Effect of fibre- and starch-rich finishing diets on methanogenic Archaea diversity and activity in the rumen of feedlot bulls

Milka Popova, Cécile Martin, Maguy Eugène, Marie-Madeleine Mialon, Michel M. Doreau, Diego Morgavi

GGAA2010 3 - 8 October 2010
Greenhouse Gases and Animal Agriculture Conference
Banff • Canada
www.GGAA2010.org

PLEASE NOTE: Authors denoted with an asterisk are registered GGAA2010 delegates

Does the complexity of the rumen microbial ecology preclude methane mitigation?

André-Denis G. Wright* and Athol V. Klieve*

Ruminant livestock are responsible for the production of a significant proportion of greenhouse gases, particularly methane (61 Tg per annum), that contribute to global warming and climate change. This methane is an end-product of the fermentation of plant material by the complex microbial ecosystem that resides in the rumen. Methanogenesis is undertaken by methanogenic archaea and represents a mechanism by which hydrogen is removed from the fermentation process in order to regenerate biochemical co-factors such as NAD+. The microbial ecosystem is very complex and involves thousands of species of bacteria, archaea, protozoa, fungi and viruses, in very dense populations (for example bacterial cells are above 10^{11} per mL of contents), interacting with feed, the host ruminant and each other. Not only is the ecosystem complex but also relatively poorly understood particularly inter-species interactions and interactions with the host. Less than 15% of the inhabitants have been cultured and characterised. However, knowledge of this ecosystem is rapidly accumulating, particularly with the advent of molecular biology and culture independent technologies. New high-throughput sequencing methodologies such as 454-pyrosequencing will greatly improve the rate of knowledge acquisition and techniques like Stable Isotope Probing will enhance the ability to unravel species inter-relationships. While we can expect an increase in knowledge of this complex ecosystem to improve our ability to predictably manipulate the ecosystem this has not prevented manipulation of the ecosystem or reductions in methane emissions in the past. Examples where this has happened are through the use of foreign and recombinant bacteria to reduce leucaena and fluoroacetate toxicity respectively, and reduction in methane emissions by changing feed and the use of feed additives (e.g. cereal grains, monensin, plant oils, BES). The challenge is to mitigate methane emissions further and this will develop as our knowledge of the intricacies of this complex ecosystem is unravelled.

Effect of fibre- and starch-rich finishing diets on methanogenic *Archaea* diversity and activity in the rumen of feedlot bulls

M. Popova*, C. Martin, M. Eugène*, M. M. Mialon, M. Doreau and D. P. Morgavi*

Methanogenesis plays an important role in hydrogen pressure maintenance in the rumen and consequently in feed digestion. Recent studies in our laboratory found that moderate supplementation with extruded linseed lipids, combined with high-starch diet, decreased methanogenesis in bulls (Eugène et al., 2010; this symposium). However, the linkage between the dietary lipid supplementation and methanogens has not been well established. The aim of this work was to compare the methanogenic community in terms of number and diversity in the rumen of bulls fed high concentrate diets supplemented or not with linseed. Twenty Charolais bulls, divided in two groups, were fed for 16 months two fattening diets containing both 87% concentrate and 13% barley straw. The concentrate of the control diet (C), richer in fibre, contained 40% NDF, 7.4% starch and 2.8% EE. The concentrate supplemented with 6% DM of extruded linseed (LS), higher in starch, contained 20% NDF, 33% starch and 4.7% EE. Bulls were slaughtered at an average age of 531 ± 45 days and 680 ± 41 kg BW. Rumen content samples were taken for microbial community analysis after slaughter. Archaeal and eubacterial

numbers were quantified by qPCR, targeting the functional mcrA gene and the rrs gene, respectively. The diversity of the rumen methanogenic community was studied using PCR-DGGE. The biodiversity was assessed by the indices of Shannon, evenness and dominance calculated from the DGGE profiles. There was no difference between diets in the number of archaea and eubacteria expressed as log copies of mcrA or rrs / g DM of rumen content ($P > 0.05$). The DGGE profile of the archaeal mcrA gene differed among individuals. A lower number of bands ($P < 0.05$) was detected in bulls fed LS compared to bulls fed C. Shannon and evenness values were also lower ($P < 0.05$) for the methanogenic community of bulls fed LS. The predominant bands were different between C and LS resulting in PCR-D GGE profiles that clustered by diet, suggesting a correlation between methanogenic diversity and linseed supplementation in diets. This study shows that dietary linseed supplementation had no effect on eubacterial and archaeal abundance in the rumen, but it significantly affected methanogenic community diversity. Further studies linking methanogens' activity (via mcrA mRNA) and other rumen microbes abundance and diversity are in progress and will allow better understanding of the microbial mechanisms of methanogenesis in cattle fed a diet supplemented or not with linseed.

Effect of C-18 long-chain fatty acids on in vitro rumen microbial populations

A. Cièslak*, M. Szumacher-Strabel*, P. Zmora, E. Pers-Kamczyc, K. Wereszka, P. Skorka and T. Michalowski

The study was carried out to investigate the effects of type and level of different C18-fatty acids (FA) on fermentation, methanogenesis and microbial population in long-term (28 days) cultures of *Entodinium caudatum* (EC), *Eudiplodinium maggii* (EM) and *Epidinium ecaudatum* (EE). The ciliates were cultured in vitro and the basal culture medium was composed of "*caudatum*" salt solution and food mixture. The control cultures were maintained on the basal medium, whereas the experimental ones were supplemented with stearic acid (SA), oleic acid (OA), linoleic acid (LN) and linolenic acid (LNA) in the proportion of 0.1, 0.5, 2.5 and 5% each. Protozoa counts and methane concentration were measured in the post-cultured medium every fourth day. After long-term cultivation, on the final 28th day, the following parameters were monitored in post-cultured medium: pH, ammonia, VFA concentration and methanogens population. Ciliates were counted under a light microscope. Methane concentration was quantified by gas chromatography. The fluorescence in situ hybridization (FISH) was used to quantify methanogens in the population. The effect of fatty acid was species dependent; however, long-term monitoring revealed that the EC and EE populations gradually adapted to the changed conditions. Fatty acids addition showed the potential to decrease methane production but it was not always related to changes in the number of methanogens. Decreased methane production was significant only for 5% LN supplement to the EC culture and for 5% OA and LNA supplements to the EE culture. Results suggest that FA have the potential to reduce rumen methane production.

Exploring rumen methanogen genomes to identify targets for methane mitigation strategies

G. T. Attwood*, E. Altermann*, B. Kelly, S. M. Leahy, L. Zhang and M. Morrison*

Methane from ruminant livestock is generated by the action of methanogenic archaea, mainly in the rumen. A variety of methanogen genera are responsible for methane production, including a large group that still lacks cultivated representatives. To be generally effective, technologies for reducing ruminant methane emissions must target all rumen methanogens to prevent any unaffected methanogen from expanding to occupy the vacant niche. Interventions must also be specific for methanogens only so that other rumen microbes can continue their normal digestive functions. Therefore, a detailed knowledge of both the diversity and physiology