EFFECT OF ORCHARDGRASS GROWTH STAGE ON POOL SIZE AND KINETICS OF DIGESTA PARTICLES IN THE RUMEN OF SHEEP

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Summary

The differences in rumen particle pool size, passage rate and rumen degradability in sheep receiving three varieties of orchardgrass hay harvested at pre-heading (H1), early-bloom (H2) and late-bloom (H3) were investigated using four ruminal-cannulated wethers (68 kg) fed 1,300 g of the hay once a day. Representative samples of whole rumen contents were collected at different times after feeding and the quantities of rumen particle pools [large particle pool (LPP), retained on a 1,180 μm sieve; small particle pool (SPP), retained on a 47 but passed a 1,180 μm sieve; and soluble fraction (SOL), passed a 47 μm sieve (SOL)] were determined by a wet-sieving technique. The following results were obtained: 1) The dry weight of whole rumen contents were significantly lower ($p < 0.05$) for H1 than for H2 or H3. The reduction rate of whole rumen contents was slightly but significantly greater for H1 than the other hay varieties. 2) The LPP disappearance rates were 26.2, 25.3 and 21.7 g DM/h for H1, H2 and H3, respectively, and no statistical differences were found among the hay varieties. Appreciable changes were not observed with SPP and SOL throughout measurements for all hay varieties; however the SPP was markedly greater ($p < 0.05$) for H2 and H3 than for H1, while SOL did not differ among hay varieties. 3) The SPP passage rate (g DM/h) and effective rumen degradability (%) for H1, H2 and H3 were, respectively, 9.7, 56.6; 16.9, 42.3; and 18.0, 28.9. The ruminal turn-over rate for SPP appeared to be higher for H1 than for the other hay varieties.

(**Key Words** : Rumen Particles, Particulate Kinetics, Orchardgrass Hay, Growth Stage, Sheep)

Introduction

Digesta clearance rate from the rumen is an important process which restricts forage intake by ruminants. This process largely depends on ruminal digestion and passage of ingested feed. Rumen solid contents are comprised of feed particles with a wide size range. Large forage particles must be comminuted to a size smaller than a critical size before they can leave the rumen (Poppi et al., 1980). However, the ruminal pool size of small particles below the critical size (able to pass a 1,180 μm screen) have been shown to make up more than 50% of the total DM in the rumen (Shaver et al., 1988; Grenet, 1989; Ichinohe et al., 1989; Okamoto et al., 1990). With such a large proportion of the rumen solids being small particles, the clearance rate of rumen digesta may be mainly influenced by the small particles removal rate.

The effect of particle size reduction rate and passage rate on the rumen digesta clearance rate do not seem to be well established. The daily changes in the ruminal pool sizes of digesta particles which differing in their rumen-escape probability may represent the net result of particulate breakdown, digestion and passage, and hence may provide useful information for particle movements in the rumen. Although several workers have reported rumen particle pool sizes and their daily changes (Poppi et al., 1981; Waghorn et al., 1986; Worrell et al., 1986; Okamoto et al., 1990), little information is available to describe the effect of particle size reduction and passage on the rumen digesta clearance for various kinds of forage.

In our previous paper (Ichinohe et al., 1994) we reported that the growth stage of orchardgrass hay greatly affected the particle size distribution of rumen digesta with time after feeding. In this paper, we have expanded our earlier studies to evaluate the effect of orchardgrass

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growth stage on the changes in rumen particle pool sizes and particle loss from the rumen.

Materials and Methods

Feeds

Three types of hay varieties (H1, H2 and H3) were prepared from 1st cut orchardgrass (*Dactylis glomerata*). The grass was harvested at three growth stages to provide differences in voluntary intake and digestibility. The hay varieties were harvested at pre-heading, early-bloom and late-bloom for H1, H2 and H3, respectively. Chemical composition (as % of DM) of H1, H2 and H3, determined via the methods of A.O.A.C. (1980) and the procedures of Goering and Van Soest (1970), was: organic matter, 90.4, 91.1 and 92.8; crude protein, 13.8, 10.0 and 7.3; neutral detergent fiber, 60.3, 69.3 and 75.6 and acid detergent lignin, 2.5, 4.4 and 5.8, respectively.

Animals, feeding and measurements

Four mature wethers, weighing 68 kg on average, which were fitted with large ruminal cannulas (75 mm o. d.), were used. The wethers were kept individually in metabolism crates under continuous lighting and had free access to water and mineral blocks. The wethers were fed hay in the long form for 3 h a day. The quantity of feed offered was 1,300 g (air dry) for H1, H2 and H3, respectively. H1, H2 and H3 hay were offered to the sheep successively. The feeding period for each hay was 36 d of which the first 7 d were for adaptation and the remaining 29 d were for measurement. During the measurement period, rumen emptying, passage rate determinations for rumen particles and rumen fluid and *in situ* incubation for the hay varieties were carried out.

Rumen sampling

On d 1 through d 17 of each measuring period, whole rumen contents were removed manually via rumen fistula. The whole rumen contents were weighed, mixed and sampled. The remainder were returned to the rumen. As have been outlined by Aitchison et al. (1986) and Ichinohe et al. (1994), we allowed a minimum interval of 72 h between each evacuation to overcome any effects of rumen emptying on subsequent measurements. Rumen samplings were conducted at 3, 7, 11, 15, 19 and 24 h after feeding. The particle size distribution of each sample was determined by a wet-sieving technique (Ichinohe et al., 1989). The rumen pool sizes of large particles (LPP, particles retained on 1,180 − 5,600 μm sieves), small particles (SPP, particles passed through the 1,180 μm but retained on 47-600 μm sieves) and the soluble fraction (SOL, passed through the 47 μm sieve) were calculated by multiplying the quantity of whole rumen contents (g DM) by the particle size distribution.

Passage rate measurements

The wethers were given a pulse dose of samarium (Sm) labeled small particles and polyethylene glycol (PEG) solution to determine the passage rate of small rumen particles and the soluble fraction, both of which are eligible for rumen escape. The three hay varieties were coarsely ground and were separated into small particles (which passed through a 1,180 and retained on a 150 μm sieve) by the wet sieving technique. The immersion method of Mader et al. (1984) was used in labeling the hay particles with Sm. On d 20 of each measuring period, the wethers were intraruminally dosed with 15 g of the Sm-labeled hay particles prepared from the respective hay varieties they were consuming. Following an intra-ruminal dose of the Sm-labeled particles, a total of 22 fecal samples were taken at increasing intervals during a 5-d collection period from each wether. Fecal samples were dried at 60℃ for 48 h in a forced-air oven, ground (1 mm screen) and wet-ashed before Sm-analysis. Fecal Sm concentrations were determined by inductively coupled argon plasma emission spectrophotometry. The passage rate constant of small rumen particles (kp, %/h) was calculated as described by Grovum and Williams (1973). On d 25 of each measuring period, PEG (30 g) dissolved 200 ml of distilled water was dosed intraruminally via cannula as a fluid phase marker. Rumen fluid samples were taken at 0.5, 3, 7, 11, 15, 19 and 24 h after the PEG dose was commenced. The PEG concentrations in the rumen fluid samples were determined by a turbidimetry (Hyden, 1955). The passage rate constant (%/h) and volume of rumen fluid were calculated as described by Hyden (1955). The rumen fluid passage rate (ml/h) was calculated by multiplying the passage rate constant by the rumen fluid volume.

Rumen degradability

The degradation characteristics of the hay varieties were determined by an *in situ* technique (Orskov and McDonald, 1979). On d 26 of each measuring period, polyester bags (5 × 10 cm, 47 μm pore size), containing 2 g of the hay (ground to pass a 1-mm screen), were placed in the rumen and two of the bags were removed from each wether at 3, 7, 11, 15, 19, 24, 48 and 72 h after incubation. The wethers being fed hays H1, H2 and H3 received bags containing the respective hay they were consuming. After removal, the bags were rinsed thoroughly with running-tap water untill wash-out water

became clear. After being washed, the bags were dried in a forced-air oven at 60℃ for 48 h to determine DM losses during incubaion. The percentage of DM disapperarnce with incubation time was described by an exponential model, $P_{(t)} = a + b(1 - e^{-ct})$ (Orskov and MeDonald, 1979). The constants a, b and c were determined using an iterative least square method. Effective degradability (DG) for each hay was evaluated according to the equation: $DG = a + (b \times c)/(c + kp)$ (Orskov and McDonald, 1979).

Statistical analyses

Results were subjected to a one-way layout design with the three stages of hay maturity as a factor. Statistical analyses were conducted by an analysis of variance, regression and the differences in mean value between each hay were compared using Duncan's multiple range test (Steel and Torrie, 1960).

Results and Discussion

Figure 1 shows the quantity of whole rumen contents (WRC, g DM) at each sampling. The WRC decreased with time after feeding (T, h) according to the following exponential equations:

H1; $WRC = 1730 \exp(-0.038\ T)$, s.e. ± 0.05, $R^2 = 0.97$,
H2; $WRC = 1665 \exp(-0.026\ T)$, s.e. ± 0.03, $R^2 = 0.97$,
H3; $WRC = 1570 \exp(-0.029\ T)$, s.e. ± 0.02, $R^2 = 0.99$.

The quantity of WRC was significantly less ($p < 0.05$) for hay H1 than for either hay H2 or H3; there was no significant difference between hay H2 and hay H3. The mean daily consumption by the wethers of hay H3 was 1,010 g (air dry); which was 23% lower than those of hays H1 and H2. Although the consumption of hays H1 and H2 were not different (1,280 and 1,170 g, respectively), the WRC was lower for hay H1 than for hay H2 for all measurements. The differences in hay consumption did not clearly affect the quantity of WRC between each of the hay varieties. The rate constants of WRC-reduction (kc, %/h) were 3.8, 2.6 and 2.9 for hays H1, H2 and H3, respectively, and was significantly greater ($p < 0.05$) for hay H1 than for either hay H2 or H3. Mean WRC-reduction (g DM/h) was calculated using following integration:

$$\int_3^{24} WRC \times kc\ dT/21,$$

where WRC is the regression equation listed above and kc is the rate constant (per h). The mean WRC-reductions were 39.6, 32.6 and 35.7 g DM/h for hays H1, H2 and H3, respectively, and was also greater ($p < 0.05$) for hay H1 than for the other hay varieties. These results were in agreement with Aitchison et al. (1986) and Grenet (1989) and suggest that the digestion rate and/or flow rate of rumen particles could be higher for hay H1 than for either hay H2 or H3. *In situ* parameters and DG values are shown in table 1. A statistical difference in *in situ*

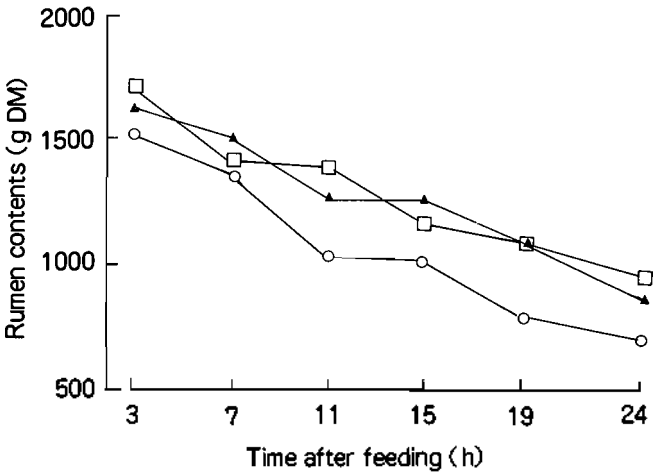

Figure 1. Weight changes in whole rumen contents with time after feeding in sheep consuming orchardgrass hay varieties (H1, H2 and H3) once a day. Mean values for four sheep. For details of the hays, see footnote in table 2.

—○— , H1: —□— , H2: —▲— , H3

TABLE 1. RUMEN *IN SITU* DEGRADABILITY[1] OF ORCHARDGRASS HAY VARIETIES

	Orchardgrass hay varieties[2]		
	H1	H2	H3
a, %	17.1 ± 2.5[3, d]	8.2 ± 1.7[e]	5.5 ± 0.4[f]
b, %	66.2 ± 3.5[d]	59.3 ± 2.7[e]	56.7 ± 5.4[e]
c, /h	0.037 ± 0.001[d]	0.039 ± 0.001[d]	0.021 ± 0.001[e]
Effective degradability (DG), %	56.6 ± 4.0[d]	42.3 ± 3.8[e]	28.9 ± 1.4[f]

[1] a, b, c are constants in the equation $P_{(t)} = a + b(1 - e^{-ct})$ (Orskov and McDonald, 1979). $DG = a + (b \times c)/(c + kp)$; kp = ruminal passage rate constant of small particles (see table 2).

[2] For detail of hay varieties, see footnote in table 2.

[3] Mean values with their standard deviations for four sheep.

[d, e, f] Means within the same row with different superscripts differ ($p < 0.05$).

disappearance rate was not similar to the WRC-reduction rate among the hay varieties, although that of the potentially degradable fraction (a + b) and the DG value were markedly greater ($p < 0.05$) for hay H1 than for either hay H2 or H3. The DG value for hay H1 was 34 and 96% greater than those for hays H2 and H3, respectively. Thus orchardgrass growth stage affected the rate of rumen digesta clearance and rumen degradation which, in turn, influenced rumen degradability of ingested hay particles.

The quantities of LPP, SPP and SOL are illustrated in figure 2 and reduction rate for each particulate pool is listed in Table 2. In accord with the WRC-reduction, the LPP declined from 683 g at 3 h after feeding to 95 g DM at 24 h for hay H1, 667 to 134 g for hay H2 and 567 to 70 g for hay H3, respectively; and regression analyses gave the following equations for LPP-reduction:

$$H1;\ LPP = 873 \exp(-0.078\ T),\quad s.e. \pm 0.03,\ R^2 = 0.91,$$
$$H2;\ LPP = 820 \exp(-0.076\ T),\quad s.e. \pm 0.03,\ R^2 = 0.99,$$
$$H3;\ LPP = 775 \exp(-0.066\ T),\quad s.e. \pm 0.01,\ R^2 = 0.99.$$

Although no significant differences were detected, the LPP quantity for all observations and its reduction rate were slightly higher for hays H1 and H2 than for hay H3. Other researcher have reported that the disappearance rate from the rumen large-particle pool (particles larger than the critical size) ranged from 4.2 to 13.0%/h in sheep (Poppi et al., 1981) and cattle (Dixon et al., 1985; Worrell et al., 1986; Bowman et al., 1991) fed all forage diets. In this study, the LPP-reduction rate for the hay varieties were within the range. The daily times spent ruminating for hays H1, H2 and H3 were 359, 390 and 498 min, respectively; it was longer ($p < 0.05$) for hay H3 than for the other hay varieties. Since rumination considerably contributes to LPP reduction (Ulyatt et al., 1986; McLeod and Minson, 1988; Okamoto et al., 1990), a significant difference in LPP-reduction/rumination time (1.55, 1.33 and 0.96 g DM/min, for hays H1, H2 and H3, respectively) between the hay varieties showed that the late-maturity hay H3 was more resistant to particle size reduction than were the other hay varieties.

In contrast to the LPP-reduction post feeding, appreciable variations in the SPP were not observed throughout the samplings. The quantity of SPP averaged 380, 613 and 681 g DM for hays H1, H2 and H3, respectively. The SPP was significantly greater for hays H2 and H3 than for hay H1 for all observations, which apparently contributed to the differences in WRC between each hay. The SPP passage rate constant and the quantity

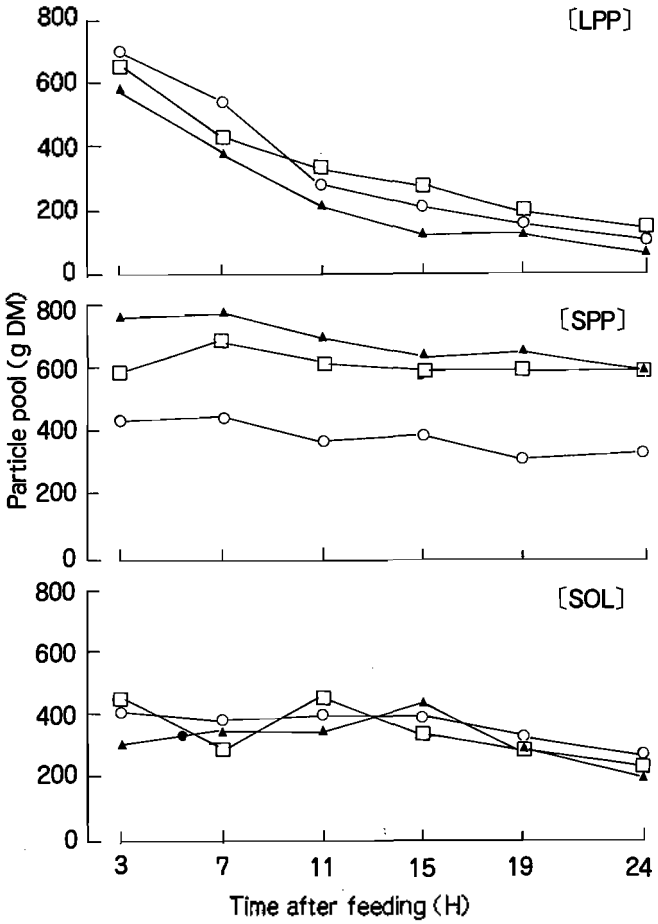

Figure 2. Weight changes in rumen particle pools with time after feeding in sheep consuming orchardgrass hay varieties (H1, H2 and H3) once a day. Mean Values for four sheep. for details of the hays, see footnote in table 2.
LPP, large particle pool (particles larger than 1,180 μm); SPP, small particle pool (particles larger than 47 but less than 1,180 μm); and SOL, soluble fractions (particles less than 47 μm).

of SPP leaving the rumen were significantly lower ($p < 0.05$) for hay H1 than for hays H2 and H3 (table 2), a result, that was not in agreement with Bowman et al. (1991) and Worrell et al. (1986); they observed that the rate of small particle passage decreased as forage maturity increased. The methodological differences in determining small particle passage might have caused the disagreement between the studies; the ruminal model Bowman et al. (1991) used to estimate particle passage was one

compartmental age-dependent model (Ellis et al., 1979), and Worrell et al. (1986) offered hay diets to animals ad libitum and obtained rumen samples via grab collection. To interpret the rumen particle leaving and entering each pool, we developed a simplified rumen model (figure 3).

TABLE 2. REDUCTION RATE OF THE RUMEN PARTICULE POOL[1] IN SHEEP CONSUMING ORCHARDGRASS HAY VARIETIES ONCE A DAY

Item	Orchardgrass hay varieties[2]		
	H1	H2	H3
LPP reduction			
Rate constant (%/h)	7.8±1.4[3]	7.6±1.5	6.6±1.1
Rate (g DM/h)	26.2±2.8[a]	25.3±2.3[a]	21.7±2.4[b]
SPP reduction			
Rate (g DM/h)	26.2	25.3	21.7
Passage			
(%/h)	2.5±0.3[a]	2.9±0.2[b]	3.0±0.2[b]
(g DM/h)	9.7±0.7[a]	16.9±1.8[b]	18.0±2.3[b]
Digestion (g DM/h)	16.5±0.9[a]	8.4±1.2[b]	3.7±1.3[c]
SOL reduction			
Passage			
(%/h)	3.6±0.2[a]	4.1±0.4[a]	5.2±0.6[b]
(g DM/h)	9.7±0.7[a]	16.9±1.5[b]	18.0±2.3[b]

[1] Large particle pool (LPP, particles retained on a 1,180 μm sieve); Small particle pool (SPP, particles retained on a 47 but passed a 1,180 μm sieve) and Soluble fraction (SOL, passed a 47 μm sieve).

[2] H1, harvested at pre-heading; H2, harvested at early-bloom and H3, harvested at late-bloom.

[3] Mean values with their standard deviations for four sheep.

[a,b] Means within the same row with different superscripts differ ($p < 0.05$).

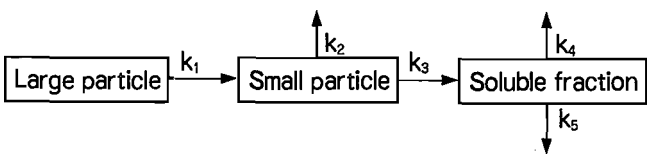

Figure 3. Flow diagram of size reduction, digestion and passage of digesta particles in the rumen. k_1 = particle size reduction rate; k_2, k_4 = ruminal passage rate; k_3 = digestion rate; and k_5 = absorption rate.

We assumed that the LPP reduces via large particle breakdown and flow into the SPP, while the SPP reduces via ruminal escape and digestion. Since McLeod and Minson (1988) reported that the rumen digestion had little effect on the size reduction of ruminal large particles, we omitted the LPP-reduction by way of the rumen digestion and developed a sequential comminution model (Faichney et al., 1989). Because the quantity of SPP was almost constant throughout the observations for all hay varieties, it is inferred that, even under the once-a-day feeding condition, a steady state existed between the LPP reduction rate (k_1) and SPP reduction rate ($k_2 + k_3$). Assuming that the net reduction rate of SPP was almost the same to that of LPP reduction, SPP turn-over rate was calculated by dividing the SPP reduction (g DM/h) by mean quantity of SPP. The SPP turn over rates (%/h) calculated for hays H1, H2 and H3 were 6.9, 4.1 and 3.2%/h, respectively. The *in situ* rate constant of rumen digestion for each hay particles (table 1) should strictly speaking only be used as an estimate of rumen digestion charcteristics of the hay, and not of small particles as is in the rumen. The reduction rate of SPP via digestion was calculated by subtracting the SPP-passage rate from the net SPP-reduction rate. The SPP reduction rates via digestion for hays H1, H2 and H3 were 16.5, 8.4 and 3.7 g DM/h, respectively, and was inferred to be highest for H1 and lowest for H3. The proportion of SPP-particles lost via digestion to net SPP-loss was accounted for 63, 33 and 17%, for H1, H2 and H3, respectively. Although passage rate of SPP lowered for hay H1 than the other hay varieties, SPP digestion rate in hay H1 estimated to exceed the SPP passage rate in hays H2 and H3, and in turn, SPP turn-over rate was supposed to be higher for hay H1 than the other hay varieties.

Although the quantity of SOL varied throughout the samplings, we observed no significant difference between the hay varieties. Digested SPP should transfer to SOL, whereas the SOL-losses occur by ruminal passage and absorption (figure 3). The estimated rumen fluid outflow rate for hays H1, H2 and H3 were 480, 520 and 650 ml/h. respectively, and was significantly higher ($p < 0.05$) for hay H3 than for hays H1 and H2 (table 2). Since the SOL outflow rate is strongly associated with the rumen fluid outflow rate (Faicheney, 1986), the SOL outflow rate should be higher for hay H3 than for the other hay varieties. Although the quantity of SPP, which transferred to SOL by digestion, should be considerably greater for hay H1 than for hays H2 and H3, no difference was observed in the SOL quantity among the hay varieties. It may be due to a higher SOL absorption rate for hay Hl than for the other hay varieties.

In this study, the different growth stages of orchardgrass did not cause a marked difference in the LPP quantity and its reduction rate. However, they did affect the SPP quantity and its manner of disappearance (i.e.

passage and digestion), and the SOL passage rate. For early maturity orchardgrass hay, the percentage of SPP reduction via rumen digestion may have compensated for the low mass of small particles leaving the rumen, which contributed strongly to the higher ruminal turn-over rate of small particles compared to late maturity hay varieties. To confirm the predictions of the ruminal model, quantitative determination of large particle pool loss by breakdown and digestion, digestion rate of small particles and absorption rate of soluble fractions are needed.

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