Determination of the Optimum Incubation Time for *In Vitro* Digestion of Rice Straw

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Abstract It is considered that rice straw which contains a large proportion of characteristic insoluble components is digested more slowly than forages of high quality, when *in vitro* digestion methods are adopted. This study aimed to determine the optimum incubation time in both the rapid cellulase method and the artificial rumen method, and the following results were obtained.

1. The *in vitro* dry matter digestibility (IVDMD) determined by the rapid cellulase method was much increased with incubation time up to two hours, but was still lower than the *in vivo* dry matter digestibility.

2. IVDMD determined by the artificial rumen method was not stabilized over 72 hours of incubation, and also it varied with the rumen fluid inocula taken from wethers under different feeding regimes.

3. IVDMD determined after the two-hour incubation by the rapid cellulase method and also that after the 72-hour incubation by artificial rumen method were highly correlated with *in vivo* dry matter digestibility. Correlation coefficients were 0.954 ($P < 0.01$) and 0.899 ($P < 0.01$), respectively.

4. It is concluded that the rapid cellulase method would be proper to estimate the nutritive value of rice straw *in vitro*, and the optimum incubation time would be two hours in applying this method to rice straw.

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Key words : incubation time, *in vitro* dry matter digestibility, artificial rumen method, rapid cellulase method, rice straw.

For the purpose of estimating the nutritive value of forages for ruminants, it has been shown that *in vitro* digestion methods can be applied. The artificial rumen method described by McDougall¹⁾ is well-known, but a new method using enzymes has been reported by ABE *et al.*^{2,3,4,5)} Furthermore, FURUYA *et al.*⁶⁾ simplified the method of ABE *et al.* and developed the rapid cellulase method. However, the forages used in these studies were usually good quality hay, and only a few data for straws are available.

TOYOKAWA *et al.*^{8,9)} applied the rapid cellulase method and the artificial rumen method to rice straw, and reported that these methods were sufficient to estimate the nutritive value of rice straw. However, since rice straw is rich in characteristic indigestible components, it is desirable to determine the optimum incubation time in these *in vitro* digestion methods. This study was conducted to determine the optimum incubation time by the successive measurement of *in vitro* dry matter digestibility

(IVDMD) of rice straw by the rapid cellulase method and the artificial rumen method.

Materials and Methods

Rapid cellulase method

Because of its simplicity and the low protein content of rice straw, the method described by FURUYA *et al.*⁶⁾ was adopted. Seven groups of rice straw harvested around Hirosaki were tested. Two of the seven groups were damaged by the cold weather in 1983. These straws were dried and ground through a 1 mm Wiley mill-screen, and then 0.4–0.5 g of ground straw was incubated in a 50 ml tube with 50 ml of 1% cellulase solution (Onozuka R-10 in pH 4.0 buffer) by shaking (100 r.p.m.) the incubator for 1, 2, 3, 4, 6 and 8 hours and consecutive IVDMD were measured.

Artificial rumen method

To carry out the incubation, urea-containing McDougall's buffer proposed by HORII *et al.* was used. Two kinds of rumen fluid inoculum were used: Inoculum A was prepared by mixing almost the same volumes of rumen fluid taken from two fistulated wethers which were fed 400 g/day of wheat bran and 500 g/day of orchardgrass hay, and given rice straw, mineral salts and water *ad libitum*. Inoculum B was prepared by the same way and using the same wethers as inoculum A, but the donors were given 400 g/day each of wheat bran and orchardgrass hay and additionally allowed to graze on an orchardgrass-pasture, with free access to mineral salts and water. The eight groups of rice straw were sampled around Hirosaki: five of them were cut at the fully ripe stage and three were cut at the tillering stage. These straws were processed in the same way as that in the rapid cellulase method and 0.4–0.5 g of substrates were incubated for 48, 72 and 96 hours.

In vivo digestion trial

In vivo digestibilities were determined for all tested straws by the total collection method using three or four wethers. To avoid residues, the amount of rice straw provided was restricted to $21 \text{ g/W}^{0.75} \text{ kg}$.

Chemical analysis

General composition, acid detergent fiber (ADF), neutral detergent fiber (NDF), cellulose and lignin were determined by official methods¹¹⁾, and crude silica was determined by the method of OKUTA *et al.*¹²⁾

Results

Rapid cellulase method

Chemical composition, *in vivo* dry matter digestibility, structural carbohydrates and insoluble components of tested rice straws are shown in Table 1 and in Table 2, respectively. Cold-damaged straws had a higher crude protein content and a lower crude ash content than normal. *In vivo* dry matter digestibilities varied from 48.1–67.7%, and the average was 57.0%, a value higher than usual. Although fractions of NDF and NDF-ADF, which are related to hemicellulose, were slightly higher, other components were at an ordinary level.

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Fig. 1 shows the change in time of IVDMD determined by the rapid cellulase method. The value increased rapidly and reached a plateau within two hours of incubation, but was much lower than the *in vivo* dry matter digestibility (Table 1).

Artificial rumen method

Table 3 shows the chemical composition of rice straw tested in this trial and their

Table 1. Chemical composition of rice straw used in the experiment by the rapid cellulase method and the *in vivo* dry matter digestibility.

Division	Chemical composition of dry matter (%)					Dry matter digestibility %
	Crude protein	Crude fat	Crude fiber	Crude ash	NFE	
Mean	6.3	1.6	30.1	15.8	46.2	57.0
Max.	10.2	2.0	32.3	19.3	52.3	67.7
Min.	4.1	1.3	28.2	11.2	43.6	48.1

Table 2. Structural carbohydrates and insoluble components of rice straw used in the experiment by rapid cellulase method experiment.

Divison	Contents in dry matter (%)						
	NDF	ADF	NDF-ADF	Lignin	ADF-lignin	Crude silica	Lignin+crude silica
Mean	69.4	40.6	28.8	9.4	31.2	10.8	20.2
Max.	72.0	44.7	31.0	11.0	34.7	14.1	23.1
Min.	65.4	36.4	27.3	8.6	27.8	6.1	14.7

Fig. 1. Changes in *in vitro* digestibility of rice straw determined by the rapid cellulase method

— mean individual

in vivo dry matter digestibilities. Table 4 shows results of the analysis for structural carbohydrates and insoluble components.

Fully ripe straw had an usual chemical composition. Tillering straw had higher contents of crude protein and crude fat, and especially the former content was almost twice as much as usual straw. Little difference was observed in the *in vivo* dry matter digestibility between fully ripe straw and tillering straw. This might be attributed to similar contents of crude fiber, crude ash and NFE, which are the main components of rice straw. Contents of structural carbohydrates and insoluble components were almost similar at two growth stages, but all components were slightly higher in fully ripe straw than in tillering except a hemi-cellulose fraction (NDF-ADF).

Fig. 2 shows the change in time of IVDMD determined by the artificial rumen method. A clear difference appeared according to the inoculum used, and IVDMD was higher at any time and more linear when rumen fluid A was inoculated. *In vitro* digestion of fully ripe straws proceeded until 72 hours of incubation when rumen fluid B was inoculated, and this trend was also seen in the case of tillering straws. The magnitude of variation of individual IVDMD was much larger when rumen fluid B was inoculated than when A was inoculated. These facts suggest that the source of the rumen fluid inoculum has an influence on the digestibility of rice straw. However,

Table 3. Chemical composition of rice straw used in the artificial rumen method experiment and the *in vivo* dry matter digestibility

Stage of harvesting	Division	Chemical composition of dry matter (%)					Dry matter digestibility %
		Crude protein	Crude fat	Crude fiber	Crude ash	NFE	
Fully ripe	Mean	4.6	1.6	30.9	17.0	45.9	47.3
	Max.	5.3	1.8	34.9	18.9	47.7	53.1
	Min.	4.3	1.3	26.5	15.2	42.7	37.7
Tillering	Mean	9.9	2.3	28.2	17.2	42.3	48.1
	Max.	11.1	2.5	30.4	17.6	42.6	51.3
	Min.	7.8	2.0	27.0	16.9	41.8	44.5

Table 4. Structural carbohydrates and insoluble components of rice straw used in the artificial rumen method experiment.

Stage of harvesting	Division	Contents in dry matter (%)						
		NDF	ADF	NDF-ADF	Lignin	ADF-lignin	Crude silica	Lignin + crude silica
Fully ripe	Mean	67.7	42.0	25.2	9.7	32.3	13.5	23.4
	Max.	73.4	46.5	26.9	11.5	35.0	15.7	24.7
	Min.	61.8	36.6	23.8	7.9	27.4	10.0	20.6
Tillering	Mean	65.4	39.4	26.0	8.2	31.2	10.7	19.1
	Max.	68.8	41.0	29.3	8.4	33.1	11.3	19.3
	Min.	60.7	37.5	19.7	7.9	29.1	9.9	18.8

In vitro Digestion of Rice Straw

Fig. 2. Changes in *in vitro* dry matter digestibility of rice straw determined by the artificial rumen method.

— rumen fluid inoculum A
 --- rumen fluid inoculum B
 — mean

IVDMD of rice straw was lower than *in vivo* dry matter digestibility, especially when inoculum B was used.

Discussion

It is considered that the values of IVDMD determined by both the rapid cellulase and artificial rumen methods will stay at a lower level than the *in vivo* digestibility even when incubated for a longer time. In the artificial rumen method, the source of the rumen fluid inoculum affected the value of IVDMD. However, the IVDMD determined at the time when digestion ceased in the artificial rumen method was higher than that in the rapid cellulase method which was only a half of the *in vivo* digestibility.

IVDMD of the rapid cellulase method was very low and stopped increasing after two hours of incubation. The correlation coefficient between IVDMD at this time and *in vivo* dry matter digestibility was $r=0.954$ ($P<0.01$). Values of IVDMD determined at one and three hours after the incubation have also highly significant correlations with *in vivo* digestibility, and the correlation coefficients were $r=0.921$ ($P<0.01$) and $r=0.959$ ($P<0.01$), respectively. Since the increase of IVDMD almost ceased after two hours of incubation, the two-hour incubation system could be recommended for rice straw as is the case for other forages. According to the rapid cellulase method, the regression equation between IVDMD for the two-hour incubation and the *in vivo* dry matter digestibility was as follows :

$$Y = 1.99 X + 17.11 \quad (Y; \text{in vivo dry matter digestibility, } X; \text{IVDMD})$$

Although the authors attempted the two-step method described by ABE *et al.*^{2,3,4,5)} with the same straws as used in this work, a lower correlation was obtained.

When the artificial rumen method was adopted, the difference in the incubation inoculum or the diet given to the donor affected the value of IVDMD, suggesting that this method is not reliable enough to estimate the nutritive value of rice straw. The consistency of the activity of inoculum is required when this method is adopted. Also, the great increase of IVDMD from 48 to 72 hours of incubation using inoculum B suggests that a longer time is required for rice straw than for forages of higher quality. Therefore, the 72-hour incubation system is recommended to stabilize dry matter digestibility of rice straw. The correlation coefficient between IVDMD of the 72-hour incubation using inoculum A and the *in vivo* dry matter digestibility was $r=0.899$ ($P<0.01$), and the regression equation was as follows :

$$Y=1.32 X+3.31 \text{ (Y ; } in \text{ vivo dry matter digestibility, X ; IVDMD)}$$

Furthermore, the correlation between values of IVDMD determined by the artificial rumen method used rumen fluid inoculum A and by the rapid cellulase method was $r=0.889$ ($P<0.01$), and the equation was as follows :

$$Y=1.26 X+15.32 \text{ (Y ; IVDMD of artificial rumen method, X ; IVDMD of rapid cellulase method)}$$

This high correlation suggests that dry matter digestibility of rice straw can be estimated by either of both *in vitro* methods.

However, in the artificial rumen method, large variations of IVDMD due to the inoculated rumen fluid make it difficult to estimate an *in vivo* nutritive value of rice straw. In adopting this method, it is necessary to use the inoculum suitable for digesting straws.

Because of its simplicity and stability, the rapid cellulase method is recommended to estimate the dry matter digestibility of rice straw *in vitro*, and a two-hour incubation is considered to be sufficient for the estimation by this method.

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稲ワラの *in vitro* 消化時間の検討

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稲ワラに *in vitro* 消化法を適用する際, その特徴的難消化性成分構成から良質の牧草などよりも分解速度が遅いため, 迅速セルラーゼ法ならびに人工ルーメン法のより適当な培養時間について検討し, 次の結果を得た.

1. 迅速セルラーゼ法の *in vitro* 乾物消化率 (以下 IVDMD と略) は培養 2 時間目まで急速に高まり, その後安定傾向を示したが, *in vivo* 消化率を大きく下まわった. 2. 人工ルーメン法の IVDMD は培養 72 時間を超えなければ安定せず, また胃液採取メソ羊の飼養

条件によって IVDMD が変動した. 3. 培養 2 時間目の迅速セルラーゼ法 IVDMD, および 72 時間目の人工ルーメン法の IVDMD は *in vivo* 乾物消化率との間でそれぞれ $r=0.954$ ($P<0.01$), $r=0.899$ ($P<0.01$) の高い相関関係を得た. 4. 稲ワラの *in vivo* 栄養価値を推定する *in vitro* 消化試験法として, 培養条件を一定化できる迅速セルラーゼ法が適切であり, 培養時間は 2 時間が適当と考えられた.

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