Digestion of Rice Straw Cell Wall Constituents in Various Rumen Conditions

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Introduction

Studies of fibrous feed seem to be valuable in many countries, since there is a tendency of keen competition in the use of concentrate between ruminants and non-ruminants or high cost aspect in the production of concentrate or grain feed. Rice straw as one of agricultural waste products found in large quantity is widely used as ruminant feed. Main components of rice straw are fibrous cell wall substances consisting of cellulose, hemicellulose, lignin and silica. Nutritional availability of rice straw observed in various feeding situations must have strong relationship with the digestion characteristics of these cell wall substances in the rumen.

Digestion of structural carbohydrates such as cellulose and hemicellulose in the rumen is a very complex process that involves some aspects of digestion physiology, nutrition and microbial activity including enzymes produced during fermentation of feed.

The objective of this study was to obtain information of rice straw cell wall digestibility in various rumen conditions.

Materials and methods

1) *Rice straw*

Rice straw used here was obtained from japonica rice produced at Ibaraki Prefecture in Japan. Its chemical composition is shown in Table 1.

Isolated cell wall was prepared by actynase treatment[1,2] and the dried sample (ICW sample) obtained was used as substrate when digestion trials were conducted in situ and in vitro.

2) *In situ nylon bag digestion of rice straw with fistulated cattle (Experiment 1)*

Approximately 0.8 g of ICW sample was enclosed in a nylon bag and was put into a rumen through fistula. Japanese black cattle fed on Italian ryegrass wafer alone were used in this trial.

Nylon bags were allowed to stand in the rumen for 2, 4, 8, 16, 24, 48 and 72 hr. After digestion in the rumen, residual dry matter, cellulose and xylan were determined.

Table 1. Content (% on dry wt. basis) of fibrous materials of rice straw used in the experiment

	Component	%
	Dry matter (DM)	
DM	Cellular contents	20.3
DM	Cell wall*	79.7
	Organic matter (OM)	
OM	Organic cellular contents	11.5
OM	Organic cell wall (OCW)*	62.5
OM	Organic cellular contents	12.0
OM	Neutral detergent fiber (NDF)**	62.0
	Acid detergent fiber (ADF)	39.1
	Cellulose	35.0
	Xylan	24.5
	Lignin	5.1
	Silica	12.9

*Enzymatic method, **Detergent method

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3) *Addition of lactic acid and in vitro ICW digestion (Experiment 2)*

Approximately 0.3 g of ICW sample was weighed into a glass tube to which 50 m*l* of basal inoculum composed of artificial saliva[12], tripticase peptone, cystein- HCL and rumen fluid was added. Lactic acid was also added to the tubes at the rate of 0, 6, 9, 12.0 and 14.4 mg per m*l* inoculum.

Incubation was carried out at 39°C for 43 hr under continuous CO_2 gas bubbling condition. After incubation, the followings were determined; pH, lactic acid, volatile fatty acids (VFAs) and residual dry matter.

4) *In situ nylon bag digestion of rice straw ICW with goat feeding on three different kinds of feed (Experiment 3)*

Approximately 1.0 g of ICW sample was enclosed in a nylon bag and was put into a rumen of fistulated goat. Following three kinds of feed named A, B and C were fed to goat, and nylon bag digestion trials were carried out for each feeding period.

The feed A was a low crude protein ration composed of 50% rice straw and 50% grass hay, and feed B was a high crude protein ration of 36% rice straw, 36% grass hay and 29% soybean meal, while feed C was a high starch ration of 21% rice straw, 21% grass hay and 57% flaked corn.

After 24 hr incubation in the rumen, residual dry matter was determined. Rumen fluid was sampled 3 hr after feeding, and immediately after the nylon bags were placed in the rumen.

In this experiment, the number and genus of protozoa in the rumen fluid were also observed together with pH and VFAs.

5) *In vivo digestion trial with goat (Experiment 4)*

Digestion trial with the total collection method was carried out with two goats when mixed feed consisting of 80% rice straw and 20% soybean meal, was fed at maintenance intake level. Some cell wall fractions described above were determined using both samples of feed and faeces.

6) *Analytical method*

Division of organic matter to cell wall (CW) and cellular contents (CC) was done by the method of Abe et al.[1] and Van Soest et al.[14].

Sample and digestion residue after incubation were treated with 72% sulfuric acid solution. The hydrolyzed solution contains some monosaccharides such as xylose and glucose originated from structural carbohydrates.

The contents of xylose and glucose in this solution were measured by the orcinol[8] and anthrone method[8], respectively. Xylan and cellulose were calculated as xylose$\times$0.88 and glucose$\times$0.9, respectively.

Acid detergent fiber (ADF), lignin, and silica were determined by successive treatment with acid detergent solution and 72% sulfuric acid solution[10]. Lactic acid was determined by the method of Barker and Summerson[5] and VFA analysis was carried out using a gas chromatography (on 5% thermon 1000 + 0.5% H_3PO_4 on chromosorb, 80/100 mesh).

Results and discussion

Rice straw contains generally 60–80% cell walls (CW) and 20–40% cellular contents (CC). Main components of CC are soluble carbohydrates and starch, which decreased in amount with time after grain harvesting.

Because the fraction CC has nutritional uniformity, digestibility or digestible amount can be estimated exactly by the application of Rucas method[3,15]. But there was no such a tendency in CW fraction.

Digestion characteristics of CW depends on its own structure explained by the degree of lignification, and other factors to which the rice straw is exposed in alimentary tract. Van Soest reported that lignin to ADF ratio influenced strongly CW digestibility[15]. The regression equation between the ratio and the CW digestibility has been recommended and presented in the USDA agriculture handbook[9]. Lignin to ADF ratio of the rice straw used here was 13.0%, and 59% NDF digestibility was calculated by this manner.

Rice straw is unique in that its high silica and high lignin contents tend to limit cell wall digestibility. It is said that there was a decrease of 3.0% digestible dry matter per 1.0% silica. Concerning lignin and silica, it is an interesting future problem to know which of them has more suppressive effect on CW digestibility, and it is also useful information that recovery percent of them in faeces was almost 100% (Table 4). We can use these materials as markers in digestion trials.

Sum of cellulose, xylan and lignin showed the value more than total organic cell wall (OCW and NDF) in Table 1, and the differences were 2.1–2.6%. Therefore cellulose and xylan described here must be regarded as crude fractions.

The objective of the Experiment 1 is to observe the potential digestibility and digestion rate of rice straw cell wall under the in situ condition. The study of Akin on grass[4] showed that at 72 hr all tissues were completely degraded and after that period a large number of bacteria have not readily attacked the lignified part of the grass.

Fig.1 shows the digestion pattern of CW, cellulose and xylan. All fractions reached near the plateau at 72 hr of digestion time. An in situ test of soybean meal carried out in parallel with the test of rice straw showed that cell wall substances of soybean meal were almost completely digested during 8 hr, whereas rice straw polysaccharides were digested very slowly. Since it took time for the bacteria to attack cell wall, enzymatic digestion of the polysaccharides may be hindered by the presence of lignin and silica.

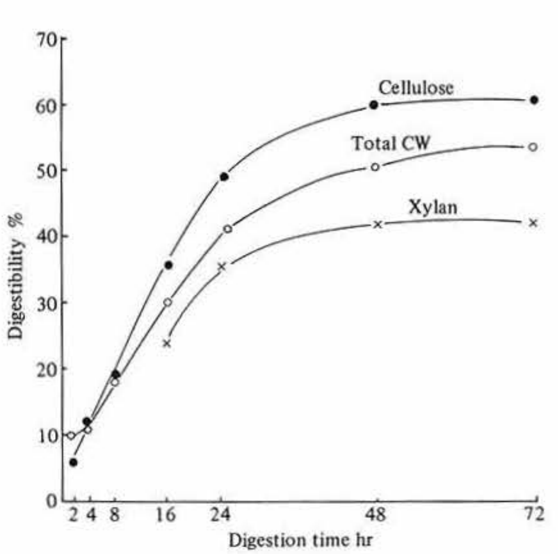

Fig. 1. Digestion pattern of rice straw cell wall and its constituents in situ

Digestibility of CW, cellulose, and xylan at 72 hr was 53.8, 61.4 and 41.6% respectively. The CW digestibility, 53.8%, gives the value of 42.9% for digestible CW from the calculation of 79.7×0.538, while the amount of digestible CC (DCC) can be predicted as 7.0% according to the equation: DCC=0.98 CC−12.9[15]. As a consequence, we can estimate that rice straw has potentially about 50% digestible dry matter.

Cellulose and xylan showed different rates of

Table 2. Digestibility of various cell wall fractions in the in vivo digestion trial of mixed feed containing predominantly rice straw

Fraction	Digestibility %
Total organic cell wall	
Neutral detergent fiber (NDF)	51.4 (48.0)*
Organic cell wall(OCW)	50.5 (45.5)*
Cell wall fraction	
Cellulose	60.8 (57.1)*
Xylan	22.0 (36.5)*
Lignin	− 0.9 (100.9)**
Silica	− 9.1 (109.1)**

* Values for rice straw, calculated by taking the values of soybean meal as 100%.
** Recovery %

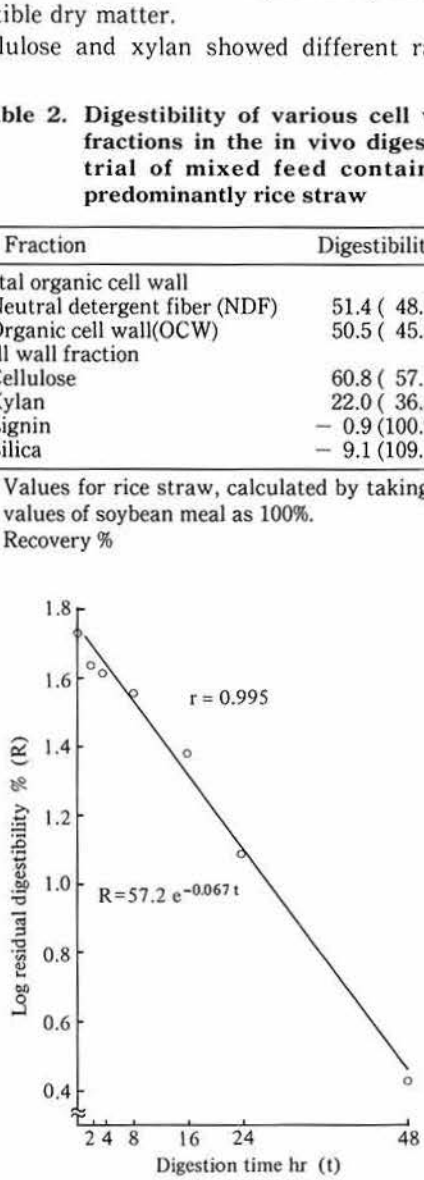

Fig. 2. Log values of residual digestibility (%) of total cell wall plotted against digestion time (hr)

digestibility and the same result was shown in the in vivo digestion trial (Table 2).

Silica moved into the rumen fluid from a nylon bag during digestion. Percent removal of silica at 4, 16 and 72 hr was 22, 33 and 43% respectively. Accompanying with digestion or solubilization of structural carbohydrates, silica might be released from cell wall.

Conversely speaking, it may be thought that remaining 57% of silica at 72 hr protects strongly the carbohydrates from cellulolytic bacteria together with lignin.

Fig. 2 shows the rate of CW digestion in situ. Linearity of the log values of residual digestibility expressed as a function of time, and the correlation coefficient ($r=0.995$) between the log values and the time indicated the first order kinetics of rice straw cell wall digestion. Generally the first order reaction can be expressed as follows:

$$R = ae^{-kt}$$

R; residual digestibility, t; digestion time,
a; potential digestibility,
k; reaction constant.

The values of a and k were 57.2 and 0.067, respectively. Half life that was the time needed for 50% digestibility of the potential was calculated as 10.3 hr from k value.

Microbial degradation of cell wall polymers depends strongly on the rumen condition. One of the depressing factors for cell wall digestion in the rumen is low pH. Although the rumen is buffered by inorganic substances such as carbonate and phosphate, acids of fermentation products sometimes can exceed the buffering capacity and pH can decline. Low pH is usually associated with an accumulation of lactic acid in the rumen.

Table 3 shows the effect of lactic acid level on pH, CW digestibility and VFA concentration in vitro. Lactic acid addition caused CW digestibility to decline and the concentration of propionic acid to increase.

The decrement of CW digestibility was extremely large at pH below 6.34, and at pH 6.14 CW digestibility was reduced to about one third of the value at pH 7.04. The growth and activity of cellulolytic bacteria would be fairly depressed at the pH regime nearly 6.1, even though they were never killed completely. On the contrary population of lactic acid degradation bacteria might be increased in the rumen.

Such low levels of rumen pH are often observed under much-concentrate feeding. Although such big difference as shown in the in vitro trial was not observed, the grain-rich ration brought about significantly lower CW digestibility as compared with the less-grain ration in the in situ experiment (Experiment 3) due to lower pH as given in Table 4.

In the Experiment 3, rice straw CW digestibility was observed by the in situ method at various rumen conditions caused by different types of feed given to goat.

The difference of feed C to feed A is only whether starch is contained or not. Crude protein is of equal level. Rumen fluid of the feed C plot had lower acetic acid and higher butyric acid concentration in comparison with the feed A plot.

Feed C also caused very high counts of protozoa predominated by Entodinium. It is well known that starch-rich ration caused Entodinium protozoa to increase in the rumen[11].

The reason for increased digestibility of feed B may be attributed to soybean meal protein. Firstly, one of the effects is explained by the increased content of crude protein in the ration itself. In a recent

Table 3. Effect of lactic acid addition on CW digestibility, pH and volatile fatty acid contents

Lactic acid mg/ml	pH		CW digestibility %	Volatile fatty acids, molar %			Final lactic acid mg/ml
	Start	Final		Acetic acid	Propionic acid	Butyric acid	
0	6.92	7.04	37.9[a)]	61.0	27.1	9.3	0
9.6	6.57	6.42	34.6[a)]	52.4	39.8	6.6	1.3
12.0	6.15	6.34	29.2[b)]	50.2	42.5	6.0	2.7
14.4	6.01	6.14	13.4[c)]	44.7	41.0	8.4	3.0

a), b), c): Values with different superscripts differ each other significantly ($P=0.05$).

Table 4. Characteristics of rumen fluid and in situ CW digestibility when three different kinds of feed were given

Item	Feed A	Feed B	Feed C
Crude protein contents, % DM	7.7	19.7	7.7
CW digestibility, %	33.0[b)]	38.5[a)]	29.0[c)]
Rumen pH	6.57	6.55	5.90
Volatile fatty acids, molar %			
Acetic acid	69.9	59.7	61.2
Propionic acid	22.2	23.2	25.1
Iso-butyric acid	—	5.0	—
Butyric acid	6.6	8.7	11.7
Iso-valeric acid	0.2	1.9	0.9
Valeric acid	0.7	1.4	0.7
Lactic acid, mg/m*l* 0.02	0.03	0.01	
Protozoa counts, 10^4/m*l*			
(Genus)			
Isotricha+dasytricha	0.7	0.5	0.5
Entodinium	5.5	3.9	58.1
Polyplastron	NS	TF	TF

a), b), c): Values with different superscripts differ each other significantly.
NS: Not seen, TF: Too few to count but present.

study on experimental methods of digestion in Japan[13)], crude protein above 12% in ration is recommended as an adequate level for obtaining normal digestibility data. The content of 7.7% in feed A might be too low for the normal growth of cellulolytic bacteria.

Secondly, there is a certain possibility that short chain fatty acids such as iso-butyric and valeric acid stimulated the cellulolytic factor of rumen microbes. Bentley et al.[6)] demonstrated that the addition of branched acids such as iso-butyric acid and valeric acid to in vitro inoculum promoted cellulose digestibility. Rumen fluid of the feed B plot had not only iso-butyric acid, but also a little higher levels of iso- and normal valeric acids compared to other feed plots.

It is thought that the source of these short chain fatty acids is mainly soybean meal. El-shazly found both straight and branched chain fatty acids among degradation products formed by rumen microorganisms from proteins and amino acids[7)].

It is concluded that the digestibility of rice straw cell wall is influenced by many complex factors particularly interaction between feed ingredients and microbial attack in the rumen.

Therefore, collaboration of many fields of research such as microbiology, ruminant nutrition, feed chemistry and others will be needed for the further progress in the study of rice straw as a feedstuff.

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