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Influence of sample temperature on the assessment of quality characteristics in undried forages by Near Infrared Spectroscopy (NIRS)

Christian Paul¹, Frauke Dietrich¹ and Michael Rode²

Abstract

The aim of establishing a continuous quality control system on forage harvesters by NIRS requires a proper understanding of the fundamental processes and effects of varying sample temperatures on NIR absorption. So, diffuse reflectance spectra of undried forages obtained at sample temperatures between 5 °C and 20 °C on two different types of NIR spectrometers are analysed to investigate the extent and causes of temperature induced peak shifts. A shift of the 1450 nm peak of these forage spectra is confirmed to correspond in magnitude and temperature dependence to the shift of the OH- first overtone region in published physico-chemical studies on pure water. Further-more peak decomposition indicates for the first time in fully hydrated plants that the shift is also caused by changes in the relative intensities of OH-bands characteristic of water molecules differing in the extent of hydrogen bond formation.

A calibration experiment was carried out on the same plant material to quantify the importance of sample temperature in the prediction of the content of dry matter (DM), crude protein (CP), crude fibre (CF), water soluble carbohydrates (WSC) and enzymatically insoluble organic matter (EIOM) of undried forages by NIRS. A calibration performed on samples at room temperature (20 °C) gave rise to systematic errors (bias) as high as 4,6 % DM when applied to validation samples maintained at 10 °C. The bias problems were avoided when a different calibration strategy – i.e. by representation of a broad range of sample temperatures in the calibration set – was followed. Analogous results were also observed for the additional quality parameters.

In their corresponding spectral range the two NIR spectrometers tested (diode array vs. scanning monochromator) provide similar spectral information and similar predictions of forage quality characteristics. These results thus provide a basis for the use and calibration of NIR diode array spectrometers on forage harvesters.

Key words: near infrared spectroscopy, sample temperature, scanning monochromator, diode array spectrometer, forage, forage quality, dry matter prediction

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Zusammenfassung

Einfluss der Probentemperatur auf die Erfassung von Qualitätsmerkmalen in ungetrockneten Futterpflanzen mittels Nah-Infrarot-Spektroskopie (NIRS)

Das Ziel einer kontinuierlichen Qualitätstkontrolle auf Futterertemaschinen mittels NIRS erfordert ein verbessertes Verständnis der grundlegenden Prozesse und Wirkungen wechselnder Probentemperaturen auf die Absorption im Nahen Infrarot.


Ein Kalibrierungsexperiment an gleichen Pflanzenmaterial diente zur Quantifizierung der Bedeutung der Probentemperatur für die Schätzung des Gehalts an Rohprotein, Rohfaser, wasserlöslichen Kohlenhydraten und der enzymatisch nicht abbaubaren organischen Substanz in ungetrockneten Futterpflanzen mittels NIRS. Eine an 20 °C-Proben erstellte Kalibrierung führte in der Validierung an 10 °C-Proben zu systematischen Fehlern bis zu 4,6 % Trockennasse. Die systematischen Fehler konnten durch eine angemessene Kalibrierungsstrategie vermieden werden, die in der Repräsentanz einer breiten Spanne von Probentemperaturen bestand. Analogere Ergebnisse wurden für die weiteren Qualitätsparameter erzielt.


Schlüsselwörter: Nah-Infrarot-Spektroskopie, Probentemperatur, Gitterspektrometer, Diodenzeilenspektrometer, Futterpflanzen, Futterqualität, Trockennmasseschätzung
1 Introduction

In the near infrared part of the electromagnetic spectrum overtones and combinations of fundamental vibrations of the OH-, NH- and CH-bonds omnipresent in the organic constituents of forages are observed. Due to the enormous complexity of these absorptions the only option for utilizing information from the near infrared for quantitative assessment of composition has been found to consist in diffuse reflectance measurements combined with empirical statistical modelling. The advantages claimed in the first application of this approach for assessing forage quality (Norris et al. 1976), i.e. “speed, simplicity of sample preparation, multiplicity of analysis with one operation, and non-consumption of sample” have all since been substantiated in numerous reports (see review by Shenk and Westerhaus 1994). In fact, Near Infrared Reflectance Spectroscopy (NIRS) can now be considered as the methodological cornerstone for comprehensive forage quality testing in routine analytical laboratories around the world. Not surprisingly the considerable speed of analysis has contributed strongly to the widespread acceptance of NIRS in forage test laboratories.

The goal of rapid forage testing might be reached even more convincingly if the usual procedure of sample drying and grinding prior to analysis could be skipped and if forages could be subjected to NIRS directly in their fully hydrated form i.e. as undried and unground samples (see Shenk 1993). The extension of this concept into on-line analysis of forages at the time of harvesting (Paul et al. 2000, Paul and Haeusler 2002) adds yet another dimension. However, if NIRS analysis of forages under field conditions is to be developed into a successful application the influence of temperature on the NIRS absorption of fully hydrated samples needs to be understood. Except for the studies by Williams et al (1982) and Iwamoto et al (1987) investigations on how temperature affects NIR diffuse reflectance spectra and quality assessments of agricultural products have not been reported in the literature.

The present study aims at the quantification of these effects and their consequences on the calibration of two different types of NIR spectrometers, i.e. a diode array instrument and a scanning monochromator.

2 Materials and methods

2.1 Plant material

A set of 94 forage samples (50 grass samples, 27 legume samples and 17 grass-legume mixtures) was available for this study. The original dry matter content of the samples, at harvest ranged from 10 to 42 %, from 10 to 32 % and from 21 to 32 % for grass, legume and mixed samples respectively. After harvesting each sample was chopped in a laboratory chopper to a theoretical chop length of 0.5 to 1.0 cm, divided into three portions of which two were deep frozen at –28 °C and one subjected to dry matter reference analysis by determining forage weight loss after oven-drying at 105 °C for 24 hours. One of the deep frozen portions was stored for later NIRS measurements, the other was freeze dried and used for a number of chemical reference analyses.

2.2 Chemical reference analyses

Water soluble carbohydrates (WSC): cold water soluble carbohydrates were quantified using anthrone according to Deriaz (1962) and given as WSC % of the dry matter.

Crude protein (CP): nitrogen was determined after boiling of the sample in concentrated sulfuric acid and catalytic conversion to ammonia according to Kjeldahl. Crude protein was calculated based on the usual conversion N x 6.25 and given as CP % of the dry matter.

Crude fibre (CF): the Weende feed fractionation method originally developed by Henneberg and Stohmann was used in its adaptation to the Fibertec system and given as CF % of the dry matter.

Enzyme insoluble organic matter (EIOM): the content of cellulose insoluble organic matter was assessed according to a modification of the method by de Boever et al (1988) and given as g EIOM / kg.

2.3 Temperature measurement

After having been taken out of the freezer at about –28 °C, the samples were allowed to reach –18 °C and filled into large rectangular sample cells (dimensions of sample cell window (202 x 47 mm) designed to fit the sample transport module of the NIRSystems 6500 instrument. During thawing of the samples (at room temperature) the actual temperature of the sample surface was measured continuously by means of a non-contact infrared thermometer (High Performance Infrared Thermometer Raynger MX4, Raytek, Santa Cruz, USA). Each sample, upon having reached the temperature threshold of +5 °C was measured first on the NIR diode array instrument and afterwards without any delay on the NIR scanning monochromator (see below). Sample temperature equilibration was allowed to proceed further and each sample was subjected to the same sequence of NIRS measurements as above upon having reached the temperature thresholds of +10 °C, +15 °C and +20 °C, respectively.

2.4 NIR diode array spectrometer

A NIR diode array spectrometer (Carl Zeiss Jena GmbH, Jena, Germany) was used which is based on an array of 128 InGaAs-diodes permitting a resolution corresponding to a data point interval of 6 nm across the spec-
tral range between 950 nm and 1700 nm (MMS-NIR 1.7) attached to a light fibre diffuse reflectance measuring head (OMK). The measuring head was contained in a self-constructed module for scanning the type of large rectangular sample cell originally designed for the NIRSystems 6500 sample transport module (see Fig. 1). Each chopped fresh forage sample was placed in this type of cell and the reflectance of each sample measured sequentially on the diode array instrument at five equidistant positions along the sample cell window (55 ms integration time) after manual movement of the sample cell. After each sample, readings from a ceramic disk were obtained for referencing and the resulting five sample scans averaged.

2.5 NIR scanning monochromator

A spectrometer (NIRSystems 6500, Silver Spring, USA) equipped with a scanning monochromator and Si and PbS detectors for reflectance measurements in the visible and near infrared region (400 – 2500 nm) was used. Each sample in the large sample cell was scanned 32 times during automated sequential upward and downward passage of the cell in front of the detector assembly in a sample transport module. Reflectance (R) data were averaged over the 32 scans and stored as log 1/R at 2 nm intervals to give a total of 1050 data points. Reflectance energy readings were referenced to alternate readings from a ceramic disk.

2.6 NIRS-Software

The NIR scanning monochromator was controlled using DOS based software by Infrasoft International (version 4.01, originally marketed by Perstorp Analytical Inc., Silver Spring, USA) and the NIR diode array instrument was controlled by ZEISS Aspect Plus software (Carl Zeiss Jena GmbH, Jena, Germany). After transformation of the NIRS data, graphical representation of the data, NIRS calibration and NIRS validation for data from both types of sources was performed by WINISI II software (version 1.50, originally marketed by NIRSystems Inc., Silver Spring, USA).

2.7 Population structuring

For both types of NIR spectrometers data sets were created which consisted of the 94 samples (see plant material) characterised by NIR spectral data obtained either at 5 °C, 10 °C, 15 °C or 20 °C and the reference data. The samples of each of the data sets were ranged according to dry matter content and subdivided by sorting every second sample into the validation set and using the remaining samples for the calibration set. Thus, across temperatures equally well balanced sample sets containing 47 samples each were available for calibration and validation for dry matter content (DM %). Two different approaches for calibration were taken: a) the single temperature calibration set contained the samples with spectral data taken at 20 °C only (n = 47; Single-T) and b) the multiple temperature calibration set contained the samples with spectral data across the temperatures 5 °C, 10 °C, 15 °C and 20 °C (n = 188; Multiple-T). To account for the fewer samples with known chemical reference values the calibration and validation sets were merged for these parameters and the evaluation for the Single-T and Multiple-T sets based on cross-validation. In this case, for later NIRS prediction, three forage groups differing in maturity were defined and formed according to dry matter content (10-20 % DM; 20-30 % DM and 30-42 % DM).

2.8 Calibration

Zeiss MMS NIR 1.7: For regression analysis the full spectral data output from 966 – 1684 nm after interpolation to 2 nm data point intervals was used, transformed into first derivative (derivative 1; gap 4; segment 4; smooth 1) and normalized by use of the standard normal variate (SNV) transformation with detrending (Det). Across the 966 – 1684 nm range 177 equally spaced NIR intensity variables were admitted for analysis which was performed using the ISI version of the modified partial least squares (MPLS) regression. Outliers were not eliminated during regression and the complexity of the final MPLS-regression model was restricted to 7 regression terms both in case of the Single-T and Multiple-T calibration.

NIRSystems 6500: For regression analysis spectral data were restricted to the PbS-range (1100 – 2500 nm) and transformed as above. Across the 1100 – 2500 nm range 173 equally spaced NIR intensity variables were admitted for analysis which was also performed by ISI MPLS
As above, outlier removal was disallowed and the MPLS-regression model restricted to 7 regression terms for Single-T as well as Multiple-T calibration.

2.9 Validation/prediction

The MPLS-calibration models for DM % established on the basis of Single-T and Multiple-T calibration for both the Zeiss MMS-NIR 1.7 and the NIRSystems 6500 were applied to the respective validation sets and the predicted DM % values regressed against those obtained by reference analysis. The validation statistics were computed as: coefficient of determination (RSQ), standard error of prediction (SEP), standard error of prediction corrected for bias (SEP(C)) and bias (reference values – NIRS predicted values). In the case of the chemical reference parameters the respective calibration models established on the basis of Single-T and Multiple-T calibration for both the Zeiss MMS-NIR 1.7 and the NIRSystems 6500 were applied to the low, medium and high DM sample sets. For bias calculations between the results of Single-T and Multiple-T calibration within group averages were computed for NIR predicted data as well as for chemical reference parameters.

3 Results and discussion

3.1 Effect of sample temperature on NIR spectra of forages

Some or even all the major absorption bands of water in the near infrared at 960 nm, 1150 nm, 1440 nm and 1930 nm are functionally relevant for the quantitative assessment of the content of water or – vice versa – dry matter in foodstuffs and feedstuffs by whatever NIR instrument. Yet, the band at 1440 nm seems better suited than others for more detailed studies on the state of water in forages. On one hand side, in this region there is less interference arising from overtones by other contributing molecular functionalities than OH-groups, especially in comparison to the situation near the OH-combination bands at 1930nm (Goddu and Delker 1960). On the other hand, the molar absorptivity of water in this region is clearly higher than at the OH-absorptions at 960 nm and 1150nm.

Average NIR spectra, representative for the grass-legume mixtures and their components in this study, show a clear maximum of 1.3 to 1.5 log 1/R at 1450 nm (Figs. 2 and 3) after spectral normalisation according to Barnes et al (1989). On the diode array spectrometer as well as on the scanning monochromator a rise in sample temperature from 5 °C to 20 °C causes a shift of this absorption maximum to lower wavelengths by about 6 nm. This value corresponds to a 30 nm shift for a temperature rise from 20 °C to 80 °C observed in transmission measurements in distilled water by McCabe et al (1970). Probably as a consequence of remaining interfering CH- and NH-bands in this region, the temperature induced variation leads an isosbestic (crossover) point of the forage spectra clearly above that noted for pure water at 1442 nm (see Iwamoto et al 1987). Whether the displacement of the isosbestic point on the diode array spectrometer to longer wavelengths in comparison to the scanning instrument is a consequence of differences in signal detection or initial spectral data treatment is a matter of ongoing research. But otherwise our results demonstrate that in strictly comparable measurements the spectral data resulting from a diode array spectrometer are essentially equivalent to those from a scanning monochromator.

The mathematical decomposition of the absorption provides an insight into the structure of water existing in forage plants. Bulk water is assumed to be structured as a consequence of hydrogen bonding in such a way that three types of molecules are possible: Water molecules with zero ($S_0$), one ($S_1$) and two hydrogen bonds ($S_2$). The extent of hydrogen bonding is temperature dependent. Buijs and Choppin (1963) assume increasing temperature to change the proportion of molecular species from $S_2$ to

![Fig. 2](image1.png)

Average grass and legume forage spectra in log 1/R (SNV + det) of water peak at 1440 nm measured at Zeiss MMS NIR 1.7 diode array spectrometer (arrows indicate direction of peak shift)

![Fig. 3](image2.png)

Average grass and legume forage spectra in log 1/R (SNV + det) of water peak at 1440 nm measured at NIRSystems 6500 scanning spectrometer (arrows indicate direction of peak shift)
towards S0. Accordingly, the OH-absorption maximum shown in Figs. 2 and 3 would consist of several definite absorption bands representative of the molecular species S0, S1 and S2. This can be confirmed by means of spectral deconvolution or differentiation. In Figs. 4 and 5 we have utilized the second derivative which achieves a separation of the log 1/R forage spectra into bands which do correspond to the S0-, S1- and S2-positions given by Iwamoto et al. (1.c.) as underlying the water absorption at 1440nm. Based on these observations the absorption shown in Figs. 2 and 3 can be interpreted as consisting of three overlapping bands (equivalent to the S0-, S1- and S2-species). A change in sample temperature does not seem to affect the wavelength position of these individual bands. It follows that the observed temperature-induced shift of the absorption maximum at 1450 nm would result from a change in the relative intensities of the three separate bands. Indeed, with increasing temperature an increase in intensity of the 1412 nm band (S0) mainly at the expense of the band at 1466 nm (S1) is observable and serves as an explanation.

It seems noteworthy that prior to this study temperature effects on the water absorption at 1440 nm appear never to have been investigated in fully hydrated plants. Furthermore, numerous reports in the scientific literature which deal with pure water are by necessity restricted to the analysis of hydrogen bridging with oxygen atoms. Despite its biological importance, hydrogen bridging with nitrogen atoms (located in proteins either as cell solutes or bound in cell walls) appears to have received very little attention. Further studies in this field are encouraged in order to expand our knowledge.

3.2 Calibration for dry matter content of undried forages

Empirical modelling is the method of choice for establishing relationships between NIR spectral data and compositional data. While for this purpose powerful mathematical algorithms are available as software tools the experimenter still needs to set up a suitable data base which puts him in a position to develop calibration equations for predicting composition rapidly from spectral data alone. In this context, the urge to bring NIRS prediction closer to field conditions certainly represents a new challenge since NIRS measurements on forages are usually performed at room temperature.

Our calibration experiment was set up in the laboratory to be able to focus solely on sample temperature. Its control in agricultural products such as forages or grains during online measurements at harvesting is economically not feasible under field conditions and provides the motive for the study presented here. Obviously a NIR diode array spectrometer as the prime candidate of instru-

Table 1

<table>
<thead>
<tr>
<th></th>
<th>Zeiss MMS NIR 1.7</th>
<th>NIRS Systems 6500</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIRS Calibration set</td>
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<td>Multiple T EQA6</td>
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<td>SEC</td>
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<td>0.95</td>
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<tr>
<td>NIRS Calibration set</td>
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<td>Multiple T EQA6</td>
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<tr>
<td>SD</td>
<td>7.60</td>
<td>7.53</td>
</tr>
<tr>
<td>SEC</td>
<td>1.34</td>
<td>1.66</td>
</tr>
<tr>
<td>RSQ</td>
<td>0.97</td>
<td>0.95</td>
</tr>
</tbody>
</table>

1 Range of sample temperatures in calibration set: 5 °C, 10 °C, 15 °C and 20 °C
2 standard deviation
3 standard error of calibration
4 coefficient of determination
5 NIRS equation established at calibration sample temperatures of 20 °C
6 NIRS equation established across calibration sample temperatures of 5 °C, 10 °C, 15 °C and 20 °C
mentation for the tough conditions of agricultural and industrial production environments needed to be considered in such a calibration exercise on undried forages. Understandably, the assessment of dry matter content as the first indicator of total nutrient content in a feedstuff receives highest attention.

The MPLS-regression equations for % DM of undried forages measured at 20 °C (Single T) on two different NIR spectrometers are established with a high degree of fit as indicated by the coefficient of determination (RSQ) of 0.96 and 0.97 for diode array and scanning monochromator respectively (see Table 1). There is a slight tendency for the standard error of calibration (SEC) for % DM to be higher for the diode array than for the scanning monochromator, with 1.56 and 1.34, respectively. When calibrations are performed across the sample temperatures of 5 °C, 10 °C, 15 °C and 20 °C again only tendencies are observed towards slightly lower RSQ values and higher SEC values as compared to calibrations at 20 °C (Multiple T vs Single T). However, as the differences in analytical performance between instruments and between NIR equations resulting from either Single T or Multiple T calibration sets are not significant in the statistical sense (p=.05), an average SEC of 1.5 % DM with about 95 % of the total observed variation in % DM being explained by the NIRS predictions can be assumed to be generally valid for the experimental situations studied here. The extreme diversity of the forage samples in terms of botanical origin, the high variability of physiological stages ranging from purely vegetative to highly differentiated forages in the generative stage and the limitations imposed by the small number of calibration samples may serve as explanations for the relatively high SEC.

The forage samples of the validation sets measured at 10 °C, 15 °C and 20 °C on the two different NIR spectrometers were predicted by the NIRS equations resulting from Single T calibrations. DM % assessed by oven-drying is plotted in Figs. 6 and 7 against NIRS Single T predicted % DM % obtained on the diode array and the scanning monochromator, respectively. While for both instruments NIRS predicted % DM values of the validation samples measured at 20 °C correspond well with those obtained by the oven-drying method, biased DM % predictions are obvious for the validation samples measured at 10 °C and 15 °C.

The extent of the bias is higher for 10 °C samples than for 15 °C samples and increases with dry matter content. As an example, 15 °C samples are predicted at 23.0 % DM at a given 25 % DM while 10 °C samples are predicted at 22.7 % DM by the Single T calibration in the case of the diode array spectrometer. The SingleT calibration established for the scanning monochromator instrument results in slightly higher bias values than the equivalent Single T calibration for the diode array on samples. By continuing the above example, 15 °C samples are predicted at 22.9 % DM at a given 25 % DM while 10 °C samples are predicted at 20.4 % DM by the Single T calibration in the case of the scanning monochromator.

A different situation emerged when the forage samples of the validation sets with sample temperatures of 10 °C, 15 °C and 20 °C were predicted by the NIRS equations resulting from Multiple T calibrations (Figs. 8 and 9). The regression lines for the relationships between %DM assessed by oven drying and % DM predicted by NIRS Multiple T do not exhibit statistical differences among each other in terms of either slope or point of intersection with the ordinate. In addition, this is analogous for both types of instruments tested. This finding clearly proves that NIRS equations are likely to possess an inherent analytical “robustness” towards variations in sample temperature only if they have been established on data sets “embodying” the whole temperature range to be encountered in the unknown samples. It also confirms in principle earlier work directed at the effect of minor temperature variations on NIRS predictions on wheat flour by Williams et al (1983). The importance of these results should be seen against the background of research and development projects initiated to enable on line, real time
NIRS predictions during the harvesting process. The temperature variation likely to be encountered during forage harvesting in the field between late spring and early autumn in Germany may span a range from 8 °C to 28 °C. So, as a matter of fact, the extremes in biased % DM predictions observed in this study might even be surpassed if analytical robustness towards the influence of temperature is not ensured already in the process of calibrating NIRS instruments.

3.3 Effect of temperature on NIRS prediction of diverse forage quality characteristics

The dependency of NIRS methods on empirical relationships between NIR spectral data and compositional data is often used as an argument against NIRS in general. But as in the situation for DM (see above) biased predictions of crude fibre (CF), crude protein (CP) and water soluble carbohydrates (WSC) as well as of the content of enzymatically insoluble organic matter (EIOM) in Tables

<table>
<thead>
<tr>
<th>Sample temperature</th>
<th>20 °C</th>
<th>15 °C</th>
<th>10 °C</th>
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<td>10 °C</td>
</tr>
<tr>
<td>10 - 20</td>
<td>26 CF¹</td>
<td>-0.6</td>
<td>-0.5</td>
</tr>
<tr>
<td>20 - 30</td>
<td>31 CF³</td>
<td>26.5</td>
<td>0.7</td>
</tr>
<tr>
<td>30 - 42</td>
<td>17 CF¹</td>
<td>28.2</td>
<td>-0.4</td>
</tr>
<tr>
<td>10 - 20</td>
<td>38 CP²</td>
<td>19.2</td>
<td>0.0</td>
</tr>
<tr>
<td>20 - 30</td>
<td>35 CP²</td>
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<td>-0.1</td>
</tr>
<tr>
<td>30 - 42</td>
<td>17 CP²</td>
<td>9.7</td>
<td>-0.2</td>
</tr>
<tr>
<td>10 - 20</td>
<td>38 WSC³</td>
<td>10.6</td>
<td>0.2</td>
</tr>
<tr>
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<td>17 WSC³</td>
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<tr>
<td>10 - 20</td>
<td>38 EIOM⁴</td>
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<tr>
<td>20 - 30</td>
<td>35 EIOM⁴</td>
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</tr>
<tr>
<td>30 - 42</td>
<td>17 EIOM⁴</td>
<td>343</td>
<td>-4.0</td>
</tr>
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</table>

1 Crude fibre (% of dry matter);
2 Crude protein (% of dry matter);
3 Water soluble carbohydrates (% of dry matter);
4 Enzyme insoluble organic matter (g/kg dry matter);
5 NIRS equation established at calibration sample temperatures of 20 °C
6 NIRS equation established across calibration sample temperatures of 5 °C, 10 °C, 15 °C and 20 °C
2 and 3 only occur as a consequence of lower sample temperatures than were present in the calibration samples. It is obvious that the Multiple-T calibration strategy - according to which a broad range of sample temperatures must be represented in the calibration set - removes the bias problems for the 10 °C- and 15 °C-samples in the validation experiment. So the failure of NIR predicted nutrient contents to reflect true composition is a consequence of the improper Single-T approach to calibration and not a demonstration of the inherent weakness of the data base dependent specification of a NIRS method.

Interestingly, the extent of the bias under the conditions above is high for CF and CP irrespective of the NIRS instrument considered. In the case of WSC, however, single-T equations give rise to highly biased predictions for 10 °C and 15 °C samples on the diode array instrument but not on the scanning monochromator. It is highly probable that this effect is due to the difference in the spectral range covered by the two instruments.

Table 3
Extent of systematic errors in NIRS assessments of diverse forage constituents within forages grouped for dry matter content after establishment of NIRS equations at a single sample temperature (Single T) and at multiple sample temperatures (Multiple T) on a diode array spectrometer (Zeiss MMS NIR 1.7)

<table>
<thead>
<tr>
<th>Sample temperature</th>
<th>20°C</th>
<th>15°C</th>
<th>10°C</th>
</tr>
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<tr>
<td>10 - 20</td>
<td></td>
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<td>30 - 42</td>
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<table>
<thead>
<tr>
<th>DM Range</th>
<th>n</th>
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<th>Single T.EQA</th>
<th>Multiple T.EQA</th>
<th>Single T.EQA</th>
<th>Multiple T.EQA</th>
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<tbody>
<tr>
<td>10 - 20</td>
<td>26</td>
<td>CF¹</td>
<td>23.1</td>
<td>0.0</td>
<td>- 0.4</td>
<td>- 4.1</td>
<td>0.4</td>
<td>- 9.4</td>
<td>0.6</td>
</tr>
<tr>
<td>20 - 30</td>
<td>31</td>
<td>CF¹</td>
<td>26.5</td>
<td>- 0.2</td>
<td>- 0.5</td>
<td>- 3.4</td>
<td>- 0.2</td>
<td>- 8.0</td>
<td>0.2</td>
</tr>
<tr>
<td>30 - 42</td>
<td>17</td>
<td>CF¹</td>
<td>28.2</td>
<td>0.3</td>
<td>0.0</td>
<td>- 1.8</td>
<td>- 0.2</td>
<td>- 8.0</td>
<td>0.4</td>
</tr>
<tr>
<td>10 - 20</td>
<td>38</td>
<td>CP²</td>
<td>19.2</td>
<td>0.2</td>
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<td>2.1</td>
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1 Crude fibre (% of dry matter);
2 Crude protein (% of dry matter);
3 Water soluble carbohydrates (% of dry matter);
4 Enzyme insoluble organic matter (g/kg dry matter);
5 NIRS equation established at calibration sample temperatures of 20°C
6 NIRS equation established across calibration sample temperatures of 5°C, 10°C, 15°C and 20°C

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