Prediction of Fermentation Parameters in Grass and Corn Silage by Near Infrared Spectroscopy

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ABSTRACT

Prediction models were developed for determination of lactic acid (Lac), acetic acid (HAc), pH, NH_3 N, and ethanol (EtOH) in grass and corn silages by near infrared (NIR) spectroscopy. Generally, Lac, pH, and NH_3 N concentrations in original sample material could be determined with a better accuracy when NIR measurements were performed on dried material rather than on wet material. The difference could only partly be explained by the repeatability error of NIR measurements for compounds stable with respect to drying. The HAc concentration was determined slightly more accurately when measurements were performed on wet material. The respective ratios between analytical concentration range expressed as standard deviation and the root mean square error of cross validation (RMSECV) were 4.9, 2.0, 3.7, and 3.1 for Lac, HAc, pH, and NH_3 N measured on dried grass silage, and 3.3 for EtOH measured on wet grass silage. The corresponding standard deviation:RMSECV for corn silage were 4.7, 1.9, 2.4, 2.9, and 4.0. The content of Lac was affected only slightly by drying at 80°C, whereas the effects on NH_3 N and HAc were more pronounced, depending on silage type.

(Key words: fermentation parameter, silage, near infrared spectroscopy)

Abbreviation key: EtOH = ethanol, HAc = acetic acid, Lac = lactic acid, NADH = reduced NAD, NIR = near infrared, RMSECV = root mean square error of cross validation, RMSEP = root mean square error of prediction, $\text{SD}_{\text{r,NIR}}$ = standard deviation of NIR measurements repeatability, SEP = standard error of prediction.

INTRODUCTION

The pH and the contents of lactic acid (Lac), VFA, NH_3 N, and ethanol (EtOH) in silage are quality parameters affecting feed intake, milk production, and milk

flavor. During the fermentation process, sugars are converted mainly to Lac and acetic acid (HAc). To obtain a stable silage with a low content of spore-forming clostridia, Lac production must be sufficiently high to ensure a relatively low pH. The growth of clostridia may otherwise increase the spore level in silage and, from direct and indirect contact contamination of milk, result in cheese spoilage because of uncontrollable gas production and off-flavor from butyric acid production. Apart from Lac, the fermentation process also results in production of VFA including HAc. Some studies indicate that feed intake may be reduced if the concentration of HAc becomes too high (Rook and Gill, 1990). However, the mechanism seems complex because the effect was not observed in other studies (Steen et al., 1998). If the fermentation process proceeds too slowly or is insufficient, an increased portion of the protein may be degraded by microbial activity, resulting in an increased level of NH_3 N. The concentration of NH_3 N may be a marker parameter for substances reducing feed intake (Rook and Gill, 1990; Steen et al., 1998). The EtOH concentration in silage is normally low, but, in some cases, it can be considerable. This may induce off-flavor in milk (Randby et al., 1998).

Because of the adverse effects of a nonoptimal ensiling process, a rapid and inexpensive analytical method is needed for estimation of silage quality. Near infrared (NIR) spectroscopy has been used successfully for many years in the determination of major chemical constituents and digestibility of forage. However, the possibility of rapid analysis of fermentation parameters by NIR spectroscopy has not been investigated to the same extent. Results on fermentation parameters obtained by calibration on wet material have been reported for grass silage (Snyman and Joubert, 1992; Sinnaeve et al., 1994; Park et al., 1998, 1999, 2002) and for alfalfa and corn silages (Reeves et al., 1989). Measurements on wet material rather than dried material permit very rapid analyses in general and introduce the possibility of analyzing volatile and especially non-ionic volatile compounds. However, practical experience has shown that it may be difficult to obtain the same prediction accuracy on the main constituents. The laboratory savings obtained by excluding the drying and grinding process

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Table 1. Composition of grass silage samples used for calibration as determined by reference methods.

Analyte	Number of samples ¹						Sample composition			
	Total	DK 2000	DK 2001	S 2001	DK 2002	DK 2003	Mean	Minimum	Maximum	SD
DM, %	392	119	140	89	21	23	40	22	79	14
NH ₃ N(DM), ² %	257	0	144	69	21	23	0.22	0.04	0.42	0.07
Lac(DM), ² %	365	119	142	60	21	23	7.8	0.08	16	3.5
HAc(DM), ² %	271	0	149	78	21	23	2.7	0.02	12	1.9
pH	379	119	145	71	21	23	4.3	3.7	6.4	0.41
EtOH(DM), ² %	279	0	146	89	21	23	0.63	0.06	5.8	0.72
CP(DM), ³ %	215	0	140	75	0	0	16	0.4	24	2.8

¹DK = Samples from Denmark; S = Samples from Sweden²NH₃ N, lactic acid (Lac), acetic acid (HAc), and ethanol (EtOH) determined in wet material and expressed as a percentage of DM.³Crude protein determined in dried material.

may also be neutralized by the introduction of other disadvantages, such as extended requirements regarding storage conditions, special sample pretreatments, difficulties in automation of the measuring process, and difficulties in subsampling for rational determination of other substances, e.g., elements by atomic spectrometry.

This study investigated the feasibility of analyzing pH, Lac, HAc, NH₃ N, and EtOH in grass and corn silage by NIR spectroscopy and compared techniques based on dried and wet material analysis.

MATERIALS AND METHODS

Samples of grass and corn silage were obtained from all regions of Denmark and southern Sweden during the period from 2000 to 2003. The samples from 2000 and 2001 were used solely for calibration (Tables 1 and 2). The samples from 2002 and 2003 were all from Denmark and were randomly split up into a calibration set (Tables 1 and 2) and a validation set (Tables 3 and 4). The samples from 2000 and 2001 included selected samples of good and poor qualities as estimated by sensoric assessments. The samples from 2002 and 2003

were taken randomly from an annual pool of 15,000 laboratory samples received during the high seasons from August to November.

The samples were equilibrated to room temperature (20 to 24°C) and carefully mixed before NIR measurement. A subsample was packed into a Natural Product Cell (Foss, Hillerød, Denmark) and measured on a NIR-Systems 6500 instrument equipped with a transport module (Foss). Reflectance measurements were performed in the wavelength range from 400 to 2500 nm. Two replicate measurements, including repacking of the sample cell, were performed on each sample.

After NIR measurement on wet material, a portion of the sample material was taken for reference analysis. Another portion of the remaining sample was dried at 80°C for 16 h and ground through a 1-mm sieve (Cyclotec 1093; Foss). The dried sample was measured in reflectance mode on a NIRSystems 5000 instrument (Foss) with spinning sample cup module. The wavelength range was 1100 to 2500 nm. The measurements were performed in duplicate with repacking of the cuvette.

The samples were analyzed for NH₃ N, Lac, HAc, pH, EtOH, and DM. Grass silage was also analyzed for CP.

Table 2. Composition of corn silage samples used for calibration as determined by reference methods.

Analyte	Number of samples ¹					Sample composition			
	Total	DK 2001	S 2001	DK 2002	DK 2003	Mean	Minimum	Maximum	SD
DM, %	227	179	10	16	22	30	22	43	3.5
NH ₃ N(DM), ² %	210	162	10	16	22	0.056	0.015	0.12	0.020
Lac(DM), ² %	202	154	10	16	22	5.8	0.23	11	1.7
HAc(DM), ² %	211	163	10	16	22	1.9	0.20	6.6	1.0
pH	221	173	10	16	22	3.9	3.7	5.0	0.15
EtOH(DM), ² %	236	188	10	16	22	0.56	0.03	3.2	0.44

¹DK = Samples from Denmark; S = Samples from Sweden.²NH₃ N, lactic acid (Lac), acetic acid (HAc), and ethanol (EtOH) determined in wet material and expressed as a percentage of DM.

Table 3. Composition of grass silage samples used for validation as determined by reference methods.

Analyte	Number of samples	Sample composition			
		Mean	Minimum	Maximum	SD
DM, %	43	40	22	79	14
NH ₃ N(DM), ¹ %	43	0.20	0.10	0.35	0.066
Lac(DM), ¹ %	43	5.6	0.08	12	3.4
HAc(DM), ¹ %	43	2.0	0.02	9	1.6
pH	43	4.6	3.8	6.4	0.53
EtOH(DM), ¹ %	43	0.52	0.01	4.0	0.56

¹NH₃ N, lactic acid (Lac), acetic acid (HAc), and ethanol (EtOH) determined in wet material and expressed as a percentage of DM.

All reference analyses, except protein, were performed on wet sample material. Crude protein N was determined by the Dumas principle (Sweeney, 1989) using a Leco CN-2000 instrument (Leco Corporation, St. Joseph, MI). Determination of NH₃ N was performed by biochemical analysis (Boehringer Mannheim, Mannheim, Germany). In the presence of glutamate dehydrogenase and reduced NAD (**NADH**), ammonia reacts with 2-oxoglutarate, whereby NADH is reduced to NAD in a stoichiometric way. The NADH concentration was measured spectrophotometrically. Lactic acid and HAc were determined by HPLC analysis of aqueous extract using a Rezex ROA column (Phenomenex, Torrance, CA), and EtOH was determined by gas chromatographic analysis of aqueous extract using a 2-m × 2-mm i.d. column packed with Chromosorb 101 (Johns-Manville, Denver, CO). Determination of pH was performed on a sample equilibrated overnight at 5°C with water. The DM content was determined from drying at 80°C for 16 h. In all cases, 2 single determinations were performed on different days. The wet samples were kept at -20°C until analysis, which was performed 2 d after NIR scanning.

Data were treated with WINISI version 1.04 software (Infrasoft International, Silver Spring, MD). The concentrations of Lac, HAc, NH₃ N, and EtOH expressed as a percentage of DM, but determined in wet material, were used for calibration and validation on dried materials. The concentrations in wet material were used for

calibration and validation on wet materials. The pH values as determined in wet materials were used in both cases. Calibration was performed by partial least squares regression on full-scan mean spectra after scatter correction with the standard normal variate transformation combined with detrend. Additional pretreatment was performed by second derivative treatment. Calibration was performed using 6 cross-validation segments. One outlier elimination pass was accepted. A relatively conservative criterion based on a T-value (residual/root mean square error of cross validation [**RMSECV**]) of 3.0 was used. The number of partial least squares factors selected was the number giving the lowest RMSECV with the fewest factors. Calculation of reference analyte concentration in DM was performed using reference-determined DM. Predicted concentration in wet material was transformed to concentration in DM using predicted DM values. The standard error of prediction (**SEP**) was calculated by the following formula (Næs et al., 2002):

$$SEP = \sqrt{\frac{1}{N-1} \sum (x_i - y_i - \text{bias})^2}$$

where $x_i - y_i$ = difference between results obtained by routine method (x_i) and reference method (y_i) on sample i .

Table 4. Composition of corn silage samples used for validation as determined by reference methods.

Analyte	Number of samples	Sample composition			
		Mean	Minimum	Maximum	SD
DM, %	37	35	28	44	3.3
NH ₃ N(DM), ¹ %	37	0.054	0.022	0.13	0.023
Lac(DM), ¹ %	37	5.1	0.81	9.6	1.5
HAc(DM), ¹ %	37	1.7	0.91	4.9	0.79
pH	37	3.8	3.6	4.3	0.16
EtOH(DM), ¹ %	37	0.89	0.02	1.5	0.37

¹Ammonia-N, lactic acid (Lac), acetic acid (HAc), and ethanol (EtOH) determined in wet material and expressed as a percentage of DM.

$$\text{bias} = \frac{1}{N} \sum (x_i - y_i) = \bar{x} - \bar{y}$$

where N = total number of samples in the test.

The root mean square error of prediction (**RMSEP**) was calculated from (Næs et al., 2002):

$$\text{RMSEP} = \sqrt{\frac{1}{N} \sum (x_i - y_i)^2}$$

The SEP and RMSEP were calculated from data obtained on test sets. The standard error of cross validation and the RMSECV were calculated in the same way as SEP and RMSEP but on data from cross validation (Næs et al., 2002).

The true accuracy of NIR spectroscopy was estimated from

$$\sqrt{\text{SEP}^2 - \text{SD}_t^2}$$

where SD_t is the total standard deviation of the final reference results used for calibration. The SD_t was calculated as the day-to-day standard deviation of the final calibration values.

The repeatability standard deviation (i.e., the variability of independent single results obtained by the same operator, using the same apparatus under the same conditions on the same test sample and in a short interval of time) and the intralaboratory reproducibility standard deviation (i.e., the variability of independent single results obtained on the same test sample in the same laboratory by different operators under different experimental conditions) were determined in accordance with ISO standard 5725-2 (International Organization for Standardization, 1994).

RESULTS AND DISCUSSION

Effect of Drying Process

Determinations of Lac, HAc, NH_3 N, and EtOH are normally performed on wet material. Drying at 80°C for 16 h completely evaporates volatile nonionic compounds such as EtOH. However, the effect is less clear for compounds forming salts. To investigate the drying effect on Lac, HAc, and NH_3 N, samples were analyzed by reference methods before and after the drying process. The possibility of predicting wet material analyses from dried material analyses was investigated for comparison with the performance obtained by NIR spectroscopy.

Lac. The results showed that the drying process has only a minor effect on the Lac concentration. The mean

loss in the drying process was 3.5%. The Lac determined in wet material by the reference method and expressed as a percentage of DM (Lac(DM)) can be related to the Lac determined in dry material (Lac(DM)_{dry}) by the equations:

$$\begin{aligned} \text{Lac(DM)} &= -0.65 + 1.15 \times \text{Lac(DM)}_{\text{dry}}; \\ r^2 &= 0.96, \\ \text{SD}_{yx} &= 0.63, n = 125 \\ &(\text{grass silage, 2 to 13\% Lac(DM)}) \end{aligned} \quad [1]$$

$$\begin{aligned} \text{Lac(DM)} &= -0.24 + 1.09 \times \text{Lac(DM)}_{\text{dry}}; \\ r^2 &= 0.84, \text{SD}_{yx} = 0.53, n = 100 \\ &(\text{corn silage, 2 to 8\% Lac(DM)}) \end{aligned} \quad [2]$$

Inclusion of pH and DM in the regression models did not improve the correlations.

HAc. The mean losses of HAc in the drying process were 57% for grass silage and 83% for corn silage. The acetic acid determined in wet material by the reference method and expressed as a percentage of DM (HAc(DM)) can be related to the HAc determined in dry material (HAc(DM)_{dry}) by the equations:

$$\begin{aligned} \text{HAc(DM)} &= 1.38 + 0.95 \times \text{HAc(DM)}_{\text{dry}}; \\ r^2 &= 0.42, \text{SD}_{yx} = 0.83, n = 100 \\ &(\text{grass silage, 0 to 10\% HAc(DM)}) \end{aligned} \quad [3]$$

$$\begin{aligned} \text{HAc(DM)} &= 0.69 + 3.5 \times \text{HAc(DM)}_{\text{dry}}; \\ r^2 &= 0.55, \text{SD}_{yx} = 0.59, n = 100 \\ &(\text{corn silage, 0 to 7\% HAc(DM)}) \end{aligned} \quad [4]$$

Inclusion of pH and DM in the regression models did not improve the correlations significantly.

NH_3 N. The mean losses of NH_3 N in the drying process were 53% for grass silage and 16% for corn silage.

The NH_3 N determined in wet material by the reference method and expressed as a percentage of DM (NH_3 N(DM)) can be related to the NH_3 N determined in dry material (NH_3 N(DM)_{dry}) by the equations:

$$\begin{aligned} \text{NH}_3 \text{ N (DM)} &= 0.121 + 0.988 \times \text{NH}_3 \text{ N (DM)}_{\text{dry}}; \\ r^2 &= 0.85, \text{SD}_{yx} = 0.036, n = 40 \\ &(\text{grass silage, 0.04 to 0.4 \% NH}_3 \text{ N(DM)}) \end{aligned} \quad [5]$$

$$\begin{aligned} \text{NH}_3 \text{ N (DM)} &= 0.009 + 1.025 \times \text{NH}_3 \text{ N (DM)}_{\text{dry}}; \\ r^2 &= 0.84, \text{SD}_{yx} = 0.007, n = 40 \\ &(\text{corn silage, 0.02 to 0.12\% NH}_3 \text{ N(DM)}) \end{aligned} \quad [6]$$

Introduction of pH and DM as variables did not improve the correlation significantly.

Table 5. Prediction of fermentation parameters and major constituents in grass silage.

Analyte	NIR ¹ mode	PLS ² factors	T outliers removed	r ² ³	RMSECV ⁴	RMSEP ⁵	SD:RMSECV and SD:RMSEP ⁶
NH ₃ N(DM), ⁷ %	Dry	10	2	0.89	0.023	0.026	3.1:2.5
	Wet	10	3	0.79	0.028	0.030	2.6:2.3
Lac(DM), ⁷ %	Dry	15	1	0.96	0.71	0.54	4.9:6.3
	Wet	11	5	0.93	0.84	0.64	4.2:5.3
HAc(DM), d ⁷ %	Dry	10	2	0.68	0.97	0.65	2.0:2.5
	Wet	10	5	0.74	0.91	0.57	2.1:2.8
pH	Dry	11	5	0.92	0.11	0.11	3.7:4.8
	Wet	15	7	0.91	0.13	0.15	3.2:3.5
EtOH(DM), ⁷ %	Wet	10	4	0.89	0.22	0.20	3.3:2.8
DM, %	Wet	6	6	0.99	0.89	1.05	16:13
CP(DM), ⁸ %	Dry	13	0	0.97	0.48		5.8
	Wet	13	6	0.93	0.78		3.6

¹NIR = Near infrared spectroscopy.²PLS = Partial least squares.³Determined from cross validation on calibration set.⁴RMSECV = Root mean square error of cross validation.⁵RMSEP = Root mean square error of prediction.⁶Sample variation SD determined by reference method.⁷NH₃ N, lactic acid (Lac), acetic acid (HAc), and ethanol (EtOH) in wet material expressed as a percentage of DM.⁸Crude protein in dried material.

NIR Predictions

Lac. Prediction of Lac was more accurate on dried sample material than wet material (Tables 5 and 6). The RMSECV values obtained by cross validation were 0.71% for grass silage and 0.36% for corn silage when

measured on dried material. The predictive capability of NIR spectroscopy is illustrated in Figure 1. The standard deviation:RMSECV for dried material were 4.9 on grass silage and 4.7 on corn silage. A lower limit of 2.0 is often used in assessment of the applicability of NIR spectroscopy for forage analyses. The corresponding ra-

Table 6. Prediction of fermentation parameters and major constituents in corn silage.

Analyte	NIR ¹ mode	PLS ² factors	T outliers removed	r ² ³	RMSECV ⁴	RMSEP ⁵	SD:RMSECV and SD:RMSEP ⁶
NH ₃ N(DM), ⁷ %	Dry	9	2	0.77	0.007	0.008	2.9:2.9
	Wet	9	1	0.72	0.008	0.009	2.5:2.6
Lac(DM), ⁷ %	Dry	10	3	0.95	0.36	0.39	4.7:3.8
	Wet	12	3	0.91	0.47	0.48	3.6:3.1
HAc(DM), ⁷ %	Dry	10	2	0.72	0.52	0.40	1.9:2.0
	Wet	10	1	0.84	0.41	0.39	2.4:2.0
pH	Dry	9	2	0.78	0.063	0.074	2.4:2.2
	Wet	9	3	0.62	0.080	0.087	1.9:1.8
EtOH(DM), ⁷ %	Wet	12	1	0.92	0.11	0.10	4.0:3.7
DM, %	Wet	6	1	0.93	0.97	1.1	3.6:3.0

¹NIR = Near infrared spectroscopy.²PLS = Partial least squares.³Determined from cross validation on calibration set.⁴RMSECV = Root mean square error of cross validation.⁵RMSEP = Root mean square error of prediction.⁶Sample variation SD determined by reference method.⁷NH₃ N, lactic acid (Lac), acetic acid (HAc), and ethanol (EtOH) in wet material expressed as a percentage of DM.

Figure 1. Correlation between Lac(DM) (lactic acid determined in wet material and presented as a percentage of DM) as predicted by near infrared (NIR) spectroscopy and Lac(DM) determined by the reference method in grass silage. The results were obtained by cross validation on the calibration set. The NIR measurements were performed on dried sample material.

Figure 2. Correlation between HAc(DM) (acetic acid determined in wet material and presented as a percentage of DM) predicted by near infrared (NIR) spectroscopy and HAc(DM) determined by the reference method in grass silage. The results were obtained by cross validation on the calibration set. The NIR measurements were performed on dried sample material.

tios for wet material were 4.2 and 3.6. The RMSECV and RMSEP values for dried corn silage appeared to be underestimated because the intralaboratory reproducibility standard deviation was on the same level (Table 7). The true accuracy of NIR spectroscopy for dried material was estimated as 0.29%.

An improvement in predictive capability from calibration on Lac determined in dry material rather than Lac determined in wet material by the reference method and expressed as a percentage of DM followed by calculations using Equations [1] and [2] could not be obtained because 1) the loss of Lac during the drying process was very low and 2) the conversion from Lac determined in dry material to Lac determined in wet material values was not characterized by a standard deviation significantly lower than the RMSECV.

The 400- to 1100-nm spectral range did not contribute significantly to the prediction accuracy on wet material. Furthermore, the accuracy could not be improved by

trimming the wavelength range to single or multiple segments showing highest correlation to Lac. The same observations were made for the other parameters calibrated.

HAc. Because most of the HAc was evaporated during the drying process, it was not expected that a good relationship between spectral data of dried samples and the HAc content in original samples would be found. This was verified by standard deviation:RMSECV of 2.0 and 1.9 for grass silage and corn silage, respectively. The predictive capability of NIR spectroscopy is illustrated in Figure 2. The high standard deviation of Equations [3] and [4] excluded the possibility of obtaining a significantly better prediction of HAc from NIR analysis of dried sample material using a calibration developed on the results for HAc determined in dry material followed by a conversion of the results using Equations [3] and [4].

Calibration on wet materials only improved the accuracy slightly. The standard deviation:RMSECV values were 2.1 and 2.4 for grass silage and corn silage, respectively.

The results show that it is difficult to extract spectral information for HAc determination. The nearly identical standard deviation:RMSECV obtained from measurements on dried grass silage and dried corn silage—although much more HAc is evaporated during the drying process in the case of corn silage—could indicate that the calibration does not measure HAc directly, but merely measures properties correlated with HAc. This conclusion is supported by results obtained from calibration on residual HAc in dried material. These cali-

Table 7. Intralaboratory reproducibility standard deviations ($SD_{R,intra}$) of single determinations performed by reference methods.

Analyte	$SD_{R,intra}$	
	Grass silage	Corn silage
NH_3 N(DM), ¹ %	0.013	0.006
Lac(DM), ¹ %	0.40	0.35
HAc(DM), ¹ %	0.25	0.14
pH	0.035	0.017
EtOH(DM), ¹ %	0.070	0.058

¹ NH_3 N, lactic acid (Lac), acetic acid (HAc), and ethanol (EtOH) determined in wet material and expressed as a percentage of DM.

Figure 3. Correlation between Lac(DM) (lactic acid determined in wet material and presented as a percentage of DM) and HAc(DM) (acetic acid determined in wet material and presented as a percentage of DM) in calibration samples of grass silage as determined by reference methods.

brations still gave standard deviation:RMSECV at the 2.0 level. The possibility that the HAc calibration solely reflects spectral data for Lac can be rejected because many of the calibration samples showed outlying features with respect to the Lac:HAc (Figures 3 and 4). The correlation plots for Lac and HAc showed similarities but also some differences in certain wavelength regions (Figures 5 and 6), which also indicates that the HAc and Lac calibration models do not extract exactly the same properties from spectral data.

Grass silage pH. Prediction of pH was better on dried material than on wet material (Table 5). The

Figure 4. Correlation between Lac(DM) (lactic acid determined in wet material and presented as a percentage of DM) and HAc(DM) (acetic acid determined in wet material and presented as a percentage of DM) in calibration samples of corn silage as determined by reference methods.

Figure 5. Correlation plots showing the correlation between near infrared spectral data and Lac(DM) (lactic acid determined in wet material and presented as a percentage of DM) (bright curve) and HAc(DM) (acetic acid determined in wet material and presented as a percentage of DM) (dark curve) in dried grass silage. Spectra were pretreated by standard normal variate transformation and second derivative treatment.

RMSECV values obtained were 0.11 for dried material and 0.13 for wet material. The corresponding standard deviation:RMSECV for dried and wet materials were 3.7 and 3.2. The accuracy of NIR spectroscopy is illustrated in Figure 7.

The correlation between pH and Lac, HAc, and DM was investigated on calibration samples using reference data. The correlation can be expressed by the equations:

$$\text{pH} = 5.28 - 1.17 \times \log(\text{Lac(DM)}); r^2 = 0.74, \text{SD}_{yx} = 0.18 \text{ and} \quad [7]$$

$$\text{pH} = 4.21 - 1.19 \times \log(\text{Lac}) + 0.014 \times \text{DM}; r^2 = 0.76, \text{SD}_{yx} = 0.18. \quad [8]$$

Figure 6. Correlation plots showing the correlation between near infrared spectral data and Lac(DM) (lactic acid determined in wet material and presented as a percentage of DM) (bright curve) and HAc(DM) (acetic acid determined in wet material and presented as a percentage of DM) (dark curve) in dried corn silage. Spectra were pretreated by standard normal variate transformation and second derivative treatment.

Figure 7. Correlation between pH predicted by near infrared (NIR) spectroscopy and pH determined by the reference method in grass silage. The results were obtained by cross validation on the calibration set. The NIR measurements were performed on dried sample material.

Inclusion of HAc(DM) and DM as factors in Equation [7] and HAc as a factor in Equation [8] did not improve the standard deviation of regression.

The high SD_{yx} values compared with the RMSECV values of 0.11 to 0.13 indicate that the pH calibration equation was not related merely to the contents of the major acids and DM.

Corn silage pH. The pH of corn silage could be predicted with RMSECV values of 0.06 and 0.08 for dried and wet materials (Table 6). The standard deviation:

Figure 9. Correlation between CP and NH_4N concentrations (percentage of DM) as determined by reference methods in grass silage.

RMSECV for dried and wet materials were 2.4 and 1.9, respectively.

The correlation between pH and Lac, HAc, and DM could be expressed by the equations:

$$pH = 4.65 - 1.02 \times \log(Lac(DM)); r^2 = 0.79, SD_{yx} = 0.076 \text{ and} \quad [9]$$

$$pH = 3.95 - 1.00 \times \log(Lac) + 0.005 \times DM; r^2 = 0.83, SD_{yx} = 0.051. \quad [10]$$

Because the SEP values related to $\log(Lac(DM))$ and $\log(Lac)$ were 0.040 and 0.045, respectively, regression

Figure 8. Correlation between NH_3N (DM) (NH_3N determined in wet material and presented as a percentage of DM) predicted by near infrared (NIR) spectroscopy and NH_3N (DM) determined by the reference method in grass silage. The results were obtained by cross validation on the calibration set. The NIR measurements were performed on dried sample material.

Figure 10. Correlation between ethanol (EtOH) predicted by near infrared (NIR) spectroscopy and EtOH determined by the reference method in grass silage (percentage of DM). The results were obtained by cross validation on the calibration set. The NIR measurements were performed on wet sample material.

Table 8. Repeatability standard deviation of NIR analyses ($SD_{r,NIR}$) including uncertainty from re-packing of sampling cells.

Analyte	Calibration performed on dry material		Calibration performed on wet material	
	Grass silage	Corn silage	Grass silage	Corn silage
NH ₃ N(DM), ¹ %	0.004	0.002	0.018	0.007
Lac(DM), ¹ %	0.14	0.12	0.31	0.34
HAc(DM), ¹ %	0.11	0.15	0.26	0.33
pH	0.021	0.034	0.062	0.039
EtOH(DM), ¹ %			0.081	0.092
DM, %			0.79	1.1
CP(DM), ² %	0.18		0.69	

¹NH₃ N, lactic acid (Lac), acetic acid (HAc), and ethanol (EtOH) in wet material expressed as a percentage of DM.

²Crude protein in dried material.

models 9 and 10 indicate that the pH calibration equation for corn silage may reflect the Lac content.

NH₃ N. Prediction of NH₃ N was slightly more accurate on dried sample material compared with wet material (Tables 5 and 6). The RMSECV values for dried material were 0.023% (grass silage) and 0.007% (corn silage). The standard deviation:RMSECV for dried material were 3.1 on grass silage and 2.9 on corn silage. The accuracy of NIR spectroscopy is illustrated in Figure 8. The corresponding ratios for wet material were 2.6 and 2.5, respectively.

As illustrated in Figure 9, the NH₃ N content could not be predicted simply from the protein content. Inclusion of the pH in a regression model did not improve the predictability. However, the possibility that other fermentation properties may correlate with the NH₃ N content, and thereby contribute to the calibration model, cannot be excluded.

Because the standard deviations for conversion of NH₃ N determined in dry material to NH₃ N determined in wet material were on the same level as or higher than the RMSECV and RMSEP values, it was not possible to obtain a better accuracy by calibration on values for NH₃ N determined in dry material followed by conver-

sion to values for NH₄ N determined in dry material values using Equations [5] and [6].

EtOH. The organic solvent EtOH is evaporated during the drying process because of its volatility and non-ionic nature. Thus, it is thus impossible to determine EtOH directly in the dried material by reference methods. An attempt to build a calibration model on dried material also showed that there were no indirect relationships between the original EtOH content and NIR spectral data of dried samples.

The EtOH content could be determined in wet silage with a standard deviation:RMSECV of at least 3, which indicates that NIR spectroscopy is suitable for determination of EtOH in silage (Tables 5 and 6; Figure 10).

Repeatability of NIR Measurements

The repeatability uncertainty of NIR measurements ($SD_{r,NIR}$) was insignificant compared with the prediction error when measurements were performed on dried material (Table 8). The $SD_{r,NIR}$ was considerably higher when measurements were performed on wet material, but still low compared with RMSECV and RMSEP values. If the SECV values were corrected for $SD_{r,NIR}$, then the differences between the accuracy obtained on dried and wet materials were reduced slightly for NH₃ N, Lac, and pH (Table 9). For protein, a 30% difference in uncertainty remained after correction.

Table 9. Standard error of cross validation corrected for the repeatability error of NIR measurements.

Analyte	Grass silage (dry/wet)	Corn silage (dry/wet)
NH ₃ N(DM), ¹ %	0.023/0.025	0.007/0.007
Lac(DM), ¹ %	0.70/0.81	0.35/0.42
HAc(DM), ¹ %	0.97/0.89	0.51/0.34
pH	0.11/0.12	0.06/0.08
EtOH, ¹ %	—/0.21	—/0.09
CP(DM), ² %	0.46/0.61	

¹NH₃ N, lactic acid (Lac), acetic acid (HAc), and ethanol (EtOH) in wet material expressed as a percentage of DM.

²Crude protein in dried material.

CONCLUSIONS

The results showed that NIR spectroscopy can be useful in the prediction of Lac, pH, NH₃ N, and EtOH in grass and corn silage. The technique is less accurate for HAc, but may be used to obtain a rough estimate of the concentration level. For compounds that are not affected by the drying process, the accuracy of NIR was significantly better when measurements were per-

formed on dried material compared with wet material. This was clearly demonstrated by the parameters Lac and protein. The difference could only partly be explained by differences in repeatability errors of NIR measurements. For NH_3N , which is moderately affected by the drying process, the slightly higher uncertainty on wet material could mainly be explained by the repeatability error of NIR measurements. For Hac, which is highly affected by the drying process, the accuracy was better on wet material, even before correction for imprecision.

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