2. After removal from the rumen, bags were rinsed in a household washing machine (the rinsing part of the wool wash programme) including three times washing for 2.5 min per washing (including the time for filling of water) using approximately 8°C water (Electrolux Wascator W75MP; AB Electrolux, Stockholm, Sweden). Thereafter, the bags were boiled for 1 h in neutral detergent solution including sodium sulphite (100 ml · g^{-1} of sample; Mertens et al., 2002), thoroughly rinsed, dried at 60°C for 24 h and weighed.

The enzymatic solubility of the forage maize samples was determined from a 2-step enzymatic *in vitro* digestion of OM (EFOS; abbreviated based on the Danish term enzym fordøjelig organisk stof) according to Weisbjerg and Hvelplund (1993). In this method, initially 500 mg of sample was subjected to a pre-treatment with pepsin-HCl (1000 ml of 0.5 M HCl and 2 g pepsin; Pepsin Orthana, 10000 NF U · g^{-1}; VWR, Kastrup, Denmark). Samples were then moved to a water bath maintained at 80°C for 45 min. The second step of digestion was performed by incubating the samples for 24 h at 40°C in 30 ml of an enzyme mixture consisting of cellulase (20 ml Cellulast, 798 EGU · g^{-1}; Novozymes, Bagsværd, Denmark), hemicellulase (10 ml Viscozyme L., 120 FBG· ml^{-1}; Novozymes, Bagsværd, Denmark), amyloglucosidase (2.135 g Megazyme E-AMGDF, 3260 U · ml^{-1}; Megazyme Int., Wicklow, Ireland) and Novozyme 51054 (17 ml, 1000 k VHCV(m)· g^{-1}; Novozymes, Bagsværd, Denmark) dissolved in 1000 ml 0.1 M acetate buffer. Samples in enzyme mixture were incubated additionally for 19 h at 60°C before washing with hot water and acetone. Finally, residues were dried overnight at 103°C weighed and thereafter combusted at 525°C for 6 h for determination of residual ash.

Rumen fluid digestible OM (VOS; abbreviated based on the Swedish term vomvätskelöslig organisk substans) was determined by a single 96-h incubation of forage samples in rumen fluid as described by Lindgren (1979). Rumen fluid digestible OM was determined from incubating 500 mg of dried forage maize samples in 49 ml buffer and 1 ml of rumen fluid at 38°C. Incubation residues were dried at 105°C for 3 h, weighed and thereafter combusted at 500°C for 40 min for determination of residual ash.

Near infrared reflectance spectroscopy predictions of iNDF

The calibration of NIRS for prediction of iNDF in Swedish farm samples ($iNDF_{NIRS-S)}$ was developed as follows. Reference samples that had been analysed at different Scandinavian research laboratories were ground through a 1.0-mm screen in a hammer mill (Kamas Slagy 200B; Kamas Kvarnindustrier AB, Malmö, Sweden) and then scanned (wavelength range 400 to 2500 nm, 2 nm intervals) with a NIR 6500 instrument (FOSS Analytical A/S, Hillerød, Denmark). Spectral data for wavelengths between 1100 and 2498 nm were transformed by applying mathematical treatment of the second order derivative (2,10,10,1 in which the values represent the number of the derivative, the gap over which the derivative is calculated, the number of points in moving average and the number of nm over which the second smoothing is applied, respectively), along with standard normal variate and detrend scatter correction (SNV&D). Samples in the calibration set consisted of fresh grass (n = 19), grass silages (n = 27), grass–clover silages (n = 16), fresh forage legumes (n = 50), whole crop cereal silages (n = 15), straw (n = 7) and forage maize samples (n = 13 of silages and n = 65 of fresh material), giving a total of 212 observations for iNDF concentrations. In all laboratories reference iNDF concentration was determined by 288-h ruminal *in situ* incubations in dairy cows using small pore size nylon bags of 12 µm according to the Nordic feed evaluation system standard (Åkerlind et al., 2011). A partial least square regression algorithm using six cross validation segments was applied for calibration and validation. The $iNDF_{NIRS-S}$ prediction was made from the dried forage maize samples ground through a 1-mm screen using a hammer mill (Kamas Slagy 200B; Kamas Kvarnindustrier AB, Malmö, Sweden) and scanned with a NIR InfraXact instrument (FOSS Analytical A/S, Hillerød, Denmark). Predicted iNDF concentrations were based on scanning across a wavelength range of 400 to 2500 nm at 2 nm intervals and using the NIRS calibration described above.

For NIRS predictions in Finland, dried forage maize samples were ground through a 0.8 mm screen with a Laboratory Mill 3100 (Danfoss AB, Linköping, Sweden) before being scanned with a NIR systems XDS (FOSS Analytical A/S, Hillerød, Denmark). Samples were scanned through a wavelength range of 400 to 2500 nm at 2 nm intervals and trimmed to a NIRS region of 1100 to 2498 nm. The NIRS calibrations for iNDF and digestible OM in the DM (D-value) were developed with WinISI™ 4

software (FOSS Analytical A/S, Hillerød, Denmark) using spectral data between 1100 and 2498 nm. A modified partial least square regression method (MPLS) using four cross-validation groups was applied for calibration and cross-validation. The external validation of D-value calibration was carried out with 50 samples not included in the calibration data set. The mathematical transformation included second order of derivative (2,4,4,1), along with SNV&D. The iNDF ($iNDF_{NIRS-F}$) and D-value predictions generated from the Finnish NIRS instrument were based on a combined silage-grass calibration consisting of samples of fresh forages (including legumes, and red clover and grass mixtures; n = 116 and 198), grass silages (n = 278 and 427), legume silages (including pure red clover and lucerne silages, and legume and grass silage mixtures; n = 41 and 59), whole crop silages (different crops and mixtures, also mixtures with pea and bean, but no forage maize samples; n = 26 and 86) and straw (n = 0 and 11), giving a total of 461 and 781 observations for iNDF and D-value, respectively. Ruminal *in situ* incubations for 288-h in dairy cows using small pore size nylon bags (6–17 μm) was used as reference method for the NIRS calibration of iNDF. *In vitro* pepsin-cellulase solubility of OM validated against *in vivo* OMD determined in sheep fed at maintenance level of feeding was used as reference method in the Finnish NIRS calibration of D-value (Nousiainen et al., 2003).

To challenge the present iNDF calibrations based on multiple species, a new NIRS iNDF calibration for the 72 forage maize samples ($iNDF_{NIRS-M72}$) was developed with the WinISI™ 4 software using Finnish NIRS scans resulting in spectral data between 1100 to 2500 nm. The calibrations were calculated with the MPLS method. Mathematical treatments of the first and second order derivatives (1,4,4,1 or 2,4,4,1) were used to enhance the spectral differences, and SNV&D was used as a scatter correction method. Calibration accuracy was evaluated by cross validation splitting data set to 4 subsets. The best NIRS calibration model was selected based on coefficient of determination (R^2), cross validated coefficient of determination (1-VR), standard error of cross validation (SECV) and the standard deviation (SD)/SECV.

Prediction equations of OMD

The OMD (given in g · kg^{-1} in all equations below) calculated from *in situ* incubation values and NDF concentrations ($OMD_{IN\ SITU}$) was used as observed values in this study. The $OMD_{IN\ SITU}$ was determined according to Huhtanen et al. (2013; equation 1):

$$OMD_{IN\ SITU} = 882 - 1.21 \times iNDF - 0.106 \times NDF \quad (1)$$

where: concentrations of iNDF and NDF are given in g · kg^{-1} DM.

Predicted OMD from EFOS values (OMD_{EFOS}) for all samples was determined according to Søegaard et al. (2001; equation 2):

$$OMD_{EFOS} = 204 + 0.727 \times EFOS \quad (2)$$

where: concentration of EFOS is given in g · kg^{-1} DM.

Predicted OMD from VOS values (OMD_{VOS}) for all samples was determined according to Lindgren (1983; equation 3):

$$OMDVOS = -20 + 0.90 \times VOS \quad (3)$$

where: concentration of VOS is given in g · kg^{-1} DM.

Predicted OMD from Finnish NIRS calibration generated D-values (OMD_{NIRS-F}) was calculated according to equation 4:

$$OMD_{NIRS-F} = \text{D-value} \times 1000/OM \quad (4)$$

where: D-value and OM are given in g · kg^{-1} DM.

Statistical analysis

All predictions were evaluated by linear regression. Observed values (y) were plotted against model predicted values (x) and a regression equation was fitted. Hypothesis testing (the slope is not different from unity) was conducted based on the parameters 95% confidence intervals. Further, predictions of forage maize OMD were evaluated by residual analysis as described by St-Pierre (2003). Predicted values were centred by subtracting the mean of predicted values from each prediction within laboratory method. This centred data points to a mean value of zero. Prediction equations were evaluated by regressing residual values (observed – predicted) on centred predicted values using the REG procedure (SAS Inc., 20020–2003, Release 9.2; SAS Institute Inc. Cary, NC). Slope and intercept of the regression lines were orthogonal and can thereby be assessed independently. Slopes and intercepts of regression equations were used to determine the presence of linear and mean biases (St-Pierre, 2003). Root mean square error of prediction (RMSEP) was calculated as:

$$RMSEP = \sqrt{[\Sigma\ (Observed - Predicted)^2/n]}.$$

Additionally, performance of the *in vitro* laboratory methods and direct D-value calibration by NIRS was further evaluated by regressing residuals vs centred forage maize NDF or starch concentration using simple regression.

Results

Digestibility related measures of the forage maize and performance of the new NIRS calibration

In situ determined and NIRS predicted iNDF values, and observed ($OMD_{IN\ SITU}$; equation 1) and predicted OMD of the forage maize across hybrids, sites and harvests are presented in Table 1. Values of OM solubility generated from the EFOS method were generally lower than the values using the VOS method. The range of *in situ* determined iNDF was not reflected in either the Finnish or Swedish NIRS predicted iNDF. The existing Finnish NIRS calibrations predicted higher minimum and maximum values of iNDF compared to the *in situ* derived iNDF. The OMD_{NIRS-F} was generally slightly lower than other values of predicted OMD.

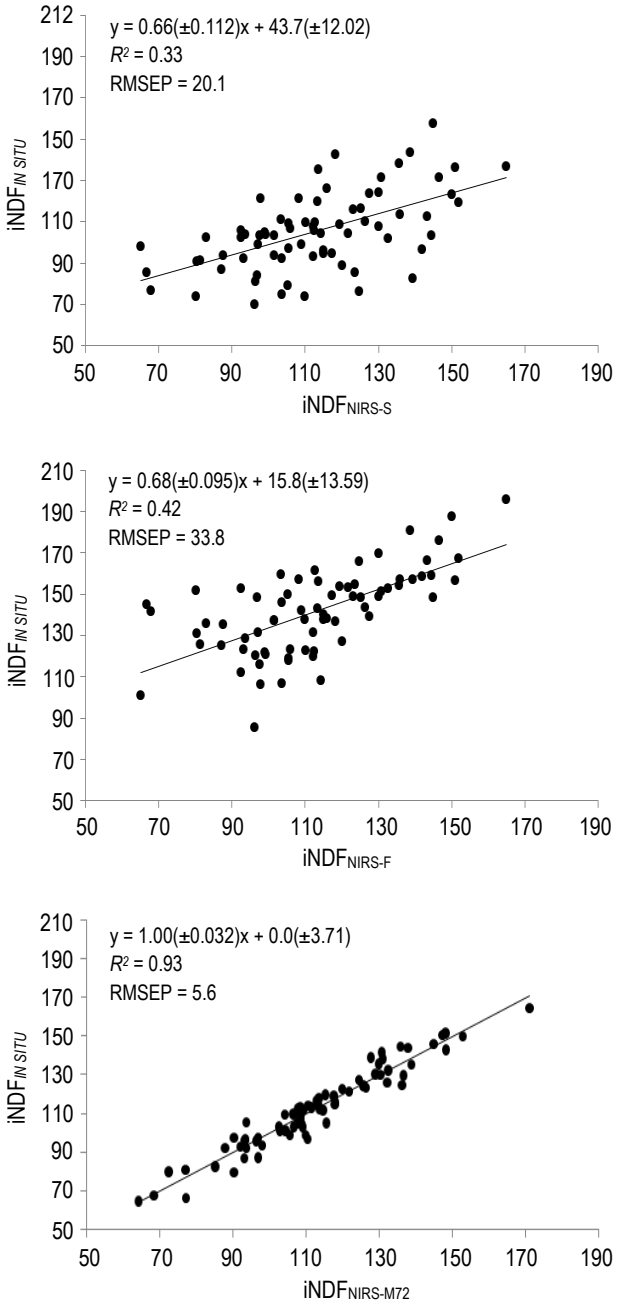

Figure 1. Relationships between predicted (NIRS-S – the Swedish NIRS calibration; NIRS-F – the Finnish NIRS calibration; NIRS-M72 – forage maize NIRS calibration) and observed (*in situ* determined) indigestible neutral detergent fibre (iNDF) for all forage maize samples (n = 72). Coefficient of determination (R^2) and root mean square error of prediction (RMSEP, $g \cdot kg^{-1}$ are given to allow evaluation of the regression equations

The best $iNDF_{NIRS}$ calibration with 72 forage maize samples was achieved using mathematical treatments of the second order derivative (2,4,4,1) and SNV&D. The R^2 value for the best calibration was 0.92, and SEC and SECV were rather low (5.9 and 10.0 $g \cdot kg^{-1}$ DM). Cross validated coefficient of determination (1-VR) was lower than R^2 of the calibration (0.78 and 0.92, respectively).The ratio SD/SECV was 2.2.

Evaluation of predictions of concentrations of iNDF

Linear regression analysis of $iNDF_{IN\ SITU}$ on NIRS predicted iNDF from Sweden and Finland and from new maize calibration ($iNDF_{NIRS-M72}$), and RMSEP are presented in Figure 1. Slope of the fitted line of observed vs model predicted was different from unity ($P < 0.05$) for both Swedish and Finnish NIRS predictions of iNDF. Slope of the fitted line of observed vs model predicted was 1.00 for $iNDF_{NIRS-M72}$. The RMSEP was greater for $iNDF_{NIRS-F}$ than for $iNDF_{NIRS-S}$, but $iNDF_{NIRS-F}$ was slightly more precise than $iNDF_{NIRS-S}$. Residual analysis showed that $iNDF_{NIRS-S}$ under-predicted $iNDF_{IN\ SITU}$ and $iNDF_{NIRS-F}$ over-predicted $iNDF_{IN\ SITU}$ ($P < 0.001$; Table 2). The bias decreased with increasing values of $iNDF_{NIRS-S}$, but that was not the case for the NIRS predictions of iNDF from Finland ($P < 0.001$; Table 2).

Table 2. Assessment of source of bias based on regression analysis of residuals of indigestible neutral detergent fibre (iNDF) and organic matter digestibility (OMD) predicted by near infrared reflectance spectroscopy (NIRS) and from different laboratory methods[1]

Predictor[2]	Intercept	SE[3]	*P*-value	Slope	SE	*P*-value
$iNDF_{NIRS-S}$	7.4	2.09	< 0.001	–0.34	0.112	< 0.001
$iNDF_{NIRS-F}$	–28.9	1.93	< 0.001	–0.32	0.095	< 0.001
OMD_{EFOS}	14.2	2.32	< 0.001	–0.28	0.076	< 0.001
OMD_{VOS}	14.2	1.95	< 0.001	0.03	0.082	0.74
OMD_{NIRS-F}	15.2	1.90	< 0.001	–0.18	0.063	< 0.01

[1] residual values were calculated as the difference between the observed and predicted values. The 288-h ruminal *in situ* determined iNDF and OMD predicted from that (Huhtanen et al., 2013) were used as observed values; [2] $iNDF_{NIRS-S}$ – from the Swedish NIRS calibration; $iNDF_{NIRS-F}$ – from the Finnish NIRS calibration; OMD_{EFOS} – predicted from the *in vitro* enzyme digestible organic matter (Weisbjerg and Hvelplund, 1993); OMD_{VOS} – predicted from rumen fluid organic matter solubility (Lindgren, 1979); OMD_{NIRS-F} – OMD predicted based on the Finnish NIRS calibration; [3] SE – standard error

Evaluation of OMD predictions

Linear regressions of $OMD_{IN\ SITU}$ values on values obtained using the other methods to determine OMD are presented in Figure 2. Slopes of the fitted regression equations were significantly different from unity ($P < 0.05$) for OMD_{EFOS} and $OMD_{NIRS\text{-}F}$ but not for OMD_{VOS} (Figure 2). The RMSEP was greatest for OMD_{EFOS} and smallest for OMD_{VOS} (Figure 2). Analysis of the residuals when comparing $OMD_{IN\ SITU}$ and predictions of OMD is presented in Table 2. Residual analysis showed that all the methods systematically slightly under-predicted $OMD_{IN\ SITU}$ ($P < 0.001$; Table 2), but the bias decreased with increasing values of OMD_{EFOS} and $OMD_{NIRS\text{-}F}$ ($P < 0.01$; Table 2). There was no significant slope bias for OMD_{VOS} ($P = 0.74$).

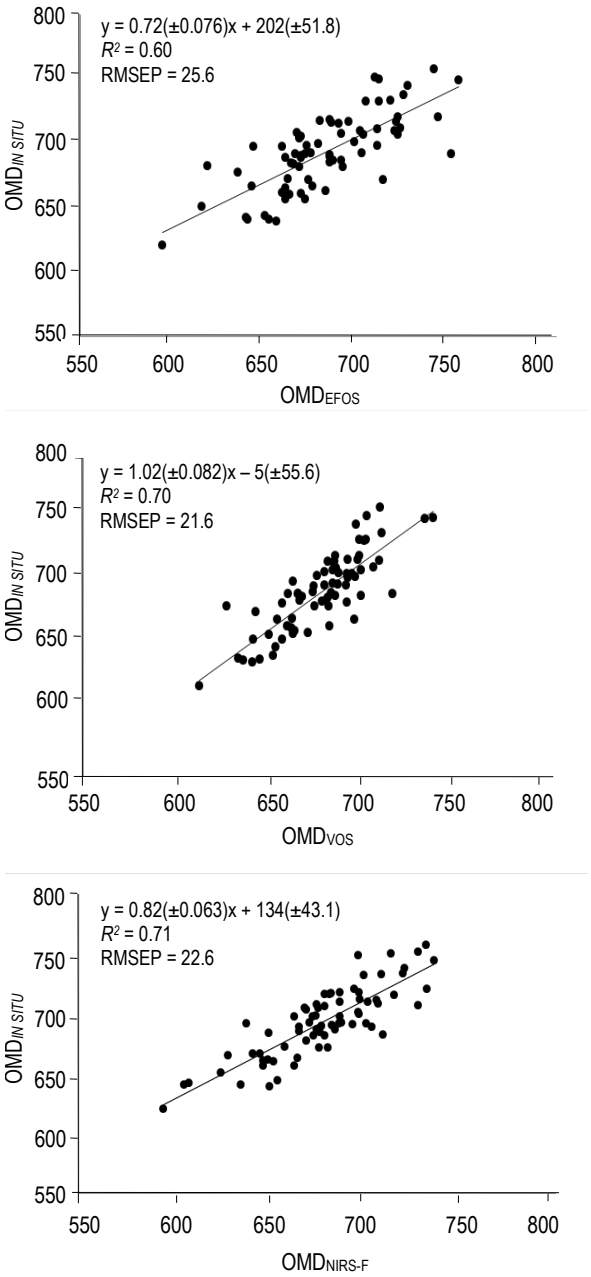

Figure 2. Relationships between predicted (EFOS – enzyme digestible organic matter; VOS – rumen fluid organic matter solubility; NIRS-F – the Finnish NIRS calibration) and observed (*in situ* determined) organic matter digestibility (OMD) for all maize hybrids (n = 72). Coefficient of determination (R^2) and root mean square error of prediction (RMSEP, $g \cdot kg^{-1}$) are given to allow evaluation of the regression equations

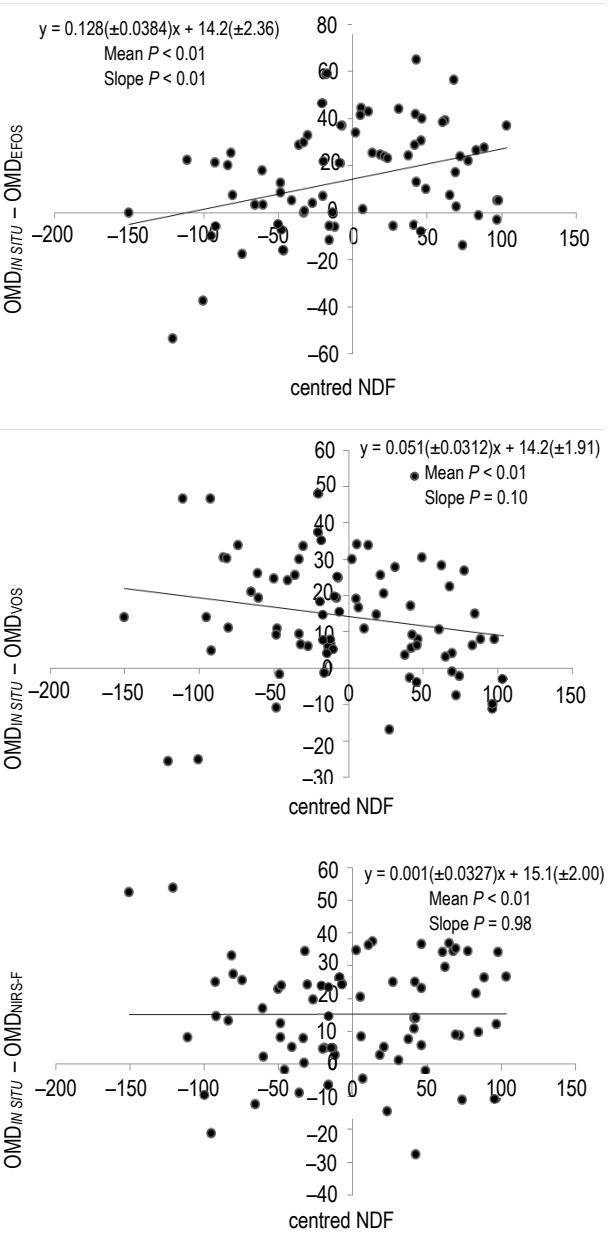

Figure 3. *Plot of residuals (observed − predicted) vs centred neutral detergent fibre (NDF) concentration in forage maize samples (n = 72). In situ determined organic matter digestibility ($OMD_{IN\ SITU}$) was used as observed values. Predicted values of organic matter digestibility were generated from enzyme digestible organic matter (OMD_{EFOS}), rumen fluid organic matter solubility (OMD_{VOS}) and the Finnish NIRS calibration of organic matter digestibility ($OMD_{NIRS\text{-}F}$). The NDF values were centered by subtracting the mean of all values from each value. P − values of mean and slope biases are given*

Effects of forage maize iNDF concentration on OMD predictions

The residual analysis of predicted OMD based on the EFOS and VOS methods generated a significant or tendency to a linear relationship to centred NDF concentrations ($P \leq 0.10$; Figure 3). Further, the analysis of residuals vs centred NDF concentrations in the forage maize samples resulted in significant mean biases for all three evaluated laboratory methods ($P < 0.01$). The results for the analysis of residuals vs centred starch concentration of all forage maize samples was a mirror image of Figure 3, i.e. indicated the same results, and are therefore not presented in the paper.

Discussion

Forage maize samples and performance of the new NIRS calibration. The objective of this study was to compare different analytical methods used for predicting OMD and iNDF concentrations in forage maize, taking into account effects of hybrid, site and maturity on the difference between observed and predicted values. Unlike other recent assessments of analytical techniques for evaluating forage quality (e.g., Gosselink et al., 2004; Krizsan et al., 2012) the plant material in this study only included one forage type. The NIRS predictive ability of feed value traits of forage maize covering a wide variation in phenological growth stages at high latitudes is not well-known. Distribution of hybrids, sites and harvesting times in this study provided a large range of agronomic performance and a novel material for evaluation of analytical methods and NIRS predictions of forage maize feed value at high latitudes. Further, the forage samples represented a wide range in attributes compared to maize grown in more southern latitudes as indicated by the variations in DM, starch and NDF concentrations (Givens et al., 1995; Givens and Deaville, 2001; Keady et al., 2008).

Prediction errors for $iNDF_{IN\ SITU}$ with $iNDF_{NIRS\text{-}M72}$ calibration were low. The calibration was made from the same samples as $iNDF_{IN\ SITU}$ was determined for. Therefore, some reservations should be made to the good performance of it. This can be seen from 1-VR that was lower than R^2 of the calibration. Further, the difference between SEC and SECV was quite large and SD/SECV ratio rather small. However, relatively small SECV, which corresponds to SE of 12.1 g · kg^{-1} in OMD (equation 1), suggests that NIRS has a great potential to predict iNDF and OMD of forage maize accurately and precisely. According to Sinnaeve et al. (1994) a SD/SECV ratio larger than 3.0 indicates that the calibration could be used for quantitative purposes, while a ratio between 2.5 and 3.0 indicates that the calibration should be used only for screening purposes. Commercial forage maize NIRS calibrations of iNDF need to be made from more samples and preferably evaluated from an independent set of data points.

NIRS predictions of iNDF. Neither Swedish nor Finnish NIRS calibration generated satisfactory predictions of the iNDF concentration in forage maize samples in this study. For the Finnish NIRS calibration of forage iNDF it can simply be explained by a lack of forage maize samples included in the calibration dataset. However, the Swedish calibration included samples of ensiled as well as fresh forage maize. Results of the Nordic ring test by Lund et al. (2004) pointed out a large between-laboratory variation in determined iNDF concentrations of different feed samples. Poor between-laboratory reproducibility of the *in situ* technique has also been reported for ruminal protein degradability (Madsen and Hvelplund, 1994). The Nordic feed evaluation system NorFor introduced a standardized protocol for *in situ* determination of iNDF in 2007 aiming to decrease variation observed between laboratories in Nordic countries (Eriksson et al., 2007). The standardized procedure of *in situ* determination of iNDF was later presented by Åkerlind et al. (2011). The procedure mainly introduced recommendations of bag cloth type, numbers of animals and length of incubation (Åkerlind et al., 2011). However, many factors still seem to contribute to variation in analytical precision of the iNDF procedure between laboratories as indicated by the most recent Nordic ring test by Eriksson et al. (2012). A decomposition of the observed variance components has not been conducted and the greatest sources of variation in the procedure have not been properly identified. Further, implementation of the standard protocol might differ between laboratories and makes it difficult to correctly interpret results of ring tests. Another major problem complicating comparisons of results obtained using different analytical procedures iNDF in ring tests, as well as in this study, is the need for relevant *in vivo* data with which to compare results with; to identify which procedure gives the most biologically correct values. A reason for the poor performance of Swedish NIRS predictions of iNDF in this study could be that reference data in the calibration originated from different laboratories using different procedures.

Despite that there were no forage maize samples in the Finnish reference data set of iNDF the Finnish iNDF calibration generated more precise predictions than the Swedish iNDF calibration. Accuracy and robustness of NIRS calibrations depend on the quality and representativeness of the calibration data set. The calibration sample population must cover all sources of variation in chemical, physical and botanical characteristics of the samples to be analysed (Deaville and Flynn, 2000). To generate reliable NIRS calibrations used for quantitative purposes all forage types to be predicted should be included in the calibration sample population. Different sample types produce different NIR spectra and therefore calibration data set must cover even small spectral variations to generate accurate and precise predictions. Strong relationships between OMD predictions obtained from the NDF and iNDF values with other laboratory methods (EFOS, VOS and direct D-value calibration) supported this suggestion (r = 0.75, 0.83 and 0.84, respectively). Furthermore, accurate and precise predictions of *in vivo* OMD using the current iNDF method (Krizsan et al., 2012) for a large sample set including maize silages support the reliability of the iNDF *in situ* method.

Reference method for *in vivo* OMD determination. The ideal reference values for OMD *in vivo* digestibility determined in sheep fed at maintenance, but for practical reasons this is not possible to apply routinely. Digestibility determinations *in vivo* require much large amount of the forage samples and are more cost demanding compared to laboratory methods. Instead of OMD determined *in vivo*, we used predictions of OMD based on forage iNDF and NDF concentrations as reference method in the present study. This decision relied on findings by Huhtanen et al. (2006) and Krizsan et al. (2012) that OMD predicted from forage iNDF concentration generated least prediction error compared to *in vivo* digestibility data. The method was also more uniform across forage types than any other laboratory method evaluated. Hetta et al. (2012) modelled *in vivo* OMD from whole plant forage maize samples based on *in vitro* gas production recordings. However, the data was not related to in animal determined *in vivo* OMD values. In a recent review by Huhtanen et al. (2013) it was concluded that predicting OMD from forage iNDF and NDF concentrations provided smaller prediction error compared to *in vivo* OMD equation that only included the concentration of iNDF. The EFOS method, which has been recommended for evaluating forage maize (Åkerlind et al., 2011), had a larger prediction error of OMD for forage maize samples in the equation developed by Søegaard et al. (2001) than the general iNDF-equation (across forage types and not forage specific) developed by Huhtanen et al. (2006).

Prediction of OMD based on EFOS. The EFOS method generated the poorest prediction of $OMD_{IN\ SITU}$ compared with the other methods evaluated in this study. The prediction error of $OMD_{IN\ SITU}$ in this study was smaller than that presented by Søegaard et al. (2001) when developing the relationship between *in vivo* OMD of forage maize and the EFOS method. Despite this, EFOS is the recommended laboratory method for whole crop maize in the Nordic feed evaluation system NorFor (Åkerlind et al., 2011). Poor prediction of $OMD_{IN\ SITU}$ might be due to limitations of enzymes compared to rumen fluid microorganisms used *in vitro*, in agreement with other recent comparisons in the literature (Koukolová et al., 2004; Jančík et al., 2011; Krizsan et al., 2012). Further, differences between determined *in vitro* and *in vivo* digestibility may vary among forage types and species. Predictions of *in vivo* OMD can theoretically be improved by developing forage-specific equations (Huhtanen et al., 2006), and even specific equations for different grass species as suggested by Jančík et al. (2011). However, such equations are not useful for evaluating diets and forage mixtures in practice on farms, and not either in plant breeding programmes for comparing individual hybrids and varieties, since the specific botanical composition and taxonomy of forages is difficult and laborious to determine.

The strong slope bias in the analysis of residuals vs centred NDF concentrations indicated that the enzyme method was not able predict accurately the effects of growing conditions, hybrid or plant maturity on OMD. Despite its poor predictive ability, the EFOS method has a major advantage in that rumen cannulated animals are not required. Thus, the technique should be easier to implement in laboratories and may be easier to standardize, like other enzymatic methods (Jones and Theodorou, 2000).

Prediction of OMD based on VOS. Although the regression equation related to the VOS technique described by Lindgren (1979) was primarily developed for predicting *in vivo* OMD digestibility of perennial grasses and legumes, in this experiment the VOS method was the best predictor of $OMD_{IN\ SITU}$. This is in accordance with previous reports that the VOS method is a good predictor of *in vivo* OMD (Krizsan et al., 2012). The under-prediction of OMD adapting the VOS technique was greater in this study than what Krizsan et al. (2012) found when comparing the predictions with values of *in vivo* OMD in maize

silage samples. The VOS technique has the advantage over other *in vitro* methods of using smaller amount of rumen fluid and longer incubation period thereby being less sensitive to fluctuations in condition of the inoculum (Krizsan et al., 2012). The tendency for a slope bias in the analysis of residuals vs centred NDF concentrations indicated that the method was affected by either growing conditions, hybrid or plant maturity.

Predicting OMD by NIRS. Prediction of $OMD_{IN\ SITU}$ from the Finnish D-value NIRS calibration displayed mean and slope biases, but prediction was almost as precise as by OMD_{VOS}. One of the limitations associated with NIRS predictions of OMD is that pepsin-cellulase OMD as reference method requires forage-specific equations (Nousiainen et al., 2003). Although NIRS calibration has performed well for a multitude of forage species (Nousiainen, personal communication), reference plant material in the Finnish calibration did not include any forage maize samples. Taking these factors into consideration, performance of NIRS predictions of *in vivo* OMD in forage maize cultivated at high latitudes have potential to be improved by extending the calibration sample set to also include forage maize samples with a wide variation in maturity. This is also supported by the results by Deaville et al. (2009), who achieved better predictive ability by NIRS of *in vivo* digestibility and metabolizable energy values than of *in vitro* traits and chemical composition data of whole crop cereals. Further, it was the technique that performed best in the analysis of residuals vs centred NDF concentrations. This indicates that the method has similar prediction error irrespective of growing conditions and plant maturity, factors that are known to influence forage maize quality (Cox et al., 1994).

Conclusions

Both Swedish and Finnish near infrared reflectance spectroscopy (NIRS) predictions of forage maize indigestible neutral detergent fibre (iNDF) concentrations were biased. None of the laboratory methods and equations relating to *in vivo* organic matter digestibility (OMD) in this study qualified for unbiased prediction of forage maize feed value. In comparison, all three prediction methods that were evaluated in this study systematically underestimated $OMD_{IN\ SITU}$ concentration in forage maize. Additionally, regression analysis of the residuals indicated that both OMD_{EFOS} and $OMD_{NIRS\text{-}F}$ were associated with slope biases, which will result in an even greater underestimation of feed value of forage maize with decreased digestibility. The NIRS was the technique least sensitive to effects of hybrid, site and maturity in its predictions of OMD. For OMD_{EFOS} and OMD_{VOS}, a larger reference data set is needed to facilitate accurate prediction of *in vivo* OMD. Based on results of this study we conclude that NIRS predictions of OMD can be a promising tool in the evaluation of feed value of forage maize at high latitudes if NIRS calibrations of iNDF including forage maize samples are generated.

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