

## Effects of Bacterial Fraction and Proportion of Silage and Concentrate on Rumen Fermentation and Gas Production Profile

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**ABSTRACT :** An *in vitro* experiment was carried out to investigate effects of solid associated (SAB) and liquid associated bacteria (LAB) and the type of incubation substrate on ruminal fermentation and gas production profiles. Bacterial fraction did not influence total numbers of bacteria. Gas production degradation parameters were significantly influenced by bacterial fraction and type of substrate ( $p < 0.05$ ). There was significant interaction between bacterial fraction and type of substrate in gas production ( $p < 0.01$ ). Total VFA concentration and acetic and propionic acid ratio were also influenced by bacterial fraction and type of substrate with little differences in individual VFA concentration. (*Asian-Aust. J. Anim. Sci.* 2004, Vol 17, No. 5 : 643-647)

**Key Words :** Gas Production, Bacterial Fraction, VFA Concentration, Interaction

### INTRODUCTION

*In vitro* incubation experiments are commonly employed by many researchers to measure digestibility and fermentation characteristics in the rumen (Tilley and Terry, 1963; Menke et al., 1979; Blummel and Ørskov, 1993; Theodorou et al., 1994). Two basic components of *in vitro* incubation studies are microbial sources and substrates for microbial fermentation. In most studies squeezed rumen fluid and a single feed have been used as a microbial source and a substrate.

It is well documented that rumen microbial population has a distinct compartment, and population in each compartment is widely variable in the number, species and enzyme activities (Lee et al., 2002; Merry and McAllan, 1983). Therefore, squeezed rumen fluid may not represent whole rumen microbial population, and *in vitro* incubation result may deviate from *in vivo* condition under this condition. Microbial population in the rumen at a given time is essentially dependent on the amount and type of substrate supplied to the incubation system (Dehority and Orpin, 1997; Fakhri et al., 1998; Krause et al., 2000). Therefore, results obtained with a single feed may not be applied to other cases where combinations of substrates are employed.

Effects of microbial preparation and substrate have been fairly well documented, but little information on interaction between microbial fraction and substrate is available in the literature. The present experiment was carried out to examine effects of bacterial fraction and proportion of a concentrate and silage on bacterial number, rumen

fermentation and gas production.

### MATERIALS AND METHODS

#### Substrate and bacterial fractions

Grass silage was prepared from the perennial ryegrass, which was wilted for 24 h, harvested using a precision chop harvester and ensiled in a roofed bunker covered with plastic sheets. Grass silages samples were freeze-dried and ground to pass through a 1 mm screen for incubation study and for chemical analysis. The concentrate was mixture of barley, beet pulp, molasses and soybean meal (Table 1). Three substrate combinations tested in the incubation studies were grass silage (S), concentrate (C) and mixture (60:40) of silage and concentrate (S+C). Rumen contents were taken from a rumen-fistulated dairy cow fed a mixture of 40% concentrate and 60% silage twice daily. To obtain liquid-associated bacteria (LAB) rumen contents was squeezed through four layers of muslin cloth and was centrifuged at 500 g for 10 min to remove particles and the supernatant fraction was recentrifuged at 27,000 g for 10 min to obtain LAB and then filtered through eight layers of cheese cloth to remove floating particles. The final liquid associated bacteria were suspended in CO<sub>2</sub>-saturated rumen buffer solution (Maeng et al., 1976) equivalent to one-half the original volume of rumen content (Figure 1). To prepare solid-associated bacteria (SAB) were suspended in CO<sub>2</sub>-saturated rumen buffer (100 g particle/320 ml), stomached two times for 5min using a Stomacher Lab-Blender (Steward & Co. Ltd., London) using sterile polyethylene bags (18×300 mm) and squeezed through eight layers of muslin cloth. The stomached rumen contents were centrifuged at 500 g for 15 min and the supernatant was filtered through eight layers of cheese cloth. After the supernatant discarded, the residue was re-suspended in CO<sub>2</sub>-saturated rumen buffer solution to adjust pH 6.5 and centrifuged again at 27,000 g for 15 min. The solid residue

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**Table 1.** Chemical composition of silage and concentrates

	Silage	Concentrate
Ingredient (g/kg fresh)		
Barley		584
Molassed sugar beet pulp		220
Molasses		81
Soybean meal		94
Mineral plus vitamins		21
Nutrient composition (g/kg DM)		
DM	291.2	878.4
OM	801.5	791.3
Nitrogen	32.3	24.6
Ether extract	35.1	27.7
NDF	549.4	187.8

was then suspended in CO<sub>2</sub>-saturated rumen buffer solution equivalent to one-half the original volume of rumen content. All procedures were carried out in a way that air contamination minimized.

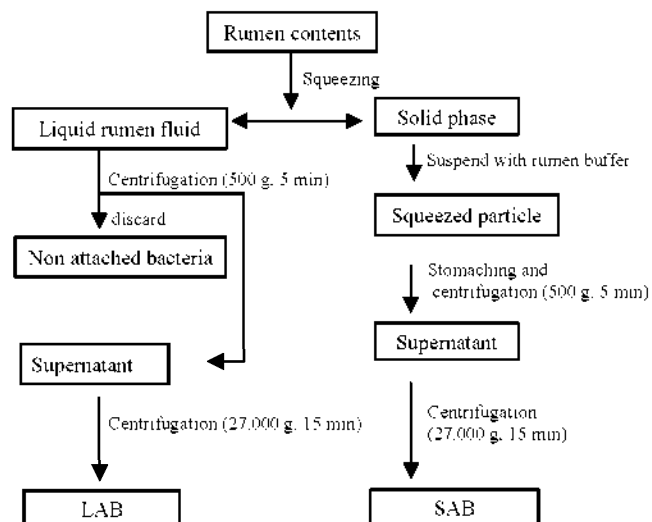
### Incubation

Incubations were carried out in triplicates in the medium (Menke and Steingass, 1988) that supported growth of all the major genera of rumen microorganisms. Culture fluid consisted of 90 ml medium and 10 ml bacterial fraction of both SAB and LAB. The amount of substrate added to each incubation bottle was 1 g (DM basis) for all treatments. Incubation was conducted at 39°C in serum bottles (Wheaton Science Ltd., Millille NJ, USA) sealed with butyl rubber stoppers and aluminum crimp seals. Serum bottles were incubated in 39°C incubator up to 72 h and measured gas production at 3 h interval.

### Analysis

Gas pressure in the headspace was read from the display unit after insertion of the hypodermic syringe needle through the butyl rubber stopper. Gas volume was determined by recording the volume of gas displaced into the syringe barrel on withdrawal of the syringe plunger until the head-space gas pressure to ambient pressure as indicated by a zero reading on the display unit (Theodorou et al., 1994). Volume was recorded using triplicate culture bottles per treatment.

Total numbers of rumen bacteria were estimated by most probable number (MPN) procedures (de Man, 1974) by using 20% clarified ruminal fluid and 80% complete medium (Dehority et al., 1989). Cellobiose (0.25%) and

**Figure 1.** Purification procedure of solid-associated bacteria (SAB), liquid-associated bacteria (LAB) of the rumen contents.

soluble starch (0.25%) were added as energy sources in the complete medium. Colonies were counted after incubation for 7 days at 39°C. Growth in the MPN medium was determined by an increase in visual turbidity. Volatile fatty acid was determined by using method of Erwin et al. (1961) with gas chromatography (HP 6890, Hewlett Packard, USA). Briefly, samples were centrifuged at 27,000 g for 20 min and 100 µl of 25% metaphosphoric acid was added to 1 ml supernatant. A standard solution of a mixture of VFA was used to calculate the concentrations of VFA in the samples.

### Curve fitting and statistical analysis

*In vitro* gas production data were fitted to the equation  $P_t = a + b(1 - e^{-ct})$  (Ørskov and McDonald, 1979).  $P_t$  was gas production at time  $t$ ,  $c$  was the rate constant of gas production and  $b$  was the asymptote of the curve when  $t$  was infinite. The potential gas production was given by  $a + b$ . Statistical analysis was carried out by using General Linear Model (SAS Institute, Inc. 1994).

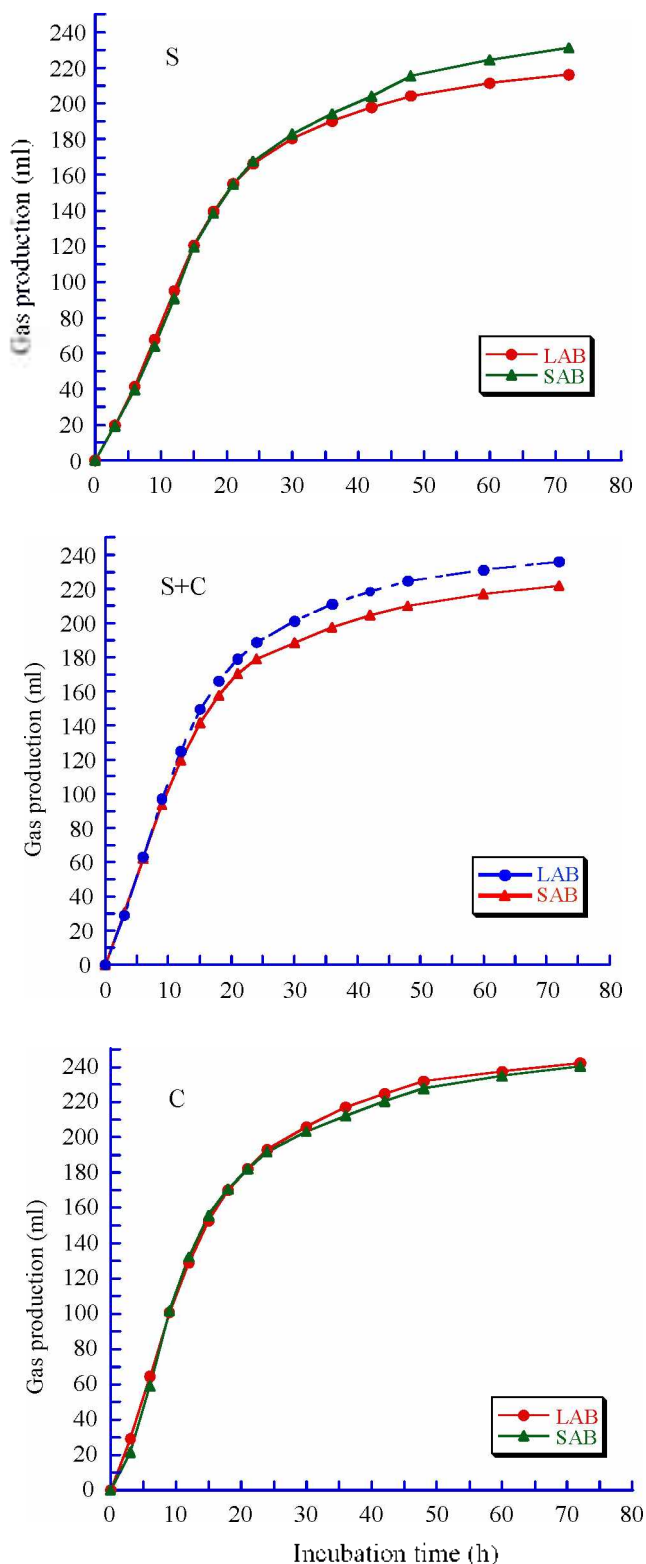
## RESULTS AND DISCUSSION

Influence of bacterial fraction and type of substrate on the pH of incubation medium was minimal (data not shown). Medium pH at the end of *in vitro* incubation was 6.33 for S.

**Table 2.** Cumulative gas production profile between SAB and LAB between grass silage and concentrate

	LAB				SAB				Significance <sup>1</sup>		
	S	S+C	C	SE	S	S+C	C	SE	B	S	B×S
b	232.46	237.99	254.98	3.55	246.67	247.86	251.30	0.91	**	**	**
c	5.44	6.35	6.53	0.17	4.79	6.39	6.80	0.30	*	**	**
R.S.D	7.30	6.32	4.93	0.36	7.28	5.07	7.34	0.39	*	*	**
R <sup>2</sup>	0.99	0.99	0.99	0.001	0.99	0.99	0.99	0.001	NS	NS	NS

<sup>1</sup>B: LAB compared with SAB; S: proportion of silage and concentrate; B×S: interaction between B and S. R.S.D: residual standard deviation, NS: not significant. \*  $p < 0.05$ . \*\*  $p < 0.001$ .



**Figure 2.** Comparison of the gas production profile between liquid associated bacteria (LAB) and solid associated bacteria (SAB).

6.30 for S+C and 6.15 for C, indicating that concentrate feed tended to depress medium pH as expected. The mean initial pH of the combined rumen medium was 6.7. There was no significant pH difference between SAB and LAB at

the end of incubation. Average total number of bacteria (data not shown) estimated by MPN procedure was  $9 \times 10^8$  bacteria per ml. and neither microbial fraction nor type of substrate significantly affected the numbers.

The gas production degradation parameters as influenced by bacterial fraction and type of substrate (Table 2). The maximum gas production represented by the asymptote of the curve, *b*, was significantly influenced by both bacterial fraction and type of substrate. SAB had higher ( $p < 0.01$ ) maximum gas production than LAB, and concentrate (C) had higher ( $p < 0.01$ ) gas production than silage (S). There was a significant interaction between microbial fraction and type of substrate in the maximum gas production. The rate constant ( $\% h^{-1}$ ) of gas production in LAB ranged from 5.44 (S) to 6.53 (C) and that in SAB ranged from 4.79 (S) to 6.80 (C).

Gas production in an *in vitro* incubation system has been known to be influenced by many factors such as medium condition, ration fed to animals for rumen fluid donation and the time of rumen content sampling after feeding (Cone et al., 1996; Cone et al., 2002; Lee et al., 2003). It is well known that SAB fraction contains a large proportion of cellulolytic microorganisms and promotes the extent of fiber digestion (Cheng et al., 1983) and hence SAB may influence gas production as was confirmed in the present study. Since SAB fraction contains more cellulolytic bacteria than LAB fraction, the effect of microbial fraction should be more pronounced from silage than a concentrate, which is also confirmed by the present data. Rymer et al. (1999) tested that the effect of method of inoculum preparation and blending using a stomacher or blender and reported that there was a tendency of increased gas production by blending although the influence of inoculum was not significant. However, Pell and Schofield (1993) did not observe similar results when blended inoculum was used.

Total gas production over incubation time by bacterial fractions was shown Figure 2. Gas production reached over 50% of its maximum at 12 to 24 h incubation. SAB produced significantly more gas than LAB when substrate was silage or a mixture of silage and a concentrate ( $p < 0.01$ ). However, SAB and LAB produced almost the same gas when the substrate was concentrate. The time required for 50% maximum gas production has been reported as 22.6 to 34.7 h depending on the nature of the substrate (Cone et al., 1997). A shorter time for 50% maximum gas production in the present study could be due to differences in substrate, microbial inoculum.

The concentrations of total and individual VFA are presented in Table 3. For both LAB and SAB fractions, acetic, propionic and butyric acids comprised about 90% of the total VFA. LAB produced the most total VFA when concentrate was a substrate, but for SAB the highest total

**Table 3.** Characteristics of volatile fatty acid (mM) on the *in vitro* fermentations after 24 h

	LAB				SAB				Significance <sup>1</sup>		
	S	S+C	C	SEM	S	S+C	C	SEM	B	S	B×S
Ace	39.97	36.14	32.33	0.47	39.28	39.83	33.23	0.97	NS	NS	NS
Pro	28.73	29.04	32.26	0.52	28.44	30.59	29.73	0.39	NS	**	NS
Buty	5.70	5.74	5.70	0.51	6.15	6.25	6.73	0.10	NS	NS	NS
Total	72.97	71.54	70.72	0.13	72.20	77.45	70.39	1.06	**	*	NS
A:P	1.31	1.25	1.0	0.03	1.28	1.30	1.13	0.03	NS	*	NS

Ace: acetate, Pro: propionate, Buty: butyrate, A:P : acetate: propionate ratio.

<sup>1</sup>B: LAB compared with SAB; S: proportion of silage and concentrate; B×S: interaction between B and S. NS-Not significant, \* p<0.05, \*\* p<0.001.

VFA concentration was obtained from a mixture of concentrate and silage. Adding concentrate reduced acetic and propionic acid ratios regardless of bacterial fraction. Bacterial fractions and types of substrate did not influence the concentration of individual VFA except for propionic acid, which was increased with higher concentrate in the incubation vessel. Senshu et al. (1980) observed that strained rumen fluid did not consist of a representative sample of the bacterial population, as it did not contain bacteria that were adhered to feed particles. They included feed particle-associated bacteria in their inoculum by successive filtering of rumen fluid, which resulted in an increase in the yield of VFA. Rymer et al. (1999) compared the preparation method of bacterial fraction (no blending, blending and stomacher) and observed no significant differences in gas production but increased concentration of VFA by blending. In practice animals consume diets containing a variety of feedstuffs, and therefore, gas production with a single natural source in the laboratory would not provide any meaningful reference.

Present results indicate that both bacterial fraction and the type of substrate influence gas production and fermentation characteristics in the rumen.

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