Near infrared reflectance spectroscopy to determine nitrogen-fractions of barley green feed and silage as affected by nitrogen fertility

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Introduction

Barley (*Hordeum vulgare* L.), as green feed (BG) or silage (BS), is used extensively in Alberta's beef and dairy industries. The content and form of plant tissue N in feeds is an important aspect of quality that has not been studied in depth, partly due to extensive analytical requirements. Management factors have been shown to influence feed quality. Calder and Macleod[1] and McKenzie *et al.*[2] reported that barley cultivars differed in their response to N fertiliser treatment and protein content.[3] Di Rienzo *et al.*[4] found that the crude protein (CP) content of barley silage ranged from 8.3% without N-fertilisation to 12.8% with 120 lb acre^{-1} of N, with dry matter (DM) and CP digestibility increasing ($p < 0.02$) as the rate of N was increased from 80 to 120 lb acre^{-1}.[4]

Protein and non-protein N (NP-N) are critical components of feed quality.[5] By-pass protein N (B2-N and B3-N) is the most desirable form of available N;[6] other forms (NP-N and B1-N) can be assimilated rapidly in the rumen[6] but are subject to loss. Van Soest states that prior to plant harvest, 60 to 80% of the total plant N is in the form of soluble protein N (B1-N).[7] McKersie[8] and McKersie and Buchanan-Smith[9] found that after harvest, during wilting and ensiling, proteinase in plant cells and bacteria hydrolyse a portion of the soluble true protein N (B1-N) to soluble NP-N. The NP-N-fraction in silage consists mainly of ammonia (NH_3), however, that in green feed contains no NH_3.[10] Excess or unutilised NH_3 will be lost as animal wastes[5,11] or as volatiles during sample handling. Thus, the type of N-fraction has a large impact on feed quality for ruminants, especially high performing dairy cattle.[5,11]

To improve feed quality through management, detailed feed characterisation is required. Near infrared (NIR) reflectance spectroscopy technology has been successfully applied to determine tissue N-fractions in alfalfa hay and silage[10] and may be suitable for testing of BG and BS. The objective of this study was to determine if NIR technology can be used to investigate the effects of N-fertilisation levels on tissue N-fractions of BG and corresponding BS.

Methodology

This paper is part of a larger study in which 388 BG and 455 BS samples, collected in 1994, 1995 and 1996, were spectrally selected and used for the development of NIR regression equations. A Foss NIRSystems Model 6500 scanning monochromator with transport module was used in conjunction with NIR 3, v. 3, ISI software. The feed quality criteria included: TS-N[10], NP-N[10] (CP-N[10] minus buffer insoluble protein-N[10] minus B1-N[10]), B1-N (Bradford method[10]), B2-N[10] (buffer insoluble but neutral

detergent soluble N[10]), B3-N[10] (the neutral detergent insoluble but acid detergent soluble N[10]) and C-N (acid detergent insoluble N).[10]

Samples were collected at the soft dough stage from two dry land sites (Pincher Creek and Barrhead—1996) and one irrigated site (Bow Island—1995 and 1996). Field level experimental design was a split plot with fertility as the main plot treatment (0, 40, 80, 120, 160 and 200 kg N ha^{-1}) and varieties (Seebe, Tukwa, AC Lacombe, CDC Earl and Leduc) as sub-plots. BG samples were collected and dried in paper sacks while BS sub-samples were collected and ensiled by packing fresh material into sealed jars in the field. The NIR equations developed in the first phase of this project were used to evaluate the effect of site, variety and N-fertiliser level on BG and BS tissue N-fractions.

Results and discussion

Accuracy of near infrared calibration equations

The accuracy of NIR determinations was measured using R^2, *SEC*, *SECV* and 1 – *VR* (Table 1). An equation with an R^2 greater than 0.9 provides an excellent, and over 0.7 a good, quantitative measure-

Table 1. Regression statistics of the NIR equations for nitrogen-fractions in barley green feed (BG) and barley silage (BS).

Constituents	Feeds	*n*[g]	Mean	*SEC*[h]	*SECV*[i]	R^2[j]	1 – *VR*[k]
TS-N%[a]	BG	365	0.66	0.10	0.11	0.91	0.90
	BS	409	1.19	0.06	0.07	0.94	0.93
NP-N%[b]	BG	360	0.43	0.09	0.10	0.81	0.76
	BS	410	1.13	0.06	0.07	0.94	0.93
B1-N%[c]	BG	367	0.12	0.03	0.03	0.78	0.74
	BS	417	0.06	0.01	0.01	0.83	0.81
B2-N%[d]	BG	369	0.59	0.08	0.08	0.79	0.76
	BS	410	0.36	0.03	0.04	0.91	0.89
B3-N%[e]	BG	373	0.23	0.05	0.05	0.76	0.73
	BS	383	0.06	0.01	0.01	0.98	0.98
C-N%[f]	BG	381	0.08	0.01	0.01	0.99	0.99
	BS	384	0.07	0.01	0.01	0.99	0.99

[a] TS-N% total buffer-soluble nitrogen % contains NP-N% plus B1-N%
[b] NP-N% non-protein–nitrogen % contains NH_3, amino acids and peptides for BS, NH_3 not for BG
[c] B1-N% buffer soluble true protein–nitrogen % which is rapidly degraded in the rumen
[d] B2-N% buffer insoluble but neutral detergent soluble protein–nitrogen % which is slowly degraded in the rumen
[e] B3-N% neutral detergent insouble but acid detergent soluble protein–nitrogen % which is slowly degraded in the rumen
[f] C-N% acid detergent insoluble protein–nitrogen % which is a fraction not degraded at all
[g] *n* number of samples used for NIR calibration (without outliers)
[h] *SEC* standard error of calibration
[i] *SECV* standard error of cross-validation
[j] R^2 correlation coefficient between chemically analysed and NIR-predicted results
[k] 1-*VR* percentage of variation in chemical analyses explained by NIR

ment (J. Shenk, Personal Communication). Correlation (R^2) values for BS were excellent by this standard, whereas R^2 values for BG were only good. The other indicators revealed a similar trend with lower *SEC*, lower *SECV* and higher 1 – *VR* in BS than those in BG. Ensiling results in a large percentage of the N being converted to NP-N, thereby reducing variation between individual BS samples. Furthermore, ensiling started immediately after harvesting in the field whereas green feed samples remained at an ambient temperature for varying lengths of time before drying, depending on the site. This may partially explain the differences in R^2, 1 – *VR*, *SEC* and *SECV* between BG and BS NIR equations. The accuracy we obtained for the barley forage is similar to the accuracy found for alfalfa hay and silage,[10] and provides a good to excellent quantitative determination of N-fractions in BG and BS.

Effect of treatments on tissue nitrogen

Nitrogen fertility had a pronounced influence on the amount and form of N stored in the feed. Higher N-application rates resulted in higher yields, higher tissue N content and a resulting higher overall N yield. Maximum yields were generally obtained with the application of 80 kg N ha^{-1} (Table 2) but nitrogen yield (particularly in BG samples from the irrigated site, for both 1995 and 1996) continued to increase at much higher rates (Figures 1, 2 and 3). Although yield varied significantly with variety[2] at different locations and in different crop years, variety had little effect on the form of tissue B1-N, B2-N ($p = 0.01$) and B3-N ($p = 0.05$) (Table 3). There was a significant difference due to site and N fertility level for all tissue N-fractions ($p = 0.01$) and due to variety for TS-N, NP-N and C-N ($p = 0.01$) (Table 3). Calder and MaCleod[1] (two cultivars of barley green feed), McKenzie *et al.*[2] (five varieties of barley green feed) and DiRienzo *et al.*[4] (barley silage) reported similar results with increases in dry matter yield and crude protein due to increased N-fertilisation rates.

Site (dry land v. irrigated) and year (Bow Island site) had an effect on tissue N quality (Figures 1, 2 and 3), due, in part, to the influence on available N by N-fertiliser and soil moisture levels at different sites. Our results (Figures 2 and 3) indicate that the desired N-fractions (B2-N and B3-N) can be increased with increased N-fertiliser application rates up to 120 kg N ha^{-1}. Above this they tend to level off except for B1-N in BG and B2-N in BG and BS harvested at Bow Island (irrigated). Otherwise, tissue N-fractions did not increase at dry land sites above 80 kg N ha^{-1}. The important B2-N and B3-N-fractions are undegraded in rumen but utilised in intestine[6]. Waldo and Jorgensen[12] stated "As milk production in a cow has increased, so has the total nitrogen (N) concentration in the feed supply as

Table 2. Increases in dry matter yield with increasing nitrogen fertility rate except after 80 N Kg ha^{-1}.

Nitrogen Fertility Rate[a]	Bow Island DMY[b] (Irrigation Site)	Pincher Creek DMY [b](Dry Land)
0	8991	6767
40	11577	8403
80	14120	8912
120	13490	8911
160	14721	8763
200	14228	8583

[a] Nitrogen fertility rate kg ha^{-1}

[b] Results of dry matter yield (DMY, kg ha^{-1}) were the average of five barley varieties, namely Seebe, Tukwa, AC Lacombe, CDC Earl and Leduc

Table 3. Tissue nitrogen-fractions show significant difference between three sites and within six nitrogen fertility treatments.

Probability	TS-N[a]	NP-N[b]	B1-N[c]	B2-N[d]	B3-N[e]	C-N[f]
Site	0.0001	0.0001	0.0001	0.0087	0.0001	0.0001
Variety	0.0001	0.0001	0.2344	0.2417	0.0390	0.0014
Treatment	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

Probability < 0.01 and < 0.05 shows significant difference at 99% and 95% confident level, respectively
[a] TS-N% Total buffer-soluble nitrogen % contains NP-N% plus B1-N%
[b] NP-N% Non-protein–nitrogen % contains NH_3, amino acids and peptides for BS, NH_3 not for BG
[c] B1-N% Buffer soluble true protein–nitrogen % which is rapidly degraded in the rumen
[d] B2-N% Buffer insoluble but neutral detergent soluble protein–nitrogen % which is slowly degraded in the rumen
[e] B3-N% Neutral detergent insoluble but acid detergent soluble protein–nitrogen % which is slowly degraded in the rumen
[f] C-N% Acid detergent insoluble protein–nitrogen % which is a fraction not degraded at all

well as more importantly N-fraction that passes through the rumen as undegraded feed protein" (B2-N and B3-N). Less favoured N-fractions, NP-N (subject to loss through excretion or in urine) and C-N (considered to be wholly indigestible),[13–15] also increased with increasing N-fertiliser level for both dry land and irrigated sites.

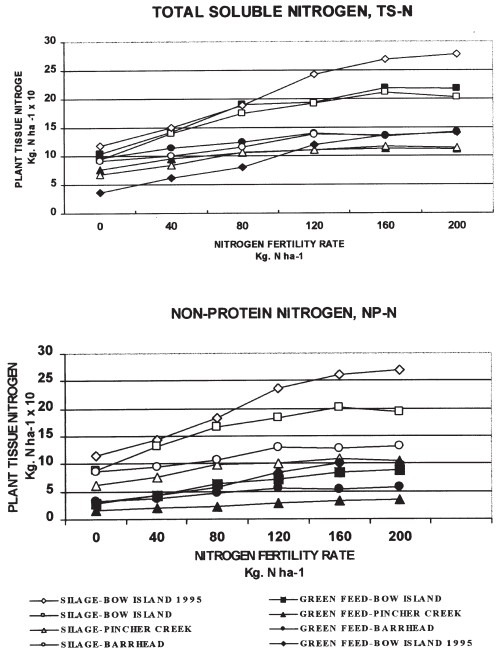

Figure 1. N-fertility increases tissue total soluble nitrogen and non-protein nitrogen in barley green feed and corresponding silage.

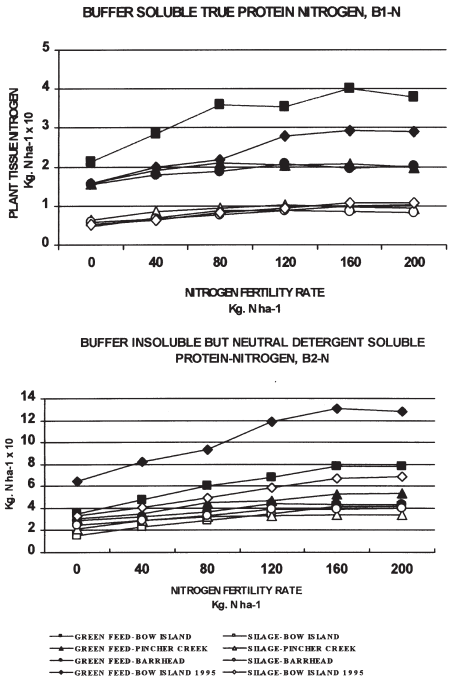

Figure 2. N-fertility increases tissue B1-N and B2-N barley green feed and corresponding silage.

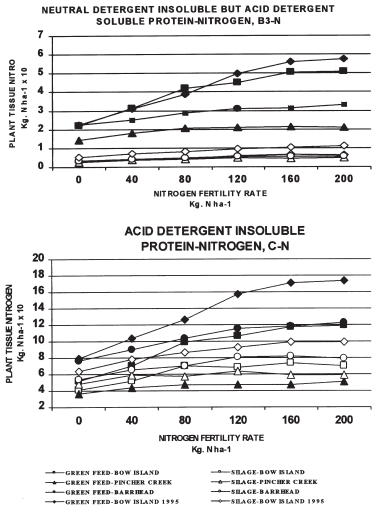

Figure 3. N-fertility increases tissue B3-N and C-N in barley green feed and corresponding silage.

Effect of preservation method on tissue nitrogen

These results clearly show that ensiling increased NP-N levels at the expense of the more desirable by-pass proteins (B2-N and B3-N). NP-N is consistently higher in BS than that in BG for all the sites tested, whereas the opposite is true for B3-N and, to a lesser extent, for B2-N. The results of this study suggest that N fertiliser should be applied at rates exceeding those producing maximum yields (80 kg ha^{-1}) for green feed crops but not silage crops. TS-N (NP-N + B1-N) is similar in both BG and BS at all sites except the1995 Bow Island site (Figure 1). More NP-N is contained in the BS than in the BG, whereas there is a greater level of B2-N in BG. Based on N-fractions, BG and BS are quite different feeds, consistent with the findings for alfalfa hay and silage.[10]

Conclusions

The amount of N-fertiliser applied is the most important fertiliser management factor determining the form of tissue N. Preservation technique is extremely important. Ensiling has a detrimental effect on the tissue N profile (feed quality) which becomes more pronounced as the N- fertiliser rate is increased. Thus, the best rate of N to apply depends on how the barley is preserved with the optimum rate for green feed being higher than for silage. This study confirms that NIR technology is very useful for evaluating the effects of management practices on barley feed quality.

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