

# Nitrogen metabolism in rumen bacteria can be characterised by their N isotopic signature

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## Abstract

The N use efficiency (NUE) of ruminants is negatively correlated to the natural  $^{15}\text{N}$  enrichment of ruminants' tissues over their diets ( $\Delta^{15}\text{N}_{\text{animal-diet}}$ ). The metabolism of rumen bacteria, which strongly discriminates against  $^{15}\text{N}$  utilisation, may contribute to this link but little is known about the role of individual bacteria. In this study, we characterised the  $\Delta^{15}\text{N}$  of three metabolically different rumen bacteria, a cellulolytic ammonia-consuming, a hyper-ammonia producing and a proteolytic organism, grown in pure culture with different N sources and levels. The N isotopic signatures significantly changed across the 3 rumen bacteria. Although we failed to observe a higher  $\Delta^{15}\text{N}_{\text{bacteria-substrate}}$  when proteolysis and ammonia production was favoured, our results confirm a strong  $^{15}\text{N}$  depletion with the ammonia-consuming bacterium. This may suggest that  $^{15}\text{N}$  depleted ammonia-consuming bacteria improves the NUE in the rumen.

**Keywords:**  $^{15}\text{N}$ , ruminant, ammonia, isotopic fractionation

## Introduction

In ruminants, the natural  $^{15}\text{N}$  enrichment of animal proteins over the diet has been proposed as a proxy for N use efficiency (Cantalapiedra-Hijar et al., 2018). The contribution of rumen microbiota to this link has been acknowledged (Cantalapiedra-Hijar et al., 2016) but the underlying mechanism remains unknown. Based on previous studies we hypothesized that the N isotopic signature of rumen bacteria ( $\Delta^{15}\text{N}_{\text{bacteria-substrate}}$ ) depends on the balance between uptake and release of ammonia (Martinez-Fernandez et al., 2018), both reactions being catalysed by enzymes discriminating against  $^{15}\text{N}$ . Because rumen bacteria differ in their N metabolism it is expected that  $\Delta^{15}\text{N}_{\text{bacteria-substrate}}$  would reflect their prevailing ability to assimilate or release ammonia. The objective of this study was to evaluate the  $\Delta^{15}\text{N}_{\text{bacteria-substrate}}$  of selected rumen bacteria grown in pure cultures according to their N metabolism.

## Material and Methods

Three different rumen bacteria strains in terms of N metabolism were chosen for this study: i) *Fibrobacter succinogenes* S85 is a cellulolytic bacterium that requires ammonia as source of N (ammonia-N consumer), ii) *Clostridium aminophilum* (DSMZ 10710) is a hyper-ammonia producing bacterium that uses protein as energy source (ammonia-N producer) and iii) *Prevotella ruminicola* (ATCC 19189<sup>TM</sup>), *Prevotella* is a predominant rumen genus that has proteolytic activity and uses either ammonia or peptides as a source of N (ammonia-N user or producer). An in vitro experiment was conducted in batch cultures (50 mL media) to test the effect of the 3 strains cultured with 2 N sources supplied at 2 levels (n = 3 x 2 x 2 conditions and 3 replicates) on  $\Delta^{15}\text{N}_{\text{bacteria-substrate}}$ . Organic (tryptone casein) and inorganic (ammonium chloride) N sources were supplied at low (1 mM) or high (11.5 mM) concentrations added to a culture medium containing 2 mM of ammonia-N as the baseline level. After 48 h of incubation at 39 °C, the fermentation was stopped by placing the bottles in ice and the content collected for N isotopic analyses from the bacterial pellet obtained through differential centrifugation.

## Results and Discussion

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Both the N level (high vs low) and source (ammonia vs protein) influenced the  $\Delta^{15}\text{N}_{\text{bacteria-substrate}}$  values (Figure 1;  $P < 0.001$ ) but in a different way depending on the specific bacterial N metabolism (Bacteria  $\times$  N source  $\times$  N level interaction effect;  $P < 0.001$ ). When the N level shifted from low to high the  $\Delta^{15}\text{N}_{\text{bacteria-substrate}}$  increased ( $P < 0.001$ ), except with *F. succinogenes* growing on ammonia (Bacteria  $\times$  N level interaction effect;  $P < 0.001$ ). Overall, our results agree with the higher  $\Delta^{15}\text{N}$  observed in ruminants fed high vs low N diets (Cantalapiedra-Hijar et al., 2018) and partially confirm that the extent of ammonia uptake and release strongly affects the N isotopic signature of rumen bacteria. When the ammonia-consumer bacterium *F. succinogenes* was grown on ammonia, the  $\Delta^{15}\text{N}_{\text{bacteria-substrate}}$  showed the lowest values ( $P < 0.001$ ) in agreement with previous in vitro results on cellulolytic rich mixed-cultures grown on ammonia (Martinez-Fernandez et al., 2018). However, we did not observe higher  $\Delta^{15}\text{N}_{\text{bacteria-substrate}}$  values when hyper-ammonia-producing *C. aminophilum* and proteolytic *P. ruminicola* were grown on protein as compared to ammonia.

**Figure 1. Nitrogen isotopic signatures ( $\Delta^{15}\text{N}_{\text{bacteria-substrate}}$ ) among rumen bacteria grown on 2 different N sources (tryptone casein vs ammonium chloride) at 2 different N levels (Low [1 mM] and High [11.5 mM]). Bars having one or more letters in common are not statistically different from each other ( $P < 0.05$ ).**

### Conclusions

Our results showed that N isotopic signatures in rumen bacteria depend on their N metabolism. In particular, a strong  $^{15}\text{N}$  depletion occurred when a bacterium specialised in ammonia assimilation was grown.

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### References

- Cantalapiedra-Hijar G., Dewhurst R., Cheng L., Cabrita A.R.J., Fonseca A., Nozière P., Makowsky D., Fouillet H., Ortigues-Marty I. 2018. Nitrogen isotopic fractionation as a biomarker for nitrogen use efficiency in ruminants: A meta-analysis. *Animal* 12(9): 1827-1837
- Cantalapiedra-Hijar G., Fouillet H., Huneau J.F., Fanchone A., Doreau M., Nozière P., Ortigues-Marty I. 2016. Relationship between efficiency of N utilization and isotopic N fractionation in dairy cows: digestion or metabolism? *Animal* 10: 221-229
- Martinez-Fernandez G., Cantalapiedra-Hijar G., Morgavi D.P., McSweeney C.S. 2018. Nitrogen isotopic signatures in mixed rumen cellulolytic bacteria differ depending on the nitrogen source. In: Proceedings of the 10th International Symposium on the Nutrition of Herbivores