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Measurement of digestibility of cell wall constituents in grass and legume forages by an artificial rumen method

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Abstract In the present study, to clarify the reason why crude fiber digestibility is generally low in legumes than that in grasses, the digestibility of crude fiber in some grass and legume forages, and the digestibility of neutral detergent fiber (NDF) and acid detergent fiber (ADF) were also determined after refining them from same forages using an artificial rumen method. The following reasons why the digestibility of fiber of legume forages is usually lower than that of grass forages will be shown; a) There is a possibility that the constituents of cell except NDF control (prevent) NDF digestibility in legume forages. b) The digestibility of ADF itself is clearly low in legume forages as compared to that in grass forages. It can be also assumed as another reason that the digestion of hemi-cellulose proceeds rapidly, and will be degradable condition at early stage of incubation. It cannot be clarified, however that the rapid progress of hemi-cellulose digestion in the early stage during incubation induces a difference in digestibility of crude fiber between legume and grass forages, or not in this study.

Keywords: artificial rumen, CWC digestion, Italian ryegrass, legume forages

Introduction

Ruminant animals are able to use efficiently fibrous constituents of their feed by a role of microbes in the rumen. Then, it could be an important factor to consider a nutritive value of feed in ruminants that an efficient use of fibrous component of feed is very important factor to enhance the utilization of feed. In general, it is well known that the digestibility of crude fiber in legumes is lower than that in grasses (Johnson et al. 1962; Nakamura, 1981a). The main of fibrous component of feed is the cell wall constituent (CWC), and its chemical composition are cellulose, hemi-cellulose and lignin. Furthermore, it has been reported that the contents of lignin, which would be indigestible by rumen microbes is relatively higher in legume forages than in grass forages (Smith et al. 1972), and this is thought to be a factor, which would prevent digestion of fibrous constituent in legumes. Another difference in the characteristics of CWC except lignin content may also be a factor affecting to digestion of crude fiber in legume forages. It can be easily presumed that the CWC content, an extent of its polymerization and/or crystallization or structural feature would be fairly different between legumes and grasses, although there is no clear evidence at the present. This is thought to influence on digestion as a difference in resistance to microbial fermentation in the rumen between legumes and grasses.

In general, the method to measure digestibility of feed is two; direct and indirect methods, and the direct method normally require a long time and a big labor cost with relatively large amount of feed. Then, to avoid those problems several indirect methods has been designed up to the present. The artificial rumen method is one of indirect method for measuring digestibility of feed, and this method is very effective to estimate the digestibility of many feed with simple short-term incubation. This method is also thought to be more available method, because there will be able to reproduce a fermentation by rumen microbes.

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In the present study, the digestibility of fibrous constituent in some grass and legume forages, and the digestibility of neutral detergent fiber (NDF) and acid determent fiber (ADF) were also determined after refining them from same forages using an artificial rumen method.

Materials and Methods

1. Forage samples

Italian ryegrass harvested at before heading stage (IBH), after heading stage (IAH) and after blooming stage (IAB) was used after sun cured as grass sample. As legume forage sample, Alfalfa hay cube (AHC), common vetch (CV) and red clover (RC) was used after sun cured except Alfalfa hay cube. They were ground using Wiley mill, and through 2 mm sieve before analysis. The samples of NDF and ADF of each grass and legumes forages were obtained according to the method of Goering and Van Soest (1970), and then, dried and ground before analysis.

2. Rumen fluid

The rumen fluid used for the artificial rumen was collected from Japanese Corriedale wether (BW, 61 kg) with rumen cannula at just before morning feed. The wether was fed mixed hay (Orchard grass predominantly) as a basal diet at a level of 2% of body weight per day. Fresh water and mineral block were available freely.

3. Equipment

The artificial rumen (Equipment) was in accordance with the method of Tilley and Terry (1963). One gram samples of forage/detergent fiber sample, 40 ml of an artificial saliva (McDougall), 10 ml of fresh rumen fluid and 1 ml of urea solution were put into a 100 ml triangular flask, and then, the flask was filling up with carbon dioxide to be anaerobic condition. Furthermore, to measure gas production a scaled syringe was set in the rubber stopper of the flask (see Fig 1). The flask was incubated during 4, 8, 12, 24 and 48 hours in the water bath (38 °C) with shaker. After each incubation time, few drops of saturated $HgCl_2$ solution were added into the flask to stop the fermentation, and then dry matter, organic matter and volatile fatty acids (VFAs) of the residue in the flask.

4. Analytical methods

Chemical composition of forage samples was analyzed according to the Association of Official Agricultural Chemists (Hoitz, 1960). Neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin and silica contents were determined according to the method of Goering and Van-Soest (1970). The VFAs production was analyzed using gas chromatography.

Results and Discussion

1. Chemical composition of forage samples

As shown in Table 1, the content of organic matter (OM) was in the range of 88～91%, and was almost similar in Italian ryegrass and legume forages. The protein (CP) content was 6.8～8.3% in Italian ryegrass, and also 17.3～22.3% in legume forages, and the value in legumes was more than 10% higher than that in Italian ryegrass. In the CWC contents, lignin tended to high in legume forages (3.0～6.9%) as compared to Italian ryegrass (2.6～3.9%). The hemicelluloses and cellulose contents in Italian ryegrass and legume forages were 24.6～30%, 15.9～21.8% and 29.2～30.7%, 20.7～24%, respectively, and silica content was 2.0～3.6% and 0.4～1.5% in Italian ryegrass and legume forages, respectively. As a whole, the

Table 1. Chemical composition of forage samples (%)

Forages	DM[1]	OM[2]	CP[3]	NDF[4]	ADF[5]	H-cellulose[6]	Cellulose	Lignin	Silica
Italian ryegrass									
Before heading	86.8	89.2	8.3	55.4	31.8	24.6	29.2	2.6	2.1
After heading	88.1	90.6	6.8	59.5	33.3	26.7	29.5	3.8	2.0
After blooming	89.2	89.7	7.6	64.6	34.6	30.0	30.7	3.9	3.6
Alfalfa	85.1	89.1[7]	17.3	52.4	30.6	21.8	24.0	6.9	1.5
Common vetch	90.0	88.4	22.3	45.0	29.1	15.9	23.8	5.3	0.4
Red clover	89.0	88.1	19.8	42.5	24.3	18.6	20.7	3.0	0.8

[1] Dry matter, [2] Organic matter, [3] Crude protein, [4] Neutral detergent fiber, [5] Acid detergent fiber, [6] Hemi-cellulose, [7] Percent per DM.

figures in Italian ryegrass were always higher than that in legume forages regardless the growth stage. These figures were in a range of NRC standard (1985), although hemicelluloses content in legume forages was slightly higher than that showed in the standard.

2. Nutrient digestibility of forage samples

As shown in Table 2, dry matter digestibility of grasses and legumes was 73～80 and 56～78% after 48 hours incubation, respectively, and there is no obvious difference of values between two types of forages.

The digestibility of NDF was shown in Table 3, and the value of Italian ryegrass after 48 hours incubation (65～71%) tended to slightly high as compared with that of legumes (53～61%). Smith *et al.* (1971) had reported that using alfalfa, orchard grass and timothy hays the digestibility of CWC after 48 hours incubation was 62～66 and 38～49% in grasses and legume, respectively, and these were comparable with the results in the present study.

The digestibility of ADF in Italian ryegrass and legumes was 62～71 and 60～64% after 48 hours incubation, respectively, and there was also no clear difference between two types of forages (Table 4).

3. Organic matter digestibility of purified CWC

As shown in Table 5, the digestibility of organic matter (OM) in purified NDF after 8 hour incubation was fairly low in Italian ryegrass (0.65～1.25%) than in legumes (6～24%), and after 48 hour incubation, the values were 19～52% in Italian ryegrass and 33～63% in legumes, in-

Table 2. *In vitro* digestibility of DM of forage samples (%)

	Incubation time (hrs)				
Forage samples	4	8	12	24	48
Italian ryegrass					
Before heading	37.43	47.05	49.89	72.67	79.60
After heading	31.50	39.40	45.40	65.80	75.90
After blooming	27.90	37.20	43.30	58.75	73.20
Alfalfa	28.80	38.35	45.05	52.80	66.40
Common vetch	16.00	24.70	44.15	49.95	55.80
Red clover	35.55	43.10	52.35	73.75	77.65

Table 3. *In vitro* digestibility of NDF of forage samples (%)

	Incubation time (hrs)				
Forage samples	4	8	12	24	48
Italian ryegrass					
Before heading	4.28	18.06	43.04	60.47	71.19
After heading	3.29	15.12	22.93	52.51	64.93
After blooming	6.98	18.55	26.31	47.85	66.28
Alfalfa	18.20	19.50	20.10	30.80	58.60
Common vetch	10.80	25.60	30.20	39.20	52.90
Red clover	18.60	19.68	20.80	38.90	61.10

Table 4. *In vitro* digestibility of ADF of forage samples (%)

	Incubation time (hrs)				
Forage samples	4	8	12	24	48
Italian ryegrass					
Before heading	4.89	18.74	30.08	58.97	70.95
After heading	3.82	11.37	19.53	51.93	65.85
After blooming	1.14	12.20	23.19	50.17	62.48
Alfalfa	3.62	11.10	20.20	51.50	62.30
Common vetch	4.09	15.20	21.20	49.60	59.90
Red clover	2.45	14.39	25.40	53.49	64.20

Table 5. *In vitro* digestibility of OM in the purified NDF of forage samples (%)

	Incubation time (hrs)				
Forage samples	4	8	12	24	48
Italian ryegrass					
Before heading	0.40	1.25	11.70	34.80	51.60
After heading	0.35	0.75	14.35	27.15	32.10
After blooming	0.15	0.65	8.50	16.85	18.45
Alfalfa	15.00	23.75	29.40	31.50	33.25
Common vetch	0.95	16.20	21.95	36.40	36.70
Red clover	1.25	5.80	10.45	44.75	62.90

dicating OM digestibility in purifies NDF is tended to be high in legumes than that in grasses. In Italian ryegrass, the rate of degradation of OM in purified NDF at late of incubation was lowered along with an advance of growth stage.

As mentioned above, NDF digestibility of forages tended to high in grasses than in legumes, but in purified NDF the digestibility of it tended to high in legumes than in grasses. This might indicate that some constituents of in the cell prevent the digestibility of NDF in legumes. Then, it will be a main factor to control crude fiber digestion in legume forages. Smith *et al.* (1971) have shown that if the sample contains more cell contents they will be easily attacked by microbes in the rumen, because physical structure of their cell wall is not well developed. It is thought to that the reason why cell constituents reduce digestion of fiber, the presence of easily degradable carbohydrate and/or protein except fiber in the feed would increase the microbes that can use those matters such as sugar and/or protein, and these microbes will compete other microbes that mainly use fiber constituent as their essential nutrients (Hoshino, 1981). The OM digestibility of purified NDF in legume forages tended to high as compared to that in Italian ryegrass at earlier stage of incubation. This is thought to be due to rapid degradation of easily degradable part of NDF in legume forages during early stage of incubation.

The OM digestibility of purified ADF with apse of time is shown in Table 6. After 48 hour incubation, the value in legumes (6.8～7.5%) tended to low as compared to that in Italian ryegrass (10.7～12.2%). Then, it is thought to be one of the reasons why the digestibility of purified ADF itself tended to low in legumes than in grasses that crude fiber digestion of legumes will be low as compared to that in grasses. This would be due to a relatively high content of lignin in legume forages than that in grass forages. Furthermore, it is assumed that the digestion of hemi-cellulose indicated as the difference between NDF and ADF, would more degradable in legume forages, because as described above, *i. e.,* OM digestibility in purified NDF tended to high in legumes than in Italian ryegrass. One of the reasons why the difference in hemi-cellulose digestion occurs, will be the difference in chemical composition of hemi-cellulose, *i. e.,* the main sugar consisted is xylose in grass forages and galactose in legume forages (Nakamura, 1981b).

It will be not ignored that there is the close relationship among hemi-cellulose, cellulose and lignin, that is, it has been reported that the digestibilities of cellulose and hemi-cellulose related to the extent of lignifications of plant tis-

Table 6. *In vitro* digestibility of OM in the purified ADF of forage samples (%)

	Incubation time (hrs)				
Forage samples	4	8	12	24	48
Italian ryegrass					
Before heading	0.65	2.20	2.95	8.65	12.15
After heading	0.00	0.18	2.20	10.50	10.70
After blooming	0.79	2.18	3.05	9.11	11.90
Alfalfa	0.00	0.15	2.90	3.88	7.52
Common vetch	0.00	0.30	2.20	4.10	6.78
Red clover	0.29	0.31	3.01	3.40	6.96

sues (Van Soest, 1967). Furthermore, it could be thought to that an physically strong fiber, which would be made by the present study, it should be thought to be necessary to discuss that an dynamics of lignin digestion in the rumen after feeding grass and legume intimate entangling of cellulose, hemi-cellulose and lignin, and it clearly prevent a degradation by microbes in the rumen (Nakamura, 1981c). In forages, although there is data about lignin digestion in the present study.

4. Fermentation products after incubation of forage samples

Total gas, acetic acid and total VFAs produced during incubation of forage samples were shown in Tables 7, 8 and 9, respectively. There are VFAs as chemical compounds and some gases as CO_2 and methane etc. as end products after degradation and/or fermentation of structural carbohydrates in feed by microbes in the rumen. In the present study, as shown in Tables 7, 8 and 9, the volume of these products during incubation increased constantly along with the lapse of time, however there is no clear difference among forages used.

Conclusion and Implications

From the results obtained in this study, the following reasons why the digestibility of fiber of legume forages is usually lower than that of grass forages will be shown. There is a possibility that the constituents of cell except

Table 7. Gas production during incubation of forage samples (ml)

	Incubation time (hrs)				
Forage samples	4	8	12	24	48
Italian ryegrass					
Before heading	3.00	6.25	10.25	13.00	15.00
After heading	3.00	3.50	6.75	12.50	12.75
After blooming	5.00	7.50	9.75	11.00	11.25
Alfalfa	3.50	6.75	9.85	11.00	11.50
Common vetch	3.50	7.05	10.50	10.75	10.75
Red clover	1.00	5.25	7.50	9.25	10.00

Table 8. Acetic acid production during incubation of forage samples (mmol/100ml)

	Incubation time (hrs)				
Forage samples	4	8	12	24	48
Italian ryegrass					
Before heading	3.00	6.25	10.25	13.00	15.00
After heading	3.00	3.50	6.75	12.50	12.75
After blooming	5.00	7.50	9.75	11.00	11.25
Alfalfa	3.50	6.75	9.85	11.00	11.50
Common vetch	3.50	7.05	10.50	10.75	10.75
Red clover	1.00	5.25	7.50	9.25	10.00

Table 9. Total VFAs production during incubation of forage samples (mmol/100ml)

	Incubation time (hrs)				
Forage samples	4	8	12	24	48
Italian ryegrass					
Before heading	1.90	3.90	5.00	7.54	8.59
After heading	2.31	3.53	5.53	8.08	10.43
After blooming	2.36	3.47	4.18	7.78	9.59
Alfalfa	2.33	3.39	3.46	6.43	7.06
Common vetch	2.69	5.03	6.20	8.39	10.20
Red clover	1.20	1.61	4.91	8.52	9.42

NDF control (prevent) NDF digestibility in legume forages. The digestibility of ADF itself is clearly low in legume forages as compared to that in grass forages.

As other reasons, it can be assumed that the digestion of hemi-cellulose proceeds rapidly, and will be degradable condition at early stage of incubation. It cannot be clarified, however that the rapid progress of hemi-cellulose digestion in the early stage during incubation induces a difference in digestibility of crude fiber between legume and grass forages, or not in this study. About this, further study is thought to be needed obviously.

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