optimise the chemical composition of an artificial saliva. Four factors were combined in a Franquart experimental design : the amount of HPO_4^{2-} (coded variable HP; -1= 0.1g l^{-1}, +1= 4g l^{-1}), the amount of HCO_3^- (coded variable HC; -1= 0.5g l^{-1}, +1= 7g l^{-1}), the amount of Cl^- (coded variable C; -1= 0.1g l^{-1}, +1= 0.5g l^{-1}), and the ratio Na^+/K^+ (coded variable R; -1= 0.5g g^{-1}, +1= 15g g^{-1}). The fermentors were supplied with 22.5g d^{-1} orchard-grass hay and 7.5g 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 1.58mmol h^{-1}. When selecting the coefficient estimates different from null at the p-value of 0.15, the polynomial for methane production rate was $Y_M = 1.54 + 0.15$ HC +0.08 C -0.14 R-0.49 HP^2 -0.38 HC^2 - 0.28 R^2 -0.31 HP*HC +0.41 HP*C +0.23 HC*C (Adj R-sq= 0.90, root MSE= 0.063mmol h^{-1}). The protozoa population density, comprised between 14 and 35 μl^{-1}, was favored by high Na^+/K^+ ratios. The model for protozoa density was Y_P= 25.7 -3.9 C +4.0 R -11.8 HC^2 +7.0 R^2 -9.8 HP*HC (Adj R-sq= 0.76, root MSE= 2.75μl^{-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 4.19g l^{-1} HCO_3^-, 1.65g l^{-1} HPO_4^{2-} and 0.22g l^{-1} Cl^- and had a Na^+/K^+ ratio of 14.2g g^{-1}, and was close to the buffer proposed by Rufener *et al.* [2].

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A phylogenetic assessment of bovine rumen methanogens during perturbation by acidosis. R Sharp[1*], CJ Ziemer[2], DA Stahl[1] (*[1]Civil Engineering, Northwestern University, Evanston, IL 60208, USA; [2]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

was positive (r=0.65 P<0.01). Values declined from 14.4 ± 2.79 (0h) to 6.8 ± 1.98μg g^{-1} rumen fluid (16h). The abundance of Eucarya increased when the cows were returned to the normal diet, reaching 40.3 ± 5.5μg RNA per g rumen fluid at 72h. Archaea decreased following the induction of acidosis, from 1.4 ± 0.4 to 0.4 ± 0.07μg g^{-1} rumen fluid at 0 and 72h respectively. The population encompassed by the families *Methanoplanaceae, Methanomicrobiaceae* and *Methanocorpusculaceae* declined rapidly during the first 16h. (0.54 ± 0.07 to 0.04 ± 0.01μg g^{-1}) and did not recover. In contrast the *Methanobacteriaceae* increased after the perturbation (24.3% of the total Archaea at 0h and 79.8% at 72h). These values were positively correlated with Eucaryal biomass (r = 0.7 P<0.01). *Desulfovibrio* sp. SSU rRNA accounted for an average 1.2% ± 0.59 of the total SSU rRNA abundance. There was a strong positive correlation between the total amounts of *Desulfovibrio* and *Methanobacteriaceae* SSU rRNA (r=0.51 P<0.01) throughout the observation period.

Intestinal ciliates and their endosymbionts from the cockroach hindgut: evolutionary aspects. AHAM Van Hoek, TA Van Alen, VSI Sprakel, JHP Hackstein, GD Vogels (*Deptartment of Microbiology and Evolutionary Biology, University of Nijmegen, NL-6525 ED Nijmegen, The Netherlands)*

Anaerobic ciliates occur in the hindgut of many cockroach species, but only in those cockroaches that also are host to intestinal methanogens. Such ciliates contain, without exception, endosymbiotic methanogenic bacteria [1]. Although all of these anaerobic ciliates seem to be related to *Nyctotherus* sp. behavioural studies (e.g. galvanotaxis) and the morphology of the methanogenic endosymbionts suggest the presence of different protists in the various cockroach species. The rDNA genes of individual ciliates and their methanogenic endosymbionts were analysed in order to study the symbioses. DNA sequencing and restriction analysis of PCR-amplified rDNA genes showed that the ciliates isolated from the various cockroach hosts did differ significantly. The endosymbiotic methanogenic bacteria of these ciliates, also, proved to be different. Thus, in contrast to the situation in aphids with their eubacterial endosymbionts [2], the phylogeny of the anaerobic ciliates and their cockroach hosts [3] does not match. Since the methanogenic endosymbionts also differ substantially, it seems reasonable to conclude that the symbioses between methanogenic bacteria, ciliates, and cockroaches evolved more than once.

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Transformation of *Butyrivibrio fibrisolvens* strains with pBH$_f$ plasmid. J Kopecný.[1], K Fliegerová[1], K Gregg[2] ([1]*Institute of Animal Physiology and Genetics, Czech Academy of Sciences, Prague 10, Uhoínìves, 104 00, Czech Republic;* [2]*Institute of Biotechnology, University of New England, Armidale, NSW 2351, Australia)*