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Predicting Chemical Compositions and Sheep Responses by Near Infrared Reflectance Spectroscopy in Forage

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Abstract

According to a newly defined index (evaluation index, E1) as proposed in this paper, near infrared reflectance spectroscopy (NIRS) has a high ability to predict accurately some forage components related to protein and fiber; furthermore, NIRS has a high potential for the direct prediction of some feeding values related with digestibility and intake as accurately as they are predicted by current laboratory methods.

Discipline: Grassland
Additional key words: evaluation index (E1), feeding value, NIRS, quality

Introduction

Near infrared reflectance spectroscopy (NIRS) has become a widely used method in various fields, especially in food industry[12]. It has three main advantages: 1) NIR measurements can be made with a high speed and be available for multiple analyses with one operation; 2) NIR instruments are simple and safe in use; and 3) NIRS is usually non-consumptive for samples and non-destructive for on-line control operations. In forage analyses, NIRS has also been used for the determination of a number of quality parameters[5,6,11,15]. Furthermore, there has been a growing interest in using it in plant breeding programs as an effective screening tool[4,7,13] because of its high capacity to analyze a large number of samples within a short period of time.

This report accounts mainly for the results of testing accuracy of prediction of forage quallity by NIRS and its acceptability for practical use. Regarding the forage quality, some chemical compositions mainly related to protein and fiber and some *in vivo* feeding values related with digestibility and intake were applied for the NIR analyses.

The technology of the NIRS method is now in the development stage; the *sleeping giant*[2] will go running at full speed in the future.

Materials and methods

1) Sampling

Ninety-nine samples of three grass species (*Dactylis glomerata* L., *Lolium perenne* L. and *Phleum pratense* L.) and fifty-two samples of three legume species (*Medicago sativa* L., *Trifolium pratense* L. and *Trifolium repens* L.) grown at the Shintoku Livestock Experiment Station, Hokkaido, Japan were collected from each harvest at different growth stages[8]. In these 151 samples, several components including chemical compositions and sheep responses were determined.

2) Determination of components

(1) Determination of chemical compositions

Samples were ground in a Wiley mill to pass through a 1.0 mm sieve, and subsequently the following chemical compositions were assayed by standard procedures: crude protein (CP), ether extract (EE), neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL). For orchardgrass samples, cellulase-soluble

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dry matter (CSDM) was also determined by hydrolysis for 16 hr with a 0.2% cellulase solution.

(2) Determination of sheep responses

The following animal feeding trials were performed by six sheep: *in vivo* dry matter digestibility (DMD), digestible crude protein (DCP), digestible cell wall substances (DCW), total digestible nutrients (TDN), *ad libitum* dry matter intake (ADMI) and digestible energy intake (DEI). DMD was measured at an *ad libitum* intake level and DCW was calculated by multiplying NDF by NDF digestibility.

3) Near infrared analysis

(1) Preparation of samples

Samples were reground through a 0.5 mm screen in a UDY cyclone mill prior to NIR measurements.

(2) Apparatus

A Neotec model 6350 MkII system was used to obtain NIR spectra of log (1/R) in the range 1,100 through 2,500 nm. The raw spectra were transformed to the second derivative spectra, because the author's preliminary study[8] indicated that the greater accuracy could be achieved by using the second derivative spectra compared with log (1/R) or first derivative spectra for the determination of some forage components.

(3) Grouping of samples

Three sample groups were made for the present study (Table 1): (A) samples of three grass species and three legume species; (B) samples of three grass species; and (C) samples of orchardgrass. In each sample group, samples for calibration sample sets were selected so that they well distributed over the range and representative in several characters of the population. The rest of the samples were used for prediction sample sets.

Table 1. Sample groups and number of samples used for calibration and prediction

Sample group	Total	Calibration	Prediction
(A) Three grass species and three legume species[a]	151	77	74
(B) Three grass species[b]	99	57	42
(C) Orchardgrass	72	41	31

a): Including orchardgrass, perennial ryegrass, timothy, alfalfa, red clover and white clover.

b): Including orchardgrass, perennial ryegrass and timothy.

(4) Calibration

In each sample group, the following three types of regression equations were derived from calibration sample sets:

$$y = c_0 + c_1 f(\lambda_1),$$

$$y = c_0 + c_1 f(\lambda_1) + c_2 f(\lambda_2), \text{ and}$$

$$y = c_0 + c_1 f(\lambda_1) + c_2 f(\lambda_2) + c_3 f(\lambda_3),$$

where y is the value of chemical compositions or sheep responses to be estimated, c_i is the intercept or constant and $f(\lambda_i)$ is the second derivative data of log(1/R) at λ_i wavelength.

As candidate equations, 24 to 33 equations were calibrated for each component. Wavelengths of λ_2 and λ_3 were selected on the basis of forward stepwise procedure.

(5) Prediction

The candidate equations were tested regarding their accuracy with prediction sample sets. An equation that had the best results of both calibration and prediction was selected for each component.

Results and discussions

1) Evaluation of accuracy and practical acceptability

There are several statistics used for assessing the accuracy and practical acceptabillity of calibration equations and prediction results[17]. In this study, standard error of calibration (SEC) and coefficient of determination (R^2) were used for assessing the accuracy of calibration equations, and standard deviation of prediction error (SDP) was used as an indicator of accuracy of prediction. The formula of SDP is as follows:

$$SDP = \sqrt{\frac{\Sigma\{(x-y) - \Sigma(x-y)/n\}^2}{n-1}},$$

where x is the predicted value, y is the actual value and $\Sigma(x-y)/n$ corresponds to bias.

Table 2 shows the calibration statistics of best calibration equations for each component in the sample group (B) of grasses and Table 3 presents the prediction statistics of those equations. The individual SDP values listed in Table 3 indicate the accuracy of prediction of each component; however, it is difficult to compare the accuracies of those compo-

Table 2. Best calibration statistics in the sample group (B) of grasses

Component		R^2	SEC[a]	Wavelengths[b]	Range
CP	(%)	0.988	0.57	3	4.9 – 22.8
EE[c]	(%)	0.733	0.62	3	1.7 – 6.2
NDF	(%)	0.974	1.51	3	32.6 – 72.0
ADF	(%)	0.962	1.25	2	18.1 – 43.5
ADL	(%)	0.895	0.57	2	1.4 – 7.7
DMD	(%)	0.876	3.60	3	42.0 – 81.0
DCP	(%)	0.982	0.60	3	1.2 – 17.7
DCW[d]	(%)	0.581	3.23	3	21.8 – 43.0
TDN	(%)	0.859	3.43	3	39.8 – 78.0
ADMI[e]	($g/W^{0.75}$)	0.769	8.14	3	29.1 – 87.1
DEI	($Kcal/W^{0.75}$)	0.845	25.7	3	49 – 295

a): Standard error of calibration, b): Number of wavelengths used in the calibration equation, c): Ether extract, d): Digestible cell wall substances, e): *Ad libitum* dry matter intake.

Table 3. Best prediction statistics in the sample group (B) of grasses

Component		SDP[a]	Bias	Range
CP	(%)	0.61	−0.23	7.6 – 21.2
EE[b]	(%)	0.45	0.17	2.8 – 5.5
NDF	(%)	1.69	0.32	44.6 – 66.3
ADF	(%)	1.09	−0.14	24.8 – 39.0
ADL	(%)	0.48	−0.00	2.4 – 7.1
DMD	(%)	2.90	−0.80	51.0 – 73.0
DCP	(%)	0.70	−0.20	3.7 – 15.9
DCW[c]	(%)	3.12	0.50	27.5 – 41.3
TDN	(%)	2.99	−0.22	51.3 – 68.4
ADMI[d]	($g/W^{0.75}$)	7.69	2.41	39.1 – 86.3
DEI	($Kcal/W^{0.75}$)	23.9	3.3	90 – 247

SDP and bias value were obtained from the prediction of the best calibration equation, data of which were shown in Table 2.
a): Standard deviation of prediction error. b), c), d): See Table 2.

nents by using SDP values alone.

In general, one of the useful statistics for evaluating relative accuracy is the coefficient of variation (C.V.), which is calculated by dividing SDP value by the mean value of the relevant component in this case. For plant breeding programs, however, the range of the component values would be more important than the mean value[14], because in the case of a wide-range component, outlier samples can be selected effectively by NIRS. On the basis of this range value, therefore, the authors defined a new index (evaluation index, EI) to compare the accuracy and practical acceptability of NIRS in predicting forage components[8]. The formula of EI is as follows:

$$EI = \frac{2 \times SDP}{Range} \times 100\ (\%)\ ,$$

where "Range" means the range of actual values of a component in the prediction samples. If the SDP values have a Gaussian distribution, SDP can be regarded as σ and about 68% of the values will be within $\pm\sigma$. The numerator "2 × SDP" estimates a major range of prediction errors. As an EI value gives a ratio of the error range to the whole range of the component, a lower EI vlaue indicates a higher accuracy and a higher practical acceptabililty in the selection of outlier samples. On the other hand, if an EI value is over 50%, the range of the prediction errors at 95% level ($\pm 2\sigma$) can be wider than the

whole range of the component; hence, the authors rated 50% of an EI value as the limit of predicting ability of NIRS.

Table 4 shows the criterion for EI. The EI values are classified into five ranks based on their accuracy and practical acceptability. The EI values for components studied were shown in Table 5. Each component tended to have a similar EI value through three sample groups; hence, their ranks in prediction could be judged as follows:

(1) CP (Fig. 1) and DCP were ranked A, which means that these components can be predicted with very high accuracy by NIRS; it would be a most practical method for predicting these components.
(2) NDF, ADF (Fig. 2) and ADL were ranked B, suggesting that NIRS can predict them with high accuracy.
(3) EE, DMD (Fig. 3), TDN, ADMI and DEI were ranked C, which suggests that accuracies for predicting them be rather limited.
(4) DCW (Fig. 4) was ranked D, suggesting low accuracy and inadequate acceptability for practical use.

2) Comparison of NIRS and chemical analysis for predicting sheep responses

The ultimate criterion for assessing the quality of forages is their potential to support animal production. In view of the time and costs needed for animal-feeding trials, current laboratory methods have been attempted to predict feeding values on the basis of chemical compositions of forages and their *in vitro* digestibility. In plant breeding programs, however, those laboratory methods are also expensive and time consuming; NIRS would be an alternative method of assessing forage quality. Even though very high accuracy in predicting feeding values by NIRS could not be obtained, as shown in Table 5, its main advantages of saving time and simplicity of procedures are still very attractive.

Taking these advantages into account, the accuracy of NIRS method was compared with the current laboratory methods[10]. Results of the prediction of *in vivo* TDN and ADMI in the sample group (C) of orchardgrass are summarized in Table 6. The SDP values in the NIRS method were rather lower than those of current laboratory methods; it is suggested that the NIRS has a capability of predicting sheep responses as accurately as they can be predicted from the current laboratory methods. Similar results were reported in some forage materials[3,16]. From these facts, it is considered that the NIRS method has a high potential for directly predicting the feeding values of forages within an acceptable level of accuracy in plant breeding.

3) Improvement in accuracy of prediction by specific calibration equations on forage species

There are two types of calibration equations in NIRS[1,6]: universal equations and specific equations. Universal equations would be originally calibrated to apply various kinds of factors, such as species, years, growth stages, harvests, methods of drying and so on. However, in the case of limiting factor to forage species, an equation which is derived from many species including grasses and legumes can be designated as a universal equation. They would be applied for various populations of any species. On the other hand, an equation derived from only one

Table 4. Criterion based on evaluation index (EI)

EI (%)	Rank	Accuracy	Practical acceptability[a)]
−12.4	A	Very high	Good enough
12.5–24.9	B	High	Good
25.0–37.4	C	Slightly high	Fair
37.5–49.9	D	Low	Poor
50.0–	E	—	Out of the question

a): Especially for plant breeding.

Table 5. Values of EI for chemical compositions and sheep responses in different sample groups (%)

Component	Sample group			Rank[d)]
	A[a)]	B[b)]	C[c)]	
CP	9.4	9.0	9.2	A
EE[e)]	25.1	33.3	30.8	C
NDF	13.7	15.6	16.0	B
ADF	20.4	15.4	16.1	B
ADL	22.4	20.2	19.4	B
DMD	30.9	26.4	27.3	C
DCP	11.7	11.5	10.7	A
DCW[f)]	— [h)]	45.2	47.1	D
TDN	38.6	35.0	25.7	C
ADMI[g)]	33.7	32.6	28.9	C
DEI	28.6	30.4	28.3	C

a), b), c): See Table 1, d): See Table 4, e), f), g): See Table 2, h): The range was overestimated.

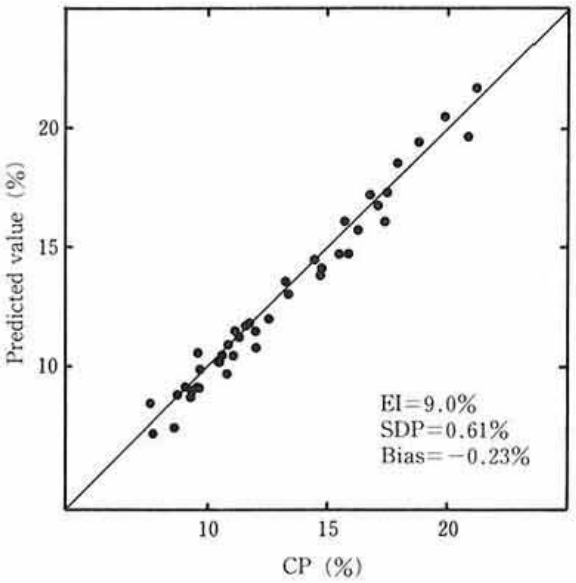

Fig. 1. Accuracy of prediction of crude protein (CP) by NIRS in the sample group (B) of grasses (EI ranking A)

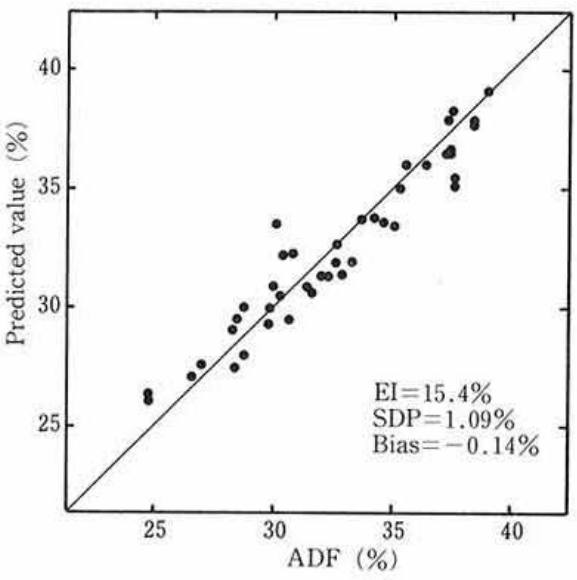

Fig. 2. Accuracy of prediction of acid detergent fiber (ADF) by NIRS in the sample group (B) of grasses (EI ranking B)

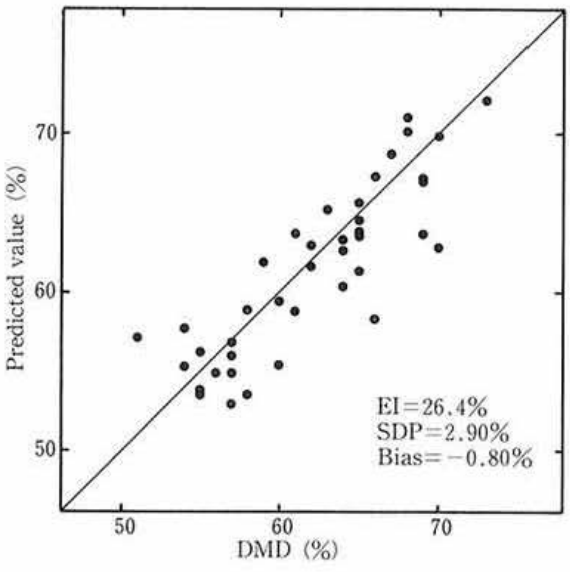

Fig. 3. Accuracy of prediction of dry matter digestibility (DMD) by NIRS in the sample group (B) of grasses (EI ranking C)

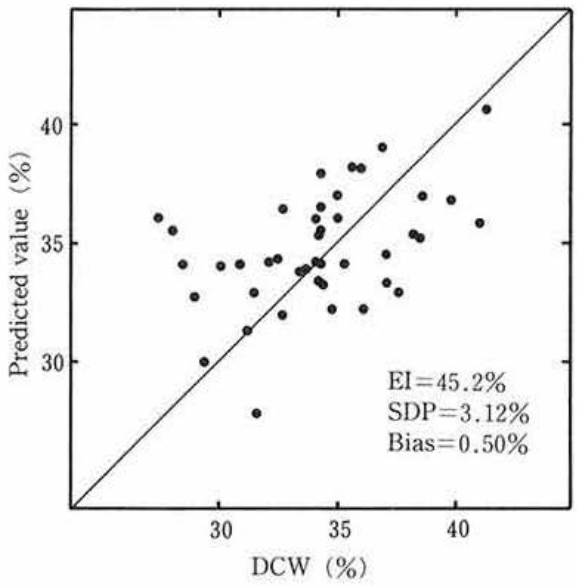

Fig. 4. Accuracy of prediction of digestible cell wall substances (DCW) by NIRS in the sample group (B) of grasses (EI ranking D)

species may be designated as a specific equation. They should be employed for closed populations of one specified species.

Table 7 shows the accuracy of prediction among these two types of equations[9]. The mean values of SDP tended to be smaller in the order of Group (A), including three grass species and three legume species; Group (B), including only three grass species; and Group (C), composed of only one grass species, especially in CP, DCP, NDF, ADF and ADL. These data indicate that the prediction errors of these components could diminish effectively by developing specific equations.

The universal equations including grasses and legumes may be useful in various types of practical applications such as an analysis of legume-grass mixture samples collected from grasslands; however, it is suggested from the results in Table 7 that in order to improve accuray, separate calibrations be the best for grass samples and for legume samples with respect to protein and fiber components. In addition, for plant breeding, it is strongly recommended to establish specific equations to obtain maximum accuracy.

Table 7. The mean values of SDP for chemical compositions and sheep responses in different sample groups

Component		Sample group		
		A[a]	B[b]	C[c]
CP	(%)	0.90	0.64	0.54
EE	(%)	0.44	0.48	0.52
NDF	(%)	2.78	1.77	1.53
ADF	(%)	1.90	1.20	1.13
ADL	(%)	0.87	0.53	0.36
DMD	(%)	3.96	3.28	3.29
DCP	(%)	1.04	0.72	0.57
TDN	(%)	3.65	3.17	2.71
ADMI	($g/W^{0.75}$)	8.57	7.81	6.73
DEI	($Kcal/W^{0.75}$)	28.2	25.4	23.3

The mean value of SDP was calculated from the average of six lowest SDP values selected from 24–33 candidate equations.
a), b), c): See Table 1.

Table 6. Best calibration and prediction statistics for estimating TDN (%) and ADMI ($g/W^{0.75}$) from the method of NIRS[a] and chemical analysis[b] in the sample group (C) of orchardgrass

Regression variables		Calibration		Prediction	
Dependent	Independent	R^2	SEC[c]	SDP[d]	Bias
TDN[e]	NIRS	0.880	2.91	2.61	0.54
TDN	CSDM[g]	0.817	3.51	3.18	−0.34
TDN	ADL	0.794	3.72	3.35	1.34
TDN	CSDM and ADL	0.845	3.27	3.02	0.41
TDN	CSDM and CP	0.824	3.49	3.06	−0.18
TDN	CSDM, ADL and EE	0.845	3.31	3.00	0.40
TDN	CSDM, ADL and CP	0.845	3.31	3.05	0.44
ADMI[f]	NIRS	0.810	7.06	7.04	2.49
ADMI	CSDM	0.593	10.06	8.70	0.76
ADMI	ADF	0.572	10.32	8.64	2.64
ADMI	CP and CSDM	0.662	9.29	7.45	1.71
ADMI	CP and ADF	0.606	10.04	7.93	2.86
ADMI	CP, CSDM and NDF	0.671	9.29	7.43	1.04
ADMI	CP, CSDM and ADF	0.662	9.41	7.45	1.59

a): A best calibration equation using the second derivative data of log(1/R) spectra at three wavelengths was selected from 30 (TDN) to 33 (ADMI) candidate equations. b): NDF, ADF, ADL, CP, EE and CSDM were determined by chemical analysis. Each component and its combination were determined on the assumption that the estimates of TDN and ADMI were independent variables. Best results were listed in this table. c): See Table 2. d): See Table 3. e): Total digestible nutrients. f): *Ad libitum* dry matter intake. g): Cellulase-soluble dry matter.

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