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 (CH₄) production during rumen fermentation is the result of the reduction of CO₂ with H₂ by methanogenic Archaea. Nitrate (NO₃) reduction is energetically more favourable than CO₂ reduction, and the presence of NO₃ in the rumen redirects H₂ from methanogenesis to NO₃ reduction, thereby decreasing CH₄ production. However, the practical use of NO₃ is precluded by the toxicity associated with its reduced intermediate, nitrite (NO₂). High concentrations of NO₂ 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 NO₃ to NO₂ than the reduction of NO₂ to ammonia (NH₃). Alcaligenes faecalis No. 4, a bacterium isolated from sewage sludge, has been shown to have the ability to convert NO₃ to nitrogen gas. Therefore, the objective of this study was to introduce A. faecalis and NO₃ into the artificial rumen in vitro system and observe if the bacteria can reduce NO₂ accumulation.

Materials and Methods
The in vitro continuous incubation system was used to monitor CH₄ and CO₂ 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 x 10⁹ cfu/ml), (3) 40 ml distilled water+2 mM NO₃ and (4) 40 ml A. faecalis culture (2.5 x 10⁹ cfu/ml)+2 mM NO₃. 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 NO₃ and NO₂. For replication, the experiment was repeated four times (n=4). Data were analysed by one-way ANOVA (CH₄ and CO₂) and two-way repeated measure ANOVA procedures (NO₃ and NO₂) using SPSS software (ver. 16.0).

Results and Discussion
Nitrate and NO₂ concentrations in the fermentation vessels did not differ (P > 0.05) between NO₃ alone and A. faecalis+NO₃ treatments after 24 h incubation. Cumulative CH₄ production (ml CH₄/24 h) was affected by treatment (P < 0.001), with cumulative CH₄ production for NO₃ 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+NO₃ treatment had a lower (P < 0.05) cumulative CH₄ production (11.5 ml) than all other treatments. Cumulative CO₂ production was not affected by treatment (P > 0.05). Although the combination of A. faecalis+NO₃ did not reduce NO₂ accumulation under the conditions of this assay, it did reduce CH₄ production by 69.6% and 77.4% compared to the addition of NO₃ alone and control treatments, respectively. Further studies are required to elucidate the synergistic mechanism in which A. faecalis+NO₃ can reduce in vitro methanogenesis.