DIVERSITY OF METHANOGENS IS INCREASED IN THE RUMEN OF SHEEP FED MONENSIN

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The knowledge of what happens to the diversity of methanogens in the rumen of sheep when the fermentation pattern changed is limited. Many studies have shown that changing rumen fermentation leads to a change in the amount of methane produced (Baker 1999). Monensin is an ionophore that has a wide range of effects on rumen fermentation (Bergen and Bates 1984). The aim of this experiment was to examine whether monensin changes the diversity of methanogens in the rumen of sheep.

Ten mature Merino wethers, fitted with rumen cannulae, were fed pellets (68% lucerne hay, 20% lupin grain, 10% molasses, 2% minerals (air dry basis)) for 6 weeks (period 1). A daily ration of 1500g was offered and withdrawn after 3 h. Five of the ten sheep received 33 mg monensin/d for 4 weeks in gelatin capsules via the cannulae immediately after feeding (period 2). The other five sheep were kept on the control diet. Samples were taken from the rumen of all sheep on the last day of periods 1 and 2 3 h after feeding. Despite many modifications to the protocol, DNA could only be extracted from six of the twenty samples, only one of which was from an animal receiving monensin. The 16S rRNA gene was amplified from the DNA using polymerase chain reaction (PCR) with methanogen-specific primers. The amplification products were cloned into *Escherichia coli* and digested with the restriction enzymes *Hae*III and/or *Sau*3A. Restriction fragment length polymorphism (RFLP) patterns were compared against an RFLP database for methanogens to presumptively identify the methanogen species present. Any RFLP patterns that did not match the database were considered new and the 16S rRNA genes were sequenced to determine their identity. The diversity of methanogens in the different samples was compared by using the Shannon index for general diversity to analyse the RFLP patterns and sequence data.

A total of 186 clones were analysed, and DNA from *Methanobrevibacter* (Mbr.) species predominated amongst them (Figure). A Shannon index of diversity was calculated from the frequency of occurrence of clones from the sheep that did not receive monensin (Figure), and the Shannon index for the clones from the sheep that received monensin was outside the 99.99% confidence interval of this. On this evidence monensin appears to increase the diversity of methanogens in the rumen of sheep.

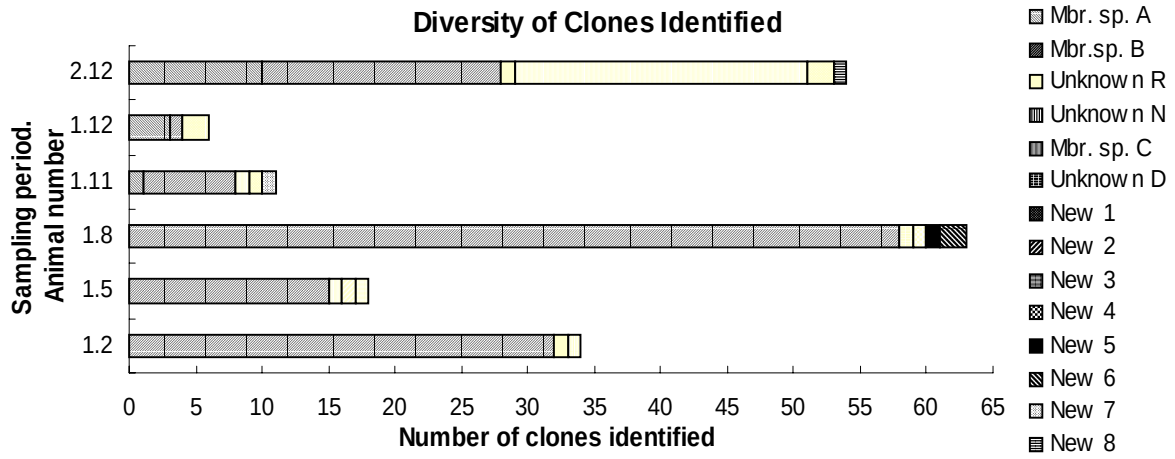

Figure 1. The numbers of 16S rRNA clones from methanogens identified using RFLP and DNA sequencing. Y-axis: The first digit refers to the period where the rumen sample was taken. The second digit(s) is the animal identification. Abbreviations: Mbr: *Methanobrevibactor.*

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