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Digestibility Estimation Based on NIRS Method Using Fecal Grab Sample

Agung PURNOMOADI*, Mitsunori KURIHARA, Takehiro NISHIDA,
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Fecal grab samples (FGS, n = 216) collected from six hours interval during first two days of digestion trials were used in this study. The trials were conducted to twenty-seven dairy cattle given three rations. The rations were Italian ryegrass combined with 40% concentrate (group 1), steamed wood at 5% (group 2) and 55% (group 3) of total dry matter supply. Near infrared reflectance spectroscopy (NIRS) was carried out to determine both the FGS and feces from total collection (FTC) to know the diurnal variation. Compositions of FGS determined by NIRS were used in digestibility estimation based on lignin indicator method (LIM). For comparison, the digestibility value of FTC was obtained from conventional analysis methods (= *in vivo* value). Diurnal variation of composition within FGS was not significant. With respect to the use of FGS composition for digestibility estimation, there was generally significantly different ($P<0.01$) from that of *in vivo*. An exception, the not significantly was obtained in the digestibility of EE (group 1), OM, ADF and energy, TDN (group 3). The range of biases of digestibilities between FGS and *in vivo* were 1.0 − 5.7, 3.5 − 8.1 and 0.6 − 7.3% for group 1, 2 and 3, respectively, and was considered related to the lignin recoveries. Standard deviations of differences between the digestibility of FGS and *in vivo* were generally averaged below 3.7% for low lignin rations (group 1 and 2), except CF (6.3%) and ADF (5.6%) in group 1, but slightly high for high lignin rations (group 3).

INTRODUCTION

It was reported that the near infrared reflectance spectroscopy (NIRS) prediction of feces and feed compositions can replace the conventional analysis to estimate the digestibility[1]. That report was used fecal samples prepared from total collection (FTC). To enlarge the application for farm using, the study using fecal grab sample (FGS) is needed. Digestibility estimation by lignin indicator method (LIM) is available for representative sample. This method is the most applicable for farms using due to easiness and the natural existence of lignin in the feed. Limitation of lignin for indicator using is reported partly digestible[2] and the variation of lignin excretion[3,4]. Variation of lignin concentration consequently changes the value of digestibility estimation. This variation may be caused by the variation of analysis, especially in particle grinding[5]. Another disadvantage of lignin analysis is time consuming which not suitable for routine and voluminous work. With known as rapid, repeatable and non chemically analysis, NIRS method may solve that problem.

The objectives of this study were to know the variation of fecal composition in six hours interval grabbing time and to obtain the representative time sampling for digestibility estimation.

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MATERIALS AND METHODS

Twenty-seven dairy cattle (lactation = 12, dry = 15) were used in digestion trials. Three rations allowed to cattle were adjusted to meet the TDN requirement based on Japanese Feeding Standard for Dairy Cattle[6]. The rations were Italian ryegrass combined with 40% of formula feed (mainly corn and soybean meal) for lactation group (group 1), steamed wood at 5% (group 2) and 55% (group 3) of dry matter supply for six and nine dry cows, respectively.

Cattle were fed twice a day at 10 : 00 and 16 : 00. Fecal grab samples were directly taken from the rectum at six hours interval for about 200 g. First grab sample was taken at 13 : 00 three hours after morning feeding. Collection of FGS was done during first two days of fecal collection period of digestion trials. Samples were directly dried at 65 ℃ for 48 hours before ground for 1 mm by Wiley mill. The FTC was obtained from five days collection of digestion trials.

Samples of FGS (n = 216) and FTC (n = 27) were subjected to NIRS to determine the composition for evaluation of diurnal variation. Composition of FGS determined with NIRS then used to estimate the digestibility by LIM. For digestibility evaluation, the digestibility value of FTC was obtained from conventional chemical analysis, termed *in vivo*. Conventional analysis for *in vivo* was determined for organic matter (OM), crude protein (CP), ether extracts (EE), crude fiber (CF) with proximate analysis methods, and energy with bomb-calorimeter[illegible]. Acid detergent fiber (ADF) was determined with that described by ABE[illegible].

Near infrared reflectance spectroscopy analysis was done using the Pacific Scientific (Neotec) 6500 series instrument equipped with ISI software in IBM compatible CPU terminal for calculation. Compositions of feces and rations were determined by NIRS using each calibration equations which have been reported[illegible].

Digestibility estimation by LIM for FGS was then compared with the *in vivo*. The GLM procedure of SAS[9] was done for variation of composition and digestibilities. The difference between FGS and FTC or *in vivo* was analyzed with paired t-tests[10].

RESULTS AND DISCUSSION

1. Composition of the rations

Chemical compositions of rations determined by conventional analysis and by NIRS are shown in Table 1. In general, the values obtained from conventional and NIRS were differing below 3%. Compared with conventional analysis results, relatively high differences were observed for CP for all groups (3.4-3.6%), EE of group 1 (9.7%), CF of group 2 (5.2%). For lignin, the differences were 3.7, 10, and 4.0% for group 1, 2, 3 respectively.

2. Variation of fecal composition

Chemical compositions of FGS and of FTC determined by NIRS method are shown in Table 2. Excretion of lignin for three groups was appeared in uniformly figure. Within two days grabbed fecal samples, the lignin content of group 1 and 3 lies above the FTC, but below the FTC in group 2. Similar pattern was also found in CP, EE, CF, ADF and energy excretion for group 1 and 3, but slightly different in group 2. Those variations were on narrow range. The width range of variation of OM, CP, EE were at 0.9-1.2, 1.0-2.2, 0.1-0.2 %, while CF, ADF, and lignin were 1.0-1.8, 1.2-2.6, 0.4-0.8% respectively for all groups. For energy, the range was observed at 30-80 cal/g.

Statistical analysis result shows that the variations of all constituents for all groups within 6 hour

Table 1 Chemical composition of rations used in this study from two analysis methods (%DM).

Composition	Group 1		Group 2		Group 3	
	Conv.[1]	NIRS	Conv.	NIRS	Conv.	NIRS
OM	92.1	92.4	90.7	90.2	95.3	95.1
CP	23.8	24.6	11.0	10.4	5.6	5.4
EE	3.1	2.8	2.9	2.9	1.8	2.0
CF	13.7	13.5	26.9	28.3	36.7	37.6
ADF	19.1	18.9	35.2	35.1	52.5	51.4
Lignin	2.7	2.6	4.0	4.4	10.1	10.5
Energy[2]	4.49	4.48	4.36	4.37	4.58	4.46

[1] Conv.: Conventional analysis methods
[2] Energy was expressed in Mcal/kg DM

interval during 2 days grab sampling were not significant. In comparison between FGS and FTC, the significant only observed for EE in group 2, and lignin in group 3 ($P<0.01$). However, if the average of FGS was calculated, some components were higher or lower than of FTC. These figures considered related to the extend of variation which was not covered during first two days. Range of difference between FGS and FTC for EE and lignin was at 0.1–0.3% and 0.7–1.2% respectively. That was very small for EE although statistically found significance. Small differences of lignin in the groups may influence the estimation of digestibility. Refer to the equations, the LIM digestibility estimation will overestimate if fecal lignin was higher or nutrient in feces was lower than that of FTC. Difference only 1% of fecal lignin will linearly result about 2.5% difference of nutrients digestibility. This different is higher than if fecal nutrient differ for 1%.

3. Digestibility estimation using fecal grab samples

Successive calculation for digestibility prediction is shown in Table 3. Digestibility estimation by LIM was calculated using NIRS predicted data of FGS, while FTC was determined using conventional analysis (= *in vivo*). In fact, it was questionable to compare the digestibility estimation using NIRS predicted value of FGS with *in vivo*. The errors from the methods of analysis and fecal collection were involved in the calculations. However, refer to previous study[1], it may be worth trying to compare directly with the apparent digestibility.

Digestibility estimation value within FGS showed no significant variation. That was according to no variations in fecal composition, as discussed previously. Digestibility estimation of FGS was observed higher than the *in vivo* for all constituents and groups, except energy in group 3. Compared with the *in vivo*, digestibilities of FGS were highly significant ($P<0.01$), except OM, ADF, and energy in group 3. These were considered the influence of lignin content of FGS which was higher than of the FTC. Lignin of rations in group 2 and 3 was overestimated, and additionally lignin content of FGS was higher than FTC. Previous study reported that digestibility estimation using NIRS predicted value tend to be higher than the *in vivo*[1], due to overestimate of lignin of feeds. In present study, the calibration equations for feeds and feces from previous study were used.

Bias of prediction was shown by the difference of digestibility between FGS and the *in vivo*. The averages of that bias for digestibility of DM, OM, CP, EE, CF, ADF, and energy for group 1 were 3.3, 3.0, 4.6, 1.0, 5.7, 5.1 and 3.6 respectively. For group 2, the biases for those constituent were 6.0, 5.7,

Table 2 Composition of fecal grab samples and feces collected from total collection (%DM)*

Composition	Fecal grab sample (FGS) 1 st day				2 nd day				FTC
	3	9	15	21	27	33	39	45	
					Group 1				
OM	86.5	86.6	86.2	86.1	87.3	86.8	86.8	87.7	86.4
CP	16.8	17.3	17.1	17.5	16.6	18.8	18.2	17.1	17.5
EE	2.4	2.5	2.5	2.5	2.4	2.5	2.5	2.4	2.3
CF	26.1	25.4	25.5	25.4	26.3	24.6	25.2	26.3	26.3
ADF	37.9	36.9	37.4	37.2	37.8	35.3	35.9	37.3	38.6
Lignin	11.2	10.9	11.3	11.6	11.7	11.1	11.0	11.2	10.5
Energy	4.47	4.51	4.47	4.48	4.51	4.55	4.53	4.55	4.56
					Group 2				
OM	84.8	84.3	84.3	84.4	84.4	84.5	84.2	84.1	81.9
CP	13.9	13.9	14.0	14.7	14.3	13.8	14.3	14.8	14.8
EE	2.7	2.8	2.8	2.9	2.8	2.8	2.9	2.9	2.6**
CF	23.8	24.0	23.5	23.1	23.6	24.1	23.0	22.2	23.9
ADF	43.6	43.6	43.4	42.7	43.1	43.7	42.7	41.9	44.6
Lignin	15.5	15.3	15.3	15.5	15.4	15.1	15.3	15.5	15.5
Energy	4.46	4.44	4.44	4.43	4.44	4.44	4.44	4.44	4.48
					Group 3				
OM	94.4	93.8	93.9	94.5	94.0	94.7	94.2	94.0	93.9
CP	7.0	7.5	7.6	7.8	7.5	8.2	7.6	7.7	7.9
EE	1.1	1.2	1.2	1.2	1.2	1.2	1.1	1.1	1.2
CF	39.8	38.8	38.6	38.7	38.9	37.9	38.6	38.9	37.1
ADF	62.0	61.4	61.3	61.2	61.4	60.8	61.3	61.2	60.5
Lignin	21.0	21.0	21.3	21.5	21.0	21.0	21.1	21.3	20.3**
Energy	4.76	4.76	4.75	4.74	4.73	4.81	4.77	4.78	4.79

* The values were determined with NIRS method ; Energy was expressed in Mcal/kg DM
** significant ($P<0.01$)

7.7, 8.1, 5.9, 3.5 and 6.6 while for group 3 were 0.9, 0.8, 7.3, 4.4, 1.8, 2.3 and 0.6, respectively. There were in small range for group 1 and 3, but relatively high in group 2. Again, the phenomena in group 2 were slightly different from other groups. Group 2 has remarkably high bias, although having a similar fecal lignin prediction to that of FTC and no variation of the compositions. As presented in Table 2, lignin contents of FGS in group 1 and 3 were higher than FTC, but in group 2 the FGS was similar or lower than of FTC. Normally, higher lignin will be overestimating the digestibility. Table 3 shows that digestibility estimations of FGS were still higher than the *in vivo*.

Standard deviations of differences (SDd) between the digestibility of FGS and *in vivo* generally were below 5%, except CF and ADF in group 1 and CP and EE in group 3. Relatively high SDd in CF and ADF in group 1 might be caused by low digestibility and low content in the rations (see Table 1). In case of CP and EE digestibility in group 3, those were possibly caused by low digestibility of CP and small content of EE in that group ration. Within FGS, constantly low SDd of digestibility indicates the small of variation and high precision of measurement.

Table 3 Digestibility calculated from fecal grab composition by LIM and its standard deviation of difference to the *in vivo*

Digestibility (%)	Fecal grab samples (FGS)								*In vivo*
	1 st day				2 nd day				
	3	9	15	21	27	33	39	45	
	Group 1								
DM	76.8	76.3	77.1	77.7	77.8	76.6	76.4	76.8	73.3**
SDd	2.7	3.0	3.0	3.1	2.8	2.9	2.9	2.6	
OM	78.3	77.8	78.6	79.2	79.0	78.0	77.8	77.9	75.1**
SDd	2.6	2.9	3.0	3.1	2.8	3.0	2.9	2.7	
CP	84.8	83.9	83.2	83.9	84.0	81.9	82.2	83.7	75.6**
SDd	3.3	4.1	4.5	3.1	3.9	3.9	4.5	2.8	
EE	80.1	79.2	80.0	80.3	80.9	78.9	79.2	80.5	78.9
SDd	2.9	3.4	3.1	2.8	2.4	2.9	3.5	2.6	
CF	55.1	55.4	56.6	58.0	56.5	57.2	56.0	54.7	49.8**
SDd	5.6	6.2	7.4	6.7	6.2	6.1	5.7	6.1	
ADF	53.7	53.8	54.7	56.3	55.6	56.3	55.5	54.2	49.5**
SDd	4.8	5.0	6.4	5.8	5.5	5.7	5.3	5.9	
Energy	76.8	76.3	77.1	77.7	77.8	76.6	76.8	76.4	73.1**
SDd	2.7	3.0	3.1	3.1	2.7	2.9	2.6	2.8	
	Group 2								
DM	71.2	70.9	70.9	71.4	71.2	70.7	71.0	71.3	65.6**
SDd	3.0	2.7	2.6	2.0	1.9	1.7	1.4	1.5	
OM	73.5	73.2	73.2	73.6	73.4	73.0	73.3	73.5	68.1**
SDd	2.6	2.4	2.2	1.7	1.6	1.5	1.3	1.4	
CP	59.2	58.8	58.7	59.4	59.1	58.4	58.8	59.3	52.0**
SDd	4.9	4.6	4.4	3.4	3.4	3.2	2.9	2.9	
EE	74.0	73.8	73.8	74.2	74.0	73.6	73.9	74.2	66.2**
SDd	2.6	2.8	2.8	2.4	2.0	2.6	2.5	2.3	
CF	75.7	75.5	75.4	75.8	75.7	75.2	75.5	75.8	70.1**
SDd	2.9	2.6	2.5	2.0	2.0	1.8	1.6	1.5	
ADF	63.4	63.1	63.0	63.6	63.4	62.8	63.2	63.5	60.4**
SDd	3.3	2.9	2.8	2.0	1.7	1.9	1.5	1.3	
Energy	70.5	70.2	70.2	70.7	70.4	69.9	70.3	70.6	64.2**
SDd	2.8	2.7	2.5	1.9	1.8	1.8	1.6	1.6	
	Group 3								
DM	49.7	49.8	50.5	50.8	49.8	49.8	50.0	50.3	49.1**
SDd	4.0	3.8	3.9	3.8	4.4	3.8	3.6	3.8	
OM	50.6	50.7	51.4	51.7	50.6	50.7	50.8	51.2	50.2
SDd	4.0	3.8	3.9	3.8	4.4	3.9	3.6	3.8	
CP	26.7	26.8	27.9	28.2	26.9	26.8	27.1	27.7	20.3**
SDd	11.0	11.3	10.7	10.9	10.9	10.9	10.3	10.4	
EE	69.0	69.0	69.4	69.6	69.0	69.0	69.1	69.3	67.9**
SDd	11.6	11.7	11.6	11.6	11.3	11.6	11.4	11.5	
CF	50.4	50.5	51.1	51.5	50.4	50.4	50.6	50.9	48.8**
SDd	4.4	4.2	4.2	4.3	4.7	4.2	4.0	4.1	
ADF	40.8	40.9	41.7	42.1	40.9	40.9	41.1	41.5	39.0
SDd	4.9	4.6	4.5	4.6	4.9	4.3	4.0	4.2	
Energy	46.0	46.2	46.8	47.2	46.1	46.1	46.3	46.7	46.8
SDd	4.4	4.2	4.3	4.1	4.9	4.4	4.0	4.3	

** Significant ($P<0.01$). SDd : Standard deviation of difference between FGS and the *in vivo*. FGS digestibility = 100 − (100 × lignin of feed / lignin of feces × nutrient of feces / nutrient in feed)

4. Lignin recovery

Differences between the digestibility of FGS and the *in vivo* may be related to the difference of lignin recoveries (further termed recovery) of the methods. Table 4 presented the recoveries of FGS and FTC. For FTC, recovery was calculated using two methods, (1) NIRS predicted data and (2) conventional analysis data of lignin. Calculation was based on the *in vivo* dry matter digestibility. Paired t-tests was carried out to analyze the recoveries compared with 100% complete recovery value.

Between the groups, recovery for FGS in group 2 showed closer to 100% than another groups. However, the recoveries for FGS in all groups were very close to 100%. The variations of recoveries obtained were still in range of reported by STREETER[1]. These facts indicated the NIRS prediction for lignin of either feces or feed were appropriate. Significances observed within grabbing time were considered the influence of diurnal variation of lignin.

Table 4 Lignin recovery calculated using lignin content of fecal grab samples determined by NIRS method

	Fecal grab samples								Total collection	
	1st day				2nd day					
	3	9	15	21	27	33	39	45	NIR	Conv.
Group 1	99.0	94.2**	97.1	105.1**	106.1**	99.3	105.2*	105.2*	107.8*	105.2
Group 2	101.3	98.7	99.2	102.5*	100.7	98.0*	98.8	92.4**	121.0**	142.4**
Group 3	98.4*	98.1**	101.5	102.1*	99.7	98.9*	104.2*	104.5*	97.8	103.1

[a] Calculation was used DM digestibility of *in vivo*.
* significant (P<0.05).
** significant (P<0.01), in comparison with 100% recovery

5. TDN calculation using fecal grab samples

Total digestible nutrients (TDN) were calculated using those digestibility estimation results and presented at Table 5. The value was compared with that obtained from widely adopted methods based on Japanese Feed Tables (JFT)[2] including manufacturer packing label.

Group 1 and 3 showed that JFT calculations were significantly high compared with that of FGS and *in vivo*. However in group 2, the significantly high compared with FGS and *in vivo* was observed. In group 1 and 3, calculation from FGS showed closer to the *in vivo* than of JFT. In group 2, FGS was similar with that of JFT, but overestimate the *in vivo*. Bias from feeding calculation using JFT was well known related to the harvesting, fertilizing and the origin. Since TDN is used as the main consideration to supply the nutrient requirement of animals, present study shows the using NIRS to measure fecal composition (as well as feeds) can be applied to allow the animal at actual requirements, better than the JFT. This is important for lactating cows, because the lack of nutrients will drop milk production. In the contrary, the oversupply of nutrient will result the loss of money.

Prediction of fecal compositions, digestibility and TDN in this study using NIRS predicted data showed that no significantly variation of lignin and nutrients at six hours interval for 2 days. Thus, in practice, fecal grab sampling can be taken any time. The significant differences between FGS and FTC may be come from the methods of fecal collection, composition analysis, and accumulation of small variation which undetected within grabbing interval. If TDN are used for final nutritive value consideration, application of LIM by using NIRS predicted value for FGS was available for farms us-

Table 5 Total digestible nutrients (TDN) calculated from FGS, JFT and *in vivo*[*]

	Fecal Grab Samples (FGS)								JFT	*In vivo*
	1st day				2nd day					
	3	9	15	21	27	33	39	45		
Group 1.	75.2	74.7	75.4	76.0	75.8	74.8	74.2	74.8	77.2	72.1
Avg diff	−3.0	−2.6	−3.3	−3.9	−3.7	−2.7	−2.6	−2.7	−5.1	
SDd	2.6	2.9	2.9	3.1	2.8	2.9	2.8	2.6	2.7	
Group 2.	69.0	68.8	68.7	69.1	68.9	68.5	68.8	69.0	68.2	64.3
Avg diff	−4.6	−4.4	−4.4	−4.8	−4.6	−4.2	−4.5	−4.7	−3.9	
SDd	2.5	2.3	2.1	1.7	1.6	1.5	1.3	1.4	1.4	
Group 3.	49.2	49.3	49.9	50.3	49.0	49.2	49.3	49.7	60.2	48.9
Avg diff	−0.3	−0.4	−1.0	−1.4	−0.1	−0.3	−0.4	−0.8	−11.3	
SDd	4.1	3.9	3.9	3.8	4.3	3.9	3.5	3.8	3.1	

[*] TDN of FGS calculated using formula TDN = DOM + 1.25 DEE which DOM and DEE were resulted from LIM ; FGS, JFT : see text ; Avg diff : average of difference ; SDd : see Table 3.

ing due to small SDd among three groups, and the bias between FGS and FTC was in range of 0.1–4.8%.

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近赤外分析法を用いる乳牛の飼料成分消化率測定における糞の採取方法の比較

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摘　要

27頭の乳牛に3種類の飼料を給与し全糞採取による消化試験を行うと同時に最初の2日間に6時間間隔で216点の糞サンプル（FGS）を採取し近赤外分析法による成分の測定を行った。飼料はイタリアンライグラス乾草と配合飼料および蒸煮木材とを組み合わせたものであり、その内容はイタリアンライグラス乾草60%と配合飼料40%（グループ1），イタリアンライグラス乾草95%と蒸煮木材5%（グループ2）およびイタリアンライグラス乾草45%と蒸煮木材55%（グループ3）の3種類であった。それらのFGSと全糞採取サンプル（FTC）の成分を，近赤外分析法（NIRS）によって測定し両者を比較した。さらに，FGSを用いてリグニンを指示物質とした消化率を求め化学分析による全糞採取法の値と比較した。6時間毎のFGS間には大きな成分含量の変動はみられなかった。FGSの消化率をFTCと比較した場合，グループ1の粗脂肪以外，およびグループ3の有機物，酸性デタージェント繊維，エネルギーの消化率以外では有意差（P＜0.01）を認めた。FGSとFTCの消化率の差の標準偏差は，グループ1の粗繊維および酸性デタージェント繊維では6.3%および5.6%であったが，グループ1，2の他成分では平均で3.7%以下であった。また，グループ3では他の2つのグループより高い値を示した。

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