

# Prediction of chemical composition of unground forage by near infrared reflectance spectroscopy

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## Introduction

Our previous study on the use of near infrared (NIR) reflectance spectroscopy for determining the chemical composition of forage showed the accuracy and suitability for farm level usage.<sup>1</sup> In Japan, the NIR method has been widely used to serve dairy farms with forage determination. Conventionally, a forage sample for NIR analysis is prepared by grinding the sample to pass through a 0.5 or 1 mm screen. This process sometimes requires a large input of work and time and it is possible to lose some components because of the heat from the grinding process. In dairy farming, forage is usually given to animals in a long form or in a 10 cm cutting. Therefore, to make taking measurements using the NIR method for forage composition simpler and more applicable, it would be useful to study the possibility of using NIR on raw forage. In this study, we studied the accuracy of prediction of the chemical composition of unground forages using NIR.

## Materials and methods

### Materials

#### *Samples for developing the calibration and validation*

This study was carried out on a total of 49 hay samples. The samples came from Japanese domestic production ( $n = 30$ ) and imported hay ( $n = 19$ ). The species were Italian ryegrass ( $n = 14$ ), timothy ( $n = 12$ ), orchardgrass ( $n = 5$ ), alfalfa ( $n = 7$ ), alfalfa cube ( $n = 1$ ), alfalfa pellet ( $n = 1$ ), oats hay ( $n = 3$ ), sudangrass ( $n = 4$ ), tall fescue ( $n = 1$ ) and bermudagrass ( $n = 1$ ). The domestic hay was collected from the National Institute of Animal Industry (MAFF), Toyama Prefectural Livestock Experimental Station, the livestock laboratory in Fukuoka Prefectural Experimental Station and a dairy farm in Saitama Prefecture. The imported hay was provided by a dairy farm in Saitama and Fukuoka prefecture.

### Methods

#### *Chemical composition and analytical methods*

The samples were analysed for moisture, crude protein (CP) and crude ash using a proximate analysis method,<sup>2</sup> organic cell wall (OCW), organic cellular contents (OCC), high digestible fibre (organic a fraction: Oa) and low digestible fibre (organic b fraction: Ob) were analysed using the enzymatic method,<sup>3,4</sup> and neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) by using the detergent method.<sup>5,6</sup>

**Table 1. Chemical components of samples (DM%).**

	Hay samples (n = 49)			Calibration set (n = 31)			Prediction set (n = 18)		
	Min.	Max.	Av.	Min.	Max.	Av.	Min.	Max.	Av.
Moisture	6.8	14.4	10.2	6.8	14.4	10.3	7.0	14.4	10.1
CP	5.7	24.0	10.7	5.7	24.0	11.1	5.9	21.6	10.1
OCW	43.5	80.2	63.5	43.5	72.5	63.3	49.5	80.2	63.9
OCC	14.2	45.8	27.6	19.7	45.8	27.7	14.2	39.7	27.5
Oa	4.9	24.4	10.6	4.9	24.4	11.3	5.2	16.0	9.4
Ob	30.1	75.0	52.9	30.1	65.0	52.0	41.1	75.0	54.5
NU	34.4	80.3	61.8	34.4	75.2	62.1	39.9	80.3	61.3
AN	25.9	49.9	38.6	25.9	45.7	38.0	31.9	49.9	39.5
ADL	2.4	9.7	6.0	2.4	9.7	5.6	4.7	9.3	6.8
Crude ash	4.0	15.9	8.9	4.0	15.9	9.1	5.1	11.6	8.6

CP: Crude protein

OCW: Organic cell wall

OCC: Organic cellular contents

Oa: High digestible fibre

Ob: Low digestible fibre

NDF: Neutral detergent fibre

ADF: Acid detergent fibre

ADL: Acid detergent lignin

#### ***NIR instrument and measurement spectra***

NIR spectra were measured using an NIRSystems 6500 spectrometer (NIRSystems, MA, USA) and the spectra were processed using NSAS software (NIRSystems, MA, USA). Hay samples were chopped at about 5 cm length to fit into the sample cell. For the measurement of NIR spectra, the reflection spectrum was measured using a glass cell sample for high moisture measurement (thickness: 1 cm, length: 20 cm). The near infrared spectrum was read at 2 nm intervals over the range of wavelengths between 1100 and 2500 nm. Spectra of each sample were recorded five times and were then averaged to obtain one spectrum for calculation.

#### ***Average of spectra***

One sample was recorded five times. Each spectrum was corrected by the average of another four spectra and was saved as a corrected spectrum for NIR analysis. This correction was done to examine the effect of sample variation and density in packing of the cell on the estimation accuracy. Calibration equations were developed and examined using these spectra. The use of the averaged spectra was also examined for the effectiveness of determination.

#### ***Calibration equations and prediction of hay composition***

31 hay samples were used for developing the calibration equation. Eighteen samples (named unknown samples) of hay were used for validation test samples. The five kinds of spectra were calculated

in second derivative form (gap 2, segment 20) and were used to develop the calibration equation using 2, 3 and 4 wavelengths by multiple linear regression. The calibration equations were developed for each component. The accuracy of each calibration equation developed by various combinations of wavelength number for each component was judged using the correlation coefficient ( $r$ ), standard error of prediction ( $SEP$ ) and ratio of standard deviation of reference data to the  $SEP$  ( $RPD$ )<sup>7</sup> between analytical data and NIR predicted data.

## Results and discussion

### Sample components

The major components in the standard samples ranged widely and the averages were, 18.3% for CP, 36.7% for OCW and 45.8% for NDF. The moisture content, ADL and ash had a much narrower range than the other components.

### The equalisation of the NIR spectrum

Table 2 shows the wavelengths of various chemical compositions as, well as their standard error of calibration ( $SEC$ ) values when predicted by NIR against laboratory analytical data. This table also

**Table 2. Number of spectrum used for averaged spectrum, selection of wavelengths and statistical evaluation of calibration test.**

Number of spectrum		Wavelength				$r_2$	$SEC_2$	$r_3$	$SEC_3$	$r_4$	$SEC_4$
		1st	2nd	3rd	4th						
CP	1	1972	2296	1348	1806	0.95	1.55	0.96	1.41	0.97	1.34
	2	1972	1714	2194	1890	0.95	1.65	0.96	1.42	0.97	1.37
	3	1972	2296	1348	1604	0.95	1.60	0.96	1.43	0.97	1.33
	4	1972	2296	1350	1780	0.95	1.54	0.97	1.37	0.97	1.33
	5	1974	1716	2196	1886	0.96	1.50	0.97	1.26	0.98	1.18
OCW	1	1232	1892	1346	2006	0.90	3.51	0.91	3.30	0.92	3.20
	2	1232	1894	2344	1824	0.90	3.40	0.92	3.28	0.93	3.13
	3	1754	1968	2112	1602	0.92	3.19	0.94	2.77	0.95	2.60
	4	1754	1968	2114	1602	0.92	3.11	0.94	2.71	0.95	2.71
	5	1754	1968	2134	1600	0.92	3.06	0.95	2.65	0.95	2.47
OCC	1	1958	2284	1556	1734	0.93	2.61	0.93	2.52	0.94	2.40
	2	1958	2284	1554	1734	0.92	2.77	0.93	2.65	0.94	2.51
	3	1958	1756	2144	1706	0.92	2.71	0.94	2.41	0.95	2.16
	4	1754	1888	2346	1818	0.93	2.60	0.94	2.42	0.95	2.21
	5	1754	1888	2346	1818	0.93	2.54	0.94	2.37	0.95	2.18

CP, OCW, OCC: As shown in Table 1

1st, 2nd, 3rd and 4th: selected wavelength for calibration,

$r_2, r_3, r_4$ : Correlation coefficient

$SEC_2, SEC_3, SEC_4$ : standard error of calibration

**Table 3. Statistical evaluation of prediction test for a number of spectrum used in averaged spectrum.**

	Number of spectrum									
	1		2		3		4		5	
	<i>r</i>	<i>SEP</i>	<i>r</i>	<i>SEP</i>	<i>r</i>	<i>SEP</i>	<i>r</i>	<i>SEP</i>	<i>r</i>	<i>SEP</i>
Moisture	0.87	1.20	0.88	1.15	0.89	1.06	0.89	1.06	0.90	1.04
CP	0.93	2.07	0.95	1.70	0.94	1.78	0.94	1.79	0.95	1.69
OCW	0.88	4.23	0.89	3.95	0.98	1.71	0.98	1.62	0.97	1.61
OCC	0.94	2.55	0.93	2.64	0.94	2.19	0.94	1.80	0.94	1.77
Oa	0.66	2.96	0.68	2.95	0.68	2.95	0.64	3.16	0.64	3.09
Ob	0.76	6.59	0.72	6.72	0.74	6.66	0.74	6.83	0.74	6.63
NDF	0.94	4.14	0.95	4.13	0.98	3.13	0.98	2.98	0.98	2.97
ADF	0.86	2.14	0.89	1.93	0.89	1.85	0.90	2.08	0.90	2.13
ADL	0.54	1.42	0.56	1.33	0.56	1.11	0.57	1.10	0.58	1.11
Crude ash	0.67	1.99	0.79	1.47	0.80	1.47	0.79	1.55	0.79	1.50

CP, OCW, OCC, Oa, Ob, NDF, ADF, ADL: As shown in Table 1

*r*: Correlation coefficient

*SEP*: Standard error of prediction

Values of *r* and *SEP* were predicted by calibration equation used three wavelengths.

showed the accuracy of prediction using calibration equations developed by five kinds of equalisation spectra of the unground hay. The multiple linear coefficient correlation (*R*) was higher as the number increased, while the *SEP* tended to decrease for CP, OCW and OCC. The most accurate calibration equation was the calibration equation developed using the average spectrum of the five repetitions.

Table 3 shows the result of estimation for the components in unknown samples. The following are the results from the calibration equation using three wavelengths. For moisture, there was no improvement in accuracy in the calculation carried out on more than two repetitions. However, for OCW, OCC, NDF, CP, ash, Oa, Ob, ADF and ADL, improved predictions were obtained when using more than three repetitions.

### Estimation accuracy of the feed ingredient

#### Precision of calibrations

Table 4 shows the result of accuracy of the calibration equations. The calibration equations were made by averaging the spectra of the five repetitions. The calibrations for OCC, NDF, ADL and ash were predicted by calibration equations using four wavelengths, while the other calibrations used three wavelengths. The precision for each component, measured by the values of *r* and *SEC*, for moisture, CP, OCW, OCC, NDF and ADF were in the range of 0.89–0.97 and 0.76–4.31, respectively. The values of *r* and *SEC* for ADL and ash were 0.79 (0.98) and 0.81 (1.58), respectively, which was inferior to the other components.

#### Selection of wavelength for measurements

The first wavelength used was 1974 nm for the CP, which was correlated with the absorption of materials derived from the NIR related to amino acid. The wavelength 1754 nm was used for OCW

**Table 4. Statistical evaluation of calibration and prediction test using the averaged spectrum in five repetitions spectrum.**

	Calibration equation										Prediction		
	Wavelengths				$r_2$	$SEC_2$	$r_3$	$SEC_3$	$r_4$	$SEC_4$	$r$	$SEP$	$RPD$
	1st	2nd	3rd	4th									
Moisture	1370	1508	1560	1236	0.92	0.83	0.94	0.76	0.96	0.76	0.90	1.04	1.83
CP	1974	1716	2196	1886	0.96	1.50	0.97	1.26	0.98	1.18	0.95	1.69	3.02
OCW	1754	1968	2134	1600	0.92	3.06	0.95	2.65	0.95	2.47	0.97	1.61	4.99
OCC	1754	1888	2346	1818	0.93	2.54	0.94	2.37	0.95	2.18	*0.95	1.75	4.01
Oa	1428	2034	2370	2200	0.90	2.10	0.92	1.90	0.94	1.67	*0.67	2.89	1.16
Ob	1228	1896	1580	2038	0.86	4.88	0.89	4.31	0.92	3.79	0.74	6.63	1.36
NU	1234	1754	1966	2076	0.94	3.47	0.96	3.03	0.97	2.82	*0.98	2.36	4.24
AN	1974	2368	1406	2430	0.80	3.17	0.90	2.34	0.94	1.90	0.90	2.13	2.28
ADL	2210	2370	1496	1616	0.72	1.09	0.79	0.98	0.83	0.90	*0.67	1.11	1.23
Crude ash	1620	1710	2248	1564	0.76	1.70	0.81	1.58	0.83	1.52	*0.83	1.55	1.42

CP, OCW, OCC, Oa,Ob, NDF, AH, AR: as shown in Table 1

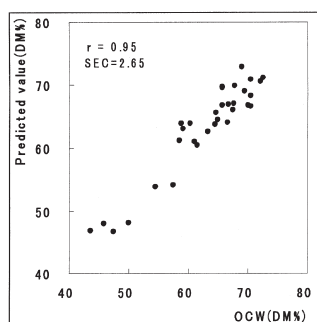
1st, 2nd, 3rd, 4th,  $r_2$ ,  $r_3$ ,  $r_4$ ,  $SEC_2$ ,  $SEC_3$ ,  $SEC_4$ : as shown in Table 2

$r$ ,  $SEP$ : as shown in Table 3

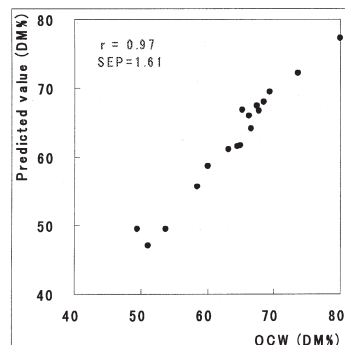
$RPD$ : Ratio of standard deviation of reference data in prediction sample set to  $SEP$

\*: Statistical evaluation of prediction test by calibration equation using four wavelengths.

and OCC, which is believed to be due to the absorption of cellulose which has a peak at 1780 nm. The first wavelengths for ADF (1974 nm) and ADL (2210 nm) were considered as binding of lignin.<sup>9</sup> For NDF,Ob and Oa, the first wavelengths used were 1234, 1428 and 1228 nm, respectively. However, it is difficult to conclude the derivation because these wavelengths are in the region of second combination/overtone absorptions.



**Figure 1. Relationship between analytical data of OCW and NIR predicted data of OCW in developing calibration equations using three wavelengths.**



**Figure 2. Relationship between analytical data of OCW and NIR predicted data of OCW by calibration equation using three wavelengths.**

#### Validation of calibration equation

Table 4 shows  $r$  and  $SEP$  of values predicted by NIR for CP, OCW, OCC and NW, which ranged between 0.95–0.98 and 1.61–2.97, respectively. These results are also presented in Figures 1 and 2. Moisture and ADF had good accuracy of estimation with  $r = 0.90$ ,  $SEP = 1.04$  and  $r = 0.90$ ,  $SEP = 2.13$ , respectively and they were slightly inferior to CP, OCW, OCC, NDF. Poor results were obtained for ADL ( $r = 0.67$ ,  $SEP = 1.11$ ). The value of RPD for CP, OCW, OCC and NDF were over 3.0 and lower for ADF and moisture at 2.3 and 1.8. For the former components, the conclusion can be made that it is possible to employ NIR feed analysis for unground forage samples. However, further study is necessary to improve the accuracy of measurement for Oa, Ob and ADL.

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