The effectiveness of *Alcaligenes faecalis* to reduce nitrite accumulation when nitrate is used to suppress *in vitro* rumen methanogenesis

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Introduction

Methane (CH_4) production during rumen fermentation is the result of the reduction of CO_2 with H_2 by methanogenic *Archaea*. Nitrate (NO_3) reduction is energetically more favourable than CO_2 reduction, and the presence of NO_3 in the rumen redirects H_2 from methanogenesis to NO_3 reduction, thereby decreasing CH_4 production. However, the practical use of NO_3 is precluded by the toxicity associated with its reduced intermediate, nitrite (NO_2). High concentrations of NO_2 in the blood of livestock can lead to methemoglobinemia, a condition caused by the oxidation of the ferrous iron in haemoglobin, rendering the molecule incapable of oxygen transport. Nitrite accumulates in the rumen due to the more rapid reduction of NO_3 to NO_2 than the reduction of NO_2 to ammonia (NH_3). *Alcaligenes faecalis* No. 4, a bacterium isolated from sewage sludge, has been shown to have the ability to convert NO_3 to nitrogen gas. Therefore, the objective of this study was to introduce *A. faecalis* and NO_3 into the artificial rumen *in vitro* system and observe if the bacteria can reduce NO_2 accumulation.

Materials and Methods

The *in vitro* continuous incubation system was used to monitor CH_4 and CO_2 during the incubation period. Dried (40 °C, 48 h), milled (1 mm apertures screen) Timothy hay+concentrate (50:50, gravimetric dry matter (DM) basis) samples (10 g) were weighted into four individual 1 L fermentation vessels together with 600 ml of a 4:1 volumetric mixture of buffer solution and rumen fluid inoculum. The latter was collected from two non-lactating fistulated cows, immediately strained through a woven nylon cloth and pooled. In addition, one of the following treatments were added to each vessel: (1) 40 ml distilled water (control), (2) 40 ml *A. faecalis* culture (2.5×10^9 cfu/ml), (3) 40 ml distilled water+2 mM NO_3 and (4) 40 ml *A. faecalis* culture (2.5×10^9 cfu/ml)+2 mM NO_3. Incubations were carried out under anaerobic conditions at 39 °C for 24 h. Samples from each vessel were collected at intervals of 0, 2, 4, 6, 8, 12 and 24 h and stored at -20 °C for later determination of NO_3 and NO_2. For replication, the experiment was repeated four times ($n = 4$). Data were analysed by one-way ANOVA (CH_4 and CO_2) and two-way repeated measure ANOVA procedures (NO_3 and NO_2) using SPSS software (ver. 16.0).

Results and Discussion

Nitrate and NO_2 concentrations in the fermentation vessels did not differ ($P > 0.05$) between NO_3 alone and *A. faecalis*+NO_3 treatments after 24 h incubation. Cumulative CH_4 production (ml CH_4/24 h) was affected by treatment ($P < 0.001$), with cumulative CH_4 production for NO_3 alone (37.8 ml) being lower ($P < 0.05$) than either the control (51.0 ml) or *A. faecalis* only (51.7 ml) treatments. *A. faecalis*+NO_3 treatment had a lower ($P < 0.05$) cumulative CH_4 production (11.5 ml) than all other treatments. Cumulative CO_2 production was not affected by treatment ($P > 0.05$). Although the combination of *A. faecalis*+NO_3 did not reduce NO_2 accumulation under the conditions of this assay, it did reduce CH_4 production by 69.6% and 77.4% compared to the addition of NO_3 alone and control treatments, respectively. Further studies are required to elucidate the synergistic mechanism in which *A. faecalis*+NO_3 can reduce *in vitro* methanogenesis.