RUMEN DEGRADABILITY OF ITALIAN RYEGRASS (*Lolium multiflorum*, L) HARVESTED AT THREE DIFFERENT GROWTH STAGES IN SHEEP

A. Fariani[1], L. Warly, T. Matsui[2], T. Fujibara and T. Harumoto

Laboratory of Animal Science, Faculty of Agriculture
Shimane University, Matsue-shi 690, Japan

Summary

This experiment was carried out in order to evaluate the chemical composition and rumen degradation characteristics of Italian ryegrass harvested at three different growth stages, i.e. pre-blooming, early-blooming and late-blooming. Degradation values were obtained by incubation of the samples using the nylon bag technique in the rumen of sheep fed a normal diet (Timothy hay with 200 g/d concentrate per head) for 12, 24, 36, 48 and 72 hours, respectively. Chemical composition of the grass showed that crude protein content declined rapidly with advancing maturity from 17.1% at pre-blooming to 9.4% and 7.8% at early-blooming and late-blooming, respectively. Neutral detergent fiber (NDF) content was highest at late-blooming (64.4%) while no difference was found among the pre-blooming and early-blooming (49.4% vs 48.3%). However, acid detergent fiber (ADF) content markedly increased from 30.0% at pre-blooming to 35.4% and 46.4% at early-blooming and late-blooming, respectively. Liguin and silica contents also increased as advancing maturity of the grass. Rumen degradation of dry matter (DM) significantly reduced ($p < 0.05$) as advancing maturity of the grass. Rumen degradation of cellulose and ADF at pre-blooming were significantly higher ($p < 0.05$) than those of early-blooming and late-blooming. However, no significant differences were observed among the early-blooming and late-blooming. With advancing maturity, rumen degradation of NDF and hemicellulose significantly reduced ($p < 0.05$) at all the incubation times.
(**Key Words**: Italian Ryegrass, Stage of Growth, Rumen Degradation, Sheep)

Introduction

Italian ryegrass (*Lolium multiflorum*, L) is an important forage as a feed for ruminant animals. In general however, utilization of the forage is closely related to its quality that has evolved as a result of interaction between genetic and environmental factors. It is also well recognized that various factors contribute to the chemical composition of forage including soil fertility, water supply, climate, stage of growth, frequency of cutting, variety and strain of the forage. Among these factors, the stage of growth or maturity is the most important factor which directly contributes to the quality of forage. Sullivan (1973) reported that the nutritive value of a forage depends upon the morphological and physiological changes which take place during each stage of growth. As the forage matures, the cytoplasmic portion of each cell becomes less important and the quantity of some of its constituents such as protein, lipids, soluble carbohydrates and soluble minerals decreases. However, the cell walls become relatively more important, and the fibrous constituents increase and become more lignified. Consequently, digestibility of the forage reduces with advancing maturity. On the other hand, although the quality of forage is very high at the early stage of growth, the yield of dry matter per unit area is very low. Therefore, in order to get more nutrients per unit area the optimum time of harvest must be considered as a result of the nutritive value and dry matter yield. Miyashige et al. (1989) suggested that the Italian ryegrass should be harvested before or around the time of heading, when an efficient utilization of crude protein in the rumen could be achieved.

Objectives of the present experiment were to evaluate the nutritive value of Italian ryegrass harvested at three different growth stages by determining its rumen degradability of fibrous components in sheep.

[1]Address reprint requests to Dr. A. Fariani, Laboratory of Animal Science, Faculty of Agriculture, Shimane University, Matsue-Shi 690, Japan.

[2]Present address: Faculty of Agriculture, Kyoto University, Sakyo-ku, Kyoto-shi 606, Japan.

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Materials and Methods

Animals and management

Three Japanese Corriedale wethers with an average initial body weight (BW) of 47.1 ± 3.6 kg were used in a 3 × 3 latin square design. They were fed with Timothy hay (2% of BW) and 200 g/d of concentrate (60% wheat bran and 40% barley) to meet the maintenance requirements. Each experimental period lasted for 4 days, consisting of 3 d for incubation of samples in the rumen and 1 d for collection of rumen fluid sample. The sheep were allowed a 7-d adjustment period to the new diet before the first experimental period was begun. Each sheep was fitted with a permanent fistula (40 mm diameter) in the dorsal sac of the rumen and were kept in the metabolism crates throughout the experimental periods. The daily allowance of hay and supplements were offered in two equal portions twice daily at 09:00 and 17:00 h. A mineralized salt block and water were freely available. A vitamin premix was given at a dose of 1 g/d per animal. To control internal parasites all the animals were treated with "Thybenzole 75%" (90 mg/kg B.W) a week before starting the experiment.

Preparation of Italian ryegrass sample

The Italian ryegrass was seeded at the end of October 1991, and the representative samples were taken from the grass harvested at three different growth stages, i.e.: pre-blooming, P-B (April 8, 1992), early-blooming, E-B (May 4, 1992), and late-blooming, L-B (May 30, 1992). The stages of growth at harvest were described according to stage of maturity terms used in the international feed names reported by Harris (1970). Where pre blooming is the stage when stem begins elongation until just before blooming. Early-blooming is the stage between initiation of bloom and stage at which one-tenth of the plants are in bloom, and late-blooming is the stage at which blossoms begin to dry and fall and seeds begin to form.

Immediately after harvesting, the samples were dried in a forced air oven at 60℃ for 24 h and ground through 1 mm screen. Chemical composition of Italian ryegrass and feeds were analyzed by the standard method of the Association of Official Analytical Chemists (AOAC, 1984), and their neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin contents were determined according to the procedures of Goering and Van Soest (1970). In order to evaluate the rumen condition, ammonia-nitrogen (NH_3-N) concentration was analyzed by the method of Oser (1955), and total volatile fatty acids (VFA_s) concentration was determined according to the method described by Morimoto (1971). Chemical composition of the diet used in this experiment is presented in table 1.

TABLE 1. CHEMICAL COMPOSITION OF DIET (% OF DM BASIS)

Constituent	(%)
Organic matter	83.3
Crude protein	7.4
Crude ash	16.7
Crude fat	2.4
Crude fiber	12.6
NFE*	60.9
NDF	61.1
ADF	37.7

* Nitrogen free extract.

Procedures for determination of rumen degradation characteristics

The nylon bag technique described by Orskov (1985) was used to obtain the rumen degradation values. The outer dimensions or size of the bags and the pore size were 8 × 12 cm and 20-40 μm, respectively. Approximately 3 g of each ground sample was placed separately into the bags and they were introduced into the rumen immediately before morning feeding. A set of 10 bags were suspended simultaneously in the rumen of each sheep, and then two of the bags (duplicate) were withdrawn at 12, 24, 36, 48 and 72 h after feeding. After withdrawal, the bags were washed for about 10 minutes, and then they were kept in a freezer at −20℃ for later analyses. After completing all the experimental periods, the bags were washed in a washing machine thoroughly for about 30 min until the rinsing water was colourless, and then dried at 60℃ for 24 h to determine DM content. The residues were analysed for NDF, ADF, cellulose and hemicellulose. The degradation values were obtained by difference in weight of the samples before and

after incubation. Characteristics of rumen degradation were determined by fitting the degradation values to the exponential equation described by Orskov and Mc Donald (1979), as follows:

$p = a + b (1 - e^{-ct})$, where;

p = rumen degradation at the time t,

a = intercept, which is highly correlated with the water soluble fraction,

b = the portion of feed which is degraded in time t,

c = the degradation rate of 'b' fraction, and

t = incubation time,

In addition, a + b (asymptote) shows the value of rumen potential degradability. Lag phase of degradation was calculated from the above equation when the rumen degradation value at the time t (p) was zero.

Statistical analysis

All data were subjected to analysis of variance for a 3 × 3 latin square, and differences among the treatment means were determined by the least significant difference method (Steel and Torrie, 1981). The constants of degradation characteristics were determined by analysis of non-linear regression (Gauss-Newton method) with the procedures of SAS (1985).

Results and Discussion

Effect of harvesting stages on chemical composition

Chemical composition of Italian ryegrass harvested at three different growth stages is shown in table 2. Crude protein (CP) content declined rapidly from 17.1% at pre-blooming to 9.4% and 7.8% at early-blooming and late-blooming, respectively. This result strongly supports the conclusion of Mellin et al. (1962) that the CP content of forage was negatively correlated with maturity. According to Ventura et al. (1975) in his experiments using Pangola digitgrass (*Digitaria decumbens*, Stent), the CP content decreased from 12.1% at 4 weeks to less than 7% at 12 weeks in first-regrowth and from 17.8% at 4 weeks to less than 7% at 8.5 and 11 weeks in second-regrowth, respectively. According to Jones and Wilson (1987) young vegetative growth is high in protein but the content rapidly falls as the proportion of leaf decreases. There is a slower decline in the protein content in the leaf than in the stem with advancing maturity reflecting the decrease in the proportion of cell contents in the stem as it lignifies and the cell walls thicken. With the exception of hemicellulose, advancing maturity was also associated with marked increases in all cell wall components, although the increasing NDF content was only observed in late-blooming (49.4 vs 64.4%). Lignin content increased from 1.3% at pre-blooming to 2.7% and 5.0% at early-blooming and late-blooming, respectively. These results were in agreement to those obtained by Van Soest et al. (1978) and Bailey (1973) that the content of cell wall constituents (CWC) was positively correlated with maturity of forage.

TABLE 2. CHEMICAL COMPOSITION OF ITALIAN RYEGRASS HARVESTED AT THREE DIFFERENT GROWTH STAGES (% OF DM BASIS)

Constituent	Pre-blooming	Early-blooming	Late-blooming
Organic matter	85.4	88.6	87.6
Crude protein	17.1	9.4	7.8
Crude Ash	14.6	11.4	12.4
Crude fiber	24.0	28.2	36.9
Crude fat	3.7	2.3	0.3
NFE	40.7	48.7	42.6
NDF	49.4	48.3	64.4
ADF	30.0	35.4	46.4
Hemicellulose	19.4	12.9	18.0
Cellulose	26.5	29.8	36.2
Acid detergent lignin	1.3	2.7	5.0
Silica	2.2	2.9	5.2

Characteristics of ruminal fermentation

It is well known that maximum ruminal degradation of forage could be achieved when condition of the rumen environment is not a limiting factor for activity of rumen micro-organisms. As shown in table 3, mean of rumen pH was 6.37 and concentrations of rumen NH_3-N and total VFA_s were 15.04 mg/100 ml and 9.87 mmol/100 ml, respectively. Satter and Slyter (1974) reported that maximum microbial growth rate should be achieved when the concentration of rumen NH_3-N was 5 to 8 mg/100 ml. In another study however, Perdok et al. (1988) has demonstrated that a much higher level of rumen NH_3-N concentration (10 to 20 mg/100 ml) was needed for maximum rumen microbial fermentation. From these results, it could be concluded that the rumen condition in the present experiment was optimum to support microbial activities.

TABLE 3. THE MEAN OF RUMINAL FLUID pH, RUMEN NH_3-N AND TOTAL VFA_s CONCENTRATIONS*

Item	Sheep 1	Sheep 2	Sheep 3	Mean ± SD
pH	6.50	6.34	6.27	6.37 ± 0.12
NH_3-N (mg/100 ml)	15.91	14.29	14.91	15.04 ± 0.82
Total VFA_s (mmol/100 ml)	9.74	9.86	10.02	9.87 ± 0.14

* Each value is the mean of 6 sampling times.

Characteristics of rumen degradation

Figure 1 shows the curves of rumen degradation of DM, while its degradation characteristics are presented in table 4. The degradation of DM expressed as amounts of DM disappearance from the nylon bags was significantly decreased ($p < 0.05$) with advancing maturity of the grass at all the incubation times. The trend of its degradation was almost similar for all the harvesting stages, where it rapidly increased associated with increasing the incubation time up to 48 h. Furthermore, the values were almost constant when the incubation time was increased from 42 h to 72 h. These findings strongly support the previous results obtained by Miyashige et al. (1989) for Italian ryegrass and Nelson and Satter (1992) for Alfalfa (*Medicago sativa*, L). As shown in table 4, soluble fraction of DM (a) was lower at late-blooming than that of pre blooming and early-blooming, while the degradable fraction of DM (b) was higher at pre-blooming compared to early-blooming and late blooming. No difference was observed in terms of degradation rate of DM (c) among pre-blooming and late blooming, but they were higher compared to that of early-blooming. Potential degradability (a + b) of DM was greatly reduced as advancing maturity of the grass. These findings suggest that both the increasing quantities and changes in composition of CWC were the main factors which depressed the degradation rates of DM in late-

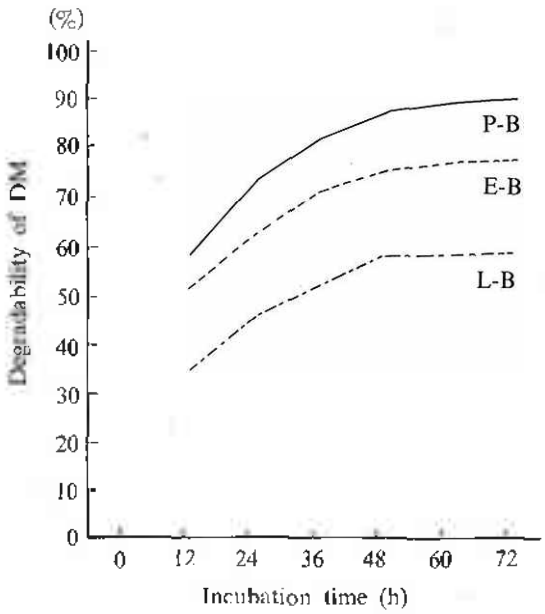

Figure 1. Effect of harvesting stages on rumen degradation of dry matter (DM), (P-B = pre-blooming, E-B = early-blooming and L-B = late blooming).

blooming stage of the grass.

With the exception of 12 and 24 h incubation times, rumen degradation of NDF significantly reduced ($p < 0.05$) as advancing maturity of the grass (figure 2). This result was in agreement with the results reported by Cleale et al. (1986) and

Panditharatne et al. (1987). As shown in table 5, the values of degradable fraction (b) and potential degradability (a + b) of NDF markedly decreased as advancing maturity of the grass. In contrast however, the early-blooming had the lowest NDF soluble fraction (2.97%) followed by pre-blooming (8.13%) and late-blooming (11.32%), respectively. The rate of NDF degradation value (c) for pre-blooming was the same as for early-blooming (0.046), but it was higher than for late-blooming (0.037). The facts that the advancing maturity has been associated with reduction of NDF degradable fraction and its potential degradability was probably caused by the increasing proportion of lignin-carbohydrate bonds, particularly hemicellulose-lignin complex.

TABLE 4. EFFECT OF HARVESTING STAGES ON THE CHARACTERISTICS OF DRY MATTER DEGRADATION ACCORDING TO THE EQUATION $p = a + b\ (1-e^{-ct})$

Harvesting stages	a	b	(a + b)*	c
	%			fraction/h
Pre-blooming	29.38	60.77	90.15	0.054
Early-blooming	30.53	47.89	78.42	0.049
Late-blooming	12.57	47.23	59.80	0.053

* Reperesent the potential degradability of dry matter.

TABLE 5. EFFECT OF HARVESTING STAGES ON THE CHARACTERISTICS OF NEUTRAL DETERGENT FIBER DEGRADATION ACCORDING TO THE EQUATION $p = a + b\ (1-e^{-ct})$

Harvesting stages	a	b	(a + b)	c
	%			fraction/h
Pre-blooming	8.13	78.65	86.78	0.046
Early-blooming	2.97	68.67	71.64	0.046
Late-blooming	11.32	52.04	63.36	0.037

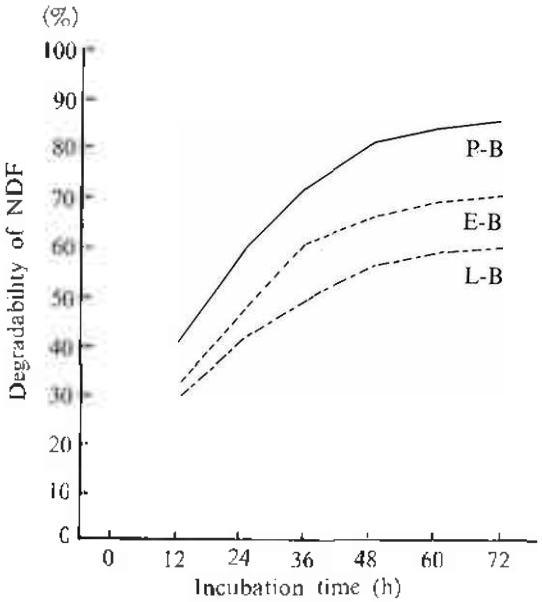

Figure 2. Effect of harvesting stages on rumen degradation of neutral detergent fiber (NDF).

The effect of harvesting stages on ADF degradation is presented in figure 3. In general, the advancing maturity of the grass has been associated with decreasing degradability of ADF. These values were markedly higher ($p < 0.05$) for pre- blooming than those for early-blooming and late-blooming. However, there was no significant difference among the early-blooming and late-blooming at all the incubation times. Characteristics of ADF degradation in the rumen are presented in table 6. Potential degradability (a + b) of ADF was highest for pre-blooming, followed by early-blooming and late-blooming, respectively. On the other hand, no difference was observed concerning rate of degradation value (c) among the three harvesting stages of the grass. It is interesting to note that the value of ADF soluble fraction (a) was negative for early-blooming (−12.07%) and late-blooming (−3.90%). According to Orskov et al. (1990) the negative value of soluble fraction (a) is indicating a lag phase

of degradation. The lag phase of ADF degradation for early-blooming and late-blooming calculated from the above exponential equation in the present experiment were 4.06 h and 1.67 h, respectively.

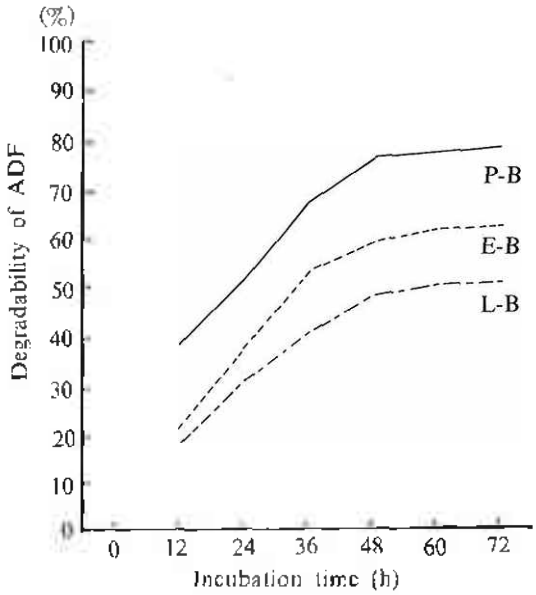

Figure 3. Effect of harvesting stages on rumen degradation acid detergent fiber (ADF).

Rumen degradation of cellulose for pre-blooming was significantly higher ($p < 0.05$) than for early-blooming and late-blooming at all the incubation times. However, with the exception for 48 h of the incubation time, no significant differences were observed among the early-blooming and late-blooming (figure 4). As can be seen in table 7, the values of soluble fractions (a), degradable fractions (b), potential degradability (a + b) and rate of degradation (c) of cellulose were greatly reduced by advancing maturity of the grass. The harvesting stages had a consistent effect on the rumen degradation of hemicellulose, in which the highest values were found at pre-blooming, followed by early-blooming and late-blooming respectively, for all the incubation times (figure 5). As shown in table 8, degradable fraction (b) and potential degradability (a + b) decreased with increasing maturity of the grass. Rate of degradation value (c) was not different among the pre-blooming and early-blooming, while the late-blooming had the lowest c value. These results indeed proved that the increasing of lignin and silica contents due to advancing maturity of the grass were associated with reduction of fiber degradation in the rumen. According to Minson (1990) much of the cellulose and hemicellulose in forage is protected from the attack of the rumen microflora by a layer of indigestible lignin that can only be disrupted by ball milling or chemical treatment. Thus the cellulose and hemicellulose can be divided into potentially digestible and indigestible fractions separated by a protective layer of lignin, in which the indi-

TABLE 6. EFFECT OF HARVESTING STAGES ON THE CHARACTERISTICS OF ACID DETERGENT FIBER DEGRADATION ACCORDING TO THE EQUATION $p = a + b\ (1-e^{-ct})$

Harvesting stages	a	b	(a + b)	c
		%		fraction/h
Pre-blooming	3.17	79.16	82.38	0.044
Early-blooming	−12.07	76.92	64.85	0.048
Late-blooming	−3.90	57.53	53.63	0.042

TABLE 7. EFFECT OF HARVESTING STAGES ON THE CHARACTERISTICS OF CELLULOSE DEGRADATION ACCORDING TO THE EQUATION $p = a + b\ (1-e^{-ct})$

Harvesting stages	a	b	(a + b)	c
		%		fraction/h
Pre-blooming	−14.21	104.39	90.15	0.064
Early-blooming	9.09	74.39	83.48	0.042
Late-blooming	17.06	61.69	78.75	0.039

gestible fractions will increase proportionally as advancing maturity of forage. The importance of lignin in the reduction of fiber digestibility is also well explained in a review reported by Jones and Wilson (1987). They showed that variation in the total content of structural constituents or their components is of lesser significance in the nutrition of the herbivore than the interrelationship between the constituents. The polysaccharides of the cell wall of herbage are totally digestible when the lignin is removed. Their association with lignin, however, hinders attack by microbial enzymes to a varying extent depending primarily on the degree of lignification. Moreover, lignin reduces the digestibility by preventing the physical attachment of bacteria to the cell walls.

In conclusion, the quality of Italian ryegrass based on its chemical composition and fiber degradation characteristics rapidly decreased as advancing maturity. Furthermore, to get an optimum rumen degradation, it is recommended to harvest Italian ryegrass at or before the early-blooming stage.

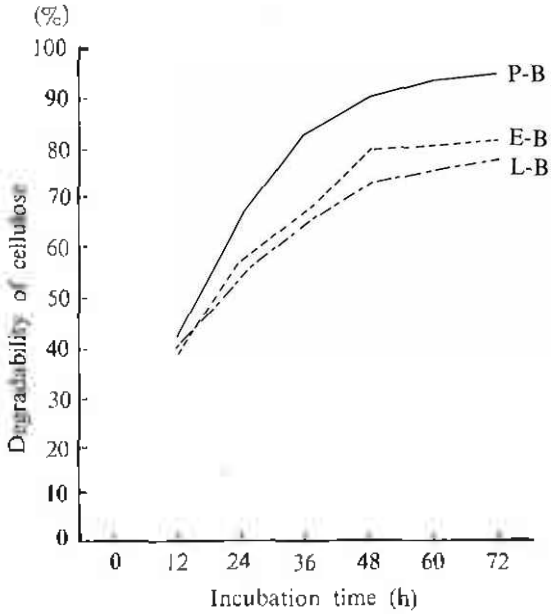

Figure 4. Effect of harvesting stages on rumen degradation of cellulose.

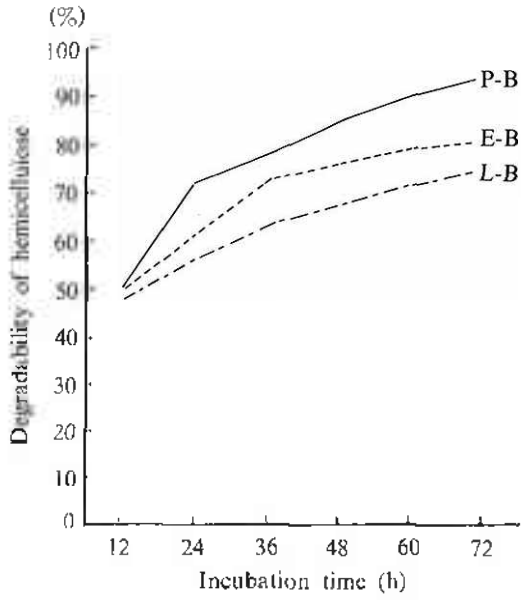

Figure 5. Effect of harvesting stages on rumen degradation of hemicellulose.

TABLE 8. EFFECT OF HARVESTING STAGES ON THE CHARACTERISTICS OF HEMICELLULOSE DEGRADATION ACCORDING TO THE EQUATION $p = a + b\ (1-e^{-ct})$

Harvesting stages	a	b	(a + b)	c
	..... %			fraction/h
Pre-blooming	19.24	75.10	94.34	0.045
Early-blooming	25.83	56.15	81.98	0.045
Late-blooming	34.43	45.40	79.83	0.027

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