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/kg0.75 and 57 g/kg0.75 for DCS, and 36 g/kg0.75 and 59 g/kg0.75 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 N2. Content of CO2 in gas composition was significantly higher at 2 M level than at 1 M (p<0.05) for both RWS and DCS, whereas CH4 showed no significant difference between feeding levels. At both feeding levels, CO2 showed a higher (p<0.05) percentage in DCS than RWS. A dilution technique by using N2 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.

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 O2, N2 and CH4 or silica gel column of 50 cm for CO2. 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 g/day between DCS and RWS at both feeding levels. Digestibilities of CP, NDF and ADF did not significantly differ between silages, although daily intake differed about 100 g/day between DCS and RWS at both feeding levels. 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 CO2 and CH4 tended to be higher and of O2 and N2 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 N2. Results were pooled and are shown in Table 3 for each treatment. The percentage of CO2 was significantly higher at 2 M level than at 1 M (p<0.05) for both RWS and DCS, whereas CH4 showed no significant difference between feeding levels with a tendency for a greater mean at 1 M level of feeding. At both feeding levels, CO2 showed a significantly higher percentage in DCS than RWS (p<0.05). The percentages of N2 and O2 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    |
| DMI, g/day                                 | 913±50 | 1,207±84 |
|                                             | 768±51 | 1,301±139 |
| CP, g/kg DM                                 | 4.2±2.3 | 57±4.1   |
|                                             | 36±2.4 | 59±6.5   |
| Digestibility, g/kg                         | 640±19 | 630±43   |
|                                             | 490±20 | 486±36   |
| OM, g/kg DM                                 | 674±24 | 661±44   |
|                                             | 551±14 | 545±32   |
| NDF, g/kg DM                                | 427±21 | 400±73   |
|                                             | 334±16 | 303±43   |
| ADF, g/kg DM                                | 355±18 | 332±67   |
|                                             | 288±31 | 270±54   |
| Intake of digestible nutrients, g/day        | 582±31 | 754±52   |
|                                             | 367±24 | 615±65   |
| DCP, g/kg DM                                | 42±2   | 61±4    |
|                                             | 28±2   | 48±5    |
| DNDFI, g/kg DM                              | 409±22 | 535±37   |
|                                             | 208±13 | 255±27   |
| DADFI, g/kg DM                              | 170±9  | 211±15   |
|                                             | 141±9  | 217±23   |
| 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: CO2 (circle), CH4 (triangle), N2 (square) and O2 (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).
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.

**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.

**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 | CO₂ | CH₄ | N₂ | O₂ |
|---------------------|-------------------------|-----|-----|----|----|
| D C S M             | 48.7±5.0                | 38.2±3.7 | 11.5±1.9 | 1.6±0.4 |
| D C S 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 |
| R W S 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.

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

$Y = 34.6 - 6.6(±0.6)\ln(X) \ r = -0.945, \ p < 0.01$

(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

$Y = 38.9 - 4.1(±0.4)\ln(X) \ r = -0.985, \ p < 0.01$

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.
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\textsubscript{2} and CH\textsubscript{4} percentages sharply decreased in the first 48 h fasting period, while N\textsubscript{2} percentage showed a sharp increase in the same period. The level of CO\textsubscript{2} percentage was less than 10\% after 48 h fasting and that of CH\textsubscript{4} percentage after 72 h fasting. The N\textsubscript{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.

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