Effect of Intraruminal Sucrose Infusion on Volatile Fatty Acid Production and Microbial Protein Synthesis in Sheep

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ABSTRACT : Effects of sucrose supplement on the pattern of VFA production and microbial protein synthesis in the rumen were examined in sheep consuming basal diet of grass silage (2.5 kg fresh wt/d) that was provided in 24 equal meals each day by an automatic feeder. Four mature wethers were allocated to four experimental treatments in a 4×4 Latin square design with periods lasting 14 days. The treatments were (1) the basal diet, (2) supplemented with 150 g sucrose and 7.0 g urea, (3) 300 g sucrose and 13 g urea, and (4) 450 g sucrose and 20 g urea given as a continuous intraruminal infusion for 24 h. All infusions were given in 2 litres of aqueous solution per day using a peristaltic pump. The effect of sucrose level on rumen mean pH was significantly linear ($p<0.01$). There were not significant differences in the concentration of ammonia-N, total VFA and the molar proportions of acetate, propionate and butyrate with the level of sucrose infusion. The molar proportions of isobutyric acid ($p<0.05$) and isovaleric acid ($p<0.001$) were significantly reduced when the infused amount of sucrose was increased. The flow of microbial N was linearly ($p<0.001$) increased with sucrose and urea level. High levels of readily fermentable carbohydrate in a ration reduced the efficiency of microbial protein synthesis in the rumen. It was demonstrated that of the individual fatty acids, only the molar proportion of isovalerate showed a significant negative correlation (R2=0.3501**) with the amount of microbial N produced and a significant positive correlation (R2=0.2735**) with the efficiency of microbial growth. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 3 : 350-353)***

Key Words : Branched-chain Fatty Acid, Grass Silage, Microbial Protein Synthesis, Sucrose

INTRODUCTION

The nutritional implications of carbohydrates have only recently emerged as important in ruminant animals. This is not restricted to structural carbohydrates, such as those in NDF, but also nonstructural carbohydrates (NSC) or readily fermentable carbohydrates (RFC), including starches and soluble sugars. In the rumen fermentation, the pattern in which VFA are produced and presented is a reflection of the RFC sources, levels and processing. However, there remains uncertainty over the extent to which factors such as the nature of the basal diet and level of addition of the RFC to the rumen can influence the composition of the VFA mixture produced.

In general, RFC results in a high concentration of propionate in the rumen. More recently, however, some results (Cushnahan et al., 1995; Huhtanen et al., 1997) showed clearly that the high residual water-soluble carbohydrate (WSC) in restrictively fermented silage resulted in low production of propionate and increased either the proportion of acetate or butyrate. It is well established that the differences in the efficiency of energy utilization or nutrient partitioning between lactation and body tissue arising from the feeding of varying silage fermentation characteristics are associated with the differences in the proportions of VFA produced in the rumen (Cushnahan et al., 1995; Huhtanen et al., 1997; Miettinen and Huhtanen, 1997).

Sucrose supplementation has been shown to be more effective than starch supplementation in reducing rumen ammonia N concentration (Chamberlain et al., 1985). Huhtanen (1987) found that ruminal ammonia N concentration and the molar proportion of isovalerate were markedly reduced and microbial N flow at the duodenum was increased linearly when the amount of sugar in the basal diet was increased. Sugar-rich feedstuffs that are by-products from the food industry are commonly used in some countries. However, there is very little information about the effect of sugars on the pattern of VFA production and microbial protein synthesis in the rumen.

This experiment was conducted to investigate the effect of sucrose supplements on the pattern of VFA production and microbial protein synthesis in the rumen. To avoid the negative effects of sucrose supplements on the ruminal fermentation, sucrose was given in the form of continuous intraruminal infusion (Kim et al., 2000).

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MATERIALS AND METHODS

Animals and management

4 mature wethers, average body weight 49 kg (S.E. 3.4) were used. Each animal was fitted with a permanent cannula into the rumen and was housed individually in a

Table 1. The chemical composition of silage (%DM)

Dry matter (%) [1]	27.3
Total-N (TN)	2.7
NPN (%TN)	78.8
Ammonia-N (%TN)	13.9
Water soluble carbohydrate	4.7
pH	3.92
Lactic acid	5.8
Acetic acid	2.7
Propionic acid	0
Butyric acid	0

[1] Toluene distillation.

metabolism crate. The basal diet was grass silage (2.5 kg fresh weight per day) that was provided in 24 equal meals each day by automatic feeder. Water was provided free choice and the animals had free access to a proprietary mineral lick.

Experimental diets and treatments

The silage was made from perennial ryegrass (*Lolium perenne)* cut at an early stage of growth and ensiled, with the addition of a commercial inoculant (Ecosyl, Ecosyl Products Ltd, Billingham, Cleveland, UK) at the manufacturer's recommended rate, in a bunker silo of 60-t capacity. The chemical composition of the silage is shown in Table 1. The basal diet was calculated to supply about 1.3 times the metabolizable energy required for maintenance (ARC, 1980).

The four experimental treatments were (1) the basal diet (BASAL), (2) supplemented with 150 g sucrose and 7.0 g urea (Sucrose 150), (3) 300 g sucrose and 13 g urea (Sucrose 300), and (4) 450 g sucrose and 20 g urea given as a continuous intraruminal infusion (Sucrose 450). All infusions were given in 2 litres of aqueous solution per day using a peristaltic pump. The experiment was designed as a 4×4 Latin square with 2 week period and the amount of urea addition was determined on the basis of the efficiency of microbial protein synthesis (30 g N/RDOM). The rumen contents obtained by suction through the ruminal cannula were taken on the final day of each experimental period at 12:00, 13:00, 14:00 and 15:00 h and the complete output of urine was collected into 100 ml of 4 M H_2SO_4 for last 5 days.

Chemical and statistical analysis

Analyses of silages and rumen contents were carried out as described by Chamberlain et al. (1992). The total purine 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 and regression procedures of Genstat 5 (Lawes Agricultural Trust, 1990).

RESULTS AND DISCUSSION

The silage was well preserved and the quality was good as revealed by a low pH and no butyric acid and low concentration of ammonia N (Table 1). Despite wilting and relatively high DM content, inoculated silage showed lactic acid fermentation and the concentration of residual water soluble sugar (WSC) was moderately low.

Mean values of rumen pH, concentrations of ammonia-N and total VFA and molar proportions of individual fatty acids are given in Table 2. The effect of sucrose level on rumen mean pH was significantly linear ($p<0.01$). However, there was no evidence of extreme ruminal fermentation even with the highest addition of sugar. The concentration of ammonia-N, total VFA and the molar proportions of acetate, propionate and butyrate were not significantly different. The molar proportion of isobutyric acid ($p<0.05$) and isovaleric acid ($p<0.001$) were significantly reduced when the infused amount of sucrose was increased.

The flow of microbial N was linearly ($p<0.001$) increased with sucrose and urea level (Table 3). Moreover, high levels of sucrose in a ration reduced the efficiency of microbial protein synthesis ($p<0.001$) in the rumen. On

Table 2. Ruminal pH, the concentrations of ammonia-N, total VFA and the molar proportions of individual VFA in sheep receiving diets of grass silage with ruminal infusion of varing amount of sucrose

	BASAL	Sucrose level			SED [1]	Significant effect	
		150	300	450		Linear	quadratic
pH	6.90 [2]	6.67	6.69	6.47	0.12	**	NS
NH_3-N (mg/L)	187.4	177.6	168.9	189.5	11.55	NS	NS
VFA (mmol/L)	9.4	10.0	10.2	10.8	1.05	NS	NS
Acetic acid (molar %)	61.0	55.4	57.8	53.0	3.05	NS	NS
Propionic acid (molar %)	25.6	31.9	28.6	33.7	4.61	NS	NS
Isobutyric acid (molar %)	1.2	0.4	0.2	0	0.31	*	NS
Butyric acid (molar %)	9.1	9.6	11.2	11.7	1.77	NS	NS
Isovaleric acid (molar %)	1.9	1.4	0.9	0.2	0.34	***	NS
Valeric acid (molar %)	1.3	1.3	1.3	1.4	0.19	NS	NS

[1] Standard error deviation. [2] Values are means of 4 samples during the day in each of four sheep.

* $p<0.05$; ** $p<0.01$; *** $p<0.001$.

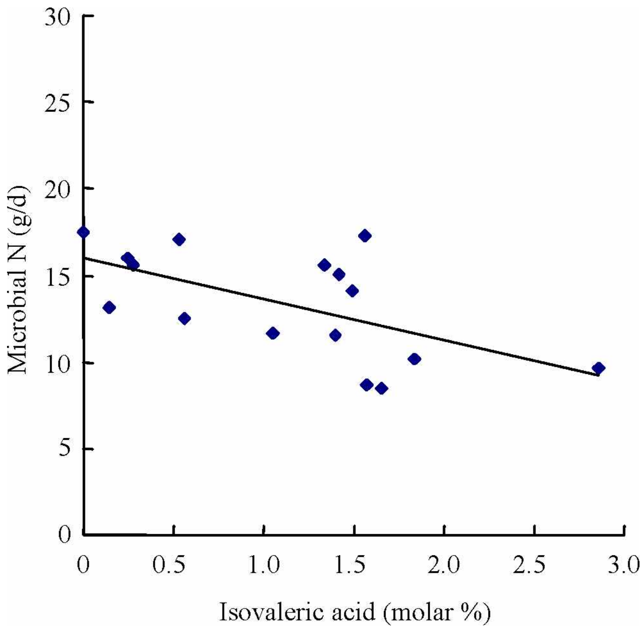

Figure 1. The relation of isovaleric acid content and microbial nitrogen in sheep receiving diets of grass silage with ruminal infusion of varing amount of sucrose. y = -2.37 (SE 0.86)x+16.06 (SE 1.16); n=16; R2 0.3501; p<0.0156.

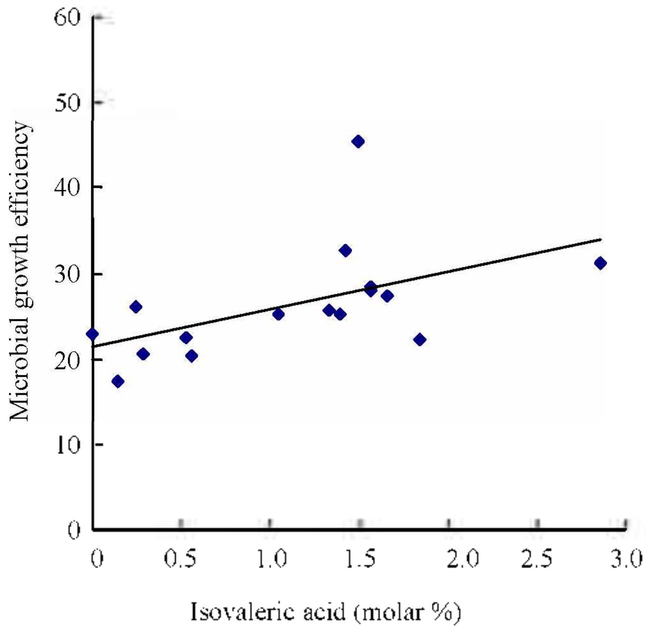

Figure 2. The relation of isovaleric acid content and microbial growth efficiency (g of microbial nitrogen/DM digestion in the rumen) in sheep receiving diets of grass silage with ruminal infusion of varing amount of sucrose. y = 4.83 (SE 1.91) x+21.40 (SE 2.57); n=16; R2 0.2745; p<0.0377.

average, microbial N production was increased by 14 g/kg sucrose infused. This is markedly lower than the value of 23 g/kg sucrose reported previously (Kim et al., 1999). The average efficiency of microbial N synthesis (g N/OM digested in the rumen (OMDR)) was 26.3 g/kg OMDR.

The degradability of silage crude protein varies with the additive used during ensilage (Thomas et al., 1980). The silage used in this experiment would have higher ruminal degradability (80%) than that of restrictively fermented silage (60-65%) because it contained about 79% of its nitrogen as non-protein form. Assuming that the silage contained around 70% of its DM as digestible OM, and assuming that about 80% 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 47 g RDN/kg OMDR). Indeed, this was a prerequisite of the experiment that an excess of RDN over the BASAL treatment was essential to allow a measurable response of MPS to the infusion of sucrose. As sucrose is highly fermentable in the rumen, for the different sucrose levels an appropriate quantity of urea was added. Consequently, the amount of microbial N synthesis was increased linearly with sucrose and urea level without decrease in ammonia-N concentration.

In this experiment, it was demonstrated that sucrose would reduced the concentration of isovalerate in the rumen. Of the individual fatty acids, only the molar proportion of isovalerate showed a significant negative correlation (R2=0.3501**) with the amount of microbial N produced (Figure 1) and positive correlation (R2=0.2735*) with the efficiency of microbial protein synthesis in the rumen (Figure 2). There was much interest in the importance of branched-chain fatty acid (BCFA) in the rumen, since isobutyric, isovaleric, and 2-methylbutyric acids are required for resynthesis of the branched-chain amino acid from which they were derived or are used for synthesis of long-chain branched fatty acids or aldehydes (Allison, 1969). Rumen fermentation of protein-free purified diets produces undetectable BCFA (Ørskov, 1982). *In vitro* fermentation systems demonstrated that BCFA supplementation could increase microbial protein synthesis, fermentation of plant cell walls and DM digestion (Cummins and Papas, 1983). Several studies with cattle on grazing rangelands and pastures consuming mature forage of moderate to low quality have shown that ruminal concentrations of some BCFA are below detectable limits (Judkins et al., 1987; Krysl et al., 1987), indicating that BCFA supplementation could be beneficial. However, many *in vivo* experiments concluded that a ruminal BCFA is not the first-limiting factor affecting the performance of ruminants fed low quality roughage sources (Hefner et al., 1985; Gunter et al., 1990).

The sucrose supplementation seemed to effectively reduce the concentration of branched-chain fatty acids (BCFA) in continuous infusion (Huhtanen, 1987) as well as in animals fed two equal daily meals (Oh et al., 1999). This holds true with supplementation of sufficient NPN source as well as supplementation of true protein (Oh et al., 1999). Huhtanen (1987) also showed similar results of change in molar proportion of isovalerate and its significant negative correlation with the amount of microbial N produced with increased sucrose level in cattle receiving diets of grass silage and barely without adding any protein source. If a lower proportion of isovalerate with sugar infusion indicates efficient incorporation of this acid by rumen microbes and if lower efficiency of microbial N synthesis is highly correlated with lower proportion of isovalerate, supplementation of BCFA other than sugar should be needed for maximum microbial protein synthesis.

It is important to consider the possibility that the well-known suppression of cellulolytic activity associated with dietary addition of RFC, such as sucrose, may be linked with a deficiency of BCFA, which deserves attention. The utilization of silage nitrogen for rumen microbial protein synthesis is improved by the supplementation of sugar. However, the practical advantages of using sugars may be limited to some extent by a reduction in fiber digestion, which leads to reduced forage dry matter intake. Therefore, further research is required to determine whether intraruminal infusion of BCFA with high level (450 g per day) of sucrose limits dry mater and fiber digestion, and the efficiency of microbial protein synthesis.

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