# Fungal secondary metabolites reduce rumen methane production in vitro and in vivo

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## Animal variation in methane production, expression of *mcrA* gene and microbial numbers in the rumen of goats fed alfalfa hay with or without oats

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This study was designed to evaluate the intra and inter animal variability of methane production and potential link with the expression of the *mcr*A gene and the numbers of the main microbial groups in the rumen of goats fed alfalfa hay with (AHO) or without oats (AH). For each diet methane production was measured in chambers during 3 consecutive days from 4 ruminally cannulated goats in two experimental periods with 15 days of difference to evaluate the persistency of the effects, resulting in 4 periods in total for the whole trial. Every measuring day rumen samples were taken 2 h after the morning feeding to quantify the expression of *mcr*A using RTqPCR and the copy numbers of 16S rRNA (for bacteria), 18S rRNA (for protozoa) and *mcr*A gene (for methanogenic archaea) by qPCR. Results show that daily methane production averaged 28.0 and 21.5 L $kg^{-1}$ DMI for diets AHO and AH, respectively with a variation within animals of 20.84 and 26.6%. However, the methane emissions estimated over the first two hours after the morning feeding were much higher than for the whole day measuring period (averaged 60.8%). The relative expression of the *mcr*A gene presented higher intra-animal variation for diet AHO (67.7%) than for AH (28.5%). The inter-animal variation averaged 19.6 and 26% for methane production and 71.8 and 84.8% for *mcr*A expression. The variation for the qPCR number of gene copies, respectively, for diets AHO and AH was 21.4 and 30.4% for total bacteria, 48.1 and 25.2% for protozoa and 33.8 and 28.2% for methanogenic archaea. Methanogenic archaeal numbers followed the same pattern as methane emissions; however, there was not agreement with the expressions leveles of the *mcrA* gene. The high inter-animal variation in the expression levels of the *mcr*A gene together with the lack of agreement with the in vivo methane emissions may preclude the use of the gene expression as methane production indicator. Furthermore, the differences among animals in feeding pattern and the diurnal variation in rumen fermentation make a single spot rumen sample doubtfully reliable. Further research to investigate the consistency of the variation over extended periods of time in order to assess the suitability of the *mcr*A gene expression as an indicator of methanogenic activity in the rumen needs to be undertaken.

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Decreasing methanogenesis without affecting fermentation and degradation of feeds in the rumen can reduce the environmental impact of ruminant production and have a beneficial effect on feed conversion efficiency. In this work metabolites produced by the fungus *Monascus* sp. were assayed for their antimethanogenic activity in vitro and in vivo. The capacity of seven strains of *Monascus* sp. to produce secondary metabolites was assessed in solid media. Monitored metabolites were: the Statins monacolin K, pravastatin, and mevastatin, and the mycotoxin citrinin. Extracts from five different selected solid media from the two strains producing the highest (HM) and lowest (LM) monacolin to citrinin ratio were tested in vitro. A commercial preparation of monacolin K was also used. Extracts in fermentation flasks were adjusted to a concentration of 20 μg $ml^{-1}$ of monacolin K. Fermentation was not negatively affected by any treatment but one HM culture decreased methane production. This extract was further assayed in three sequential batch incubations where a marked decrease in methane was observed in the second and third batches. In contrast, methane produced in flasks with pure monacolin K was not different from controls. Rice on which the selected *Monascus* sp. was grown also decreased methane production when used as substrate for in vitro incubations. The effect of *Monascus*-treated rice on methane production was then assayed in vivo. Six wethers were adapted for several weeks to a

diet containing rice and hay (1:1 ratio). Rice was then replaced by treated rice and given to animals for nearly two weeks. Animals were monitored for a further two weeks after the treatment. Daily methane emissions decreased by 30% after 2-3 days into the treatment and remained low throughout the administration period. This change was associated with lower acetate to propionate ratio and lower numbers of methanogens in the treatment period as detected by qPCR (copy number of mcrA $g^{-1}$ rumen content). In contrast, no changes in the methanogenic community were observed by DGGE. Total bacteria and protozoa were marginally or not affected by the treatment. Methane emissions and the acetate to propionate ratio remained numerically lower in the two weeks post-treatment as compared to measures before treatment. Metabolites produced by *Monascus* appear to have an inhibitory effect upon methanogens and decreased methanogenesis in vitro and in vivo without any apparent negative effect on rumen fermentation. This strategy needs to be further explored and could be an abatement option under certain feeding situations.