Estimation of Rumen Gas Volume by Dilution Technique in Sheep Given Two Silages at Different Levels of Feeding

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ABSTRACT : The gas dilution technique was used to evaluate the possibility of estimating the volume of gaseous phase in the rumen from its composition in sheep given rice whole crop silage (RWS) or dent corn silage (DCS) at a level of maintenance (M) or 2 M, and in the course of fasting. The rumen gas composition was determined at 2 and 7.5 h after morning feeding. Nitrogen gas was injected by using an airtight syringe into the rumen immediately after collecting the rumen gas sample as a control. Then rumen gas samples were collected at 5, 10, 20, 40 and 60 min. after injection. Dry-matter intakes were 42 g/kg$^{0.75}$ and 57 g/kg$^{0.75}$ for DCS, and 36 g/kg$^{0.75}$ and 59 g/kg$^{0.75}$ for RWS, at 1 M and 2 M levels, respectively. Animals ingested both silages about 20% less than expected at 2 M level. The rumen gas composition did not differ significantly between 2 h and 7.5 h after feeding except for N_2. Content of CO_2 in gas composition was significantly higher at 2 M level than at 1 M ($p<0.05$) for both RWS and DCS, whereas CH_4 showed no significant difference between feeding levels. At both feeding levels, CO_2 showed a higher ($p<0.05$) percentage in DCS than RWS. A dilution technique by using N_2 injection is not appropriate for the determination of gas production *in vivo*, unless the rate of rumen gas turnover is considered. Changes in composition at fasting indicate that the rumen fermentation may reach the lowest level after 72 h fasting for sheep given silage as their sole diet. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 3 : 380-383)***

Key Words : Rumen, Gas Dilution Technique, Fasting, Silage, Sheep

INTRODUCTION

Recently, consumption rates of rice per capita have been decreasing in the regions of northeast and southeast Asia (Ito, 2000). Japan has started studying the effective usage of rice crops to maintain the environment and the productivity of the paddy field such as grazing and whole crop silage for animal feed. The evaluation of the nutritive value of forage, however, is time consuming and accompanied by laborious work. *In vitro* gas production has been used as a measure to evaluate fermentation status (Abdulrazak et al., 2000). The method for estimation of energetic value of feed from the data on chemical composition of feed and *in vitro* gas production has been presented (Menke and Steingass, 1988). It has been demonstrated that *in vivo* rumen gas can be successfully collected from calves with and without rumen cannulae using an airtight syringe (Sumio et al., 1983). It may be convenient for evaluation of ruminal fermentation under a given feeding regimen that *in vivo* gas production is to be determined by a simple method. The *in vivo* gas production may be calculated by changes in the volume of rumen gaseous phase of an animal under a given feeding regimen.

The present study was designed to evaluate the possibility for estimating the volume of gaseous phase in the rumen and to determine gaseous composition in the rumen of sheep given rice whole crop silage or corn silage at a level of maintenance or twice maintenance, and in the course of fasting.

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MATERIALS AND METHODS

Four Corriedale wethers weighing 60 kg on average were individually kept in a metabolism crate. Diets were offered to sheep twice a day at 09:00 and 17:00 in equal portions of daily allowance with free access to water and mineralized salt block. Diets offered were dent corn silage (DCS) and rice whole crop silage (RWS) both harvested at yellow ripe stage, and were given to animals at maintenance (M) and twice M (2 M) levels for 2 weeks of each period under Latin square design. The last 5 days of each period were used as a comparison period for digestion trial. Samples of silage offered, feed refusals and feces were collected in a comparison period and analyzed for dry matter (DM), organic matter (OM) and proximate composition by the official methods of AOAC (1970). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined by the method described by Goering and Van Soest (1970). The pH of silage was measured with a glass-electrode pH meter (HM-5A, TOA Electronics). The rumen gas composition was determined on the day before the end of the comparison period at 2 and 7.5 h after morning feeding, and the volume of gas phase on the last day at 2 h after morning feeding using an airtight syringe (Sumio et al., 1983). Nitrogen gas was used as an inert gas for dilution to estimate gaseous volume in the rumen. The nitrogen gas was injected into the rumen immediately after collecting rumen gas samples as a control. Subsequently, rumen gas samples were collected at 5, 10, 20, 40 and 60 min. after injection of nitrogen gas. The composition of rumen gas was determined chromatographically using 2 sets of LPG analyzers combined in one system with helium gas as a carrier. Each analyzer was equipped with either molecular sieve 5A column of 200 cm in length for determination of O_2, N_2 and CH_4 or silica gel column of 50 cm for CO_2. After the last period of the treatment, all animals were fasted for 5 days. The rumen gas samples were collected every 24 h for the first 3 days of fasting and on the last day. Samples were analyzed for their composition as described above to determine the change in gas composition for the estimation of fermentation in the rumen.

RESULTS

Proximate composition of DCS and RWS was similar with a tendency for higher fibrous fractions and lower CP and NFE in RWS than DCS, as shown in Table 1. The pH was satisfactorily low in both silages with a tendency for a higher value for RWS. Table 2 shows mean dry-matter intake (DMI) and digestibility of nutrients for each treatment. The DMI on the basis of metabolic body size on 2 M was higher by 15 and 23 units over 1 M level of DCS and RWS feeding, respectively. There was no significant difference in DMI on the basis of metabolic body size between silages, although daily intake differed about 100 g/day between DCS and RWS at both feeding levels. Animals ingested both silages about 20% less than expected at 2 M level, although weight gain occurred in this period. The digestibility of nutrients showed no significant differences between levels of feeding in both silages. Digestibilities of CP, NDF and ADF did not significantly differ between diets, whereas those of DM and NFE were higher for DCS than RWS.

The concentrations of CO_2 and CH_4 tended to be higher and of O_2 and N_2 lower at 2 h after feeding in both 1 M and 2 M levels as shown in Figure 1. There was, however, no significant difference in rumen gas composition between 2 h and 7.5 h after feeding except for N_2. Results were pooled and are shown in Table 3 for each treatment. The percentage of CO_2 was significantly higher at 2 M level than at 1 M ($p<0.05$) for both RWS and DCS, whereas CH_4 showed no significant difference between feeding levels with a tendency for a greater mean at 1 M level of feeding. At both feeding levels, CO_2 showed a significantly higher percentage in DCS than RWS ($p<0.05$). The percentages of N_2 and O_2 were not significantly different between feeding levels or diets, although RWS tended to be greater than

Table 1. Proximate composition and pH of dent corn silage (DCS) and rice whole crop silage (RWS)

	DCS	RWS
Dry matter, g/kg	304	307
OM, g/kgDM	945	867
CP, g/kgDM	95	83
NFE, g/kgDM	597	441
NDF, g/kgDM	437	551
ADF, g/kgDM	239	407
ADL, g/kgDM	26	56
pH of silage	3.8	4.6

Table 2. Dry-matter intake (DMI), digestibilities of nutrients and the intakes of digestible components for dent corn silage (DCS) and rice whole crop silage (RWS)

	DCS		RWS	
	M	2M	M	2M
DMI, g/day	913±50	1,207±84	768±51	1,301±139
$g/kg^{0.75}\cdot day$	4.2±2.3	57±4.1	36±2.4	59±6.5
Digestibility, g/kg				
DM	640±19	630±43	490±20	486±36
OM	674±24	661±44	551±14	545±32
CP	480±67	534±24	447±18	445±31
NFE	750±15	742±41	614±13	587±28
NDF	427±21	400±73	334±16	303±43
ADF	355±18	332±67	288±31	270±54
Intake of digestible nutrients, g/day				
DOMI	582±31	754±52	367±24	615±65
DCPI	42±2	61±4	28±2	48±5
DNFEI	409±22	535±37	208±13	255±27
DNDFI	170±9	211±15	141±9	217±23
DADFI	78±4	96±6	90±6	143±15

Values are means with standard deviation.

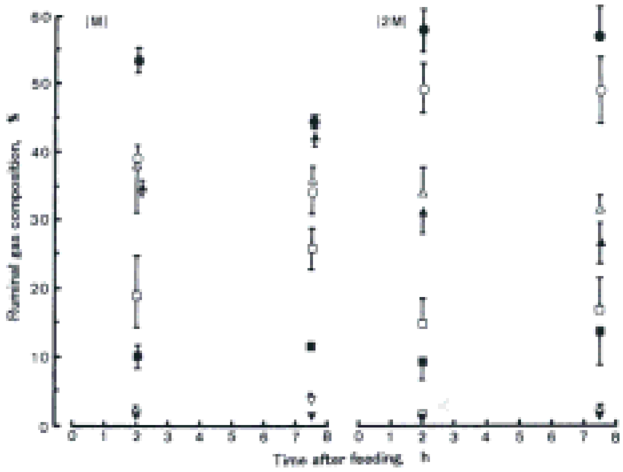

Figure 1. Changes in the composition of the ruminal gas phase of sheep given two different silages at the level of maintenance (M) and twice maintenance (2 M) at 2 and 7.5 h after morning feeding. Legend : CO_2 (circle), CH_4 (triangle), N_2 (square) and O_2 (inverse triangle), and feeding of rice whole crop silage (open symbol) or dent corn silage (solid symbol). Vertical bars show standard deviation of each mean (n=4).

Table 3. The gas composition (%)† in the rumen of wethers given silage diets at maintenance (M) and twice maintenance (2 M) levels

Silage feeding level	Components of rumen gas			
	CO_2	CH_4	N_2	O_2
D C S				
M	48.7±5.0	38.2±3.7	11.5±1.9	1.6±0.4
2M	57.4±4.9*	28.8±4.0	11.9±5.3	1.9±0.9
R W S				
M	36.7±4.5	37.3±5.7	22.6±5.4	3.4±1.2
2M	49.0±4.5*	33.2±3.5	15.6±4.7	2.2±1.0

† Values are expressed as mean±SD.
* p<0.05.

DCS. The N_2 and O_2 also tended to be higher at 1 M level than 2 M in RWS. Hydrogen gas was not detected in the present study.

Changes in N_2 gas percentage after the injection into the rumen of sheep given DCS is shown in Figure 2. The N_2 gas concentration showed curvilinear changes with time after injection. The regression equations were highly significant. The reduction rate of the percentage at 2 M level (Figure 2a) was greater than that at 1 M level (Figure 2b). The regression equation, however, revealed that the equation may not be extrapolated to the time at zero. Linear regressions, therefore, were calculated and equations were significant.

The rumen gas volume was, then, estimated using linear equations, which turned out to have a diverse range from 267 ml at 1 M level feeding of RWS to 2,000 ml at 2 M level of RWS. For DCS feeding, the estimated volume ranged from 603 ml at 2 M to 1,667 ml at 1 M level.

The rumen gas showed a great change in its composition after 24 h fasting (Figure 3) except for N_2 which increased 2 fold at maintenance after 24 h fasting. The percentage of CO_2 decreased to the level of about 10% after 48 h after feeding and thereafter stayed at about the same level. Similarly, CH_4 reduced its percentage with the time of fasting to the level of 2% at 113 h fasting. The percentage of N_2 increased to the level of a little less than 70% at 48 h fasting and to about 80% at 72 h fasting.

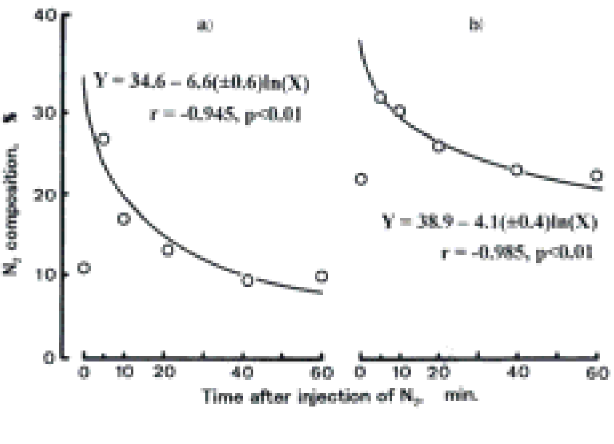

Figure 2. Typical changes in N_2 gas percentage after the injection into the rumen of a sheep.
(a): N_2 gas percentage after the injection of 47 ml N_2 in the rumen of sheep given dent corn silage at the level of 2 M at 2 h after feeding
$Y=34.6-6.6(\pm0.6)\ln(X)$ $r=-0.945$, $p<0.01$
(b): N_2 gas percentage after the injection of 27 ml N_2 in the rumen of sheep given dent corn silage at the level of M at 2 h after feeding
$Y = 38.9 – 4.1(\pm0.4)\ln(X)$ $r=-0.985$, $p<0.01$

DISCUSSION

For 2 M feeding level, DMI on the basis of metabolic body size increased to 140 to 160% of 1 M level, which may have contributed to a small and insignificant change in the digestibility of nutrients between feeding levels in both silages. Feeding levels in the present study, therefore, did not significantly affect the digestibility of nutrients, but a higher level of feeding tended to reduce digestibility for fibrous fractions in both silages. Although CP digestibility did not differ significantly for both silages and those for fibrous fractions tended to be higher in DCS than RWS, the digestibility of DM was higher in DCS than RWS. This higher digestibility of DM has been attributed to a higher NFE digestibility in DCS than RWS.

Rumen gas composition was not significantly different between 2 and 7.5 h after feeding. This result agreed with the results obtained up to 8 h after feeding in a dairy cow given alfalfa hay and grain (Dukes, 1955). Hydrogen however, was not detected in the present study because of sole forage feeding without concentrate supplementation. A

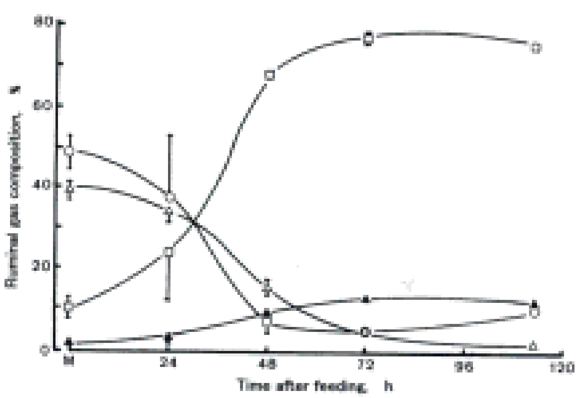

Figure 3. Changes in the percentage of CO_2 (circle), CH_4 (open triangle), N_2 (square) and O_2 (solid triangle) in the ruminal gas phase of sheep fasted for 113 h.
M stands for maintenance feeding. All symbols show mean composition (n=4) with standard deviation. Curvilinear lines are drawn by inspection.

higher CO_2 composition on 2 M level of feeding is attributed to possibly a greater substrate supply for fermentation at a higher feeding level (Table 2). Similarly a greater CO_2 percentage in DCS than RWS is also attributed to a higher DM or OM digestibility in DCS than RWS (Table 2). The digestibility of fibrous fractions tended to be higher at 1 M level of feeding for both silages, with resultant tendency towards higher CH_4 in overall composition at 1 M level of feeding. A higher percentage of N_2 or O_2 in RWS than DCS is probably a result of difference in fermentation levels in the rumen between RWS and DCS. Since the digestibilities of NFE and fibrous fractions for DCS were greater than those for RWS, this difference might have resulted in a greater production of CO_2, which produced a greater suppression of the composition of N_2 or O_2 in DCS than RWS.

The estimation for volume of gaseous phase in the rumen showed inconsistent results because of a higher variation in the estimates of rumen gas volumes ranging about 300 ml to 2,000 ml, which suggests a higher rate of gaseous turnover in the rumen. Besides expulsion by eructation or belching, gases produced in the rumen are also eliminated through the lung after being absorbed in the blood. The rates of gaseous turnover, however, can not be determined reliably in the present study because measurements were not carried out on the frequency of belching and the rate of gaseous absorption in the blood. The study on eating and rumination behavior of cattle revealed significant diurnal fluctuations of rumination patterns (Metz, 1975). The frequency of rumination has been reported to range from 11 times to 17 times/day among 8 cattle. This variation in the rumination frequency may cause variation in the frequency of eructation by an animal, which leads to a varied rate of gaseous turnover in the rumen. Kurihara et al. (1999) reported that daily methane production by Brahman cattle ranged from 65 g/kg of digestible organic matter intake (DOMI) to 75 g/kg of DOMI under feeding regimens of tropical grasses. Assuming that these results are applicable to ovine species, daily methane production was predicted to be 5 l for RWS at 1 M feeding level to 13 l for DCS at 2 M by using the results of the digestion trials in the present study. Daily CO_2 production would be speculated to be about the same for RWS at 1 M or about 26 l for DCS at 2 M from the results of the rumen gas composition presented in Table 3. These variations in gas production, therefore, might have caused a wide variation in gas reserve in the rumen of sheep under different feeding regimens.

From the discussion above, it is concluded that the dilution technique may not be suitable for the estimation of rumen gas volume, unless the gaseous turnover rate is considered.

CO_2 and CH_4 percentages sharply decreased in the first 48 h fasting period, while N_2 percentage showed a sharp increase in the same period. The level of CO_2 percentage was less than 10% after 48 h fasting and that of CH_4 percentage after 72 h fasting. The N_2 percentage also reached to the level of 80% after 72 h fasting, which is the same as the level in the atmosphere. These results indicate that ruminal fermentation decreased to the lowest level after 72 h of fasting. These results agree well with those reported for calves at the age of 4-6 months (Sekine and Asahida, 1990). It is therefore concluded that sheep may reach a quasi-postabsorptive state after 72 h of fasting, when they were given silages as their sole diet.

REFERENCES

Abdulrazak, S. A., E. A. Orden, T. Ichinohe and T. Fujihara. 2000. Chemical composition, phenolic concentration and *in vitro* gas production characteristics of selected Acacia fruits and leaves. Asian-Aust. J. Anim. Sci. 13:935-940.

AOAC. 1970. Official Methods of Analysis. 11th ed. AOAC. Washington DC.

Dukes, H. H. 1955. The Physiology of Domestic Animals. Comstock Publ. Assoc., Ithaca, New York. p. 382

Goering, H. K. and P. J. Van Soest. 1970. Forage Fiber Analyses (Apparatus, Reagents, Procedures, and Some Application). Agriculture Handbook No. 379. Agricultural Research Service, U. S. D. A. Washington DC.

Ito, S. 2000. World Grain Statistics and Graphics. Food and Agriculture Series No. 49. National Food Promotion Association. Tokyo. (In Japanese)

Kurihara, M., T. Magner, R. A. Hunter and G. J. McCrabb. 1999. Methane production and energy partition of cattle in the tropics. Br. J. Nutr. 81:227-234.

Menke, K. H. and H. Steingass. 1988. Estimation of the energetic feed valune obtained from chemical analysis and *in vitro* gas production using rumen fluid. Anim. Res. Develop. 28:7-55.

Metz, J. H. M. 1975. Time patterns of feeding and rumination in domestic cattle. Mededelinge Landbouwhogeschool (Communications Agricultural University), Wageningen 75-12:1-66.

Sekine, J. and Y. Asahida. 1990. Energy metabolism of calf. JARQ. 24:141-148.

Sumio, Y., M. Okubo, J. Sekine and Y. Asahida. 1983. Collection of rumen gases from young calves. Jpn. J. Zootechnol. 54: 60-61. (In Japanese)