Dietary nitrates decrease methane emission by inhibiting rumen methanogenic archaea without influencing nitrate reducing bacteria

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Nitrate supplementation in diets is a promising strategy for reducing enteric methane emissions from ruminants. In this work, we focused on rumen methanogens and nitrate-reducing bacteria (NRB) in order to better understand the effects of this additive on these microbial populations.

Four dry Holstein cows were randomly assigned to four dietary treatments in a 4 x 4 Latin square design: 1) control (CON, 50% hay and 50% concentrate), 2) control with 3% calcium nitrate (NIT), 3) control with 4% linseed oil (LIN), and 4) control with 3% calcium nitrate and 4% linseed oil (NITLIN). At each experimental period, methane was quantified in open chambers (week 4) and rumen content was sampled 3h postfeeding (week 5). DNA and RNA were co-extracted and used in qPCR for microbial quantification and genes expression analysis. Targeted genes were: *rrs* (total bacteria), *mcrA* (methanogens) and *narG*, *napA*, *nirk* (NRB).

Compared to CON, methane yield decreased by 21%, 17% and 32% with NIT, LIN and NITLIN, respectively ($P<0.01$). Quantities of total bacteria and NRB were unaffected by treatments ($P>0.05$) whereas methanogens numbers were reduced by 6.6%, 5.6% and 4.7% with NIT, LIN and NITLIN ($P=0.01$). Expression of *mcrA* was reduced by 66% with NIT and 56% with NITLIN compared to CON or LIN ($P=0.04$). The *narG* activity was similar among treatments ($P=0.86$) and expression of *napA* and *nirk* was never detectable. These results show that methane mitigation by dietary nitrates could be attributed to a partial inhibition of methanogens without influencing the population of NRB.