JARQ 31, 55–63 (1997)

Application of Near Infrared Reflectance Spectroscopy to Forage Analysis and Prediction of TDN Contents

Masahiro AMARI and Akira ABE

Department of Animal Nutrition, National Institute of Animal Industry
(Tsukuba, Ibaraki, 305 Japan)

Abstract

Near infrared reflectance spectroscopy (NIRS) method was used to predict the chemical composition and total digestible nutrients (TDN) content of forage. A total of 388 out of 503 samples were used to develop the calibration equation. The samples consisted of temperate grass in the form of hay (n = 126), silage (n = 120), and its hay-silage mixture (n = 60), and corn silage (n = 142). The remaining 115 forages samples including temperate grass hay (n = 58), silage (n = 39), and corn silage (n = 18) were used to validate the developed calibration equations. These samples were analyzed in duplicate for moisture, crude protein (CP) and crude fiber (CF) contents by the proximate method. Organic cellular contents (OCC), organic cell wall (OCW), low digestible fiber fraction (organic b fraction: Ob), and high digestible fiber fraction (organic a fraction: Ob) were determined by an enzymatic method, while acid detergent fiber (ADF) was determined by a detergent method. Accuracy of the predictions was assessed by the correlation coefficients (r) and standard error of prediction (SEP). Two prediction methods of the TDN by NIRS were compared. In the first method, NIRS values were extrapolated to the TDN value calculated by the following equations (indirect method): (1) equation in Adams method, (2) equation in Martin method, (3) Donker equations, (4) equations based on enzymatic analysis, and (5) equation for detergent method. In the other method, the in vivo TDN was directly calibrated (direct method). *In vivo* value was obtained from digestion trials carried out in sheep. In the prediction by NIRS method, the r and SEP values of hay were 0.98 and 0.96 for CP, 0.79 and 2.95 for CF, 0.92 and 2.44 for OCC, 0.91 and 3.01 for OCW, 0.81 and 4.80 for Ob, 0.74 and 2.44 for ADF, respectively. Similar values were obtained for grass silage. However, lower r and higher SEP values were observed in corn silage compared with the hay samples used in this study. These results indicated that the moisture, CP, CF, OCC, OCW, Ob and ADF contents in forage samples can be predicted by using NIRS techniques with an acceptable accuracy. For the TDN, in the case of the indirect method, an r value of over 0.7 and a standard error (Se) of 1.5-3.8 were obtained from calibration based on the equations of Adams, Martin, and enzymatic analysis in hay. However, for corn silage a very low r value, below 0.5, and Se values of 0.8-3.8 were obtained. In the direct method of TDN calibration, correlation coefficients over 0.8 and standard error of 2.3-3.4 for all the feed groups were recorded.

Discipline: Animal industry
Additional key words: corn silage, grass silage, hey

Introduction

Successful results in using the NIRS method for forage composition and its nutritive value including total digestible nutrients (TDN) have been reported in recent years. These studies were based on chemical analysis results or the TDN content obtained from digestion trials. The equation for the estimation of the TDN was an alternative method to cut the costly, laborious and time-consuming methods involved in digestion trials. Calibration of the NIRS using the reference data obtained from these equations is possible but may result in higher bias than by using reference data from *in vivo* determinations because of the accumulation of bias from each component used in the equation.

Successful results from the NIRS were obtained in one species of forage samples. However, in practice, feeds given to animals consist of a combination of many kinds of feeds differing in processing. Physical structure of feed is known to affect the NIRS method. Mixing or processing of feeds may affect the reliability of this method. Based on these 2 facts, this study aimed at evaluating the reliability of the NIRS method for studies on the chemical composition, including total digestible nutrients (TDN), of grass in the form of hay, silage and its mixture.

Materials and methods

1) NIRS apparatus

The NIRS apparatus used was Neotec FQA model 51A, which is connected to an N300 computer terminal. The computer was equipped with NSAS software.

2) Samples for developing the calibration equation

Samples for developing the calibration equation (standard samples) consisted of temperate grass hay (hay; n = 126), temperate grass silage (grass silage; n = 120), corn silage (n = 142) and hay-grass silage mixture (n = 60 of each). Composition of grass hay and grass silage used was as follows:

(1) Hay: orchardgrass (n = 7), Italian ryegrass (n = 66), rice straw (n = 6), orchardgrass-dominant mixture (n = 23), others (n = 24).

(2) Grass silage: orchardgrass (n = 19), Italian ryegrass (n = 13), and grass-dominant mixture (n = 63), others (n = 25).

(3) Hay-grass silage mixture: Separate calibration for hay, grass silage, hay-grass silage mixture, and corn silage was developed in standard samples.

3) Samples for validation

Fifty-seven samples of hay, 39 samples of grass silage and 18 samples of corn silage were used for validation test of calibration (unknown samples) as follows:

(1) Hay: orchard grass (n = 10), Italian ryegrass (n = 9), tall fescue (n = 6), perennial ryegrass (n = 6), alfalfa (n = 6), fall panicum (n = 7), and grass-dominant mixture (n = 7).

(2) Grass silage: orchard grass-timothy 50-50 mixture (n = 32), grass-dominant mixture (n = 5), timothy (n = 1), and alfalfa (n = 1).

4) Chemical composition and analytical methods

The samples were analyzed for the moisture, crude protein (CP), ether extract (EE), crude fiber (CF) and crude ash contents by a proximate method[13], organic cellular contents (OCC), organic cell wall (OCW), low digestible fiber fraction (organic b fraction: Ob), high digestible fiber fraction (organic a fraction: Oa) by an enzymatic method[4], and acid detergent fiber (ADF) by a detergent method[17]. In case of corn silage, starch content was also determined. *In vivo* data for unknown samples were observed from digestion trials carried out in sheep.

Table 1. Various regression equations used for TDN estimation in hay and corn silage

	Methods*		Regression equation
Hay	Adams method[6]	Grass	TDN = 50.41 + 1.04 × CP − 0.07 × CF
		Mixed	TDN = 65.14 + 0.45 × CP − 0.38 × CF
		Alfalfa	TDN = 74.43 + 0.35 × CP − 0.75 × CF
	Martin method[12]		TDN = 96.35 − 1.15 × ADF
	Enzymatic method[2]	Grass or mixed	TDN = 1.111 × (OCC + Oa) + 0.605 × Ob − 18.8
		Alfalfa hay	TDN = 1.021 × (OCC + Oa) + 0.498 × Ob − 16.1
Corn silage	Adams method[6]		TDN = 77.07 − 0.75 × CP − 0.07 × CF
	Donker method[8) a)]		TDN = 90.25 − 1.175 × CF
	Donker method[8) b)]		TDN = 79.56 − 0.3353 × CF + 0.7006 × CP − 0.0538 × (CF × CP)
	Enzymatic method[1]		TDN = 0.545 × OCC + 1.413 × Oa + 26.4
	Summation method[1,15]		TDN = 0.860 × (OCC + Oa) + 0.574 × Ob + 0.996 × EE − 8.9
	ADF method[14]		TDN = 89.89 − 0.752 × ADF
	Feed table[15]		TDN = DCP[c)] + 2.25 × DEE + DNFE + DCF

* Numbers indicate the references used.
a): Donker method with simple regression equation.
b): Donker method with multiple regression equation.
c): Digestibility of proximate components in feed table was used to estimate the TDN.

5) Particle size of the samples

All the samples were ground using a Cutting mill with a 1 mm screen for chemical analysis, and an Udy cyclone mill to pass a 0.75 mm screen for NIRS analysis.

6) Accuracy of prediction and estimation of TDN content

(1) Accuracy of the prediction

Accuracy of the predictions was evaluated by the correlation coefficients (r) and standard error of the prediction (SEP) between values on a laboratory analytical basis (LAB) and contents predicted by near infrared reflectance spectroscopy (NIR value).

(2) Estimation method of TDN contents

Two prediction methods were compared to estimate the TDN value by NIRS. In the first method the NIR value was extrapolated to TDN calibration equations (indirect method). In the other method, direct calibration of *in vivo* TDN was applied (direct method). The calibrations for hay and corn silage in the indirect method are shown in Table 1. In the direct method, calibrations of *in vivo* TDN contents were performed in feed groups consisting of hay, grass silage, corn silage and mixed hay-grass silage.

Results and discussion

1) Precision of calibration

(1) Components in samples

Contents of major components in the standard samples ranged widely in temperate hay, amounting to 20.2% for CP and 43.2% for OCW. The same trend was noted in grass silage. Furthermore, in corn silage, the contents widely ranged with a value of 36.4% for OCW and 33.9% for starch. Ether extracts (EE) showed a very narrow range, as each feed contained a small amount of EE. In the hay-silage mixture, these components were as widely distributed as in hay and silage. Compared with the distribution of various components in unknown samples, the same trend as that of standard samples was noted for hay, grass silage and corn silage.

(2) Selection of wavelength for measurements

Table 2 shows the wavelengths of various components in hay as well as the correlation coefficient (r) and standard error (Se) of LAB and NIR values. The first wavelength used was 1,756 nm for the moisture content, 2,149 nm for the CP content, 2,258 nm for the EE content, and the same peak at 2,281 nm for the CF, OCW and ADF contents, respectively. These wavelengths were correlated with the absorption derived from the chemical structure of each component[9,11,18,19]. The absorption was considered to be associated with the OH group in the case of moisture, NH of amino group in CP, CH bonding for fat in EE, and CH bonding for cellulose in CF, OCW and ADF. However, absorption in the case of crude ash, OCC, Oa and Ob was not associated with the chemical structure of the components. It is worth mentioning that for the second and third wavelengths the absorption was not associated with the chemical structure of the components.

Table 2. Selected wavelengths and statistical evaluation for calibration of hay

Chemical components a)	Wavelength (nm)			r b)			SEC b)		
	1st	2nd	3rd	r_1	r_2	r_3	SEC_1	SEC_2	SEC_3
Moisture	1756	1629	1744	–	0.95	0.95	–	0.68	0.67
Crude protein	2149	–	–	0.98	–	–	0.88	–	–
Ether extract	2258	2021	2182	0.45	0.49	0.50	0.87	0.86	0.85
Crude fiber	2281	2332	1591	0.92	0.94	0.95	1.76	1.48	1.37
Crude ash	1606	2005	2313	0.68	0.72	0.79	2.02	1.90	1.71
Organic matter	1605	2005	2313	0.68	0.72	0.79	2.02	1.90	1.71
OCC	2279	1931	2026	0.93	0.95	0.96	2.78	2.46	2.21
OCW	2281	1650	1946	0.91	0.94	0.96	3.43	2.88	2.39
Oa	2214	1725	1746	0.67	0.75	0.79	3.58	3.20	3.00
Ob	2212	2023	1935	0.87	0.93	0.94	5.73	4.29	3.97
ADF	2281	1590	2332	0.91	0.94	0.95	2.24	1.91	1.66

a): OCC; Organic cellular contents, OCW; Organic cell wall, Oa; High digestible fiber fraction, ash-free, Ob; Low digestible fiber fraction, ash-free, ADF; Acid detergent fiber.

b): 1, 2, 3; r and SEC using 1st wavelength, using 1st and 2nd wavelengths, and using 1st, 2nd and 3rd wavelengths for 1, 2, 3, respectively.

Table 3. Ranges of chemical components, correlation coefficients and standard error of samples used to develop the calibration equations

(DM%)

Chemical components[a)]	Hay (126)[b)]			Grass silage (120)			Mixed[c)] (120)			Corn silage (142)		
	Range	r[d)]	SEC[e)]	Range	r	SEC	Range	r	SEC	Range	r	SEC
Moisture	2.3–12.7	0.95	0.67	3.3–12.9	0.74	1.05	3.3–12.9	0.80	1.12	5.4–11.9	0.73	0.95
Crude protein	3.6–23.8	0.98	0.88	4.3–23.9	0.90	1.42	3.6–17.6	0.92	1.32	4.8–11.9	0.90	0.44
Ether extract	1.2– 5.8	0.50	0.85	1.3– 6.8	0.76	0.62	1.2–5.8	0.66	0.80	1.5– 5.7	0.65	0.47
Crude fiber	14.6–40.8	0.95	1.37	18.2–43.1	0.92	1.64	23.7–43.1	0.89	1.75	15.4–35.2	0.91	1.26
Ash	3.7–18.1	0.79	1.71	4.4–24.5	0.81	1.86	3.7–18.9	0.77	1.86	3.6– 9.6	0.70	0.91
Organic matter	81.9–96.3	0.79	1.71	75.5–95.6	0.81	1.86	81.1–96.3	0.77	1.86	90.4–96.5	0.70	0.91
OCC	12.0–54.0	0.95	2.46	12.7–44.5	0.92	2.32	12.7–39.2	0.92	2.46	21.8–59.1	0.94	2.09
OCW	37.0–80.2	0.96	2.39	47.2–81.1	0.92	2.52	51.7–80.2	0.92	2.56	36.6–73.0	0.93	2.00
Oa	6.2–35.1	0.79	3.00	3.7–24.0	0.64	3.83	3.7–26.3	0.71	2.96	3.3–19.0	0.75	1.28
Ob	14.6–73.3	0.94	3.79	24.2–73.0	0.89	4.01	30.4–73.3	0.91	3.35	28.4–58.5	0.91	2.18
ADF	28.0–49.7	0.95	1.67	22.9–48.6	0.92	1.86	28.0–49.7	0.89	1.92	20.6–45.3	0.91	1.53
Starch	–	–	–	–	–	–	–	–	–	0.5–34.4	0.91	2.89

a): As shown in Table 2.
b): ( ); Number of samples.
c): Mixed samples; Consisted of 60 hay and 60 grass silage samples.
d): r; Correlation coefficient.
e): SEC; Standard error of calibration.

Multiple regression used in the NIRS calculation combined with selection of the wavelengths enabled to obtain a high r value. The wavelengths, however, sometimes were not related to the chemical structure of the measured components, because the selected wavelength aimed only at achieving a high r value in the sample population. Thus, the precision may not always be attained if the equations from standard samples were applied to determine the components with a certain chemical structure in unknown samples.

Hence, the wavelength corresponding to the chemical structure of the respective components should be selected not merely based on the high r value. A wide absorption not affected by changes of wavelengths, and without sharp and small peaks should be selected as the derivations of the peaks may change depending on the temperature and moisture conditions[10,20]. These factors reduce the precision of calibration.

(3) Precision for each component

The range of forage samples used for the calibration, and their correlation coefficient (r) and standard error of calibration (SEC) are presented in Table 3. The values of correlation coefficients for CP, OCC, CF, OCW, Ob and ADF in each feed group were higher than 0.9. High correlation was also noted for starch in corn silage. A high correlation, 0.95, was obtained for moisture of hay, while in other feed groups lower values (0.73–0.80) than that of hay were recorded.

Lower values were observed for the other components, i.e. 0.50–0.76 for EE, 0.70–0.81 for crude ash, and 0.64–0.79 for Oa in every feed group. In the feed groups, very high values of 0.94–0.98 were observed for the moisture, CP, OCC and fibrous components in hay, which was also the highest within the components of every feed group. In the feed groups other than hay, the values were about 0.90 for CP, OCC and fibrous components. For the standard error (Se), corn silage showed a very small value of 0.44 for CP. Low r value was noted in every feed group for EE, while the Se value ranged from 0.47 to 0.87. On the contrary the r value was high for Ob, but the Se value was the highest among the components, ranging from 2.18 to 4.01.

These results suggest that a very high precision can be obtained for CP, with high r and small Se values. Ether extract with a lower content and a narrow range of components showed small Se and low r values. Correlation was low, because crude ash, which is an aggregate of various organic materials, can hardly be estimated with a particular wavelength. In the near infrared region, no absorption wavelength of the atomic group forming crude ash has been identified. Small r value of Oa was related to the high digestibility in ruminant animals. The chemical structure of Oa should be clarified and the precision of calibration should be improved, since Oa plays an important role among the fibrous

Table 4. Ranges of chemical components and *in vivo* TDN, correlation coefficients and standard error of prediction obtained from validation of the developed calibration equations

(DM%)

Chemical components[a)]	Hay (58)[b)]			Grass silage (39)			Mixed[c)]				Corn silage (18)		
	Range	r[d)]	SEP[e)]	Range	r	SEP	r_1[f)]	SEP_1	r_2[g)]	SEP_2	Range	r	SEP
Crude protein	6.4–26.9	0.98	0.96	8.1–21.0	0.92	1.40	0.98	0.81	0.93	1.38	6.3–10.0	0.57	0.82
Crude fiber	22.2–40.2	0.79	2.24	24.9–44.0	0.88	2.25	0.75	2.43	0.89	2.19	17.1–30.9	0.74	2.54
OCC	15.9–43.4	0.92	2.44	15.9–40.1	0.81	3.17	0.90	2.75	0.84	2.91	29.3–57.5	0.88	3.14
OCW	45.3–77.3	0.91	3.02	49.5–78.8	0.86	3.18	0.83	4.09	0.81	3.69	37.8–61.3	0.82	3.41
Ob	31.0–67.6	0.81	4.80	34.6–75.9	0.92	3.71	0.89	4.24	0.89	3.84	28.9–44.4	0.73	3.05
ADF	26.0–47.7	0.74	2.95	30.9–52.6	0.82	3.08	0.73	2.95	0.79	3.29	20.7–37.0	0.82	2.58
In vivo TDN[h)]	47.7–71.5			44.4–74.7							59.9–71.6		
Mean of TDN	60.2			57.8							66.9		

a): As shown in Table 2. b), c), d): As shown in Table 3.
e): SEP; Standard error of prediction.
f): 1; Statistical evaluation of hay sample prediction, using the calibration of mixed samples for hay and grass silage.
g): 2; Statistical evaluation of grass silage sample prediction, using the calibration of mixed samples for hay and grass silage.
h): TDN; Total digestible nutrients.

components in forage.

(4) Validation of calibration

Table 4 shows the range of forage samples used for the validation, and their correlation coefficient (r) and standard error of prediction (SEP). Each component showed a high correlation for CP, OCC and fibrous components and a low correlation for EE, crude ash and Oa. Components with high correlations showed a decreasing trend compared with r and Se in the calibration. When the CP of hay and grass silage in the unknown samples was estimated using the calibration for a mixed feed with hay and grass silage, the r value was higher, ranging from 0.98 and 0.93, respectively. In grass silage, the r and Se values of CP, OCC and fibrous components were in the range of 0.81–0.92 and 1.40–3.71, respectively, revealing large differences from the value of the calibration. In corn silage, the CP showed a low correlation of 0.57, due to the narrower component range than that of other feeds (3.7%). Generally in corn silage a low correlation of the components was noted, as the component range was narrower than that of hay.

Furthermore, in the prediction for the mixed feed, a similar trend was noted compared with the prediction of hay and grass silage for various components. The present study shows that a single calibration equation from either hay and silage can be used presumably because hay with a high moisture may undergo fermentation during storage. This process is similar to the ensilage process. Although, the form of silage and hay is different, the origin and the composition in dry matter are similar. Thus, the calibration developed from mixed hay and silage can be used for hay or silage.

To improve the estimation for EE and Oa where a low correlation coefficient of calibration (0.7) was obtained, study of these chemical structures is required. Furthermore, for CP, OCC, and the fibrous components, the precision should be enhanced by detailed classification of forage and analysis of the relation between the chemical structure and absorption characteristics.

2) Estimation of the content of total digestible nutrients (TDN)

(1) TDN in test samples

The highest, lowest and mean values of the content of *in vivo* TDN in the test samples in hay were 71.5, 47.7, and 60.2%, respectively, while in grass silage the values were 74.7, 44.4, and 57.8%, respectively. Thus, the distribution of the TDN values was wide in both hay and grass silage. However in corn silage the values were 71.6, 59.9, and 66.9%, respectively, with a narrower range than that of hay and grass silage.

(2) Estimation of TDN by regression equation

(A) Estimation of TDN in hay

Table 5 shows the results of TDN estimations using various estimation formula in Italian ryegrass. There were appreciable differences when the r and Se values of TDN and *in vivo* TDN were estimated by the methods of Adams[6)] and Martin[12)], namely 0.83 and 0.81 as well as 2.7 and 2.9, respectively. When the regression equation by enzymatic analysis (enzymatic method) was employed, the content of

Table 5. Range, mean, correlation coefficients and standard error for TDN in hay and corn silage determined with the estimation methods in comparison with the *in vivo* method

Estimation methods	Range	Mean	r	Se
Italian ryegrass				
In vivo	53.2 – 68.3	59.9	–	–
Adams	56.8 – 67.3	62.2	0.83	2.7
Martin	51.6 – 60.0	56.3	0.81	2.9
$Enzymatic_1$	54.2 – 61.0	57.1	0.81	2.9
$Enzymatic_2$	60.6 – 67.3	64.4	0.86	3.8
Corn silage				
In vivo	59.9 – 71.6	66.9	–	–
JFT[a)]	63.3 – 71.7	68.3	0.06	3.4
Adams	68.3 – 70.5	69.3	0.10	0.8
$Donker_3$[b)]	60.4 – 71.2	66.1	0.28	3.5
$Donker_4$[b)]	66.4 – 72.7	69.2	0.23	2.6
ADF	66.4 – 74.6	69.8	0.37	3.1
$Enzymatic_1$[c)]	60.9 – 68.5	65.6	0.49	2.5
$Enzymatic_2$[c)]	59.0 – 66.6	63.8	0.17	3.3
Summation	61.3 – 69.5	65.6	0.27	2.3

a): JFT; Japanese feeds tables.
b): 3,4; Donker method with simple regression equation and with multiple regression equation, respectively.
c): 1,2; Oa was obtained by subtracting Ob from OCW of NIR and by direct determination of NIR, respectively.

Oa was obtained in 2 ways by NIRS: by the direct method employed to obtain Oa (Oa method) and by the indirect method by subtraction of Ob from OCW (OCW-Ob method). The latter was used because a higher precision for estimation can be obtained with OCW and Ob, while the precision in Oa is low by NIRS. Based on the enzymatic methods (Oa method and OCW-Ob method), the r and Se values of TDN as well as the TDN content were estimated to be 0.86 and 0.81, and 3.8 and 2.9, respectively, the values being similar to those obtained by the methods of Adams and Martin. Compared with the estimated values, the maximum and minimum values of *in vivo* TDN as well as their difference were 68.3, 53.2, and 15.1%, respectively. On the other hand, by the Adams method the values were 67.3, 56.8, and 10.5%, while by the Martin method they were 60.0, 51.6, and 8.4%, respectively. In the enzymatic method of Oa the values were 67.3, 60.6, and 6.7%, and by the OCW-Ob method, 61.0, 54.2, and 6.8%, respectively. The values were underestimated as a whole by the Martin method. Meanwhile, when Oa was obtained from the difference between OCW and Ob, the content of TDN was comparatively underestimated. In addition, when Oa obtained by the NIR value was used, since the value of Oa was biased and overestimated, the content of TDN was also overestimated. Based on these results, the maximum or the minimum values by the Martin method were smaller than those of *in vivo* TDN. All the values recorded were underestimated. Based on the difference between the maximum and the minimum values, the values were smaller than those of *in vivo* TDN by each method, showing that the content of TDN was evaluated uniformly.

Table 6 shows the estimated TDN content for each species of temperate grass hay. Firstly, in grasses of pure sward and orchardgrass the r value was about 0.85 in all the methods. By the OCW-Ob enzymatic method, the value was 0.56, being lower than that by the other 2 methods. For Italian ryegrass, the

Table 6. Correlation coefficients and standard error of TDN of hay determined with the estimation methods in comparison with the *in vivo* method

	Number of samples	Adams		Martin		Enzymatic			
		r[a)]	Se[b)]	r	Se	r_1[c)]	Se_1	r_2[d)]	Se_2
Orchard grass	10	0.85	3.2	0.84	3.2	0.56	4.9	0.86	3.0
Italian ryegrass	9	0.83	2.7	0.81	2.9	0.81	2.9	0.65	3.8
Tall fescue	6	0.86	2.8	0.89	2.5	0.72	3.8	0.73	3.7
Perennial ryegrass	6	0.77	3.8	0.97	1.5	0.88	2.9	0.94	2.1
Alfalfa	6	0.70	4.7	0.65	5.1	0.89	3.1	0.89	3.0
Fall panicum	7	0.90	1.2	0.96	0.8	0.27	2.7	0.49	2.5
Mixed hay	7	0.71	5.1	0.62	5.7	0.62	5.7	0.45	6.5
Grass hay	34	0.62	3.9	0.78	3.1	0.58	4.0	0.76	3.2
Grass and mixed hay	41	–	–	0.75	3.7	0.61	4.4	0.71	3.9

a): r; Correlation coefficients.
b): Se; Standard error.
c): 1; Oa was obtained by subtracting Ob from OCW of NIR.
d): 2; Oa was obtained by direct determination of NIR.

same figure as that for orchardgrass was noted. In perennial ryegrass, high correlations above 0.9 were noted by the Martin and Oa methods, but a lower value than that obtained by the other 2 methods was obtained by the Adams method. In tall fescue, the value was about 0.8 by the methods of Adams and Martin and 0.7 by the enzymatic method, the values being lower than those for other species of grasses. In 34 forage grasses of pure sward and 41 grasses of mixed sward, the r value was about 0.6–0.8, the latter showing a lower correlation than the former by each method.

As for Se, the values ranged from 2.7 to 3.8% by the Adams method, 1.5–3.2% by the Martin method, 2.9–4.9% by the OCW-Ob method, and 2.1–3.8% by the Oa method for forage grasses of pure sward. In the grasses of mixed sward, there was a very large error of 5.1–6.9% by each method. In alfalfa, the correlation was higher by the 2 enzymatic methods, but lower by the methods of Adams and Martin. For Se, there was a larger error by each method compared with that of forage grasses.

In the case of fall panicum, a high correlation was noted by the methods of Adams and Martin, with very small standard errors of 1.2 and 0.8%, respectively. By the enzymatic method, the r value was very low, while the Se value was small, 2.5 and 2.3 in the Oa and OCW-Ob methods, respectively.

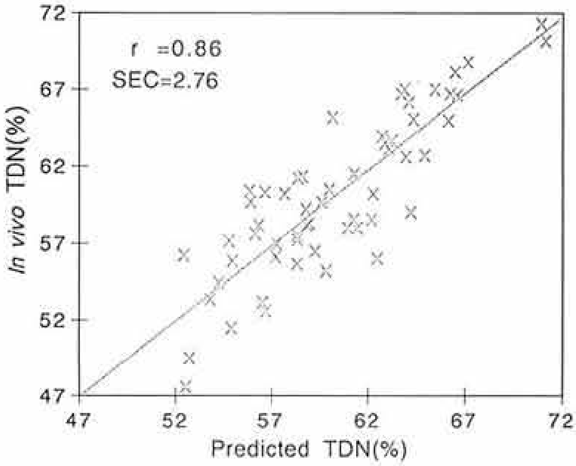

Fig. 1. Relationship between *in vivo* TDN and predicted TDN in hay using multiple reflectance data employing 3 wavelengths

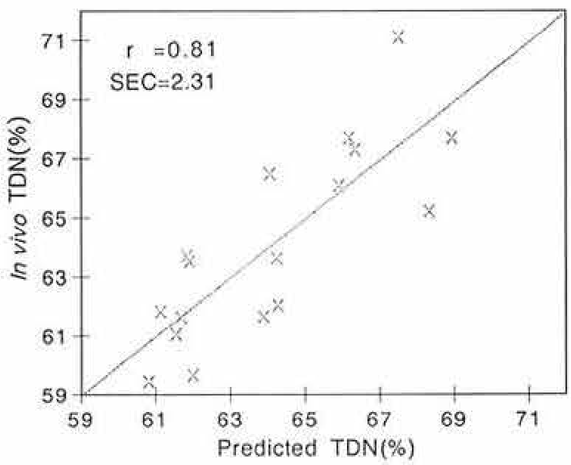

Fig. 3. Relationship between *in vivo* TDN and predicted TDN in corn silage using multiple reflectance data employing 3 wavelengths

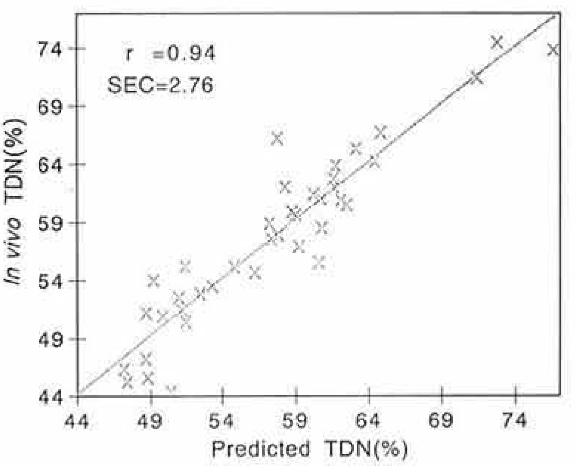

Fig. 2. Relationship between *in vivo* TDN and predicted TDN in grass silage using multiple reflectance data employing 3 wavelengths

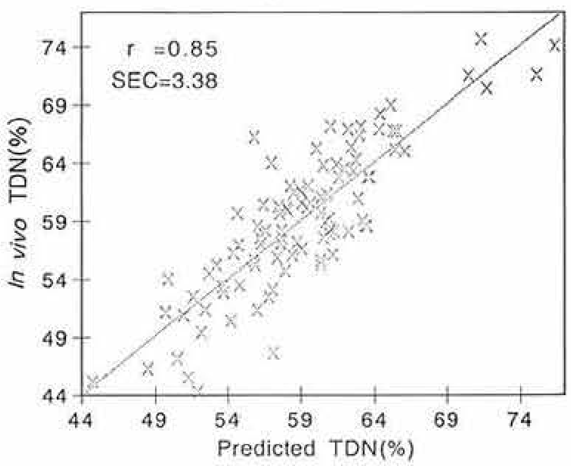

Fig. 4. Relationship between *in vivo* TDN and predicted TDN in mixed forage hay plus grass silage using multiple reflectance data employing 3 wavelengths

Table 7. Selected wavelengths and statistical evaluation of *in vivo* TDN calibration test

	n	Wavelength (nm)			r[a]			SEC[b]		
		1st	2nd	3rd	r_1[c]	r_2	r_3	SEC_1[c]	SEC_2	SEC_3
Hay	57	1645	1251	2007	0.77	0.83	0.86	3.34	2.96	2.76
Grass silage	39	1645	2308	1675	0.86	0.92	0.94	3.91	2.91	2.76
Corn silage	17	2252	1711	2208	0.59	0.69	0.81	2.74	2.54	2.31
Mixed samples[d]	96	1647	2315	1666	0.83	0.84	0.85	3.60	3.45	3.38

a): r; Correlation coefficients.
b): SEC; Standard error of calibration.
c): 1, 2, 3; r and SEC using 1st wavelength, using 1st and 2nd wavelengths, using 1st, 2nd and 3rd wavelengths for 1, 2, 3, respectively.
d): Mixed forage consisting of hay and grass silage.

Values of TDN estimated in individual samples by the method of Martin and enzymatic method in forage grasses of pure sward were lower than those of the mean *in vivo* TDN. In grasses of the mixed sward, the values were high by the Adams method, low by the Martin method, and close to the mean *in vivo* TDN by both the Oa and OCW-Ob enzymatic methods. For alfalfa the values were high by the methods of Adams and Martin, and low by the enzymatic method. For fall panicum, the TDN value by the Adams method was close to the mean *in vivo* TDN value, while it was lower than the mean *in vivo* TDN value by the Martin and enzymatic methods.

(B) Estimation of TDN content in corn silage

In corn silage, the TDN content estimated by the NIR value resulted in a very low correlation with the *in vivo* TDN by all methods. Thus, estimated values in the individual samples were recorded, and the results are shown in Table 5. According to the estimation based on the NIR value, the method relating to digestibility in the Feed Standard Tables[15] and that of Adams[6] showed uniform levels. By the methods using Donker's multiple regression equations[8], and that using ADF[14], the TDN content by the NIRS value was higher than that of the *in vivo* TDN. By Donker's simple regression equation[8], enzymatic methods[1], and the Summation[1,5], the difference between the kinds of feed was distinct, reflecting the *in vivo* level. Furthermore, by the use of the OCW-Ob method *in vivo* TDN levels were rather lower.

Regression equation for TDN was obtained from the contents of CP or various fibrous components in the feed which were estimated by NIRS. As stated above, there were samples or sample groups showing a fairly high precision unlike other groups. Since the TDN content was estimated using the equations, there were errors in estimation due to the accumulation of errors from analysis methods (LAB vs NIR), and the equations themselves. Many studies have indicated that a definite estimation error should be considered in regression equations[7,12].

3) Calibration of in vivo *TDN content*

To estimate the TDN content, calibrations of *in vivo* TDN content were made by NIRS for each feed group. The results are shown in Figs. 1–4. In these calibrations, the correlation coefficients between *in vivo* TDN contents and NIRS prediction were high. The r and Se values for hay, grass silage, corn silage and mixed hay-grass silage were 0.86 and 2.76, 0.94 and 2.76, 0.81 and 2.31, and 0.85 and 2.31, respectively.

Suitable wavelengths for calibration of each feed group are presented in Table 7. Wavelength of calibration was 1,645 nm for hay, grass silage and mixed hay-grass silage and 2,252 nm for corn silage. The wavelength of 1,645 nm was considered to originate from the chemical bond of aromatic molecule combination[15] or $=CH_2$[8,15] or $-CH_3$[7,8,15], and that of 2,252 nm from C-H[7] of the chemical bond in starch. Although the relation between selected wavelength and *in vivo* TDN contents can not be defined based on the data obtained in this study, it was considered to be applicable to the direct estimation of TDN content by NIRS.

In conclusion, the error in the indirect method is compounded by 2 errors caused by: (1) estimation of component contents by NIRS and (2) estimation of TDN contents by the developed calibration equation. Therefore, a similar study should be conducted for combined samples on which digestion trials are carried out in cattle.

References

1) Abe, A. (1988): Feed analyses based on the carbohydrates and its application to the nutritive value of feeds. *Mem. Natl. Inst. Anim. Ind.*, **2**, 46-64 [In Japanese].
2) Abe, A. et al. (1985): Assessment of total digestible nutrients (TDN) of hays from its chemical composition. *Jpn. J. Zootech. Sci.*, **56**, 12-19 [In Japanese with English summary].
3) Abe, A. & Iwasaki, K. (1984): Starch estimation method by enzymatic analysis of corn silage, formula feed and ingredients of formula feed. *J. Jpn. Grassl. Sci.*, **29**, 350-353 [In Japanese with English summary].
4) Abe, A. & Horii, S. (1979): Comparison of detergent method and enzymatic method for the determination of cell wall constituents of feed samples. *J. Jpn. Grassl. Sci.*, **25**, 70-75 [In Japanese with English summary].
5) Abe, A. & Nakui, T. (1979): Application of enzymatic analysis to the prediction of digestible organic matter and to the analysis of the changes in nutritive value of forages. *J. Jpn. Grassl. Sci.*, **25**, 231-240 [In Japanese with English summary].
6) Adams, R. S. et al. (1964): New relationships for estimating TDN content of forage from chemical composition. *J. Dairy Sci.*, **47**, 1461.
7) Barber, W. P., Adamson, A. H. & Altman, J. F. B. (1984): New methods of forage evaluation. *In* Recent advances in animal nutrition. eds. Haresign, W. & Cole, D. J. A., Butterworths, England, 161-176.
8) Donker, J. D. & Naik, D. G. (1979): Predicting total digestible nutrients and estimated net energy of dairy ration from chemical component. *J. Dairy Sci.*, **62**, 424-432.
9) Goddu, R. F. (1960): Near-infrared spectrophotometry. *Adv. Anal. Chem. Instrum.*, **1**, 347-417.
10) Iwamoto, M. & Uozumi, J. (1985): Near infrared spectrophotometry for nondestructive measurement of food quality. *Nippon Shokuhin Kogyo Gakkaishi.*, **32**, 685-695 [In Japanese].
11) Law, D. P. & Tkachuk, R. (1977): Near infrared diffuse reflectance spectra of wheat and wheat components. *Cereal Chem.*, **54**, 256-265.
12) Martin, N. (1985): Agricultural extension service, University of Minnesota, No. 2637.
13) Morimoto, H. (1971): Experimental method of animal nutrition. Yokendo, Tokyo, 280-304 [In Japanese].
14) Nakui, T. et al. (1980): The influence of varieties and weather on nutritive value and yield of corn silages. *Res. Bull. Hokkaido Natl. Agric. Stn.*, **126**, 149-162 [In Japanese with English summary].
15) National Research Council of Agriculture, Forestry and Fisheries (1980): Standard tables of feed composition in Japan. Central Association of Livestock Industry, Tokyo [In Japanese].
16) Norris, K. H. (1984): Modern methods of food analysis IFT basic symposium series. Reflectance spectroscopy. The AVI Publishing, Westport, 178.
17) Van Soest, P. J. (1963): Use of detergents in the analysis of fibrous feeds. I. Preparation of fiber residues of low nitrogen content. *J. Assn. Off. Anal. Chem.*, **46**, 825-829.
18) Wetzel, D. L. & Mark, H. (1978): Spectroscopic cellulose determination as a criterion of flour purity with respect to bran. *Cereal Foods World*, **22**, 481.
19) Wheeler, O. H. (1959): Near infrared spectra of organic compounds. *Chem. Rev.*, **59**, 629-666.
20) Williams, P. C., Norris, K. H. & Zarowski, W. S. (1984): Influence of temperature on estimation of protein and moisture in wheat by near-infrared reflectance. *Cereal Chem.*, **59**, 473-477.

(Received for publication, November 10, 1995)