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The Change of Feeding Behavior and Intra-Ruminal Property with Intake of White Clover on Sheep

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Summary

The present study was carried out to investigate the changes of feeding behavior, ruminal fermentation and physical property of ruminal fluid when ruminants ingested a large amount of white clover. Four Suffolk and Corriedale crossbred wether sheep (mean BW 38.0 kg) fitted rumen fistula was used for the experiment in metabolic cages. Eating and rumination time, ruminal VFAs and NH_3N concentrations, protozoal density, and foam stability of ruminal fluid were investigated with the lapse of time after switching to fresh white clover feeding from fresh orchardgrass feeding for 72 hr.

Eating and rumination time significantly decreased after switching to white clover feeding. Ruminal propionic acid, butyric acid, NH_3N and protozoal density significantly increased on and after 48 hr from switching to white clover feeding. Furthermore, foam stability of ruminal fluid significantly increased on and after 48 hr from switching to white clover feeding. These changes on feeding behavior and ruminal phenomena might occur also in ruminants by ingesting a large amount of white clover on grass/clover pasture. In particularly, the increase of foam stability formed in the rumen might influence the digestion and absorption of nutrients in herbage except bloat effect. It is important to clarify not only the change of ruminal fermentation but also physical function of stable foam formed in the rumen on the utilization of nutrients in herbage.

White clover (*Trifolium repens* L.) is the most popular legume introduced in temperate pasture. White clover is high quality in nutrient and easily digested in the rumen as compared with grass (1, 2), however, occurrence of ruminal bloat is concerned on it's grazing use (3). Ogura and Sugawara (4) reported that white clover took a high voluntary intake than orchardgrass under free choice of white clover and orchardgrass by sheep when their appetite had uplifted, and it was suggested that a large quantity of white clover might be grazed in a short time by animals in such the time as immediately after changing paddock or setting up the grazing on mixed grass/white clover pasture. Dobson (5) indicates the importance of investigation on physiological changes in the period of dietary transition and grazing in association with metabolic disorders of animals. In such situation that a large quantity of white clover is ingested by animals in a short time, metabolic disorders as hypomagnesemia and bloat may occur in ruminants. It is important to clarify the metabolic change in ruminants when a large amount of white clover was grazed, in order to promote grazing use of white clover. In this study, the changes of feeding behavior, ruminal fermentation and foam stability of ruminal fluid were investigated when a large amount of white clover was ingested.

Materials and Methods

Animals and management

The orchardgrass (*Dactilis glomerata* L., cv. Hayking) and white clover (*Trifolium repens* L., cv. California Ladino) were mowed from monocultured grasslands, respectively, at the Kawatabi Experimental Farm of Tohoku University. Forages were harvested daily and fed to animals on fresh.

Four 8-months old Suffolk and Corriedale crossbred wether sheep (mean BW 38.0 kg) fitted a rumen fistula (26 mm i.d.) were kept in metabolic cages and used for the experiment. Before the experiment, the wethers were fed a mixture containing several grass species.

The experiment started on 10 October 1994 and consisted of 6 days, with day 1 through day 3 for offering of orchardgrass and day 4 through day 6 for offering of white clover. Herbage intake was measured every 2 hrs for *ad libitum* intake by deduction of remaining from offering herbage. Water and trace mineralized salt were available continually.

Chemical analysis of herbage

The forage samples were dried at 70°C for 48 hr to determine dry matter (DM), and ground with a Wiley mill to pass 1-mm screen for subsequent analysis. Crude protein (CP) was measured using the macro Kjeldahl method (6). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were measured by the method of Abe (7). Energy content was analyzed by using Yoshida bomb calorie meter-H.

Observation of feeding behavior

During the experiment (for 6 days), behavior of animals was recorded with two video cameras and time lapse video decks. After the experiment, eating time and rumination time was measured by 1 minute scan sampling.

Analysis of ruminal fluid

Ruminal contents were collected at 0 (immediately before white clover feeding), 2, 4, 6, 12, 18, 24, 36, 48, 60, and 72 hr after switching to white clover feeding, and 50 ml of fluid fractions were sampled by straining through two layers of gauze. The pH of fluid fraction was measured by Horiba F-7ssII pH meter immediately after sampling and fluid samples were prepared for subsequent analysis of volatile fatty acid (VFA), ammonia N (NH_3N) and protozoal density.

For the ruminal VFA measurement, 1 ml of the fluid was centrifuged at 12,000×g, 4°C for 10 min, and 0.5 ml of 5N-phosphoric acid (contained 60 mM crotonic acid) was added to 0.5 ml of the supernatant. The mixture was centrifuged again at 12,000×g, 0°C for 10 min, and the supernantants were stored at −35°C for VFA analysis. Ruminal VFA concentrations were determined by using Yanagimoto G-3800 gas chromatography equipped with a flame ionization detector. A glass column (225 cm×3 mm i.d.) packed with Shimadzu FAL-M on Shimalite TPA was used for chromatography of VFAs. Analysis was carried at 158°C of oven temperature; and 50 ml/min of carrier gas flow (N_2).

For ruminal NH_3N, 2 ml of ruminal fluid was acidified with 2 ml of 5N-H_3PO_4 and stored at −35°C until analysis by CONWEY's method (8).

For measurement of protozoal density in the ruminal fluid, 2 ml of ruminal fluid was mixed with 2 ml of MFS solution (35% hormaldehyde 100 ml+distilled water 900 ml+NaCl 8.0 g+methyl green 0.6 g) and stored at 4°C. Protozoa number of 10 μl of stored sample was counted by microscope with a magnification of 150.

Measurement of foaming of ruminal fluid

20 ml of ruminal fluid taken into a plastic centrifuge tube (50 ml) was foamed by mixing with a homogenizer (Sanshin Kogyo Ltd.) at 10,000 rpm for 1 min at room temperature. The height of foam formed in the tube was measured at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30 and 60 min after mixing. Foaming power was regarded as foam height at 0 min and foam stability was estimated by disappearance of foam height as the lapse of the time after mixing.

Statistics

All data were analyzed by using analysis of variance. Factors in the model were sheep and the time after switching to white clover feeding from orchardgrass feeding. Duncan's multiple range test was used when the effect of time after switching the herbage was significant ($p<0.05$).

Results

Chemical composition of herbage

Chemical composition and gross energy content of herbage are shown in Table 1. Orchardgrass was higher in NDF and ADF than white clover, whereas white

TABLE 1. *Chemical Composition and Energy Content of Herbage.*

Herbage	Moisture (%)	% of day matter (DM)			Energy (MJ/gDM)
		CP	NDF	ADF	
Orchardgrass	77.4	15.1	58.6	33.4	19.65
White clover	89.2	26.7	26.6	21.9	19.79

clover was higher in moisture and CP. Energy content was nearly equal in both herbages.

Herbage intake

The change of DM intake of white clover after switching from orchardgrass was shown at 2 hr intervals in Fig. 1. The highest rate of intake was appeared immediately after switching to white clover feeding, that is, 157 gDM/head at 0–2 hr. After that, DM intake decreased to 22 hr, and increased again to 44 hr. DM intake at 44–46 hr was 159 g/head which was approximate equal to that immediately after switching to white clover feeding from orchardgrass feeding. Thereafter, the rate of white clover intake showed little variation in the range of 62 to 100 gDM/head.

In sole feeding of orchardgrass or white clover, average DM intake of herbage

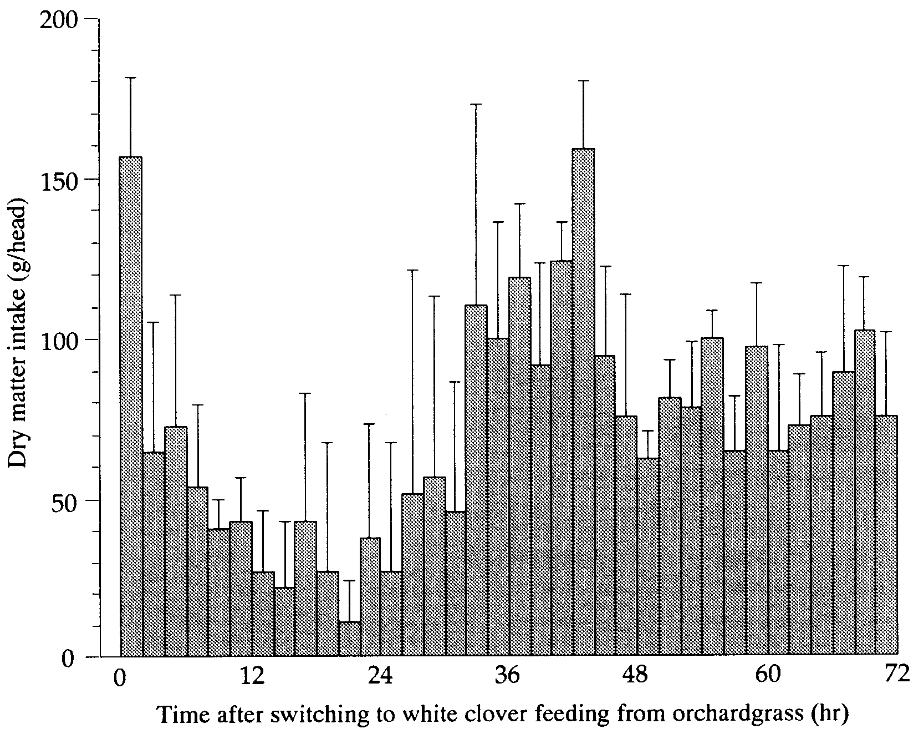

FIG. 1. The change of mean (±SD) dry matter intake of herbage by sheep after switching to white clover feeding from orchardgrass feeding.

was 870 gDM/head/day in white clover and 1,250 g/head/day in orchardgrass, respectively.

Eating and rumination time

The changes of eating time and rumination time of sheep per day were shown in Table 2. In orchardgrass feeding, eating time and rumination time were 9.0-10.7 hr and 7.7-8.5 hr, respectively. However, after switching to white clover feeding, both eating and rumination time decreased significantly ($p<0.05$). The rate of rumination time/eating time in day 2 and day 3 of white clover feeding also significantly decreased ($p<0.05$) comparing with the rate in orchardgrass feeding.

The changes of ruminal phenomena

The pH of ruminal fluid was shown in Fig. 2. Ruminal pH, which was 6.5

TABLE 2. *Eating Time (ET), Rumination Time (RT) and RT/ET Ratio of Sheep with Intake of White Clover.*

	OG[1)]	WC-day 1	WC-day 2	WC-day 3
ET (hr)	$9.95 \pm 1.27^{A2)}$	4.93 ± 1.49^{C}	6.80 ± 2.32^{B}	6.14 ± 1.49^{BC}
RT (hr)	8.00 ± 0.81^{A}	4.71 ± 0.45^{B}	3.06 ± 0.47^{C}	3.38 ± 0.42^{C}
RT/ET	0.82 ± 0.16^{B}	1.01 ± 0.23^{A}	0.50 ± 0.21^{C}	0.58 ± 0.18^{C}

1) OG : orchardgrass, WC : white colver.
2) Mean values ± SD (n=4). Mean values within same rows with different subscripts are significantly different ($p<0.05$).

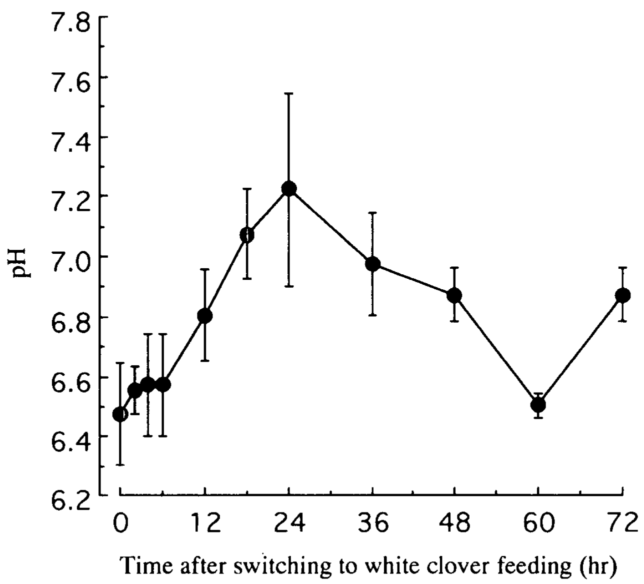

FIG. 2. The change of ruminal pH of sheep with intake of white clover.

in orchardgrass feeding (=0 hr) increased to 7.2 at 24 hr after white clover feeding. Thereafter, pH lowered to 6.6 at 60 hr. Although pH varied from 6.5 to 7.2 with intake of white clover, it was not regarded as abnormal change on rumen function.

The changes of ruminal VFA concentrations were shown in Fig. 3. VFA concentration decreased coincidentally from 0 hr to 24 hr after switching to white clover feeding with decrease of DM intake of white clover. After 24 hr, VFA concentrations turned to increase, and total VFA at 48 hr was significantly higher than that at 0 hr ($p<0.05$), and acetic acid concentration at 48-60 hr rose up to nearly same level as that at 0 hr ($p>0.1$). The concentrations of propionic acid and butyric acid were significantly higher on and after 48 hr than at 0 hr ($p<0.05$). Consequently, the rates of propionic acid and butyric acid to toal VFA were also significant higher on and after 48 hr than 0 hr ($p<0.05$).

As shown in Fig. 4, ruminal NH_3N concentration varied scarcely during 0 to 24 hr, ranging from 17.4 to 20.4 mg/dl. But it turned to increase after 24 hr and peaked at 38.6 mg/dl of 60 hr.

Protozoal density in the ruminal fluid also increased with the course of white clover intake (Fig. 5). Especially, the rate of increase was high at 0 to 24 hr and 48 to 72 hr. Protozoal density was significantly higher at 60 and 72 hr than 0 hr ($p<0.05$).

The foam stability of ruminal fluid was shown in Fig. 6. The foam heights at 0 min after mixing showed little difference among every samples. Consequently, foaming power of ruminal fluid was nearly constant throughout the experiment. Foam stability did not rise until 36 hr after white clover feeding, but on and after 48 hr, it significantly increased ($p<0.01$).

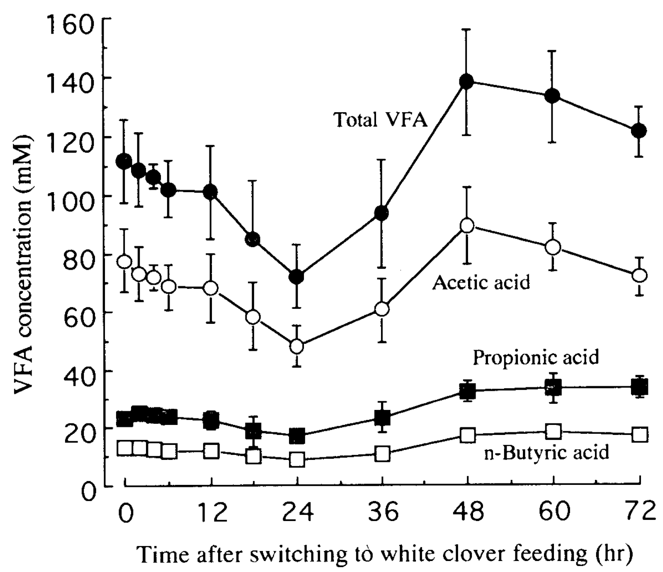

FIG. 3. The change of ruminal VFA concentrations of sheep with intake of white clover.

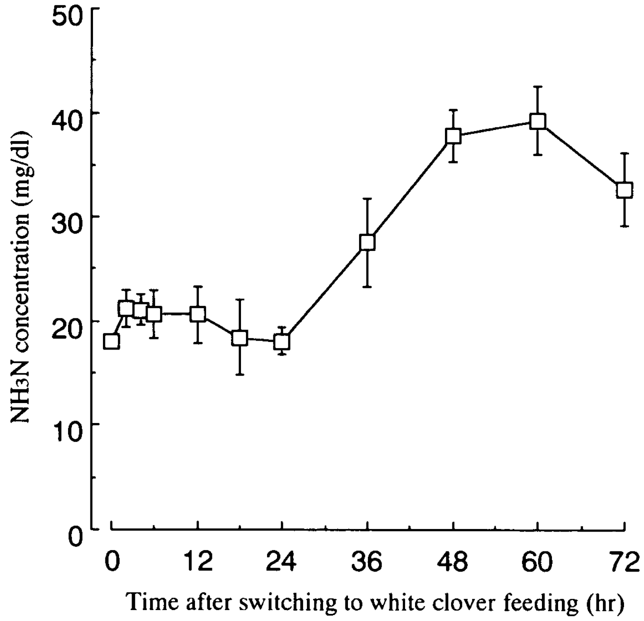

FIG. 4. The change of ruminal NH_3N concentration of sheep with intake of white clover.

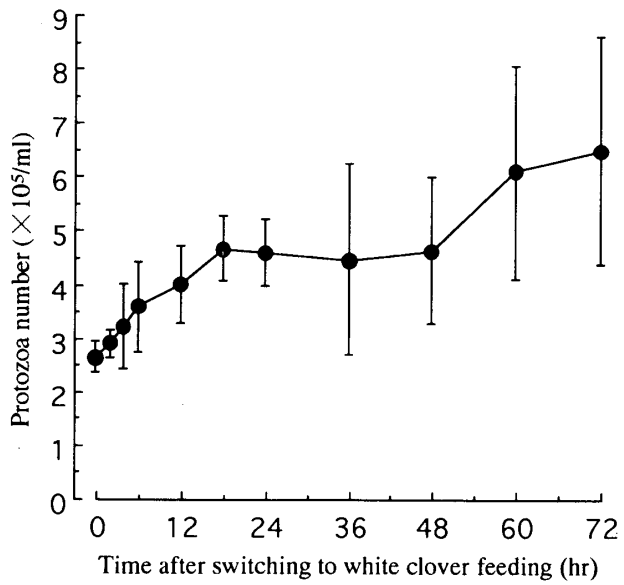

FIG. 5. The change of protozoa number in ruminal fluid of sheep with intake of white clover.

Discussion

This study is to investigate the changes on feeding behavior and characteristics of ruminal phenomena when white clover was fed freely to ruminants.

Eating time and rumination time reduced from day 1 after switching to white clover feeding from orchardgrass feeding (Table 2). The rumination time is

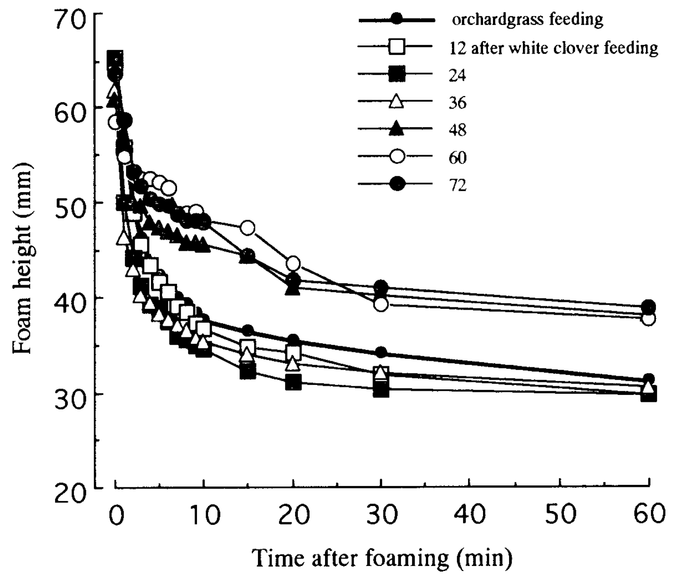

FIG. 6. The change of foaming power and foam stability of rumen fluid of sheep with intake of white clover.

known to be correlated with the contents of cell wall constituents of feed (9, 10). The reduction of rumination time with intake of white clover may be due to lower fiber content and higher digestibility in white clover than that in orchardgrass. However, the rate of RT/ET in white clover-day 1 was significantly higher than that in orchardgrass. That is because the degree of decrease of RT was lower than that of ET in white clover-day 1. The long RT in white clover-day 1 may be attributed to the remaining of orchardgrass which was fed prior to feeding of white clover in the rumen.

The concentrations of ruminal propionic acid, butyric acid and NH_3N increased with intake of white clover (Figs 3 and 4). These results agree with the result of Annison (11), who observed the increase of ruminal propionic acid and NH_3N when feeds were transferred from hay to lush spring grass. These changes of ruminal fermentation proceed in ingestion of a large amount of readily fermentable protein and non-protein substances. Grubb (12) also showed that the concentrations of propionic acid and butyric acid was increased with an abrupt change of diet from whole roughage to high concentrate. Beever *et al.* (13, 14) reported higher ruminal NH_3N level when steers were given white clover than when given perennial ryegrass. The increase of ruminal propionic and butyric acid, and NH_3N concentration observed in this study appear to be due to rapid degradation of soluble carbohydrate and readily fermentable protein contained richly in white clover by rumen microorganisms.

Protozoal density in the ruminal fluid was increased with the intake of white clover (Fig. 5). It is known that protozoal density in the ruminal fluid increase when the available energy intake is raised (15, 16). As shown in Table 1, gross energy contents in white clover was nearly equal to that in orchardgrass, but the fiber content in white clover was lower than that in orchardgrass. In this experiment, the high protozoal density in ruminal fluid after switching to white clover feeding may be due to increase of supply of readily fermentable substances to rumen microorganisms. Furthermore, protozoa number was not constant through the experimental period. Grubb (12) observed that protozoal density reached a plateau on about 5 days after changing whole hay to high concentrate. In the present work, if white clover was fed for additional term, further increase of protozoal density would be expected.

From the results as described above, the changes of ruminal fermentation were ascertained to be able to be caused easily by ingesting white clover in ruminants.

However, the changes of ruminal property appeared in not only ruminal fermentation, but also the foam stability of ruminal fluid (Fig. 6). Many substances such as water-soluble protein and saponin (17), which are contained in plants and increase the foam stability of ruminal fluid, are known. In this experiment, the increase of the foam stability of ruminal fluid with the intake of white clover might be due to participation of the substances released into the ruminal fluid with disruption of plant tissue. In addition, protozoal polysaccaride and protein produce very rigid foams (18, 19). Thus, the increase of the density of rumen protozoa with intake of white clover (Fig. 5) might contribute to produce stable foams of ruminal fluid.

In this experiment, bloat did not appear in the tested animals, but highly stable foam after white clover intake was observed in all sheep with white clover feeding. The formation of the stable foam must be because of ruminal fermentation products of white clover and foamy substances into the rumen from plant tissue.

It can be considered that the majority of ruminal fluid exists as foams in the rumen when a ruminants is ingesting a large amount of white clover. Clarke and Hungate (20) found that clover protein was degraded rapidly, but the degradation was retarded after denaturation by foaming. This gives a possibility that foaming of ruminal fluid may affect at least to the digestion of soluble protein eluted from herbage. Furthermore, it can be expected that the foam affects the action of rumen microorganisms and dynamics of fluid and absorption of nutrient such as VFAs in the rumen. Then, whether ruminal bloat occurs or not, the foaming of ruminal fluid may influence the digestion and absorption of nutrients in herbage. The clarification on the relation between the foaming of ruminal fluid and degradation and adsorption of nutrients seems to be useful on nutritional physiology in ruminants.

The present results showed the significant changes in eating time and rumination time, ruminal fermentation, and physical property of ruminal fluid with intake of a large amount of white clover. In the grazing on clover/grass mixed sward or clover dominant sward, a large amount of clover may be ingested animals in the time such as immediately after changing paddock or setting up the grazing as suggested by Ogura and Sugawara (4). But they also suggested that substances contained in white clover might regulate the intake of white clover itself. The regulation of white clover intake in ruminants, if it exists, might be associated with the changes of intra-ruminal propetry observed in this experiment. On the other hand, foam stability of ruminal fluid is closely related with bloat. Further works are needed to know the effects of metabolic changes with the intake of a large amount of white clover on the voluntary intake and utilization of nutrients of herbage in addition to induction of ruminal bloat, in order to promote the grazing use of white clover by ruminants.

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