Determination of feed quality for barley hay and silage by near infrared reflectance spectroscopy

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Introduction

Barley (*Hordeum vulgare L.*) hay and silage are used extensively in cattle and sheep diets in Western Canada. We have demonstrated that near infrared (NIR) is a useful technique for determining the chemical composition and *in situ* degradability of these feed stuffs.[1] However, barley hay and silage were not separated into different calibration sets in this research and, in addition, barley straw was also included in the calibration samples. We therefore hypothesised that the accuracy of the NIR procedure could be improved by using specific samples for calibration rather than using broad-based calibration equations. Although Abrams *et al*.[2] and others have reported that broad-based equations have potential to offer accuracy comparable to more local equations, use of specific calibration sets generally improve accuracy of the NIR procedure.[3,4]

The protein requirements of ruminant animals are now expressed in terms of metabolisable protein,[5–7] which considers both the ruminal degradability of feed stuffs and the amount of microbial protein produced in the rumen. Degradability of feed protein is highly variable across and within different feeds. The NIR procedure has been successfully used to predict protein solubility and *in situ* degradability in the rumen[1,8–10] but in degradability measurements are time-consuming and expensive. Chemical fractionation of protein,[11,12] therefore, is now being used to obtain estimates of ruminal protein degradability.[7]

One objective of this research was to determine the extent to which the accuracy of NIR procedures could be improved by using specific calibration equations within forage types rather than broad-based calibrations based upon combined barley hay and barley silage samples. In addition, the usefulness of NIR in determining protein and nitrogen fractions in barley hay and barley silage was examined.

Materials and methods

The 388 barley hay and 455 barley silage samples used in this study were obtained from fertility and maturity studies in the 1994, 1995 and 1996 crop years. A Foss NIRSystems Model 6500 (Foss NIRSystems, Silver Spring, Maryland, USA) scanning monochromator with transport module, using Infra-Soft-International (ISI, Port Matilda, Pennsylvania, USA) software, NIR 3 version 3.11, was used. Feed quality criteria included: acid detergent fibre (ADF), neutral detergent fibre (NDF), lignin, non-structure-carbohydrate, crude fat, ash, calcium, phosphorus, crude protein, phosphate buffer-sol-

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uble and insoluble protein and crude protein bound with ADF or NDF. Total buffer-soluble nitrogen (TSN), non-protein nitrogen (NPN), buffer-soluble true protein nitrogen (B1-N), buffer-insoluble but neutral detergent-soluble protein nitrogen (B2-N), neutral detergent-soluble but acid-detergent-solutble protein nitrogen (B3-N), and acid detergent-insoluble nitrogen (C-N) were also examined.

The procedures for determination of forage constituents, other than protein, are given in Hsu *et al*.[12] Protein and nitrogen fractions were determined according to procedures of Licitra *et al*.[11]

The NIR calibrations (equation development) were obtained using four cross-validation groups when all samples were combined and in a fertility study and five groups in the maturity study with modified partial least squares (PLS) using wavelengths between 1100 and 2498 nm. During calibration, samples that did not fit (± 3 standard deviations) in the regression lines for spectra (*H*) or chemical analysis (*T*) were considered outliers. Malahanobis distance, $H = 25$ and $T = 2.5$ were used a cut off distance for identifying outliers during calibration. The critical "*X*", 5, was chosen to eliminate samples with unusual spectrum and $F = 10$ was set, where *F* was the value chosen to minimise over-fitting. The statistical criteria used to verify the accuracy of the NIR calibration equations were correlation coefficients (R^2) between NIR predicted values and chemically quantified results and standard error of cross-validation (*SECV*). These procedures have been described more fully in Hsu *et al*.[1] Means, *SD*, relative standard deviations (*RSD*) and standard errors of analysis (*SEA*) were also used to evaluate the accuracy of chemical analysis.

Results and discussion

Precision of laboratory measurements

The precision of the laboratory procedures for measurement of the chemical composition of standard feeds is given in Table 1. Measurements of neutral detergent-insoluble nitrogen and acid detergent insoluble nitrogen, particularly for barley silage, were somewhat variable as evidenced by the relatively high coefficient of variation. The lower precision with these samples was related to the low absolute amounts of these substances in the feed but may also have been caused by sampling errors associated with the larger particle size in the barley silage or lack of precision in the chemical procedure itself.

Prediction of structural and non-structural carbohydrates, fat and inorganic elements

The NIR procedure was useful for estimating composition of all barley hay and barley silage structural and non-structural carbohydrates, fat and inorganic elements, since values of greater than 0.7 were obtained with most calibration samples (Table 2). Exceptions were for the crude fat in silage samples when all samples, or samples from the fertility study, were examined, lignin for barley hay in the fertility study, phosphorus in all types of forage and in the fertility study and some forage constituents in the maturity study. The very low values (< 0.5) which were obtained when NIR was used to predict lignin, fat, ash and calcium in the maturity study were probably because of the low number of samples.

The values for ADF were lower (0.77 to 0.93 v. 0.98) and *SECV* were higher (1.05 to 1.35 v. 1.06) in this study (Table 2) than in our previous study[1] when barley straw, barley hay, and barley silage were included in the calibration set. However, lignin predictions were somewhat similar (R^2 values of 0.13 to 0.91 v. 0.59 and *SEC* 0.57 to 0.93 v. 0.63 for the current and previous studies, respectively). The *SECV* for calcium and phosphorus ranged from 0.05 to 0.12 and from 0.01 to 0.03, respectively (Table 2). Corresponding standard errors of prediction obtained by Jones *et al*.[13] were 0.10 and 0.02. Shenk and Westerhaus[14] reported standard errors of validation of 0.15 to 0.18 and from 0.03 to 0.04, respectively, for these elements in hay and haylage samples. These data suggest that the NIR procedure can provide useful information concerning the mineral composition of barley forages.

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Table 1. Accuracy of chemical analysis for quality assurance and quality control samples of barley silage and alfalfa hay.

Component	Feeds	n[b]	Mean	*SD* (+/–)	*RSD*[c] (% mean)	*SEA*[d]
Dry matter[a]	Barley silage	18	6.38	0.16	2.44	0.037
%	Alfalfa hay	18	4.87	0.05	0.95	0.011
Crude protein	Barley silage	18	10.78	0.26	2.41	0.061
%	Alfalfa hay	18	20.89	0.48	2.28	0.011
Total soluble nitrogen as protein	Barley silage	18	7.22	0.36	4.96	0.084
%	Alfalfa hay	18	7.91	0.30	3.83	0.071
Buffer insoluble protein	Barley silage	18	3.35	0.11	3.30	0.026
%	Alfalfa hay	18	1.25	0.38	3.04	0.089
Buffer soluble protein	Barley silage	18	0.69	0.27	3.90	0.006
%	Alfalfa hay	18	2.84	0.15	5.91	0.035
Neutral detergent fibre	Barley silage	10	46.41	0.24	0.52	0.076
%	Alfalfa hay	10	37.51	0.38	1.02	0.120
Neutral detergent insoluble nitrogen,	Barley silage	10	1.88	0.02	8.82	0.005
%	lfalfa hay	10	0.68	0.03	4.75	0.010
Acid detergent fibre	Barley silage	10	27.59	0.29	1.05	0.091
%	Alfalfa hay	10	27.39	0.21	0.76	0.066
Acid detergent insoluble nitrogen	Barley silage	10	0.14	0.02	12.98	0.005
%	Alfalfa hay	10	0.23	0.10	3.67	0.003

[a] Dry matter = 100 – moisture %
[b] Number of samples
[c] Standard deviation/mean × 100 %
[d] Standard error of analysis $(SD^2/n)^{1/2}$, %

Comparisons of accuracy of NIR for predicting structural and mineral components of barley hay and silage, using calibrations based upon specific forage types or upon broader-based calibrations with both hay and silage, can be made from data in Table 2. For all samples, the combination of the numerically highest R^2 value and the lowest *SECV* occurred for ash when barley hay samples were evaluated, and for ADF, NDF, lignin, nonstructural carbohydrates and calcium when barley silage was evaluated. In no case were the combined calibrations based upon barley hay and barley silage superior according to both these criteria. Generally, similar results were obtained within the fertility study. In contrast, within the maturity study, ADF, NDF and lignin were predicted with the highest degree of accuracy when calibrations were based upon the combined set of barley hay and barley silage samples. The reason why the combined sample sets resulted in better predictions in the case of the maturity study was related to the low numbers of samples in the calibration set; the increase in sample numbers more than compensated for any loss in accuracy by using the broader-based calibration equation. Our results are, therefore, in general agreement with the literature in that accuracy of predictions is generally greater when specific, rather than broad-based NIR calibration sets are used.[3,4,15,16] Abrams *et al.*[2] however, reported that broad-based equations have the potential to offer accuracy comparable to more local equations when hay samples were collected from 50 states. Similarly, Mathison *et al.*[17] reported that, with the exception of NDF, accuracy of NIR prediction of barley straw was not enhanced when

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Table 2. Comparison between R^2 and *SECV* with different sample sets used in NIR calibration for dry matter, structural and non-structural components, fats and minerals.

Analysis		All samples			Fertility study			Maturity study		
Component	Type	n[f]	R^2	*SECV*	n	R^2	*SECV*	n	R^2	*SECV*
DM[a], %	Barley hay	373	0.93	0.50	328	0.96	0.39	52	0.99	0.15
	Silage	417	0.88	0.47	329	0.76	0.51	51	0.98	0.37
	Combined	812	0.88	0.60	716	0.92	0.51	102	0.96	0.43
ADF[b], %	Barley hay	383	0.91	1.25	321	0.89	1.35	52	0.77	1.32
	Silage	240	0.92	1.05	209	0.89	1.31	51	0.87	1.18
	Combined	634	0.91	1.30	528	0.92	1.33	104	0.93	1.13
NDF[c], %	Barley hay	383	0.73	2.46	330	0.73	2.50	50	0.82	1.69
	Silage	239	0.92	1.54	204	0.90	1.83	50	0.78	1.89
	Combined	839	0.92	2.34	535	0.93	2.37	103	0.94	1.18
Lignin, %	Barley hay	91	0.71	0.62	58	0.65	0.93	26	0.13	0.71
	Silage	86	0.91	0.62	57	0.88	0.57	26	0.40	0.66
	Combined	176	0.84	0.76	115	0.82	0.81	59	0.78	0.72
CFAT[d], %	Barley hay	356	0.82	0.09	302	0.92	0.06	51	0.15	0.19
	Silage	124	0.49	0.25	105	0.62	0.26	18	0.90	0.33
	Combined	488	0.90	0.15	408	0.93	0.13	78	0.80	0.21
NSC[e], %	Barley hay	379	0.72	2.50	329	0.68	2.64	51	0.93	1.68
	Silage	405	0.98	0.91	380	0.98	0.95	49	0.79	2.00
	Combined	810	0.95	2.19	703	0.96	2.06	106	0.96	2.18
Ash, %	Barley hay	373	0.88	0.43	321	0.86	0.43	54	0.85	0.45
	Silage	421	0.77	0.46	203	0.81	0.51	53	0.14	0.65
	Combined	625	0.80	0.47	517	0.86	0.43	105	0.77	0.51
Calcium, %	Barley hay	376	0.82	0.07	319	0.80	0.07	51	0.78	0.07
	Silage	416	0.83	0.05	382	0.84	0.06	54	0.11	0.12
	Combined	803	0.69	0.07	712	0.75	0.07	104	0.65	0.09
Phosphorus, %	Barley hay	376	0.73	0.03	324	0.66	0.03	52	0.93	0.01
	Silage	414	0.71	0.02	389	0.59	0.02	43	0.90	0.02
	Combined	818	0.65	0.03	711	0.67	0.02	104	0.90	0.02

[a] Dry matter
[b] Acid detergent fibre
[c] Neutral detergent fibre
[d] Crude fat
[e] Non-structural carbohydrate
[f] Number of calibration samples

straw alone was used in calibration in comparison to when barley hay and barley silage were also included.

Prediction of protein and nitrogen fractions

The NIR procedure was useful for estimating the composition of all barley hay and barley silage protein and nitrogen fractions since values of greater than 0.7 were obtained for all fractions (Tables 3

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and 4). Again, the very low values (0.01) obtained for buffer insoluble protein (Table 3) and for B2-N (Table 4) in the maturity study were probably related to the small sample size.

There are very few studies in which protein fractions have been measured using the NIR procedure. Hansen *et al*.[9] measured soluble protein with NIR and Antoniewicz *et al*.[10] and Waters and Givens[8] have applied the procedure to *in situ* degraded protein. The R^2 values were lower and the *SECV* were higher for crude protein in this experiment (Table 3) than obtained by Hsu *et al*.[1] when barley straw, barley hay and barley silage were included in the calibration set. The accuracy of NIR predictions of protein fractions (Table 3) were, however, similar to those obtained by Hsu *et al*.[12] with alfalfa hay and silage. Thus *SECV* for crude protein, soluble protein, insoluble protein, acid detergent-insoluble protein and neutral detergent-insoluble protein were 0.41–0.52 vs 0.73–0.77, 0.05–0.15 vs 0.11–0.18, 0.24–0.40 vs 0.56–0.69, 0. 07–0.36 vs 0.51–1.38 and 0.04–0.09 vs 1.12–3.70 in the current study when all samples were included in the calibration set (Table 3) and the previous study, respectively. The R^2 values were, however, higher for these parameters in the Hsu *et al*.[12] study because of the greater range in concentrations in the alfalfa forages which come from farmers around the whole province. For NPN, B1-N, B2-N, B3-N and C-N fractions, the *SECV* were: 0.07–0.10 vs. 0.11–0.11, 0.01–0.03 vs. 0.02–0.04, 0.04–0.08 vs 0.08–0.11, 0.00–0.05 vs 0.06–0.09 and 0.00–0.01 vs 0.03–0.03 for all samples in the current study (Table 4) and the previous study, respectively. Thus, in general, *SECV* were lower in the current study which was probably a reflection of the lower crude protein and individual protein or nitrogen fraction of the barley hay and silage than that of the alfalfa hay and silage.[12]

Table 3. Comparison of coefficient of determination (R^2)and standard error of cross-validation (*SECV*) with different sample sets used in NIR.

Protein fractions		All samples			Fertility study			Maturity study		
Component	Type	n^f	R^2	*SECV*	n^f	R^2	*SECV*	n^f	R^2	*SECV*
CP,[a]%	Barley hay	367	0.96	0.48	316	0.96	0.44	51	0.95	0.69
	Silage	418	0.97	0.41	386	0.96	0.48	51	0.77	0.58
	Combined	808	0.95	0.52	705	0.95	0.52	102	0.93	0.52
SPROT,[b] %	Barley hay	367	0.78	0.15	319	0.82	0.15	53	0.95	0.09
	Silage	417	0.83	0.05	389	0.76	0.07	53	0.67	0.05
	Combined	791	0.82	0.11	688	0.82	0.11	106	0.94	0.09
IPROT,[c] %	Barley hay	364	0.90	0.40	318	0.91	0.39	52	0.89	0.67
	Silage	414	0.93	0.24	385	0.93	0.26	49	0.01	0.82
	Combined	794	0.95	0.39	707	0.95	0.39	95	0.93	0.58
NDIP,[d] %	Barley hay	375	0.78	0.36	322	0.72	0.36	53	0.99	0.21
	Silage	239	0.88	0.07	207	0.83	0.10	52	0.47	0.06
	Combined	610	0.88	0.28	507	0.87	0.26	101	0.98	0.17
ADIP,[e] %	Barley hay	381	0.68	0.09	327	0.62	0.09	54	0.92	0.09
	Silage	243	0.91	0.04	207	0.91	0.05	54	0.95	0.04
	Combined	634	0.73	0.08	530	0.87	0.26	104	0.92	0.06

[a] Crude protein
[b] Buffer soluble true protein
[c] Buffer insoluble protein
[d] Neutral detergent insoluble protein
[e] Acid detergent insoluble protein
[f] Number of calibration samples

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Table 4. Comparison of coefficient of determination (R^2) and standard error of cross-validation (*SECV*) with different sample sets used in NIR calibration for nitrogen fractions.

Protein fractions		All samples			Fertility study			Maturity study		
Component	Type	n^g	R^2	*SECV*	n^g	R^2	*SECV*	n^g	R^2	*SECV*
TSN[a]	Barley hay	365	0.91	0.11	314	0.93	0.10	51	0.86	0.15
	Silage	409	0.94	0.07	382	0.94	0.08	52	0.61	0.12
	Combined	787	0.92	0.12	8,6	0.94	0.11	103	0.89	0.15
NPN[b]	Barley hay	360	0.81	0.10	312	0.83	0.08	52	0.84	0.16
	Silage	410	0.94	0.07	384	0.93	0.08	52	0.63	0.12
	Combined	795	0.96	0.09	697	0.96	0.09	104	0.89	0.16
BI-N,[c] %	Barley hay	367	0.78	0.03	319	0.82	0.03	53	0.95	0.02
	Silage	417	0.83	0.01	388	0.76	0.01	53	0.67	0.01
	Combined	791	0.82	0.02	689	0.82	0.02	107	0.93	0.02
B2-N,[d] %	Barley hay	369	0.79	0.08	324	0.77	0.08	50	0.91	0.10
	Silage	410	0.91	0,04	388	0.88	0.04	48	0.01	0.13
	Combined	796	0.84	0.08	701	0.83	0.07	98	0.79	0.10
B3-N,[e] N	Barley hay	373	0.76	0.05	321	0.71	0.06	54	0.98	0.03
	Silage	383	0.98	0.00	355	0.98	0.00	52	0.79	0.01
	Combined	768	0.91	0.03	671	0.93	0.03	103	0.98	0.03
C-N,[f] %	Barley hay	381	0.68	0.01	328	0.61	0.01	54	0.92	0.01
	Silage	384	0.99	0.00	365	0.99	0.00	54	0.95	0.01
	Combined	809	0.78	0.01	709	0.77	0.01	105	0.90	0.01

[a] Total buffer-soluble nitrogen (%) contains non-protein nitrogen (%) plus buffer-soluble true protein–nitrogen (%)
[b] Non-protein nitrogen (%) contains NH_3, amino acids and peptides
[c] Buffer-soluble true protein nitrogen (%) which is rapidly degraded in the rumen
[d] Buffer-insoluble, but neutral detergent-soluble, protein nitrogen (%) which is slowly degraded in the rumen
[e] Neutral detergent-insoluble, but acid detergent-soluble, protein nitrogen (%) which is slowly degraded in rumen
[f] Acid detergent-insoluble protein nitrogen (%) which is fraction not degraded in the rumen
[g] Number of calibration samples

When samples from both the fertility and maturity study were combined, the lowest *SECV* were obtained for all protein and nitrogen fractions when barley silage was evaluated (Tables 3 and 4). The *SECV* were generally higher and values lower with barley hay, with the combination of hay and silage calibration sets being intermediate. The reason for the lower accuracy of the NIR procedure with barley hay than with barley silage is not readily apparent. In general, the accuracy of NIR for predicting protein and nitrogen fractions was similar when both studies were combined and in the fertility study, which reflects the fact that the fertility study provided most of the samples. Accuracies of the NIR procedure were generally reduced in the maturity study when the calibration set included only a few samples.

Conclusions

In conclusion, the NIR procedure was found to be useful for the prediction of structural and non-structural components, minerals and nitrogen fractions of barley hay and barley silage. Accuracies of prediction were generally enhanced when calibration sets were based upon either hay or silage samples rather than upon calibration sets in which both types of samples combined. Exceptions

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were, however, noted for the protein and nitrogen fractions in barley hay, where better predictions were generally obtained when calibrations were based upon a combined sample set.

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