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# Application of Near Infrared Reflectance Spectroscopy to Predict Fecal Composition and Its Use for Digestibility Estimation

Agung PURNOMOADI, Mitsunori KURIHARA\*,  
Takehiro NISHIDA\*, Masaki SHIBATA\*<sup>1</sup>,  
Akira ABE\* and Ken-ichi KAMEOKA

Faculty of Agriculture, Tokyo University of Agriculture,  
Setagaya-ku, Tokyo 156

\* National Institute of Animal Industry, Tsukuba Norin Kenkyu Danchi,  
Ibaraki-ken 305

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**Abstract** Two experiments were carried out to investigate the applicability of near infrared reflectance spectroscopy (NIRS) to predict the chemical composition of feces, and thereby to estimate digestibility based on a lignin indicator method. Ninety five fecal samples collected from digestion trials using dairy cattle were subjected to NIRS for the prediction of chemical composition. Three methods to determine feed digestibility were compared, namely by digestion trial (*in vivo*), by the lignin indicator method using data from chemical analysis (LIGLab), and by the lignin indicator method from NIRS prediction (LIGNIR). Digestibility was evaluated for three groups of feeds based on the type of feedstuff used in the ration. The groups were Italian ryegrass only (IRO, n=20), Italian ryegrass-concentrate ration (IRCT, n=16), and Italian ryegrass-steamed wood ration (IRSW, n=12). The rations were adjusted so as to meet the total digestible nutrients requirement of the Japanese Feeding Standard. This study showed that fecal composition could be accurately predicted by NIRS. The values obtained by the NIRS prediction method for unknown samples and the respective values obtained by chemical analysis were highly correlated for acid detergent fiber, crude fiber, lignin and ether extract; the correlation coefficients (r) were 0.98, 0.98, 0.97 and 0.96, respectively. Correlation coefficients for crude protein, organic matter and energy were 0.91, 0.91 and 0.82, respectively. With respect to digestibility estimation, the value for the LIGLab and LIGNIR estimations and that for the *in vivo* were very similar. The difference between the LIGLab and LIGNIR values and the *in vivo* value was below 3%, the standard deviation of difference was less than 5%. The results show that digestibility estimation using lignin determined by NIRS as an indicator was useful for the routine evaluation of nutritive values because it is simple, fast and accurate.

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**Key words** : NIRS, Fecal composition, Lignin, Digestibility, Dairy cattle

A number of studies to determine the nutritive values of feed using near infrared reflectance spectroscopy (NIRS) have been carried

out. The success in predicting the chemical composition of various feedstuffs by NIRS<sup>3,23)</sup> advanced the application of this method to the

<sup>1)</sup> Present address : Agriculture, Forestry and Fisheries Research Council, Ministry of Agriculture Forestry and Fisheries, Chiyoda-ku, Tokyo 100

prediction of nutritive values such as total digestible nutrients (TDN) and available energy contents<sup>1,21</sup>. All these studies were carried out directly with feeds in which the *in vivo* digestibility was previously known. However, digestibility is affected by the condition of the animal used, variation between animals as well as feeding level<sup>16</sup>. Therefore, it is possible for the same ration fed to different animals to have different digestibilities, and ideally, digestibility should be individually estimated.

The ideal way to measure digestibility is by the digestion trial method in which feed intake and the nutrients ingested can be accurately measured. However, this method is expensive and laborious. An alternative method in digestibility measurements is the indicator method in which lignin is used as an indicator. This method does not require the total collection of feces, but only needs a representative fecal sample. Only by measuring the percentage of lignin in feed and feces, can the digestibility of the feed be estimated.

Given that the chemical composition of feeds are easily and routinely predicted using NIRS, it may be worth trying to use the same approach to estimate the chemical composition of feces. One extra advantage of this approach is that it takes into account the fraction of ingested nutrients and energy lost in the feces, which accounts for the largest part of feed losses. In ruminants these losses reach 40-50% and 20-30% in case of roughage and concentrate, respectively<sup>7</sup>. Combined with the efficient indicator method, NIRS may be used to generate a faster and more accurate estimation of feed digestibility from individual animals.

The aims of this study were to validate the use of NIRS for the prediction of the chemical composition of feces, and to evaluate the accuracy of digestibility estimation using lignin predicted by NIRS.

## Materials and Methods

### Experiment 1.

Ninety-five fecal samples from a digestion trial using dairy cattle were used in this study. All cattle were fed at the total digestible nutrients (TDN) requirement level of the Japanese Feeding Standard for Dairy Cattle<sup>20</sup>. The samples were dried at 60°C for 48 hours before they were ground with a Wiley mill to pass through a 1.00-mm screen. These processed samples were used for chemical analysis to determine organic matter (OM), crude protein (CP), ether extract (EE), crude fiber (CF)<sup>16</sup>, acid detergent fiber (ADF), acid detergent lignin (lignin), and silica<sup>1</sup>, as well as energy<sup>18</sup>.

Analysis by NIRS was done by a Pacific Scientific (Ncotec) model 6500 (Perstorp Analytical, Silver Spring, MD) instrument equipped with ISI software (InfraSoft International, Port Matilda, PA) for analysis. The samples were scanned over the range 1,100-2,500 nm. The spectral data were collected at wavelength intervals of 2 nm, and the 700 data points obtained for each sample were stored in the computer as absorbance values. These values were expressed as  $\log_{10}(1/R)$ , where R is the reflectance. The second derivative of  $\log 1/R$  values were used to derive relationships with the chemical compositions<sup>23</sup>. To achieve the optimum wavelengths for predicting the components, a stepwise multiple-linear-regression program was used.

Forty-nine samples chosen randomly from the 95 fecal samples above were used as a standard sample to develop a NIRS calibration equation. This equation was then used to predict the chemical contents of the remaining 46 fecal samples (unknown samples). The calibration was done with a maximum of four wavelengths. The least number of wavelengths was used for measurement when an increase in the number of wavelengths gave no increase in significance of the R value nor any decrease in the standard error of calibration.

### Experiment 2.

It is well known that prediction by NIRS will be maximal if the calibration set samples and unknown samples are the same or of high homogeneity<sup>27</sup>. This is because, the feedstuffs were separated into two big groups to develop the calibration equation.

Twenty-five samples of forage including hay, silage, and steamed wood, and 10 samples of concentrate including grains and soybean were used to develop an NIRS calibration equation for forage and concentrate respectively. Due to the limited number of feed samples used, the validity of the developed equations was not tested. Both equations were directly used to determine OM, CP, EE, CF, ADF, lignin, and energy contents for 10 feedstuffs (7 Italian ryegrass, 2 concentrate and 1 steamed wood samples). These feedstuffs were the main components used to make the 48 rations with known *in vivo* digestibilities. Subsequently the values observed from the 10 feedstuffs were used to calculate the chemical composition of the 48 rations. Based on types of feedstuffs used in the rations, three groups were designed for evaluation. Group I was Italian ryegrass only (IRO) composed of three kinds of Italian ryegrass fed to dairy cattle (n = 20). Group II was a mixture of Italian ryegrass and concentrate (IRCT) composed of (1) a combination of Italian ryegrass and soybean, (2) a combination of Italian ryegrass and commercial formula feed, and (3) a combination of Italian ryegrass and soybean and commercial formula feed. These rations were fed to dairy cattle (n = 16). Group III was a mixture of Italian ryegrass and steamed wood (IRSW) at 5 and 55% fed to 12 dairy cattle. The separation of rations for evaluation was made to reduce the influence of feedstuff type on digestibility estimation, because of different lignin recoveries<sup>15</sup>. These rations were allowed at the TDN requirement level of the Japanese Feeding Standard for Dairy Cattle<sup>20</sup>. Finally, the digestibility of each ration was calculated

by the lignin indicator method using the formula<sup>11</sup>:

$$\text{digestibility} = 100 - \{100 \times (\% \text{ lignin in feeds} / \% \text{ lignin in feces}) \times (\% \text{ nutrient in feces} / \% \text{ nutrient in feeds})\}$$

Two approaches were applied to estimate digestibility by the lignin indicator method and both were compared with *in vivo* digestibility values for their accuracy. The first approach was to calculate the chemical composition of feed and fecal samples obtained from chemical analysis (LIGLab). The second one was to calculate those samples from NIRS data (LIGNIR).

Digestibility values estimated using LIGLab, LIGNIR and *in vivo*, were compared for OM, CP, EE, CF, ADF and energy. The differences between individual digestibility values estimated by LIGLab or LIGNIR and *in vivo* were calculated. These differences were tested for significance using paired *t*-tests<sup>28</sup>.

## Results and Discussion

### Experiment 1.

The ranges and means of all constituents for the standard and unknown samples are shown in Table 1. Regarding the range of composition, values of the standard samples were slightly wider than those of the unknown samples.

Wavelengths observed for calibration of standard samples are listed in Table 2. With respect to organic matter, the 1st to 4th absorbance wavelengths were found at 2,418, 2,206, 1,144 and 1,956 nm, respectively. The first absorbance was in the 2,410-2,460 nm region, considered to be the second overtone of C-H deformation<sup>19</sup> and this was related to cellulose. Another report<sup>8</sup> found that the first two wavelengths were absorbance values for the detergent fiber component. The 3rd wavelength was close to 1,143 nm, characteristic of aromatic structures<sup>25</sup> which is thought to be lignin<sup>22</sup>. The 4th wavelength which was close to 1,960

Table 1. Means and ranges of constituents for calibration of standard samples and prediction of unknown fecal samples

Constituents	Standard (n=49)		Unknown (n=46)	
	Mean	Range	Mean	Range
Organic matter	85.1	66.2-94.1	85.7	70.8-94.5
Crude protein	12.6	4.9-25.3	12.0	5.7-23.8
Ether extracts	1.9	0.5-3.0	1.8	0.4-3.5
Crude fiber	29.9	19.2-45.9	31.3	18.7-41.2
Acid detergent fiber	47.5	27.4-68.1	48.5	34.4-64.2
Energy	4,412	3,276-4,897	4,439	3,756-5,065
Lignin	14.7	9.6-25.3	14.6	9.5-27.6
Silica	6.3	0.9-27.4	5.9	0.8-16.4

n = number of samples

Table 2. Wavelengths used and correlation coefficients of each constituent for fecal calibration of standard and prediction of unknown fecal samples\*

Constituents	Wavelength (nm)				Standard (n=49)		Unknown (n=46)	
	1st	2nd	3rd	4th	R	SeC	r	SeP
Organic matter	2,418	2,206	1,144	1,956	0.957	1.29	0.911	0.35
Crude protein	2,186	2,090	2,396	—	0.988	0.70	0.912	0.09
Ether extracts	1,470	2,334	1,690	—	0.983	0.14	0.956	0.02
Crude fiber	2,332	1,820	2,452	1,954	0.983	1.22	0.976	0.19
Acid detergent fiber	2,300	1,330	—	—	0.987	1.45	0.979	0.12
Energy	2,420	1,572	2,036	1,602	0.931	70.6	0.819	21.1
Lignin	2,388	1,760	2,318	—	0.971	1.07	0.972	0.15
Silica	2,220	1,372	1,694	—	0.925	0.86	0.792	0.06

\* R : correlation coefficient from multiple regression ; r : correlation coefficient from simple regression ; SeC : standard error of calibration ; SeP : standard error of prediction.

nm, was for protein<sup>25)</sup>.

Three wavelengths at 2,186, 2,090 and 2,396 nm were recorded for the calibration of CP. The 1st wavelength was protein at 2,180 nm<sup>25)</sup>. The 2nd wavelength was considered to be cellulose at 2,088 nm<sup>25)</sup>, while the 3rd wavelength at 2,396 nm was close to the 2,380 nm of hemicellulose<sup>25)</sup>.

Three wavelengths at 1,470, 2,334 and 1,690 nm were recorded for the calibration of EE. The 1st wavelength was 19 nm different from the 1,489 nm of cellulose<sup>25)</sup>. The 2nd and 3rd wavelengths were very close to 2,336 and 1,685 nm which are known to be the absorbance of cellulose and lignin<sup>22,23)</sup>, respectively. These

wavelengths demonstrated that dominant fecal fibrous components were used in determining ether extract. Ether extracts in feces is the smallest constituent and does not clearly appear in log 1/R. Consequently, the wavelength properties of EE might be concealed by the more dominant spectrum of cellulose.

Crude fiber (CF) was well predicted by using four wavelengths at 2,332, 1,820, 2,452 and 1,954 nm. The first two wavelengths were close to the absorbance of cellulose, at 2,336 and 1,820 nm<sup>25)</sup>. The 3rd and 4th wavelengths were close to the 2,461 nm absorbance of starch and 1,960 nm absorbance of protein<sup>25)</sup>. The first



two wavelengths are known to be the most important as cellulose is the most important component of CF. The 3rd wavelength, that of starch, was considered to be important, as starch is structurally similar to cellulose. The 4th wavelength, that of protein, apparently has no relation with CF, and was simply used to improve the correlation coefficient in this regression.

Only two wavelengths at 2,300 and 1,330 nm were used for the calibration of ADF. The first wavelength was close to the 2,294 nm of neutral detergent fiber (NDF)<sup>23)</sup>. The second wavelength was related to that of hemicellulose at 1,360 nm<sup>25)</sup>. Hemicellulose with cellulose and lignin form NDF, but hemicellulose does not form a part of ADF which contains just cellulose and lignin. The appearance of NDF or hemicellulose absorbance in ADF was related to the fact that both cellulose and hemicellulose are carbohydrates in which they have the same absorbance region.

For lignin, three wavelengths were observed, which were 2,388, 1,760 and 2,318 nm. The 1st and 2nd wavelengths were close to the reported absorbances of *in vitro* dry matter digestibility (IVDMD) at 2,386 nm<sup>13)</sup> and at 1,759 nm<sup>29)</sup>, respectively. The 3rd wavelength was considered to be hemicellulose, which is usually observed at 2,314 nm<sup>25)</sup>.

The four wavelengths observed for energy were 2,420, 1,572, 2,036 and 1,602 nm. The first wavelength was the same as the first wavelength of organic matter, which was considered to be cellulose. The 2nd wavelength was close to either the reported 1,570 nm of cell wall<sup>6)</sup> or the 1,580 nm of cellulose<sup>25)</sup>. The 3rd wavelength at 2,036 nm was 14 nm different from 2,050 nm, which is the absorbance of protein<sup>12)</sup>. The fourth wavelength at 1,602 nm was considered to be the absorbance of organic matter. That was close to the 1,606 nm absorbance of organic matter<sup>3)</sup>, and the 1,610 nm absorbance for IVDMD, the first wavelength from five wavelengths reported by Holeček *et al.*<sup>13)</sup>.

The four wavelengths observed here were in agreement with the fact that organic matter is closely related to energy<sup>5)</sup>.

For silica, three wavelengths were observed at 2,220, 1,372 and 1,694 nm. The first wavelength was close to the reported 2,212 and 2,214 nm of the digestible fraction of cell wall<sup>3)</sup>. The 2nd and the 3rd wavelengths were close to the 1,365 nm of cellulose<sup>25)</sup> and 1,685 nm of lignin<sup>22,25)</sup>, respectively.

Correlation coefficients (R) between the values obtained by chemical analysis method and those obtained by NIRS for all constituents in the standard samples were observed to be more than 0.90. The highest R, 0.988, was found for CP while the lowest, 0.925, was for silica. As presented in Table 2, R values for the fibrous fractions were very close to one, being 0.983, 0.987, and 0.971 for CF, ADF, and lignin, respectively. High R values were also found for OM, EE and energy contents, being 0.957, 0.983 and 0.931, respectively. For validity of equations developed, the prediction equations in the standard samples were tested for further application.

The prediction equation obtained from the standard samples was used to determine the constituents of 46 unknown samples. Except for CF and lignin, the simple correlation coefficient (r) and standard errors (SeP) were generally lower than those for the standard samples. Because of the higher similarity of R values for lignin in the standard and unknown samples, it may lead to a possibility of lignin as an indicator for digestibility estimation.

The decrease in the correlation coefficient values of ADF, EE, OM and CP observed from the calibration of standard samples compared to that from the prediction of the unknown samples was small. A remarkable decrease of the correlation coefficient was observed for silica (from 0.925 to 0.792) and energy (from 0.931 to 0.819). This result shows that silica was poorly predicted. It agrees with the finding that minerals are known to have no

absorption bands in the near infrared region<sup>27)</sup>. In case of energy, using only four wavelengths, which is the maximum capacity of instrument used, may not satisfy the requirements for fecal prediction because of spectral complications resulting from the wide possibility of combinations. This was due to the fact that energy is the heat contributed from all chemical components in the samples.

Reviewing the absorbances observed, the wavelengths appropriate for feces were somewhat different from that usually observed for feed. The wavelengths observed for all constituents in feces were dominantly in the cellulose spectra for predicting chemical composition. Reference to wavelengths obtained only from feed made it difficult to conclude whether the wavelengths obtained were appropriate for feces. However, the obtained wavelengths were still related to the chemical composition predicted.

**Experiment 2.**

The ranges and means of feedstuffs for NIRS calibration in this study are presented in Table

3. In forage, high R values were found for OM, CP, CF, ADF, and lignin, ranging from 0.978 to 0.986. Lower R values were found for EE and energy, being 0.834 and 0.770, respectively. In addition, in concentrate, high R values were also found for OM, CP, EE, ADF, energy, and lignin, ranging from 0.992 to 0.997. A lower R value, 0.907, was found only for crude fiber. The high calibration R values for lignin in both forage and concentrate suggests that lignin in feed may be well predicted and can be used for digestibility estimation based on lignin.

The chemical compositions of 48 rations, classified into three groups, IRO, IRCT, IRSW are presented in Table 4. The chemical composition of the feedstuff groups were calculated using the data obtained from chemical analysis and from NIRS prediction of the 7 roughages, 2 concentrates and 1 steamed wood components. Referring to mean values, only small differences between the two methods of calculation were observed for all constituents. Relatively large differences were observed only in ADF of IRO and CF of IRSW, being 1.7 and

Table 3. Means, ranges and wavelengths used for NIRS calibration of feedstuff samples<sup>\*)</sup>

Constituents	Mean	Range	Wavelength (nm)				R	SeC
			1st	2nd	3rd	4th		
Roughage (n=25)								
Organic matter	90.3	87.9-99.6	1,486	2,266	—	—	0.978	0.61
Crude protein	9.2	0.1-12.4	2,188	1,228	—	—	0.986	0.54
Ether extracts	2.6	0.8-3.4	1,684	1,286	1,220	2,040	0.834	0.46
Crude fiber	31.1	24.7-47.8	2,184	1,606	2,360	—	0.984	1.01
Acid detergent fiber	39.4	30.8-68.1	1,740	2,378	—	—	0.981	1.75
Energy	4,305	4,177-4,770	1,822	1,692	2,322	1,964	0.770	83.7
Lignin	6.2	3.0-16.5	2,378	1,670	—	—	0.981	0.64
Concentrate (n=10)								
Organic matter	93.2	92.8-94.3	1,142	2,258	1,490	2,032	0.922	0.07
Crude protein	19.1	14.4-50.7	2,132	—	—	—	0.993	1.41
Ether extracts	3.0	1.7-3.5	1,974	2,256	1,394	2,446	0.986	0.09
Crude fiber	5.6	4.2-7.6	1,328	1,808	1,140	—	0.907	0.43
Acid detergent fiber	9.7	7.8-13.6	1,212	2,152	1,992	—	0.995	0.22
Energy	4,398	4,308-4,864	2,132	1,682	—	—	0.997	12.1
Lignin	1.8	0.3-3.5	1,722	1,520	1,190	1,810	0.992	0.12

<sup>\*)</sup> R : correlation coefficient from multiple regression ; SeC : standard error of calibration.

Digestibility estimation by NIRS predicted data

Table 4. Means of chemical composition of the ration groups used in this study as calculated with chemical analysis data and NIRS predicted data (%DM)\*

Constituents	IRO (n=20)		IRCT (n=16)		IRSW (n=12)	
	Lab	NIR	Lab	NIR	Lab	NIR
Organic matter	90.0	89.9	92.1	92.4	93.0	92.7
Crude protein	9.0	9.1	23.8	24.6	8.3	7.9
Ether extracts	2.8	2.7	3.1	2.8	2.4	2.5
Crude fiber	30.3	30.7	13.7	13.5	31.8	33.0
Acid detergent fiber	36.4	38.1	19.1	18.9	43.9	43.2
Energy <sup>1)</sup>	4,266	4,289	4,487	4,482	4,470	4,415
Lignin	5.2	5.7	2.7	2.6	7.1	7.5

\*<sup>1)</sup> Feed composition calculation of 48 rations used the data of 7 roughage, 2 concentrate and 1 steamed wood feed obtained from chemical analysis (Lab) and NIRS prediction (NIR); IRO: Italian ryegrass only, IRCT: Italian ryegrass-concentrates, IRSW: Italian ryegrass-steamed wood.

<sup>1)</sup> Energy expressed in cal/g DM.

1.2%, respectively. Other constituents were below 0.6%. For energy, a large difference was only observed for IRSW, being 55 cal/g.

The mean digestibility calculated from the three methods as well as the standard deviation of difference (SDd) between both LIGLab or LIGNIR, and that *in vivo* are shown in Table 5. The evaluation was done for 3 groups separated on the basis of type of feedstuffs.

In general, the estimated values were slightly higher than the *in vivo* values. A comparison between LIGLab and LIGNIR, for the IRO and IRSW groups indicates that the LIGLab estimated value was higher than that of LIGNIR. However, the contrary was observed for the IRCT group. These figures may be caused by the NIRS predicted data for lignin of the rations (Table 4). Due to the equation for digestibility estimation used, the estimation value will be lower if the value of lignin of feces is low or lignin of feeds is high.

For a comparison of the means between the digestibility estimated by LIGNIR and *in vivo*, the difference observed in DM and OM were 2.3, 1.4, 2.0 and 3.0, 1.1, 1.7% for the IRO, IRCT, IRSW groups, respectively. These differences are relatively small. For CP and EE digestibility, the differences of means were 1.2, 1.6, 5.8 and 0.9, 0.5, 4.8% for the IRO, IRCT and IRSW

groups, respectively. The different values observed for the IRO and IRCT groups were small, but were relatively high for the IRSW group. Small differences were observed in CF and ADF digestibility, being 0.5, 0.6, 2.8 and 2.1, 0.3, 1.2% for the IRO, IRCT and IRSW groups, respectively. For the digestibility of energy, the differences of means for IRO, IRCT and IRSW groups were 2.4, 1.5 and 1.6%, respectively. From these differences, the digestibility estimated with LIGNIR were very similar to the values obtained *in vivo*, except for the digestibility of CP and EE for the IRSW group. For comparison, Penning and Johnson<sup>26)</sup> using acid insoluble ash (AIA) as a marker observed the mean differences of OM digestibility at 0.9 and 1.8% for sheep fed ryegrass at 15 and 25 g per kg live weight, respectively.

Referring to the standard deviation of difference (SDd) between the estimation values of LIGLab or LIGNIR and that *in vivo*, the values were generally below 5% for the IRO and IRCT groups, except for the digestibility of CP of the IRO group. For the IRSW group, the SDd was relatively high. The accuracy of digestibility estimations of similar studies to the present experiment were expressed by the residual standard deviation (RSD) which was observed as the differences between the *in vivo* value and

Table 5. Digestibility value estimated from LIGLab and LIGNIR in comparison with the *in vivo* method

Digestibility of constituents	LIGLab		LIGNIR		<i>In vivo</i>	
	Mean	SDd	Mean	SDd	Mean	Range
Italian ryegrass only (IRO, n=20)						
Dry matter	53.3	4.849	49.6*	4.261	51.9	39.1-68.4
Organic matter	55.6	4.508	51.4**	4.346	54.4	41.3-71.7
Crude protein	46.1	6.180	43.1	6.118	44.3	32.8-58.0
Ether extracts	66.5	3.455	66.2	3.243	65.3	54.2-74.9
Crude fiber	50.4*	4.514	48.5	4.505	48.0	31.2-68.3
Acid detergent fiber	38.3	4.790	36.9	4.827	39.0	22.7-59.9
Energy	52.6	4.877	48.8*	4.217	51.2	38.1-68.8
Italian ryegrass-concentrates (IRCT, n=16)						
Dry matter	72.6	2.312	73.5*	2.266	72.1	65.3-77.6
Organic matter	75.1	2.100	75.8*	2.110	74.7	68.9-79.0
Crude protein	72.8	2.536	73.7	3.402	72.1	57.2-83.1
Ether extracts	76.9	4.133	78.2	2.665	77.7	63.2-82.1
Crude fiber	52.1	2.454	52.5	3.457	51.9	44.6-57.8
Acid detergent fiber	47.0	4.851	47.5	3.656	47.8	35.7-56.2
Energy	72.7	2.222	73.6*	2.116	72.1	65.8-77.2
Italian ryegrass-steamed wood (IRSW, n=12)						
Dry matter	62.0*	5.977	58.9	4.300	56.9	44.5-67.4
Organic matter	63.4*	5.516	60.3	4.375	58.6	45.1-70.2
Crude protein	45.3**	8.165	43.7**	5.673	37.9	15.9-55.0
Ether extracts	71.0*	6.333	71.1**	3.340	66.3	62.2-71.8
Crude fiber	62.7**	4.751	60.8**	3.246	57.9	34.0-71.8
Acid detergent fiber	53.9**	4.551	50.0	2.652	48.8	29.9-62.4
Energy	60.5	5.875	56.5	5.748	54.9	40.1-66.8

\* Significant (P<0.05), \*\* significant (P<0.01); SDd: standard deviation of difference between estimation values of the LIGLab or LIGNIR and the *in vivo*. Dry matter digestibility = 100 - (100 × lignin feed/lignin feces).

the estimation adjusted value resulting from the regression equation. These RSD values represented the estimation variation associated with the techniques. Standard deviation of differences and RSD are comparable because they are both calculated by the difference between values determined *in vivo* and those estimated. The RSD of dry matter (DM) digestibility estimated using lignin determined by three methods ranged from 2.4 to 4.5<sup>17)</sup>, while the same estimation made using cellulase digestion methods was in the range of 2.5 to 2.7<sup>11)</sup>.

For OM digestibility, Navaratne *et al.*<sup>21)</sup>

reported the prediction of roughages (grass and straw) by various methods. These methods used AIA as a marker (RSD=4.06), rumen fluid (RSD=3.78), pepsin-cellulase (RSD=5.09) and nylon bag methods (RSD=4.08). If the comparison is done based on type of feed, RSD values observed for the grass group using AIA marker, rumen fluid, pepsin-cellulase and nylon bag methods, however, were lower by 1.78, 1.66, 1.40, and 1.24 than the results of the present experiment, respectively. The estimation of OM digestibility in this study was also similar to that reported by Aerts *et al.*<sup>2)</sup> who used two-stage *in vitro* digestibility method.

A relatively high SDd was observed for digestibility of CP in the IRO and IRSW groups, and for digestibility of energy in the IRSW group. These estimations are less accurate than other components, although this is statistically not significant. The high SDd of CP digestibility observed in the IRO and IRSW groups were merely caused by the wide range of CP digestibility for the low CP content in the feed (both groups were below 10% CP). However, evaluation of CP can be neglected for ruminants due to the involvement of non protein nitrogen (NPN) in the calculation<sup>21</sup>. Meanwhile, the high SDd for digestibility of energy in the IRSW group was considered to be influenced by the poor energy prediction of the feedstuff in that group (see Table 4). A comparison of observed SDd with the RSD reported from the various methods above indicates that the estimation method used in this study was apparently favorable.

The most favorable estimation of digestibility in the IRCT group, as indicated by the smallest bias and SDd among the rations, was related to the accuracy of lignin prediction of feedstuffs. Since usual farming management would use a combination of roughage and concentrate, this digestibility estimation shows the applicability for farms. To improve the accuracy of this method, a larger sample number and wider range of feedstuffs for developing NIRS prediction equations should be considered.

In practice, only DM digestibility<sup>30)</sup> or OM digestibility<sup>2)</sup> would be used for evaluating feeding management. The differences between the lignin indicator methods and the *in vivo* method were related to the fact that lignin was partially digestible<sup>10,11,15)</sup>. Van Soest<sup>30)</sup> noted that the limitation of using lignin as a digestibility indicator is limited because of the large inter-species variation. However, this can be overcome by using fairly similar feedstuffs, although under these conditions standard error is still about 3% of digestibility.

Thus, the results above show that this digestibility estimation method is sufficiently reliable.

Digestibility estimation by LIGNIR shows the potential for individual and routine measurement. Near infrared reflectance spectroscopy prediction of the chemical composition of feces and feed as well as the application of NIRS prediction for digestibility estimation may be useful for the evaluation of feedstuffs on a practical farm basis, provided some correction factors or equations are devised to minimize the difference between *in vivo* and that estimated by LIGNIR.

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