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Two Methods of Near Infrared Reflectance Spectroscopy for Determining the Digestibility and Energy Value of Feeds

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Abstract Two groups of rations with known *in vivo* digestibility and energy values were used in this study. These rations were composed of Italian ryegrass only (IRO, n=45), and combination of Italian ryegrass and concentrates (IRC, n=58). The IRC ration was obtained from two batches, namely IRC-1 and IRC-2 consisting of 42 and 16 samples, respectively. Two methods of near infrared reflectance spectroscopy (NIRS ; direct and indirect method) were carried out for comparison with the *in vivo* value. Samples (30 each) of IRO and IRC-1 group rations were randomly chosen to develop the NIRS calibration equations for estimating digestibility and energy value. These equations were used to determine the digestibility and energy value of the remaining 15 samples of IRO and 12 samples of IRC-1 (direct method). Also, digestibility of those remaining samples were calculated based on the lignin indicator method using feeds and fecal components determined by NIRS (indirect method). Comparison of the value determined by direct and indirect methods with the *in vivo* of those remaining samples called validation test. Same comparison was conducted to IRC-2 ration samples for application test. The direct method of NIRS showed reliability only for ration at the same batch with that used in developing the calibration. However, the indirect method showed the potential and reproducibility for determination of digestibility and energy values of ration either originated from the same or different batch with that used in developing calibration.

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Key words : NIRS, Digestibility, Energy value, Dairy cattle

Application of near infrared reflectance spectroscopy (NIRS) method for prediction of digestibility and energy value of feeds[1,5,7] were done by directly calibrating the feeds to the references of the *in vivo* value. The reliability of the calibration equation depends on similarity of samples used for developing the calibration and the one to be predicted[16]. However, the digestibility of feeds is complex[2,11], because various factors are involved such as animal factor[11], feeding level used[8] and interaction of feedstuff within the ration[4]. Therefore, large number of samples covering these factors are required for developing an acceptable calibration equation. An alternate method to cope with this problem is by employing the compositions of feed and feces determined by NIRS[15]. Advantages of this method are that it does not require the reference data of digestibility or energy value from the energy balance trials; and it is possible to cover the factors affecting digestibility by including fecal data in calculation. Moreover, this method had been shown to have an appropriate accuracy for determination.

The objective of this study was to compare direct and indirect methods of NIRS for the estimation of digestibility and energy value of feeds.

Materials and Methods

A total of 103 ration samples with known digestibility and energy values collected from energy balance trials with dairy cattle were used in this study. These rations were Italian ryegrass only (IRO, n=45), and the combination of Italian ryegrass and concentrates (soybean, corn, or manufactured concentrates; IRC, n=58). Combinations of the feedstuffs in the IRC were varied. In case of IRC, it comes from two batch samples, namely IRC-1 (n=42) and IRC-2 (n=16). Ration samples of IRC-1 were used for developing the calibration equation and for validation, while the IRC-2 was used for the application test only. Origin, chemical compositions and ratio of Italian ryegrass and concentrate of IRC-1 and IRC-2 given to animals were similar. The difference between IRC-1 and IRC-2 was the harvest season for grass and the variation of grains used for manufacturing the concentrates. All rations were adjusted to meet the total digestible nutrients (TDN) requirements of Japanese feeding standard[9]. Digestibility were evaluated for dry matter (DM), organic matter (OM), crude protein (CP), ether extracts (EE), acid detergent fiber (ADF) and TDN. Energy value was evaluated for the digestible energy (DE) and metabolizable energy (ME).

NIRS prediction

Prediction of nutrient digestibility by NIRS was carried out by a Pacific Scientific (Neotec) model 6500 (Perstorp Analytical, Silver Spring, MD) scanning monochromater instrument attached to an IBM computer. Scanning was done on the range of 1100-2500 nm at 2 nm intervals. Calculation was done for the second derivatives of log 1/R values. This instrument was equipped with ISI (Infra Soft International, Port Matilda, PA) program in spectral calculation. Calibration equations were carried out with multiple step-wise regression to achieve the highest correlation regression (R) and the lowest standard error (Se). Samples were ground to pass a 1 mm screen.

NIRS-direct method

Direct method is a conventional method of NIRS which is done by directly determining the nutritive value of feeds using calibration equation. In this study, the calibration equations were developed with the reference data obtained from energy balance trials. Calibration equations for each group ration were developed using each 30 samples randomly chosen from 45 samples of IRO and 42 samples of IRC-1. Grouping was done to achieve a maximal prediction of the developed calibration for similarity of samples used[6,16]. Range of chemical composition of samples for developing calibration is presented in Table 1. Wavelengths for calibration were selected for maximum four wavelengths due to the limitation of equipment used. In this method, preparation of samples for NIRS analysis were made in a mixed form in proportion as fed to the cattle.

NIRS-indirect method

Indirect method is the estimation of digestibility and energy values by estimation equation using NIRS predicted value. The estimation was based on lignin as an indicator. Because the method was carried out using NIRS predicted value of feeds and fecal constituents, it was considered to be able to cover the animal factors affecting the digestibility. NIRS calibration equations used for determining constituents of feeds and feces following the report in previous study[15]. Metabolizable energy and the TDN content of the indirect method were calculated by formula,

$$ME = -0.33 + 0.958\ DE^{10)};$$

$$TDN = DOM + 1.25\ DEE$$

Components used in these formulas, DE, digestible organic matter (DOM), and digestible ether extracts (DEE) were obtained by multiplying the contents of energy, OM, and EE of feeds determined by NIRS with those of digestibility estimated value.

Validation of the methods

Validation of the direct or indirect methods were done for determination of the remaining 15 samples of IRO group, and 12 samples of IRC-1 group. Ranges and mean values of samples used in validation are presented in Table 1. Digestibility by the direct method for each group was determined with each developed calibration equation. These values were compared to that calculated by indirect method.

Application test

In conventional method of NIRS, reliability of developed calibration equation was based on magnitude of correlation coefficient and standard error of prediction obtained from validation test[13,16]. Application test was done to evaluate the reliability of that calibration equation for samples from different batch. This test was done for IRC group only because IRO group samples was not provided.

Statistical analysis

Paired t-tests[17] was carried out to analyze the significance between the methods and the *in vivo* results. Precision of the methods was evaluated from the residual standard deviation (RSD) between the NIRS prediction methods and the *in vivo* value.

Results and Discussion

Means and ranges of samples used for developing calibration equation and validation. In general, the ranges of calibration were wider than the validation as presented in Table 1. The ranges observed in IRO group were wider than that of the IRC.

Wavelengths used for the calibration and its validation for each group are presented in Table 2. In the present study, evaluation of wavelengths was pointed to the first two wavelengths due to the importance related to the components predicted[13]. Clark and Lamb[2] introduced the evaluation based on the wavelength region. They noted that digestibility measurements (*in vivo* and *in vitro*) is complex and do not measure functional groups (C-H, N-H, etc.) as in the determination of chemical composition. They categorized wavelengths into the nearest 100 nm range. For example, the wavelengths from 1750 to 1849 nm were categorized as 1800-nm region, and etc.

Dominant wavelengths obtained in IRO group in the 1st and 2nd measurement were 1200, 1500 and 2400-nm region. As the 1200-nm region is known as the second overtone area[14], it can be estimated that the first overtone occurs in 1700-nm area[12]. Thus, included in this region is the 1st wavelength of EE digestibility. This 1700-nm region was related to fiber and protein, and often used for digestibility determination of legumes and grasses[2]. The region of 1500-nm is related to the easily digestible materials such as, protein and soluble carbohydrates[2], while 2400-nm region is known as the starch and cellulose absorbance[14]. Wavelengths obtained in those components were in the proper regions.

In the IRC group, the 1st and 2nd wavelengths were dominated by 2000 and 2100-nm region. These regions are known as the amide group area and starch[14]. This was correlated to the fact that IRC group contained more protein and starch due to the presence of concentrate.

The correlation coefficient of calibrations in IRO group was higher than that of the IRC which ranged between 0.770 and 0.946, while IRC ranged between 0.580 and 0.921. The lower correlation coefficient for IRC group may

Table 1. Means and ranges of digestibility value of the rations used for developing the calibration and for validation

Constituents[1)]	Calibration (n=30) Mean	Calibration (n=30) Range	Validation Mean	Validation Range
Italian ryegrass only (IRO group)				
Digestibility (%)			n=15	
DM	56.5	26.5 -75.4	54.3	36.1 -67.6
OM	59.8	29.3 -76.4	56.6	41.7 -70.9
CP	41.1	15.9 -58.0	40.5	28.7 -51.1
EE	67.4	58.4 -78.8	66.6	54.2 -72.2
CF	52.7	15.7 -74.2	51.2	32.0 -75.3
ADF	44.1	10.1 -71.0	43.7	25.4 -69.0
Energy value				
TDN	56.3	27.7 -78.5	52.8	39.2 -66.9
DE	2.27	1.10- 3.11	2.39	1.63- 2.96
ME	1.90	0.67- 2.85	1.96	1.23- 2.50
Italian ryegrass and concentrate (IRC-1 group)				
Digestibility (%)			(n=12)	
DM	69.7	62.3 -76.1	72.2	66.5 -77.6
OM	72.5	65.5 -79.9	74.9	69.8 -79.9
CP	61.1	34.1 -79.6	72.2	66.5 -77.6
EE	69.1	61.5 -76.0	76.7	62.9 -81.1
CF	51.2	41.5 -65.5	53.4	44.6 -65.5
ADF	46.4	28.2 -56.0	50.0	42.0 -56.2
Energy value				
TDN	69.1	61.5 -76.0	71.6	66.0 -76.0
DE	3.03	2.71- 3.37	3.20	2.90- 3.48
ME	2.58	2.16- 2.91	2.73	2.37- 3.00

1) TDN was expressed as %DM intake ; DE, ME were expressed as Mcal/kgDM

be related to the limitation of wavelengths used for the more complex spectra of mixed form samples. Another possible reason is related to the broad combination of feedstuffs in small reference data for calibration (n=30). It is well known that combination of two or more feedstuffs in the ration resulted in an interaction in digestibility[4,11]. This phenomena in the living systems may not be completely interpreted only in small number of references data or four wavelengths used.

Furthermore, the developed calibration equation of each group was subjected to validation test and are presented in Table 2. Generally, the correlation coefficient value and standard error of prediction (SeP) in the validation were lower than that of the calibration. Based on the r and SeP values, the calibration equations for digestibility of CP and EE in IRO group seem not reliable for further use. Similarly, it was observed in the digestibility values of OM, EE, CF and ADF in IRC group. However, because those low correlation coefficient values may be related to the narrow range of the reference values used for validation, further evaluation of the difference from the *in*

Table 2. Wavelengths used for calibration and its validation for unknown set samples of the IRO and IRC group[a)]

Constituents[1)]	Wavelength				Calibration		Validation	
	1st	2nd	3rd	4th	R	SeC	r	SeP
Italian ryegrass only (IRO group)								
Digestibility (%)								
DM	1158	2440	1692	2216	0.913	5.2	0.910	4.7
OM	1158	1366	2396	1982	0.939	4.4	0.848	5.9
CP	2066	2394	2278	1658	0.770	5.8	0.302	5.7
EE	1694	2390	1156	1716	0.871	2.2	0.199	4.5
CF	1796	1894	2234	1408	0.929	5.6	0.770	9.4
ADF	2358	1150	1282	1516	0.917	5.3	0.820	8.8
Energy value								
TDN	2332	1558	1158	2128	0.946	3.8	0.835	5.6
DE	2284	1944	2464	2150	0.889	0.2	0.776	0.3
ME	2240	2440	1488	1966	0.807	0.3	0.867	0.2
Italian ryegrass and concentrate (IRC-1 group)								
Digestibility (%)								
DM	1474	2148	1744	2196	0.640	2.6	0.559	3.2
OM	2038	2148	1396	1582	0.682	2.5	0.410	3.0
CP	1730	1486	1222	2204	0.921	2.9	0.881	4.6
EE	1284	1538	2212	1608	0.686	3.9	0.366	6.1
CF	2020	1842	1672	1268	0.580	4.5	0.073	5.6
ADF	2296	2148	1368	1748	0.601	4.3	0.041	5.3
Energy value								
TDN	2038	2148	1396	1582	0.717	2.4	0.498	3.0
DE	2122	1172	1390	2204	0.766	0.1	0.653	0.1
ME	2446	1938	1450	2146	0.766	0.1	0.720	0.1

[1)] See Table 1.
[a)] Calibration set was used for 30 samples, while validation set was used for 15 samples of IRO and 12 samples of IRC-1 ;
R : Correlation coefficient from multiple regression, r : correlation coefficient from simple regression, SeC : standard error of calibration, SeP : standard error of prediction.

vivo value was done.

Simultaneously those values obtained from the direct and indirect methods were compared with those of *in vivo*. Comparison are presented in Table 3. The estimated values by the direct and indirect methods were generally in the same level with the *in vivo*. The difference of means between the value estimated by the methods and the *in vivo* showed that the direct method was generally smaller than that of indirect method. However, these biases were less than 5%. It can be explained that the direct method of NIRS was based on the strength of correlation regression between the reference value and the selected wavelengths of samples in batch used in developing calibration equation. Consequently, the mean value of the direct method will be closer to the mean of reference value.

In some cases statistically different results were observed. From the IRO group, the digestibility of DM, OM, ADF, and DE and the

Table 3. Means and residual standard deviation between the NIRS methods and the *in vivo* for the two groups rations[a)]

Constituents[1)]	NIRS direct		NIRS indirect		*in vivo*	
	Means	RSD	Means	RSD	Means	Range
IRO group (n=15)						
Digestibility (%)						
DM	54.0	4.6	49.9**	4.1	54.3	36.1 -67.6
OM	55.5	5.9	51.6**	3.7	56.6	41.7 -70.9
CP	38.8	5.7	41.6	5.1	40.5	28.7 -51.1
EE	66.2	4.5	67.1	3.4	66.6	54.2 -72.2
CF	48.5	9.4	49.4	4.9	51.2	32.0 -75.3
ADF	44.6	8.8	39.0**	5.9	43.7	25.4 -69.0
Energy value						
TDN	53.2	5.6	48.5**	3.5	52.8	39.2 -66.9
DE	2.07**	0.30	2.11**	0.30	2.39	1.63- 2.96
ME	1.71**	0.23	1.69**	0.29	1.96	1.23- 2.50
IRC-1 group (n=12)						
Digestibility (%)						
DM	71.7	3.2	73.9	3.9	72.2	66.5 -77.6
OM	74.3	3.0	76.5	3.5	74.9	69.8 -79.9
CP	67.2	4.6	71.1	5.5	68.2	50.5 -80.5
EE	77.6	6.1	79.1	6.8	76.7	62.9 -81.1
CF	52.0	5.6	55.2	5.7	53.4	44.6 -65.5
ADF	46.9*	5.3	51.1	5.5	50.0	42.0 -56.2
Energy value						
TDN	71.0	3.0	73.2	3.5	71.6	66.0 -76.0
DE	3.15	0.16	3.22	0.15	3.20	2.90- 3.48
ME	2.66	0.14	2.76	0.14	2.73	2.37- 3.00

[1)] See Table 1.
[a)] RSD : Residual standard deviation, *significant ($P<0.05$), **significant ($P<0.01$) ;

TDN estimated using indirect method were found significantly different ($P<0.01$). In IRC group, significances ($P<0.05$) were observed only in the digestibility of ADF estimated by direct methods. However, the residual standard deviation (RSD) of the indirect method was lower than the direct method in IRO group, but was similar in IRC group.

This validation test showed that conclusion made based on the correlation coefficient and standard error of prediction values, as usually done in direct method, is not completely correct as observed in the ME of IRO group. Also, this comparison showed the accuracy of both direct and indirect methods were similar and appropriate to estimate the *in vivo* value.

Application test

The application test was done using IRC-2 and are presented in Table 4. Compared with the *in vivo*, almost all the estimated values of two methods were significantly different ($P<0.01$). In general, the difference of the methods showed that the indirect method was smaller than the direct ones. The direct method showed inaccuracy for estimation of OM, EE, CF, and ADF digestibilities as well as the TDN,

Table 4. Means and residual standard deviation between the NIRS methods and the *in vivo* of application test rations[a]

Constituents[1]	NIRS direct		NIRS indirect		*In vivo*	
	Means	RSD	Means	RSD	Means	Range
Italian ryegrass and concentrate, (IRC-2, n=16)						
Digestibility (%)						
DM	73.8	1.6	74.2	1.4	70.7	68.0-73.6
OM	61.9	1.0	76.5	0.9	74.9	73.1-76.7
CP	66.3	1.9	71.2	1.9	69.4	64.9-72.2
EE	95.1	4.6	76.5[ns]	5.3	75.3	64.5-81.0
CF	2.9	2.6	72.8	2.2	68.2	63.0-71.9
ADF	33.7	2.3	63.3*	2.1	61.6	58.2-65.3
Energy value						
TDN	57.8	3.0	73.0*	2.7	75.4	69.7-78.6
DE	3.31*	0.10	3.12	0.08	3.25	3.05-3.36
ME	2.93	0.19	2.64*	0.17	2.78	2.22-2.97

[1] See Table 1.
[a] Statistical analysis by paired *t*-test were significant ($P<0.01$) between the methods and the *in vivo*; ns : not significant, * : significant ($P<0.05$)
RSD : Residual standard deviation.

with the biases of 13.0, 19.8, 65.3, 27.9, and 17.6%, respectively. In the indirect method, biases for estimated values were considerably small. The highest bias was 4.6% of CF digestibility, while other constituents were the range of 1.2-3.5%.

In the prediction of DE and ME value, the direct method differed at 0.06 and 0.15 Mcal/kg, or if expressed in percentage were 1.9 and 5.4%, respectively. Differences of these values resulting from the indirect method were 0.13 (4.7%) and 0.14 (5.0%) Mcal/kg, respectively. These results showed that both methods were able to determine the DE and ME values, but the accuracy should be improved by more references data. The RSD of DE and ME determined by indirect method was different. This fact indicated that the linear equation for the ME determination using the DE value was not able to cover the variation from animal factor resulting *in vivo* value. However, those biases were small and just higher than 4% as limit value of bias in farm usage recommended by Van Es[18]. By adopting that value, the estimation of digestibility value obtained from the indirect method was in the reasonable range for farm use.

Evaluation of IRO (validation test), IRC-1 (validation test) and IRC-2 (application test), showed that the RSD of the indirect methods was smaller or similar with the direct method. These facts, however, showed that the indirect method has the reproducibility for digestibility estimation method.

This study showed that indirect method of NIRS has a potential for digestibility and energy value determination. Referring to the result of the application test, the indirect method was better than the direct method. This is in contrast to the statement of Coleman and Murray[3] that direct prediction by NIRS method was better than the secondary prediction using chemical composition as intermediate. This is because digestibility and energy value is affected by complex interaction between/within the animal and feeds[11] which may not be detected by the direct method.

Stability of precision indicates high reproducibility of the method as it is important for routine application in farm management. Moreover, the result of this study show that indirect method was superior than direct method to cover the interaction factors occurring in animal due to the inclusion of fecal data in the estimation. The bias observed in this study can be reduced by developing a more accurate calibration equation for feeds and fecal components including lignin by increasing the number of samples for calibration.

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近赤外分析法による飼料の消化率とエネルギー価の 2推定方法の比較

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イタリアンライグラス乾草のみを給与した消化試験試料（IRO 区，n=45）とイタリアンライグラス乾草と配合試料とを混合給与したロットの異なる2グループよりなる消化試験試料（IRC 区，n=42 および 16）とを用いて，近赤外分析法を活用した飼料消化率およびエネルギー価の2種類の推定方法について比較検討した．推定方法は，それぞれの試料区毎に30点の消化試験成績を用いて消化率とエネルギー価の検量線を作成し，これを用いて直接，消化率とエネルギー価を推定する方法（直接法）と，リグニンを含む飼料成分を近赤外分析法によって求め，その成績に基づいてリグニンを用いた指示物質法により推定する方法（間接法）の2方法である．両推定方法の信頼性の検討は，それぞれの方法による推定値と *In vivo* 値とを同一の飼料区の残りの試料（IRO 区 15 点，IRC 区 12 点）を用いて比較することによって行った．さらに IRC 区のロットの異なる 16 点の試料を用いて，両方法の応用性についても検討した．その結果，直接法は同一ロットの試料に対しては十分な信頼性を有するものの，異なるロットに対しては偏差が大きかった．一方，間接法は，直接法に比較して *In vivo* 値との差および残差標準偏差（RSD）が直接法よりも小さく，いずれのロットの試料に対しても十分な信頼性を有することが示され，本方法が個体毎の消化率の評価法として活用できることが明らかになった．

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