

## Effect of monensin on methane production in sheep

	<i>CO<sub>2</sub>/CH<sub>4</sub> in rumen gases</i>		
	<i>T<sub>0</sub> h</i>	<i>T<sub>2</sub> h</i>	<i>T<sub>5</sub> h</i>
Before monensin treatment	1.22 <sup>a</sup>	2.31 <sup>b</sup>	2.18 <sup>a</sup>
During monensin treatment	1.26 <sup>a</sup>	2.84 <sup>a</sup>	2.34 <sup>a</sup>
After monensin removal	1.31 <sup>a</sup>	2.39 <sup>b</sup>	2.25 <sup>a</sup>
RSD	0.20	0.30	0.27
Monensin effect	0.34	0.00	0.28

RSD : Residual Standard Deviation. Means with different superscripts are significantly different ( $P \geq 0.05$ )

were fed the control diet. The antibiotic was added to the diet (25mg kg<sup>-1</sup>) during period 2 for 86 days, and it was withdrawn during period 3 (50 days). Rumen gases were analysed by gc [4]. Analysis of variance with two controlled effects (day and animal) was applied. The ratio CO<sub>2</sub>/CH<sub>4</sub> in rumen gas significantly increased immediately after monensin was given and remained higher throughout period 2. It significantly decreased as soon as monensin was withdrawn during period 3 and almost immediately fell to the mean value of period 1. However, these effects were observed only during the 2h following feed intake and were not evident between T<sub>5</sub> and the next meal (T<sub>0</sub>). In conclusion, methanogenic bacteria did not adapt to monensin during the 86-day treatment and recovered immediately after removal of the antibiotic.

1. Mbanzamihigo L, Van Nevel CJ, Demeyer, DI (1995) *Reprod Nutr Dev* 35, 353-365
2. Johnson DE, Aboomar JS, Saa CF, Carmean BR (1994) *Proc XIII<sup>th</sup> Symp on Energy Metabolism of Farm Animals*, (JF Aguilera ed), pp 339-342, EAAP Pub 76, CSIC, Spain
3. Kirchgessner M, Windisch W, Müller HL (1995) *Proc VIII<sup>th</sup> Int Symp on Ruminant Physiology*, (WV Engelhardt, S Leonhard-

marek, G Breves, D Giesecke, F Enke, eds), Verlag, Stuttgart, Germany

4. Jouany JP, Senaud J (1979) *Ann Biol Anim Biochim Biophys* 19, 1007-1010

**Effect of minerals on methane production and protozoa numbers in continuous culture of rumen microorganisms.** L-P Broudiscou, Y Papon<sup>1</sup>, AF Broudiscou<sup>2</sup> (<sup>1</sup>INRA, Station de Recherches sur la Nutrition des Herbivores, Centre de Clermont-Theix, 63122 Saint Genès-Champanelle, France; <sup>2</sup>LMRE, Université d'Aix-Marseille III, 52 avenue Escadron Normandie-Niemen, 13013 Marseille, France)

In ruminants, the minerals supplied by the diet or salivary secretion act on rumen microorganisms directly by providing them with enzymatic cofactors and essential elements for biosynthesis, by modifying rumen pH and osmolality, and indirectly by affecting the host digestive physiology, e.g. rumen content dilution rate and absorption rate of metabolites. In the present study, six dual outflow continuous fermentors [1] were used to quantify the direct response of mixed rumen microbes to a change in the input of minerals, and to

optimise the chemical composition of an artificial saliva. Four factors were combined in a Franquart experimental design: the amount of  $\text{HPO}_4^{2-}$  (coded variable HP;  $-1 = 0.1 \text{ g l}^{-1}$ ,  $+1 = 4 \text{ g l}^{-1}$ ), the amount of  $\text{HCO}_3^-$  (coded variable HC;  $-1 = 0.5 \text{ g l}^{-1}$ ,  $+1 = 7 \text{ g l}^{-1}$ ), the amount of  $\text{Cl}^-$  (coded variable C;  $-1 = 0.1 \text{ g l}^{-1}$ ,  $+1 = 0.5 \text{ g l}^{-1}$ ), and the ratio  $\text{Na}^+/\text{K}^+$  (coded variable R;  $-1 = 0.5 \text{ g}^{-1}$ ,  $+1 = 15 \text{ g}^{-1}$ ). The fermentors were supplied with  $22.5 \text{ g d}^{-1}$  orchard-grass hay and  $7.5 \text{ g d}^{-1}$  ground barley during 3 seven-day periods. Using the coded factors, second order polynomial models were fitted to the data by multiple linear regression.

The action of minerals on methane production and protozoa involved to an equal degree several experimental factors with a nonlinear behavior. The amount of methane produced ranged from  $0.85$  to  $1.58 \text{ mmol h}^{-1}$ . When selecting the coefficient estimates different from null at the  $p$ -value of  $0.15$ , the polynomial for methane production rate was  $Y_M = 1.54 + 0.15 \text{ HC} + 0.08 \text{ C} - 0.14 \text{ R} - 0.49 \text{ HP}^2 - 0.38 \text{ HC}^2 - 0.28 \text{ R}^2 - 0.31 \text{ HP} \cdot \text{HC} + 0.41 \text{ HP} \cdot \text{C} + 0.23 \text{ HC} \cdot \text{C}$  (Adj R-sq=  $0.90$ , root MSE=  $0.063 \text{ mmol h}^{-1}$ ). The protozoa population density, comprised between  $14$  and  $35 \mu\text{l}^{-1}$ , was favored by high  $\text{Na}^+/\text{K}^+$  ratios. The model for protozoa density was  $Y_P = 25.7 - 3.9 \text{ C} + 4.0 \text{ R} - 11.8 \text{ HC}^2 + 7.0 \text{ R}^2 - 9.8 \text{ HP} \cdot \text{HC}$  (Adj R-sq=  $0.76$ , root MSE=  $2.75 \mu\text{l}^{-1}$ ). The composition of a mineral base was optimized by the technique of desirability function maximization, to simultaneously sustain the *in vitro* maintenance of protozoa and the activity of methanogens. The resulting artificial saliva contained  $4.19 \text{ g l}^{-1} \text{ HCO}_3^-$ ,  $1.65 \text{ g l}^{-1} \text{ HPO}_4^{2-}$  and  $0.22 \text{ g l}^{-1} \text{ Cl}^-$  and had a  $\text{Na}^+/\text{K}^+$  ratio of  $14.2 \text{ g}^{-1}$ , and was close to the buffer proposed by Rufener *et al.* [2].

1. Hoover WH, Crooker BA, Sniffen CJ (1976) *J Anim Sci* 43, 528-534
2. Rufener WH, Nelson WO, Wolin MJ (1963) *Appl Microbiol* 11, 196-201

**A phylogenetic assessment of bovine rumen methanogens during perturbation by acidosis.** R Sharp<sup>1\*</sup>, CJ Ziemer<sup>2</sup>, DA Stahl<sup>1</sup> (<sup>1</sup>*Civil Engineering, Northwestern University, Evanston, IL 60208, USA;* <sup>2</sup>*Animal Science, University of Minnesota, 130 Haecker Hall, 1364 Eckles Avenue, St Paul, MN 55108, USA;* \**Current address: Dunn Clinical Nutrition Centre, Cambridge, CB2 2DH, UK*)

The rumen microbial community is complex and we have little information on the diversity of contributing populations, their activities or possible interactions. Comparative sequencing and molecular probes have provided an important set of tools for describing and quantifying the diversity of ruminal populations, it is however, only the first step. To move beyond the descriptive, it is essential that ruminal population dynamics be related to ruminal processes. In this study we characterised a well recognised disruption of normal ruminal processes resulting from acidosis using group specific small sub-unit (SSU) ribosomal RNA (rRNA) probes together with chemical measures of population abundance.

Populations of Eucarya, Archaea, *Methanobacteriaceae*, *Methanomicrobiales* and *Desulfovibrio* sp. were quantified with SSU rRNA-targeted probes. Cows were fasted for 12h before introducing the acidotic diet. Rumen pH decreased from  $6.53$  to  $5.44$  within 10h after first feeding the acidotic diet. Correlation between pH and the quantity of Eucaryotic SSU rRNA