# Effects of the Pattern of Energy Supply on the Efficiency of Nitrogen Utilization for Microbial Protein Synthesis in the Non-Lactating Cows Consuming Grass Silage

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**ABSTRACT** : Effects of the pattern of energy supply on the efficiency of nitrogen utilization for microbial protein synthesis (MPS) were examined in cows consuming grass silage (7.1 kg DM/d) and supplement of 1 kg sucrose per day given as an intraruminal infusion. Three non-lactating cows received three experimental treatments in a 3×3 Latin square design with each period lasting 14 days. The treatments were (1) the basal diets of silage alone given in one meal each day at 09:30 h (BASAL), supplemented with (2) 1.0 kg sucrose given a 4-h infusion starting at 09:30 h (SYNC), (3) 1.0 kg sucrose given a continuous infusion for 24 h (CONT). Compared with BASAL, sucrose infusions altered ($p<0.05$) the pattern of variation in ruminal pH and the concentration of ammonia at 4 h after feeding but none of the sucrose treatments resulted in any changes in the ruminal concentration of VFA. All sucrose treatments increased ($p<0.05$) MPS relative to BASAL by 14% and 33% for SYNC and CONT, respectively, and that for CONT was greater ($p<0.05$) than for SYNC. It is concluded that synchronization of energy and nitrogen over the shorter term has no further advantage of the efficiency for MPS relative to CONT. (*Asian-Aus. J. Anim. Sci. 2000. Vol. 13, No. 7 : 962-966*)

**Key Words** : Energy Supply Pattern, Grass Silage, Microbial Protein Synthesis

## INTRODUCTION

Ammonia is the most important nitrogen precursor for microbial protein synthesis (MPS) under the practical dietary condition, especially when diets containing a high concentration of ruminally degradable nitrogen compounds are fed. Since energy is also one of the most limiting factors for microbial growth, increasing the ruminal availability of energy or synchronizing the rates of energy and nitrogen release are considered by some extent to be important for enhancing the efficiency of ammonia utilization for MPS (van Vuuren et al., 1990; Chase, 1993; Beever, 1996). However, there is little clear evidence in agreement with the benefit of synchrony in terms of MPS over the asynchrony of energy and nitrogen release (Henning et al., 1993; Chamberlain and Choung, 1995). The need for synchrony has been still considered especially important with diets based on grass silage because the ruminal degradation of silage crude protein, which contains a large proportion of free amino acids and small peptides, can lead to marked asynchrony of energy and nitrogen release (Beever and Cottrill, 1994).

From an experiment with cows receiving grass silage alone or supplemented with intraruminal infusion of sucrose in various degree of synchrony, Kim et al. (1999a) provided a strong argument against the conventional view. They postulated that the lack of effect of synchrony would be ascribed to the modest level (about 15% of DM) of fermentable carbohydrate in the experimental diets and then designed an experiment to test this hypothesis (Kim et al., 1999b). The results obtained from the experiment suggested that the degree of synchrony in the ruminal release of energy and nitrogen is likely to influence MPS only with certain diets such as those containing high concentrations of readily fermentable carbohydrate (about 30% of DM). However, these studies failed to obtain data in agreement with the suggestion (Henning et al., 1993) that dietary manipulation should be aimed at first obtaining the most even ruminal energy supply pattern. Infusion time for synchrony in the experiments was over two 6-h period to avoid extremes of fermentation, which would lead to no differences of MPS between synchrony and continuous infusion.

The aim of the present study was to investigate the reponse of MPS to energy supply pattern in a given time. To do this, non-lactating cows fed grass silage once a day to supply more energy and nitrogen in a shorter term than the previous experiment (Kim et al., 1999a).

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## MATERIALS AND METHODS

### Animals and management

Three non-lactating Friesian cows, average weight 532 (SE±21.4) kg were used. They were housed in individual stalls. The animals fed grass silage once daily at 09:30 h and water was freely available at all times.

### Experimental diets and treatments

The basal diet was grass silage at 25 kg fresh weight (7.1 kg DM) per day, which was calculated to supply about 1.4 times the metabolizable energy (ME) required for maintenance (ARC, 1980). The silage was made from perennial ryegrass (*Lolium perenne*) cut at an early stage of growth and ensiled with addition of formic acid (3 liters/ton) in a bunker silo of 60 t capacity. The chemical composition of the silage is shown in table 1. The material was well preserved with a low pH and no butyric acid but the concentration of residual sugar was moderately high.

**Table 1.** The chemical composition of silage (% DM) unless stated otherwise

| | |
|---|---|
| DM (%) | 28.2 |
| Organic matter | 90.2 |
| Total-N (TN) | 3.2 |
| NPN (% TN) | 67.3 |
| $NH_3$-N (% TN) | 11.8 |
| Water-soluble carbohydrate | 12.6 |
| NDF | 54.8 |
| ADF | 30.6 |
| pH | 4.01 |
| Lactic acid | 3.3 |
| Acetic acid | 1.2 |
| Butyric acid | 0 |
| Ethanol | 0.5 |

Three experimental treatments were (1) the basal diet (BASAL), supplemented with (2) 1.0 kg sucrose given a 4-h intraruminal infusions starting at 09:30 h each day, and (3) 1.0 kg sucrose given a continuous intraruminal infusion (CONT). All infusions were given in 4 litres of aqueous solution per day using a peristaltic pump (Watson Marlow Ltd, Falmouth, UK). Since the amount of water infused, over a 24-h period, represented around 10% or so of likely rumen volume, and was most unlikely to influence rumen outflow rate (Harrison et al., 1975), animals on the BASAL treatment were not given an intraruminal infusion of water.

### Experiment plan and sampling procedures

The experiment was designed as a 3×3 Latin square with 2-week periods. The complete output of urine was collected into 500 ml of 4 M $H_2SO_4$ on days 11, 12 and 13 of each period. Urine was collected via a bladder catheter implanted on day 10 of each period. Samples of rumen contents were taken, by suction, on the last day of each period at 09:00 (before feeding); 10:30; 11:30; 13:30 and 15:30 h

### Chemical and statistical analysis

All analyses of silages and rumen contents were carried out as described by Chamberlain et al. (1992). The total purin derivatives (PD) in urine were determined, as an allantoin, by the method of Borchers (1977). Xanthine, hypoxanthine and uric acid were converted to allantoin using the enzymic procedure described by Fujihara et al. (1987). The supply of microbial nitrogen to the small intestine was calculated from the urinary output of PD using the equation of Susmel et al. (1994).

The results were statistically analysed using the ANOVA directives of Genstat 5 (Lawes Agricultural Trust, 1990).

## RESULTS AND DISCUSSION

Daily mean values for ruminal variables are shown in table 2. Compared with the basal diet, sucrose infusions reduced the mean pH, but there were no statistically significant differences among treatments. The mean ammonia concentration for BASAL was high and there were significant ($p<0.05$) reductions in concentration when sucrose infusions were given. However, there were no significant differences between

**Table 2.** Rumen fermentation characteristics in cattle receiving a basal diet of grass silage with or without (BASAL) intraruminal infusion of sucrose given synchronously (SYNC) and continuously (CONT)

| | BASAL | SYNC[1] | CONT | SED[2] |
|---|---|---|---|---|
| pH | 6.71 | 6.35 | 6.39 | 0.16 |
| $NH_3$-N (mg/L) | 293.7 | 247.7 | 248.5 | 8.39* |
| Total VFA (mmol/L) | 11.3 | 11.3 | 11.4 | 0.98 |
| Acetic acid (molar %) | 57.8 | 55.6 | 57.4 | 0.60 |
| Propionic acid (molar %) | 27.8 | 27.1 | 26.7 | 0.64 |
| Isobutyric acid (molar %) | 0.1 | 1.3 | 0 | 0.10 |
| Butyric acid (molar %) | 11.0 | 14.2 | 13.1 | 1.18 |
| Isovaleric acid (molar %) | 1.7 | 1.5 | 1.4 | 0.20 |
| Valeric acid (molar %) | 1.5 | 1.5 | 1.5 | 0.20 |

[1] Infusion for 4 h after feeding. [2] Standard error deviation; * $p<0.05$.

the sucrose treatments in their effects on mean ammonia concentration. With regard to the total VFA and the molar proportions of VFA in the rumen, the sucrose treatments did not alter the VFA pattern seen with the BASAL. When values for ruminal pH and concentrations of ammonia for individual sampling times were considered (figure 1), the variables at 4 h after feeding were significantly different, especially the concentration of ammonia for sucrose treatments was lower ($p<0.05$) than that for BASAL. The calculated amount of microbial nitrogen entering the small intestine was greater ($p<0.05$) for SYNC relative to BASAL and that for CONT was greater ($p<0.05$) than for SYNC (table 3).

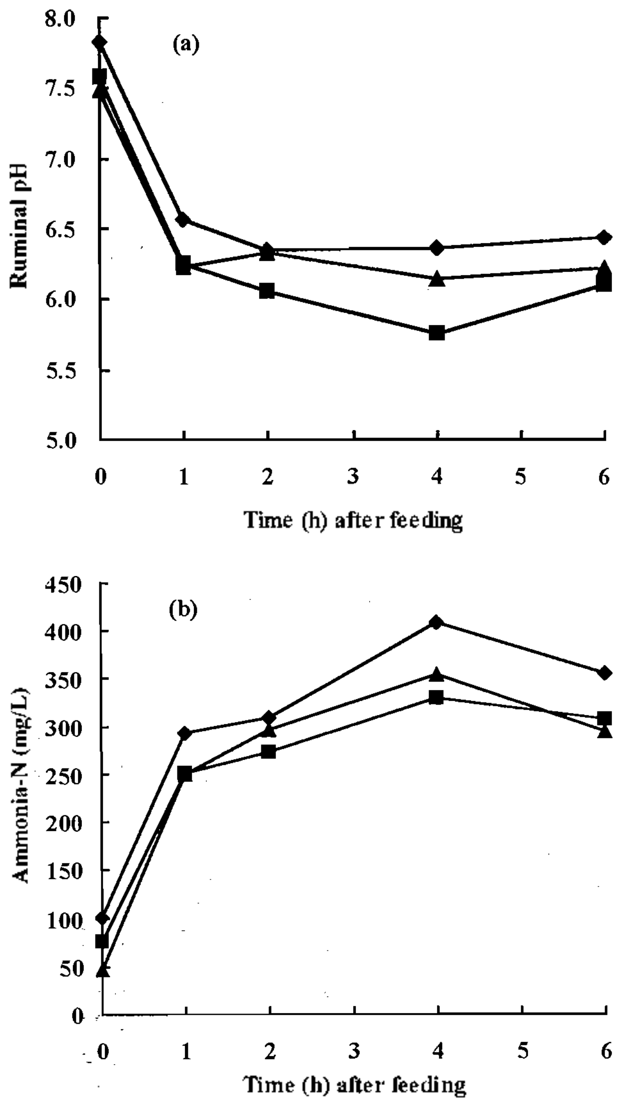


**Figure 1**. Variation in ruminal pH (a) and concentrations of ammonia (b) in cattle receiving a basal diet of silage with or without (◆) intraruminal infusions of sucrose given synchronously (■) or continuously (▲)

**Table 3.** The daily output of total purine derivatives (PD) in urine and calculated amount of microbial nitrogen entering the small intestine in cattle receiving a basal diet of grass silage with or without (BASAL) intraruminal infusion of sucrose given and synchronously (SYNC) continuously (CONT)

| | BASAL | SYNC[1] | CONT | SED[2] |
|---|---|---|---|---|
| PD output (mmol/d) | 170.1 | 188.3 | 211.5 | 6.55* |
| Microbial N (g/d) | 107.6 | 123.1 | 143.0 | 5.60* |
| Microbial growth efficiency[3] | 25.6 | 29.3 | 34.0 | 1.33* |

[1] Infusion for 4 h after feeding. [2] Standard error deviation.
[3] g of microbial nitrogen/DM digested in the rumen (OMDR).
* $p<0.05$.

The degradability of silage crude protein varies with the additive used during ensilage and the results with formic acid indicated that the amount of degradable crude protein is closely related to the proportion of true protein in the silage crude protein (Thomas et al., 1980). The silage used in this experiment would have lower ruminal degradability (60-65%) than that of extensively fermented silage (80%) because it contained about 67% of its nitrogen as non-protein form. Assuming that the silage contained around 70% of its DM as digestible OM, and assuming that about 65% of organic matter (OM) digestion occurred in the rumen (ARC, 1984), it can be calculated that there was a large excess of ruminally degraded nitrogen (RDN) relative to the amount of OM digested in the rumen (OMDR) for the BASAL treatment (about 49 g RDN/kg OMDR). Assuming complete digestion of sucrose in the rumen, for the two sucrose treatments there would still be a substantial excess of RDN, amounting to about 36 g RDN/kg OMDR. Indeed, this was a prerequisite of the experiment in that an excess of RDN on the BASAL treatment was essential to allow a measurable response of MPS to the infusion of sucrose.

In the present study, the decisions to feed silage and to infuse sucrose for 4 h once a day were taken with the aim of increasing the amounts of nitrogen and energy over 4 h post-feeding than those seen previously in cows consuming diets of grass silage twice a day (Kim et al., 1999a). The results show, in contrast to the previous report, high concentrations of ammonia after 4 h post-feeding for all three treatments (figure 1). Continuous supply of energy to the rumen resulted in higher MPS ($p<0.05$) than the same amount of energy supplied in a 4-h period (table 3). This may be explained by the fact that the abundance of sucrose would lead to extremes of fermentation in the rumen directly after infusion for SYNC, which in turn cause the increase in ruminal lactic acid concentration. Lactate production leads to a lower energy yield of ATP per unit of glucose fermented campared to VFA (Strobel and Russell, 1986) and consequently low microbial growth efficiency. Although lactic acid concentration was not estimated, it may not be difficult with above notions to say that it caused abundant sucrose to be utilized inefficiently for MPS for SYNC in this experiment.

Currently, much emphasis is given (Beever and Cottrill, 1994; Stern et al., 1994) to the importance of synchronizing ruminal availabilities of energy and nitrogen to ensure efficient MPS, especially with diets based on grass silage (Beever, 1996). However, from a experiment with cows receiving grass silage alone supplemented with intraruminal infusion of sucrose in various degree of synchrony, Kim et al. (1999a) concluded that there was no advantage to synchronizing energy and nitrogen release over the asynchrony. They postulated that apparent lack of effect of the degree of synchrony on MPS derives from the ability of rumen bacteria synthesizing intracellular storage polysaccharide against the fluctuating supplies of available carbohydrate with diets low in fermentable carbohydrate. With diets rich in fermentable carbohydrate, the synchrony had marked effect on MPS (Kim et al., 1999b) because the capacity of ruminal bacteria for storage of intracellular polysaccharide must be limited (Russell, 1998). It should be important to define the critical level of readily fermentable carbohydrate in the diet beyond which ruminal synchrony influences MPS.

However, none of these two studies obtained superiority of even ruminal energy supply for MPS suggested by Henning et al. (1993). Infusion time over two 6-h period for SYNC in the experiments (Kim et al., 1999a, b) would avoid extremes of fermentation and which in turn lead to no differences of MPS between SYNC and CONT. Comparing results of the previous studies and the present experiment, it is evident that synchrony of high energy and nitrogen over the shorter term has no further advantage of the efficiency of microbial growth relative to CONT.

In a practical sitution, frequency of feeding is one of the more easily modified feeding parameters. It would be logical to expect that a higher feeding frequency leads to higher utilization of ruminal energy and nitrogen for MPS through the more balanced rumen fermentation. The results obtained by Shabi et al. (1998) support this hypothesis. As compared to twice daily feeding, four daily feedings resulted in an increased microbial dry matter and CP flow to the abomasum. However, Robinson and Sniffen (1985) did not find any increase in microbial flow as a result of more frequent feeding. It must be recognized that interpreting the results of experiments in which feeding pattern has been altered is not always straightforward, and this is especially true when the diet is rich in readily fermentable carbohydrate.

The present study suggests that a more accurate estimation of microbial efficiency may be obtained by taking into account not only the feeding frequency and the amount of readily fermentable carbohydrate but also those of protein component of the diet. Robinson and McQueen (1994) published a finding that comes close to the clearest interpretation. When dairy cows fed two protein supplements either twice or five times daily, there was no effects on the diurnal variation in ruminal concentration of peptide which may act as a pool of nitrogen for microbial growth. Further carefully planned experiments are needed before the question can be answered clearly.

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