

日本畜産学会報 第69巻 第3号 平成10年3月25日発行 (毎月25日発行) (通巻第560号) 昭和40年1月20日第3種郵便物認可

ISSN 0918-2365  
CODEN: NICKA3

# ANIMAL SCIENCE AND TECHNOLOGY

**Vol. 69 No. 3, March 1998**

**日本畜産学会報**

(社) 日本畜産学会

JAPANESE SOCIETY OF ZOOTECHNICAL SCIENCE

# Animal Science and Technology

Official Journal of Japanese Society of Zootechnical Science

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**Abbreviation** : Anim. Sci. Technol. (Jpn.)

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略 称 : 日 畜 会 報

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# Prediction of Feed Digestibility Using Differences in NIRS Spectra between Feeds and Feces at a Determined Region of Wavelength

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(Received August 13, 1997)

**Abstract** A total of seventy-two pairs of feed and fecal samples collected from the digestion trials of dairy cattle were used in the study to predict dry matter digestibility (DMD), organic matter digestibility (OMD) and total digestible nutrients (TDN) using near infrared reflectance spectroscopy. Calibration and prediction were made from the difference of second derivative spectra between feeds and feces. From these spectra, 31, 28 and 13 samples were separated for calibration, prediction and test, respectively. Wavelengths of 1878, 2172, 2278 and 2362 nm were selected from determined region at 1900-, 2200-, 2300- and 2400-nm for developing the calibration equations for DMD, OMD and TDN. Correlation coefficient for DMD, OMD and TDN of the calibration were 0.95, 0.92 and 0.95, respectively, while the standard error were 2.9 for the first two nutrients and 2.8 for TDN. Whereas, *r* values (and standard error) for the prediction equations were 0.89 (3.5), 0.92 (3.32), and 0.91 (3.16), while for the test samples, were 0.97 (1.96), 0.97 (1.88) and 0.96 (1.94), respectively. This study showed the possibility and applicability of predicting digestibility and TDN using the spectra difference of feeds and feces through the wavelengths from four determined regions above 1900-nm.

*Anim. Sci. Technol. (Jpn.) 69 (3) : 253-259, 1998*

**Key words** : NIRS, Digestibility, Dairy cattle

Studies using near infrared reflectance spectroscopy (NIRS) for predicting the digestibility of feeds have been widely reported<sup>1,2,8,11)</sup>. Majority of these studies were performed on feed samples with reference data of *in vitro* or *in vivo* digestibility. Digestibility in ruminants is a complex phenomenon resulting from the interaction between the animals, rumen microorganisms, feeds and feeding level<sup>6)</sup>. This interaction, however, cannot be detected from

feed spectra only. Moreover, the NIRS spectrum of feeds contains information of different chemical groups at the same wavelength<sup>7)</sup>. This overlapping varies the obtained wavelengths for predicting nutritive value of feed as summarized by Clark and Lamb<sup>3)</sup> and could lead to misinterpretation and low reliability of data in application. Ideally, prediction of digestibility is taken from the spectra of the digested feed fractions. However, disappear-

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ance of these portions in the digestive tract does not warrant estimation. Since, feed spectra represent feed composition, while fecal spectra represent the indigested fraction of feed, the digested fraction spectra can be obtained by subtracting fecal spectra from the feed spectra.

This study was conducted to examine the possibility and the reliability of the use of spectra difference for digestibility determination. In addition, the selected wavelength in this study was fixed based on the most correlated region<sup>3)</sup>.

**Materials and Methods**

A total of seventy-two pairs of feed and fecal samples collected from the digestion trials of dairy cattle fed with Italian ryegrass based diets such as : Italian ryegrass as a sole feed (n =32), Italian ryegrass combined with concentrate (commercial feed, n=27) and Italian ryegrass combined with steamwood (n=13). The diet of Italian ryegrass and concentrate was given in two ratios 70 : 30 and 40 : 60, while the diet of Italian ryegrass and steamwood was given in 55 : 45 and 95 : 5 ratios. The diets contained the total digestible nutrients (TDN) requirement level following Japanese Feeding Standard for Dairy Cattle<sup>5)</sup>.

Feed, feed orts and fecal samples collected from digestion trials were dried at 65°C for 48 hours, ground to pass a 1 mm screen for chemical analysis to determine the dry matter and organic matter digestibilities and TDN. These digestibilities and TDN were then used as ref-

erence data for developing and validating the calibration of NIRS analysis.

The seventy-two spectra differences obtained from those pairs samples were separated. Fifty-nine difference spectra obtained from Italian ryegrass sole feed and Italian ryegrass-concentrate were used for developing the calibration equation and for the prediction. From these, thirty-one spectra were chosen randomly for the calibration, while the remaining (n=28) was used as the prediction samples for validating the calibration. The spectra difference obtained from Italian ryegrass-steamwood were used as test samples. These test samples were quite different from the samples used in the calibration and prediction which were used to verify the developed calibration equation in application, since the calibration and the prediction originated from same population. The composition of diets and the separation for NIRS analysis are shown in Table 1.

Thorough mixing and proper sampling procedure of experimental diets were conducted prior to NIRS analysis. Analysis by NIRS was done by a Pacific Scientific (Neotec) model 6500 (Perstorp Analytical, Silver Spring, MD) equipped with ISI software (InfraSoft International, Port Matilda, PA). The samples were scanned over the range of 1100-2500 nm. The spectral data were collected at wavelength intervals of 2 nm, and the 700 data points obtained from each sample were stored in the computer as absorbance values. These values were expressed as log [1/R], where R is the

*Table 1.* The composition of feeds used for developing calibration and for prediction and for test

Feed	Ratio	n	Calibration	Prediction	Test
Italian ryegrass		32	18	14	—
Italian ryegrass-Concentrate	70 : 30	9	5	4	—
	40 : 60	18	8	10	—
Italian ryegrass-Steamwood	55 : 45	7	—	—	7
	95 : 5	6	—	—	6
Total		72	31	28	13

reflectance. The difference spectra was obtained by subtracting the second derivative spectra ( $\Delta^2 \log [I/R]$ ) of feces from that of feeds. To achieve the optimum prediction, a step-wise multiple regression procedure<sup>13</sup> was used in calculation. The final form of calibration equation will be,

$Y = a + b(X_1) + c(X_2) + d(X_3) + e(X_4)$ , where ; the Y is variable to be predicted which commonly obtained by reference method ; the a, b, c, d and e are the coefficients ; and the X is wavelength.

The wavelength  $X_1$ ,  $X_2$ ,  $X_3$  and  $X_4$  were selected in determined four regions above 1900-nm, i.e. 1900, 2200, 2300, and 2400-nm, respectively, based on the study of Clark and Lamb<sup>3</sup>. These regions of wavelengths were rounded to the nearest 100 nm (e.g. all wavelengths from 1850 to 1949 were categorized as 1900). The spectra above 1900-nm region was used because the region below 1900-nm is mainly dominated by the overtones of similar chemical bond and it is weaker than those above 1900-nm.

At the region of 1900-nm, there is a strong relationship between water and other chemical components of living systems including the presence of starch and protein bonds<sup>3</sup>. The 2200-nm region is frequently used for protein determination in forages, and the 2300-nm region is associated with more fibrous portion of plant material and the nitrogenous material associated with the fiber. The 2400-nm region contains the fibrous carbohydrate fraction and selected most often for determining digestibility of forages<sup>3</sup>. Assuming that the dry matter digestibility (DMD), organic matter digestibility (OMD) and TDN are highly correlated, the wavelengths obtained from DMD calibration were used for developing calibration equation of other constituents.

The reliability of calibration equation was judged based on coefficient correlation ( $r$ ), standard error of prediction (SEP) and RPD value (the ratio of standard deviation of refer-

ence data in prediction set to the observed standard error prediction)<sup>14</sup>.

## Results and Discussion

The study made use of the second derivative form of the feeds and fecal spectra differences to predict nutrient digestibility. With this approach the basic problem of overlapping peaks and very large baseline variation as influence by particle size was eliminated<sup>4,8</sup>.

The second derivative measured by subtracting feed and fecal spectra are presented in Fig. 1. Absolute peak of feed spectra were generally higher than those of feces. This was because the height of NIRS absorbance represents the organic substances in the samples<sup>7</sup>.

Wavelengths obtained from the four determined regions at 1900, 2200, 2300 and 2400-nm were at 1878, 2172, 2278 and 2362 nm, respectively. These wavelengths were then used to estimate the constituents of prediction set samples and test set samples. The range and standard deviation of digestibilities and TDN in these samples are presented in Table 2. The obtained correlation coefficient, standard error and RPD of the calibration, prediction and test samples are presented in Table 3.

The prediction data showed slightly lower  $r$  value while the SEP were higher compared from those obtained in the calibration. These facts may lead to the conclusion that the developed calibrations were unstable and unreliable when applied. However, because the spectra used in this study were generated from various feeds, the judgement should not be addressed to the correlation and standard error only but also to the absolute value of prediction. Therefore, the RPD value was used because in some cases it is difficult to judge the validity of the developed calibration if the low correlation value of the prediction were accompanied by low standard error, or the high correlation value were accompanied by high standard error.

The further use of the calibration equation

developed in this study could be justified by the obtained RPD values for predicting DMD, OMD and TDN, *i.e.* 2.92, 3.09 and 3.10, respectively. Williams<sup>14)</sup> classified the RPD value for judgement where 2.5-3.0 is regarded as adequate for rough screening, value above 3.0 as

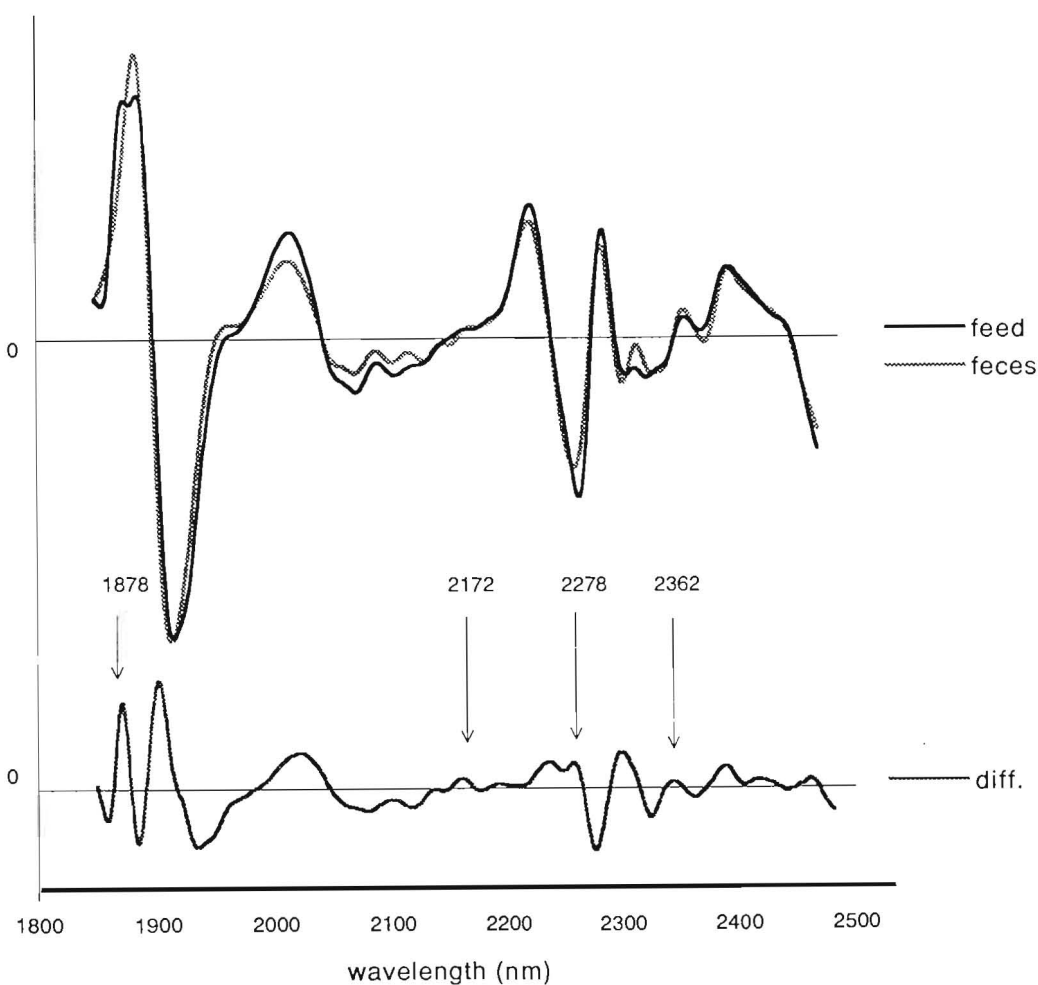


Fig 1. Second derivative spectra of feed, feces (up) and the spectra difference of feed and feces (bottom).

Table 2. Range, mean and standard deviation of digestibility and TDN values for calibration, prediction and test samples

	Calibration (n=31)			Prediction (n=28)			Test (n=13)		
	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range
DMD	62.5	9.5	44.3 - 76.1	63.1	10.2	39.2 - 75.1	56.5	9.9	44.5 - 67.4
OMD	65.7	9.8	47.1 - 79.9	65.7	10.3	41.3 - 78.5	58.2	9.9	45.1 - 70.2
TDN	61.9	9.4	44.1 - 76.0	62.0	9.8	38.4 - 74.8	55.9	8.5	44.3 - 66.5

DMD : dry matter digestibility, OMD : organic matter digestibility, TDN : total digestible nutrients.

### Difference Spectra for Predicting the Digestibilities

*Table 3.* Coefficient correlation, standard error and RPD of calibration, prediction and test samples for predicting digestibilities using difference spectra

	Calibration		Prediction			Test		
	R	SEC	r	SEP	RPD	r	SEP	RPD
DMD	0.949	2.89	0.894	3.50	2.92	0.965	1.96	4.64
OMD	0.915	2.93	0.915	3.32	3.09	0.968	1.88	5.25
TDN	0.950	2.82	0.912	3.16	3.10	0.963	1.94	4.36

R : coefficient correlation obtained from multiple regression ; r : coefficient correlation obtained from simple regression ; SEC, SEP : standard error of calibration, prediction and test samples ; RPD : ratio of standard deviation of reference data in prediction set to SEP ; DMD, OMD, TDN : see Table 1.

satisfactory for screening, the values of 5 and upward are suitable for quality control analysis, and values of above 8 are excellent and can be used in any analytical situation. Based on this classification, the developed calibration equations employed to predict digestion coefficients of various diets were within the range of satisfactory for screening.

Results obtained using the developed calibrations showed relatively high for DMD, OMD and TDN with almost identical value of 0.96. On the other hand, SEP were lower than 2 for all nutrients. The RPD revealed values of 4.64, 5.25 and 4.36 for DMD, OMD and TDN, respectively. Considering the limitation of the study, i.e. less number of observations and different diets used in the calibration and prediction for measurements, findings suggests applicability of calibration equation with at least 50 similar test samples to attain a more reliable prediction values<sup>(2)</sup>.

From the result of present study, two conclusions can be made. Firstly, the prediction of digestibility using the spectra difference of feeds and feces showed high accuracy even with a wide variations in the type of feeds and animal spender used in the measurement. It was because the spectra employed was able to segregate undigested fraction present in feces. Secondly, the stable accuracy of the calibration developed using four regions wavelengths of 1900-, 2200-, 2300- and 2400-nm showed that the digestibility of feeds could be measured

directly and precisely due to eliminating errors caused by animal factors.

Previous studies<sup>9,10)</sup> showed that the animal factor in the digestibility can be measured by using lignin predicted by NIRS as an indicator. However, the method needs an accurate lignin calibration for feed and feces. In view of the wide variety of feeds in the farm, and the absence of calibration equation for lignin this method can be an alternative.

### Acknowledgements

This work was supported by the Program for Promotion of Basic Research Activities for Innovative Biosciences (PROBRAIN). The authors are grateful to Ms. M. Someya for the chemical analysis assistance.

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飼料と糞の特定波長域の近赤外スペクトルの  
違いに基づく消化率の推定

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乳牛を用いた72組の消化試験の飼料および糞を用いて乾物消化率(DMD)，有機物消化率(OMD)およびTDNの近赤外分光法による推定方法について検討した。推定式の作成は飼料と糞の2次微分スペクトルの差をもとに，1900，2200，2300，2400 nmの領域から各1波長を選択することによって行った。検量線の作成には31組の試料を使用し，その適合度の検定には同一ロットの28組を，さらに異なるロットの試料による検定として13組を用いた。検量線の相関係数(標準誤差)は，DMD，OMD，TDNの順に0.95(2.9)，0.92(2.9)，0.95(2.8)であり，同一ロットおよび異なるロットの検定用試料に対する適合度も良好であった。以上のことから，飼料と糞のスペクトルの差を用いて飼料の消化率を推定することが可能であること，1900 nm以上の領域の近赤外スペクトルの利用が有効であることが示された。

日畜会報，69(3)：253-259，1998

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