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Title: Effects of grass hay proportion in a corn silage–based diet on rumen digesta kinetics and digestibility in dairy cows

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Running Head: RUMEN KINETICS OF GRASS HAY IN CORN SILAGE DIET

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ABSTRACT

In this study, we aimed to evaluate the effects of six levels of orchardgrass hay (GH) proportion (0%, 10%, 20%, 30%, 40% or 50% of dry matte) in finely chopped corn silage (CS)-based diets on digesta kinetics of CS and GH in the rumen. Six non-lactating, rumen-cannulated Holstein cows were used in a 6 × 6 Latin square design. Ruminal digesta kinetics was measured by ruminal dosing of feed particle markers (Dy for CS, Er for GH) followed by fecal sampling. The increase of GH proportion had a quadratic effect ($P < 0.01$) on total tract digestibility of neutral detergent fiber (NDF) and acid detergent fiber. The proportion of GH did not affect the particle size distribution of rumen digesta, total weight of dry matter, or NDF in the rumen. The rates of large particle size reduction in the rumen for CS tended to increase linearly with increasing GH proportion ($P = 0.077$). A quadratic effect ($P < 0.05$) was found with increasing the GH proportion for the ruminal passage rate of small GH particles, but not for CS particles. The results suggested that associative effects between CS and GH could be generated on rumen digesta kinetics when cows were fed a CS-based diet with an increased proportion of GH.

Key words: corn silage, dairy cow, digestibility, grass hay, passage rate

INTRODUCTION

Whole corn plants used for ensiling are chopped into short lengths, as a long forage chop length can result in poor silage fermentation due to difficulties in packing and maintenance of anaerobic conditions (Johnson *et al*. 2003). Fine chopping of corn plants for ensiling also prevents selective ingestion of concentrate in total mixed rations, which can cause large diurnal variations in rumen acid production and result in subacute ruminal acidosis (Van Soest 1994).

The physical form of roughage plays an important role in stimulating chewing and maintaining ruminal functions. Forages with short particle lengths can result in reduced chewing, saliva production, and rumen pH (Beauchemin *et al.* 2003; Krause *et al*. 2002; Stone 2004), which cause depression of fiber digestibility (Rojas-Bourrillon *et al.* 1987; Bal *et al.* 2000) and may also reduce the retention time of feed particles in the rumen (Wyburn 1980; Varga & Kolver 1997), thereby decreasing dry matter (DM) digestibility (Allen & Mertens 1988). The negative effects of fine chopping during silage preparation on digestibility have been shown to be mitigated by long grass hay (GH) supplementation, which effectively maintained an optimal ruminal function in cows fed on a finely chopped corn silage (CS)-based diet (Couderc *et al*. 2006) and a finely chopped alfalfa silage–based diet (Beauchemin *et al*. 1994).

The appropriate addition of GH in a CS-based diet can recover the depression of digestibility caused by fine chopping of corn plants, but excess addition can depress the ruminal passage rate of CS, thereby reducing silage DM intake. Recent reports have shown no effect of GH addition on the ruminal passage rate of finely chopped CS in cows fed on CS‑based diets supplemented with 5% to 10% GH (Couderc *et al*. 2006; Castro *et al*. 2010). The supplementation of GH in these studies may be insufficient to

alter the ruminal passage rate of CS. It remains unclear whether the ruminal passage rates of CS are affected by supplementation with greater than 10% GH in a CS-based diet.

Associative effects between two forages on digestion kinetics in the rumen, which impact DM intake and diet digestibility, have been reported for digestion rate (Andrighetto *et al*. 1993) and passage rate (Moseley & Jones 1979). Bhatti *et al*. (2008) reported that alfalfa hay addition in an orchardgrass hay-based diet in beef cattle increased the passage rate of small orchardgrass particles, as well as the disappearance rates of DM and neutral detergent fiber (NDF) disappearance in the rumen. These findings suggest that the ruminal kinetics of two forages in a diet are not independent and could be associatively interrelated. However, no previous studies have reported an interrelationship between CS and GH in terms of ruminal kinetics.

The objective of this study was to determine the effects of 0% to 50% supplementation of GH in a CS-based diet on digestibility and ruminal digesta kinetics in non-lactating dairy cows. The tested hypothesis was that the increased GH proportion would decrease the CS ruminal passage rate by synchronizing it with the intrinsically slow passage of GH, thereby increasing ruminal CS digestibility.

MATERIALS AND METHODS

Cows and treatments

This experiment was conducted at the Experimental Farm of Hokkaido University, Sapporo, Japan. The methods of feeding management and ruminal cannulation surgery of cows used in this study were approved by the Animal Care and Welfare Committee of Hokkaido University.

Six rumen-cannulated, non-lactating Holstein cows (777.4 ± 79.7 kg body weight) were assigned to one of the six dietary treatments in a 6 × 6 Latin-square design. Each experimental period consisted of a 9-day adaptation period and a 5-day collection period. The dietary treatments consisted of six proportions (on a DM basis) of CS and GH in total rations of: (1) CS 100%; (2) CS 90% + GH 10%; (3) CS 80% + GH 20%; (4) CS 70% + GH 30%; (5) CS 60% + GH 40%; and (6) CS 50% + GH 50%. The corn silage used in the experiment had been preserved in a banker silo. Whole corn plants were harvested at one-half milkline stage of maturity and chopped at a theoretical length of 9 mm with a forage harvester 6 months before the experiment was initiated. The orchardgrass hay was harvested at flowering stage and chopped at a theoretical length of 60 mm by using a chopper machine. Two equal portions of the experimental diets were offered at 07.30 and 19.30 hours. Two forages were mixed thoroughly immediately before each feeding. The total feed allowance was fixed at 10 kg of DM per day per cow, and the cows had free access to fresh water and trace mineral salt blocks. To measure the daily fecal excretion of cows, 50 g of an external marker was administered immediately before each feeding of the treatment diets throughout the experiment. The marker was prepared from beet pulp pellets labeled with La using the immersion method, as described by Mader *et al.* (1984).

Data collection and sample analysis

The weight of refusals was measured before every morning feeding. Samples of feed offered and refused were collected during the sample collection period. The actual intake was determined by the difference between the feed offered and refused during the sample collection period.

Eating time and ruminating time per day were measured by recording eating and

ruminating activity with a video tape recorder on the third and fourth day of each experimental collection period.

On the first day of each collection period, Co-EDTA (5 g/250 mL water) was administered into the rumen at 07.00 hours to measure the ruminal liquid passage rate. Approximately 100 mL of rumen fluid was then collected eight times at 3-h intervals. Rumen fluid samples were sucked with a syringe through a catheter inserted into the rumen, which was fixed on the rumen cannula. The pH of the ruminal fluid was measured immediately after sampling by a digital pH meter (B-212; Horiba, Kyoto, Japan). A 1-mL subsample of each rumen fluid sample was mixed with 0.1 mL of 25% metaphosphoric acid, and the resultant fluid samples were centrifuged at 28,000 $\times$ *g* for 10 min at 4°C. The supernatants were used for volatile fatty acid (VFA) analysis by gas chromatography (GC-20; Shimadzu, Kyoto, Japan) by using a capillary column (ULBON HR-20M; Shinwa Chemical Industries, Kyoto, Japan) with a column temperature of 150°C, injection temperature of 150°C, and He as carrier gas. Another 0.1-mL subsample was mixed with 20% NaCl and then analyzed for ammonia-N as described by Wetherburn (1967). A third 10-mL subsample of ruminal fluid was placed in a vial and dried for the analysis of Co.

A pair of markers (100 g DM for each), Dy-labeled CS and Er-labeled GH, were prepared by the methods of Mader *et al*. (1984) and dosed into the rumen through a cannula on the first day of each collection period at 08.00 hours to measure the ruminal passage parameters of CS and GH particles. The cows in the 100% CS treatment were dosed with only Dy-labeled CS. Fecal samples were collected every 3 h for 42 h, and every 6 h until 96 h, after the dosing of markers.

The digesta weight in the reticulorumen was measured by manually emptying it on

the fifth day of the collection period at 14.00 hours. The total rumen digesta of each cow was manually mixed and a subsample was taken. The subsample was dried at 60°C for 48 h and ground through a 1-mm screen. The weight distribution of different particle sizes of the rumen digesta was determined with another wet subsample by the wet sieving method with sieves of 1.18- and 0.15-mm aperture.

Samples of offered feed, feces, rumen digesta, and sieved rumen digesta were dried at 60˚C for 48 h and ground through a 1-mm screen for subsequent chemical and marker analyses. Feed and fecal samples were analyzed for DM, crude ash, crude protein (CP), starch, ash-free NDF, ash-free acid detergent fiber (ADF).Ash-free acid detergent lignin (ADL) content was analyzed only for feed samples. Determination of NDF content was also conducted for rumen digesta samples. DM, CP, and crude ash were determined according to the AOAC methods (1990). NDF, ADF, and ADL were measured according to the methods described by Goering and Van Sorest (1975). Starch was determined by the perchloric method (Viles & Silverman 1949; Yemm & Willis 1954). Concentrations of rare earth elements (Dy, Er, and La) in the feces and La-labeled beat pulp pellets, as well as that of Co in the dried rumen fluid, were determined using an inductively coupled plasma spectrometer (Eran DRC-e, Perkin-Elmer, United States) after digestion with 2:1 nitric acid:perchloric acid.

Dried and ground samples of CS and GH were subjected to measurements of *in vitro* digestibility of DM and NDF. Triplicate 1.0-g samples were incubated with ruminal fluid at 39°C for 96 h using the procedure described by Theodorou *et al.* (1994). The rumen fluid was taken from cows fed on grass hay and concentrate formula feed. At the end of the fermentation period (96 h), the samples were filtered with preweighed glass filters. These samples were oven-dried at 103°C for 24 h and weighed, and DM

digestibility were then calculated. Samples of residues in the glass filters were analyzed for NDF.

Calculation of data and statistical analysis

The nutrient digestibility in the total digestive tract was calculated from the nutrient intake and fecal excretion. Fecal DM excretion (kg DM/day) was calculated using the following equation: fecal DM excretion = (mg of La dosed per day)/(mg of La per kg of fecal DM). Ruminal liquid passage rate was estimated by the following exponential equation: $Y=Ce^{-kt}$, where Y = Co concentration at time t (h), C = the initial concentration of Co in the rumen, and k = fractional rate of liquid passage from the rumen. The rate constants for ruminal passage of CS and GH were estimated using the two-compartment, gamma age-dependent and independent (G4G1) model described by Pond and Ellis (1988). The mean outflow rates from the age-dependent compartment ($k1=0.4085686\cdot\lambda$, λ: rate parameter for gamma distributed residence time) and the rate constant of outflow from the age-independent compartment (k2) were estimated by fitting a marker excretion curve to the model using the NLIN procedure of SAS (SAS 2004). The rates of k1 and k2 can be regarded as the rate of size reduction of large particles into small particles and the rate of passage of small particles from the rumen, respectively. The compartmental (ruminal) mean retention time was also calculated as $4/\lambda+1/k2$.

All data were subjected to ANOVA analysis using the GLM procedure of SAS (SAS 2004). Linear and quadratic orthogonal contrasts were tested using the contrast statement of SAS (SAS 2004). For digesta kinetics data, the differences between GH and CS were also tested with the GLM procedure of SAS (SAS 2004). Unless otherwise stated, significance was declared at $P < 0.05$.

RESULTS

The chemical compositions of the two forages are presented in Table 1. The CP content of GH was slightly higher than that of CS, but its contents of DM, NDF, ADF, and ADL was moderately higher than that of CS. The starch content of CS was 21.1% of DM, and no starch was detected in GH. *In vitro* DM digestibility at 96 h was 4% lower than that of CS; however, *in vitro* NDF digestibility for GH at 96 h was 10% greater than that of CS.

The dry matter intake (DMI) and total tract apparent nutrient digestibility of diets are shown in Table 2. A slight decrease in DMI was observed with the increased GH inclusion ($P < 0.01$). The CP digestibility was not affected by the treatments, whereas DM, organic matter (OM), NDF, and ADF digestibility had quadratic effects with an increase in GH proportion ($P < 0.05$). The DM and OM digestibility decreased with increasing GH proportion from 0% to 40% but increased from 40% to 50% GH. The lowest digestibility of NDF and ADF was observed at a lower GH proportion than those of DM and OM. The NDF digestibility was constant from 0% to 30% GH but there was a 10.3 percentage units increase from 30% to 50% GH. The ADF digestibility slightly decreased for a 3.6 percentage units from 0% to 30% GH, but a 10.1 percentage units increase was observed from 30% to 50% GH. Increased GH proportion linearly increased starch digestibility ($P < 0.01$). The difference in starch digestibility between 50% GH and 0% GH was 9.8 percentage units.

Table 3 shows eating time, ruminating time, and total chewing time. The eating time per day and per NDF intake increased linearly ($P < 0.01$) and quadratically ($P < 0.05$) as the GH proportion increased although only linear effect was significant ($P < 0.01$) for eating time per DM intake. The ruminating time per day and per DM intake

linearly increased ($P < 0.01$), whereas ruminating time per NDF intake was linearly decreased ($P < 0.01$). This increase in time by increasing GH proportion from 0% to 50% was larger for eating than for ruminating. Although the total chewing time per day and per DM intake also showed linear and quadratic increase ($P < 0.01$) with increasing GH proportion, the total chewing time per kilogram of NDF intake was not affected by the GH proportion.

The weight and the particle size distribution of rumen digesta are shown in Table 4. The increase in GH proportion increased the fresh rumen digeta weight ($P < 0.01$). However, no significant effects on DM and NDF weights of rumen digesta with increasing the GH proportion were observed. The proportions of particle dry weight retained on a 1.18 mm sieve, < 1.18 but > 0.15 mm sieve, and < 0.15 mm sieve were not affected by GH proportion.

The results for pH, NH_3-N, and VFA, and passage rate of rumen liquid are presented in Table 5. The concentration of NH_3-N was relatively low in all treatments, and it was not affected by the GH proportion. The total VFA concentration was not affected by the treatment, but ruminal pH was significantly affected by GH proportion in both linear and quadratic manners ($P < 0.05$). Significant linear effects ($P < 0.01$) were observed for molar proportions of acetate, propionate, and butyrate. The molar proportion of acetate increased with GH proportion, whereas that of propionate and butyrate decreased with increasing GH proportion. The passage rate of rumen liquid linearly decreased ($P < 0.05$) with increasing the proportion of GH.

The results for the ruminal digesta kinetics of the two forage sources are shown in Table 6. The rates of large particle size reduction (k1) for both CS and GH particles were unaffected by GH proportion in the CS-based diets, although a non-significant

trend of linear increase ($P = 0.077$) was observed for CS. A significant quadratic effect ($P < 0.05$) due to increasing GH proportion was detected for GH particles in the rate of small particle passage from the rumen (k2); however, no effect was observed in k2 for CS particles. The k2 of GH particles increased from 10% to 20% GH and decreased from 20% to 50% GH. The mean retention time (MRT) of GH particles in the rumen also displayed significant quadratic ($P < 0.05$) effects due to increasing GH proportion. This had an inverse effect on k2. When comparing these parameters between GH and CS, significant difference ($P < 0.05$) for k1 and MRT was detected only at 10% GH.

DISCUSSION

Dry matter intake and rumen fill

Generally, an increased feed intake increases passage rate of feed particles in the rumen (Van Soest 1994). As the objective of current experiment was to detect whether the increased addition of GH in the CS-based diet could alter the ruminal passage rate of CS and GH, it was needed to exclude the effect of feed intake on rumen passage rate. Considering the results of previous studies (Couderc *et al*. 2006; West *et al*. 1997), the large variation in feed intake with changing the GH proportion was fully expected also in the current experiment when cows were offered diet *ad libitum*. Thus the current study was conducted under the conditions restricted the dietary supply. However, as small amounts of GH refusal were recorded at 30%, 40%, 50% GH treatments, the increased proportion of GH in the diet slightly decreased the total DMI.

Shaver *et al*. (1986) found that the effects of forage physical form on ruminal fill were small when using alfalfa hay with low fiber content. However, the influence of forage physical form on rumen fill and intake may be larger when mature (high fiber)

forages are fed (Heaney *et al.* 1963). In the current experiment, DM and NDF weight of rumen digesta which is considered to reflect the rumen fill did not differ among treatments. Although the grass hay used in the current experiment had a high NDF content, the ingestion of GH did not increase the DM and NDF weight of rumen digesta regardless of its proportion in the diet.

The weight of fresh rumen digesta increased with increasing GH proportion in the current study. This was caused by the increased liquid pool size of rumen, because the DM weight of rumen digesta was constant among GH treatments. This increase of the rumen liquid pool size was likely associated with a decreased in liquid passage rate from the rumen with increasing proportion of GH. The enlarged rumen liquid pool size with increasing GH proportion could have arisen from an increase in saliva production due to the prolonged total chewing time per day and/or from an increase in water intake due to the increased ingestion of GH with less moisture.

Particle size reduction and chewing

The eating time per day, in the current study largely increased with increasing the proportion of GH. This result indicated that cows spent a comparatively longer time to breakdown GH particles via ingestive chewing than to breakdown CS particles. A significant role of ingestive chewing is to make bolus which cows can easily swallow by reducing the size of feed particles. Cows fed diet with a greater proportion of CS may require less eating time as CS particles are small enough to swallow, and their size could be reduced much more easily than GH particles. Indeed, the time required to eat 1 kg DM of CS was less (7.1 min) than that required for 1 kg DM of GH (23.2, 28.9, 24.2, 24.7, and 20.8 min as calculated by subtracting the eating times of 0% GH from the eating times of 10%, 20%, 30%, 40%, and 50% GH, respectively). When eating time

was expressed per kg NDF intake, it still showed a linear increase with increasing GH proportion. This indicated that the eating time was prolonged more than the increase of NDF intake. Therefore, the prolongation of time for ingestive chewing with GH inclusion is controlled by not only NDF intake but also other factors such as rigidity of GH particles for breakdown GH.

In the current study, the rumen digesta was taken at the time (14.00 hours) when nearly the entire morning diet (07.30 hours) had been consumed by cows. Therefore, the particle size distribution of the rumen digesta strongly reflects the extent of particle size reduction via ingestive chewing. However, no difference in particle size distribution was observed among the treatments. This result indicates that the added GH in the CS-based diets was reduced in size to a similar extent via ingestive chewing despite its increased proportion in the diet.

The rumination time per day was increased by increasing the proportion of GH in the diet. Contrary to this, Beauchemin and Buchanan-Smith (1989) reported that adding 21, 32, and 43% long alfalfa hay to an alfalfa silage-based diet did not increase rumination time of dairy cows fed diets *ad libitum*. Similar to the current results, increased rumination time was reported when dairy cows were fed a finely chopped CS-based diet supplemented with 3kg/day unprocessed alfalfa hay (Fischer *et al.* 1994) and when 5% long hay was included in CS-based diet for dairy cows (Couderc *et al.* 2006). These inconsistent results regarding rumination time may arise from differences in total DMI or hay length. The extent of particle size reduction via ingestive chewing is a significant factor, because large particles that escape the breakdown into small sizes by ingestive chewing must be brokendown by rumination to sufficiently small particles to pass the rumen. Therefore, the lesser extent of particle size reduction during ingestive

chewing increases the need for rumination chewing and extended rumination time. In the current study, although the particle size distribution of rumen digesta showed a similar extent in particle size reduction during ingestive chewing regardless of dietary proportion of GH, the ruminatiton time per day and per kilogram of DM intake showed linear increase with increasing GH proportion. This indicated that an increase in GH particles in ruminal large particles pool could increase the need for rumination chewing and extend the rumination time. However, the increased rumination time with increasing GH proportion (42 min/day) was less than one-half of that for eating (102 min/day). Moreover, when expressed per kilogram NDF intake, rumination time decreased linearly decrease with increasing the GH proportion. Thus, the increased proportion of GH particle in the CS-based diet did not prolong as much the time for rumination as for eating.

The mean outflow rate of the age-dependent compartment (k1) is considered to reflect the rate of particle size reduction for the large particle pool in the rumen (Pond & Ellis 1988). In the current study, the k1 for GH was unaffected by GH proportion. This result indicated that GH inclusion in the diet did not alter the rate of ruminal particle size reduction of GH, regardless of the proportion of GH. Although rumination time per DM intake linearly increased with increased proportion of dietary GH, this increase was not significant to alter the k1 of GH.

Although non-significant, an increase in k1 was observed for CS with increasing the proportion of GH. This result indicates that an increase of GH particles in regurgitated bolus in response to the increasing GH proportion accelerated the breakdown of CS particle during rumination chewing. As GH contains more NDF and ADL than CS, the force to breakdown with rumination chewing might be required more

for GH than for CS. When cows chew on CS with as much force as GH, the breakdown of CS during rumination can be accelerated by increasing the dietary proportion of GH. Moreover, such acceleration can also be expected during ingestive chewing.

Digestibility and ruminal passage rates

In the current study, there was a quadratic effect of GH proportion in the diet on the NDF and ADF digestibility. A decrease in digestibility was observed for NDF and ADF with increasing the GH proportion to up to 10% and 30%, respectively. The increase in proportion of GH to more than 30% increased the digestibility of both fibers. Although we presumed that GH inclusion in the CS-based diets would decrease the passage of CS due to an intrinsically slow passage rate of GH, thereby improving the fiber digestibility of CS, there was no effect of dietary GH proportion on the ruminal passage rate of CS small particles (k2). Furthermore, k2 and MRT did not differ significantly between GH and CS. Therefore, the change of NDF and ADF digestibility by increasing the dietary GH proportion could not be attributed to the ruminal digestion of CS. Fleck *et al*. (1988) suggested that the low NDF and ADF digestibility in native tall grass hay may not be related to the ruminal passage rate but instead to the high starch content of the ration and the consequent fluctuation in ruminal pH and reduction in rumen cellulolytic activity. In the current experiment, molar proportion of acetate, propionate, and butyrate in rumen fluid were altered because of GH proportion, thus reflecting the fiber and starch contents in the diets. However, the ruminal pH altered within a relatively stable range (6.77 to 6.89), which was unlikely to depress ruminal cellulolytic activity even at 0% GH diet with the highest starch content.

The alteration of NDF and ADF digestibility with GH proportion can be

attributed rather to the quadratic response of k2 of GH particles. The increase of GH proportion from 10% to 20% increased k2 of GH particles, but it decreased at dietary levels of GH greater than 30%. The increased ruminal passage rate of GH particles from 10% to 20% GH did not cause a decrease in digestibility of NDF and ADF. The GH intake in this range of dietary GH proportion was not sufficient to decrease the fiber digestibility of diet. However, the decreased passage rate of GH particles in the diet with 20% to 50% GH could increase digestibility of NDF and ADF at 30% to 50% GH. Thus, the improved fiber digestibility with an increase of GH proportion from 30% to 50% was driven not by changes in the CS passage rate but by decreasing the ruminal passage rate of GH *per se* in the rumen. Moreover, the *in vitro* NDF digestibility at 96 h was greater for GH than for CS. Therefore, the inclusion of GH, which has a relatively higher potential digestibility of NDF than CS, could increase the total dietary fiber digestibility in the rumen as dietary GH proportion increases. This effect was emphasized by a decrease of ruminal passage rate of GH with increasing the GH proportion above 20% in the diets. The improvement of ruminal fiber digestibility with increasing GH proportion paralleled the increased ruminal acetate concentration.

In previous reports, partial replacement of reed canary grass hay by CS caused a significant increase in digestibility of dietary constituents in dairy cows (Fenner & Barnes 1966), and the positive effects on digestibility of the CS-based diet were greater when sheep were supplemented with a low-quality grass silage rather than a medium-quality grass silage was added (Vranic *et al*. 2008). West *et al.* (1997) also reported that the apparent NDF digestibility of dairy cows was greater in CS-based diets containing 15% and 30% Bermudagrass hay than in the CS-based control diet without hay. The results in the current experiment indicate that the observations presented in

previous reports might not result from a decreased CS passage rate due to grass hay or silage addition. In agreement with our results, Couderc *et al.* (2006) and Castro *et al.* (2010) reported that the ruminal passage rate of CS was unaffected by 5% and 10% long hay addition to CS-based diets for dairy cows. However, Obitsu *et al*. (2009) showed a decreased ruminal passage rate for both CS and alfalfa hay particles and also an increased ruminal NDF digestibility when dietary chopped alfalfa hay proportion in the CS-based diet increased from 20% to 60% in steer.

The extent of entrapment of small particles into the rumen mat was suggested to relate to a specific gravity of particles in the rumen (Sutherland 1988; Poppi *et al*. 2001). In the current experiment, an increased proportion of rumen mat could be expected with increasing the GH proportion from 20% to 50%. The increased mat proportion may cause an increase in the entrapment of small GH particles in the rumen, thereby reducing the ruminal passage rate of small GH particles. Wattiaux *et al*. (1991) showed that the specific gravity of CS particles in the rumen was greater than that of alfalfa hay. The ruminal small CS particles, in the current experiment, could escape an entrapment into the rumen mat due to their high specific gravity resulting in a constant passage rate regardless of GH proportion. Although it is generally accepted that an increase in indigestible fiber content in diets reduces ruminal passage rate of solid digesta, this was not the case in our study, because the indigestible NDF content in the diet decreased with increasing the GH proportion. Further studies of the associative effects of rumen passage kinetics between forages and the relation to the rumen mat formation and consistency are required.

The appropriate proportion of GH for actual feeding of lactating dairy cows is a vital consideration. To determine the appropriate proportion of GH for a practical dairy

feeding system, a comparison between 20% GH and 50% GH must be conducted regarding DMI and milk yield, as well as ruminal passage rate. The actual effective level of GH proportion in practical feeding must be determined to maximize the voluntary feed intake and performance of lactating dairy cows.

Conclusion

The current study tested whether the ruminal passage rate of CS particles in a CS-based diets would decrease with increasing dietary GH proportion by means of synchronization with the intrinsically slow passage rate of GH. This hypothesis was rejected, as the ruminal passage rate of CS particles was not fractuated by GH proportion and hardly differed from that of GH. On the other hand, this study found a quadratic change of ruminal passage rate for GH particles and a trend of linear increase of size reduction rate for CS particles with increasing GH proportion. Therefore, it can be concluded that the associative effects on the ruminal particle kinetics between CS and GH are generated when cows are fed CS-based diets with increasing proportion of GH. This study also showed that the increased fiber digestibility with increasing GH proportion from 30% to 50% in CS based diets. The increase in dietary fiber digestibility with GH proportion may be attributable to both of the intrinsic digestibility and the decreased ruminal passage rate of GH.

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Table 1 Chemical composition of corn silage and grass hay

	Corn silage	Grass hay
DM, % of fresh matter	32.4	90.1
OM, % of DM	94.3	93.4
CP, % of DM	7.6	9.3
NDF, % of DM	41.7	77.0
ADF, % of DM	25.0	45.4
ADL, % of DM	2.9	5.9
Starch, % of DM	21.1	0.0
96-h in vitro DM digestibility, %	74.4	70.4
96- h in vitro NDF digestibility, %	59.4	70.8

DM: dry matter; OM: organic matter; CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; ADL: acid detergent lignin.

Table 2 Dry matter intake and total tract digestibility of non-lactating Holstein cows fed corn silage-based diets supplemented with 0 to 50% (dry matter basis) grass hay

	Grass hay proportion (%)							*P*-value	
	0	10	20	30	40	50	SEM	Linear	Quadratic
DM intake, kg/day	10.0	10.0	10.0	9.8	9.6	9.8	0.09	0.002	0.634
Digestibility, %									
DM	59.6	58.4	57.6	55.7	54.7	59.2	1.36	0.209	0.038
OM	62.1	61.0	60.1	58.1	57.0	61.2	1.32	0.108	0.045
CP	47.8	46.9	48.3	47.0	45.4	51.4	1.64	0.388	0.147
NDF	45.3	44.3	45.2	45.6	47.4	55.9	1.93	0.001	0.009
ADF	46.0	43.8	43.2	42.4	44.7	52.5	1.83	0.035	0.002
Starch	83.2	89.4	90.7	90.8	89.3	93.0	1.74	0.003	0.157

DM: dry matter; OM: organic matter; CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; ADL: acid detergent lignin.

Table 3 Chewing activities of non-lactating Holstein cows fed corn silage-based diets supplemented with 0 to 50% (dry matter basis) grass hay

	Grass hay proportion (%)						SEM	*P*-value	
	0	10	20	30	40	50		Linear	Quadratic
Eating time									
min/day	70	94	128	148	165	172	7.8	<0.001	0.044
min/kgDM intake	7	10	13	15	18	18	0.9	<0.001	0.059
min/kgNDF intake	17	21	27	30	32	30	1.8	<0.001	0.012
Ruminating time									
min/day	432	432	469	462	490	474	10.3	<0.001	0.234
min/kgDM intake	43	43	47	47	51	49	1.1	<0.001	0.235
min/kgNDF intake	104	95	97	91	94	83	2.3	<0.001	0.710
Chewing time									
min/day	501	526	596	610	655	647	12.5	<0.001	0.028
min/kgDM intake	50	53	60	62	69	66	1.4	<0.001	0.044
min/kgNDF intake	121	117	124	121	126	113	3.0	0.611	0.070

Table 4 Rumen digesta weights and particle size distribution in the rumen of non-lactating Holstein cows fed corn silage-based diets supplemented with 0 to 50% (dry matter basis) grass hay

	Grass hay proportion (%)							*P*-value	
	0	10	20	30	40	50	SEM	Linear	Quadratic
Rumen digesta weight									
Fresh weight, kg	93.8	99.7	99.8	103.8	102.3	104.2	1.26	<0.001	0.031
Dry weight, kg	11.5	11.9	11.5	11.6	12.0	11.8	0.39	0.526	0.916
NDF weight, kg	8.2	8.5	8.4	8.4	8.7	8.2	0.28	0.518	0.550
Particle size distribution, %									
>1.18mm	33.1	33.0	35.4	31.4	28.9	32.2	2.28	0.295	0.903
<1.18mm, >0.15mm	31.3	35.5	35.1	31.0	33.5	29.4	2.00	0.253	0.115
<0.15mm	35.7	31.5	29.5	37.6	37.5	38.4	2.92	0.120	0.232

Particle size distribution: dry matter basis

Table 5 Ruminal fermentation parameters and ruminal liquid passage rate of non-lactating Holstein cows fed corn silage-based diets supplemented with 0 to 50% (dry matter basis) grass hay

	Grass hay proportion (%)						SEM	*P*-value	
	0	10	20	30	40	50		Linear	Quadratic
pH	6.77	6.79	6.76	6.77	6.78	6.89	0.028	0.013	0.013
NH_3-N, mg/dL	6.37	5.19	4.77	5.70	6.02	5.86	0.67	0.881	0.188
Total VFA, mmol/dL	6.67	6.78	6.71	6.91	7.20	6.50	0.227	0.746	0.204
Acetate, mmol/100mmol	66.1	67.5	67.9	70.3	69.9	70.8	0.72	<0.001	0.205
Propionate, mmol/100mmol	17.1	16.1	16.4	15.8	15.7	15.8	0.49	0.003	0.237
Butyrate, mmol/100mmol	12.2	12.1	11.5	9.9	10.5	9.6	0.53	<0.001	0.580
Liquid passage rate, %/h	9.87	9.41	9.99	8.92	8.91	9.15	0.339	0.041	0.726

Table 6 Ruminal digesta kinetics of finely chopped corn silage and grass hay

	Grass hay proportion (%)							*P*-value	
	0	10	20	30	40	50	SEM	Linear	Quadratic
Corn silage particle									
k1, %/h	9.68	9.31	10.28	10.34	11.46	11.69	1.062	0.077	0.718
k2, %/h	3.14	3.25	3.41	3.12	3.06	2.95	0.142	0.152	0.140
MRT, h	51.2	49.9	46.6	48.7	48.2	49.9	1.98	0.566	0.161
Grass hay particle									
k1, %/h		8.37	10.51	9.51	10.45	10.84	1.242	0.235	0.745
k2, %/h		3.33	4.77	4.03	3.67	2.73	0.54	0.195	0.047
MRT, h		53.3	44.1	49.4	49.7	55.8	2.649	0.230	0.021

k1: mean rate of size reduction for large particle in the rumen

k2: passage rate of small particles from the rumen

MRT: ruminal mean retention time of particle

和文抄録

コーンサイレージ主体飼料中のイネ科乾草割合が消化率とルーメン内容物動態に及ぼす影響

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ルーメンカニューレ装着ホルスタイン種乾乳牛 6 頭を供試し、細切コーンサイレージ（CS）主体飼料中の切断イネ科乾草（GH）割合が消化率とルーメン内容物動態に及ぼす影響を検討した。CS および GH の設定切断長はそれぞれ 9 ㎜および 60 ㎜であった。CS 主体飼料中に GH の混合割合を乾物で 0%, 10%, 20%, 30%, 40%, 50%とする飼料を 6 × 6 ラテン方格法により配置し試験を実施した。Dy で標識した CS および Er で標識した GH をルーメン内に投与し、投与後 96 時間までに糞を採取した。糞中の各標識元素の排出曲線からルーメン内容物の通過動態を解析した。GH 割合の増加によって中性デタージェント繊維および酸性デタージェント繊維の全消化管消化率は２次曲線的な変化を示した($P < 0.01$)。GH 割合はルーメン内容物の総 DM 重量および粒度分布に影響しなかった。CS の大飼料片のルーメン内微細化速度は GH の増加により増加する傾向が見られた（$P = 0.077$)。GH の小飼料片のルーメン内通過速度には GH 割合の増加に伴う 2 次曲線的な増加の影響が認められたが($P < 0.05$)、CS の小飼料片の通過速度には GH 割合は影響を及ぼさなかった。以上の結果から，細切 CS 主体飼料中の GH 割合を変えることにより，ルーメン内での CS の微細化速度と GH の通過速度が変動することが示唆された。