Effect of 2-bromoethanesulfonic acid, a methanogen inhibitor, on fermentation in the rumen and hindgut. KG De Graeve [1], JP Grivet [2], M Durand [3], D Demeyer [1] *([1] Onderzoekscentrum voor Voeding, Veeteelt en Vleestechnologie, Proefhoevestraat 10, 9230 Melle, Belgium; [2] CNRS, Centre de Biophysique Moléculaire, Université d'Orléans, Av de la Recherche Scientifique, 45071 Orléans Cedex 2; [3] INRA, Laboratoire de Nutrition et Sécurité Alimentaire, 78350 Jouy-en-Josas, France)*

Introduction

Stoichiometry of rumen and hindgut fermentation differs as, in contrast to rumen fermentation hindgut fermentation uses part of the metabolic hydrogen in CO_2 reduction to acetate (De Graeve *et al*, 1990). To investigate factors that determine the balance between methanogenesis and reductive acetogenesis in the rumen and hindgut, incubations were performed with a specific methane inhibitor; 2-bromoethanesulfonic acid (BES) (Sparling and Daniels, 1987) and with the addition of $^{13}CO_2$.

Materials and Methods

Hindgut and rumen contents of cattle (10 g + 40 ml buffer solution) were incubated (24 h) under CO_2 with addition of BES (20 mM). Other *in vitro* incubations were performed with pig hindgut washed cell suspensions (WCS) (5 ml) with 50 mM NaH $^{13}CO_3$ added under an atmosphere of CO_2 (20%) and H_2 (80%) (De Graeve *et al*, 1990). BES was added (20 mM) and ^{13}C-incorporation was determined with a Bruker AM 300 NMR-spectrometer.

Results and Discussion

Addition of BES to rumen incubations shifted the fermentation from acetate (786 and 551 µmol produced/incubation flask –BES and +BES respectively) to the more reduced end products, propionate (201–247 µmol) and butyrate (356–420 µmol), with a reduction of total VFA production, a total inhibition of methane and an accumulation of H_2. In the hindgut, however, an opposite effect was observed. Total inhibition of methanogenesis was accompanied by a stimulation of total VFA production, mainly due to an increase in acetate (589–670 µmol) while H_2 did not accumulate. This suggests that the addition of BES in the hindgut stimulated reductive acetogenesis, confirmed by $^{13}CO_2$-incubations with pig hindgut WCS. BES inhibited methanogenesis totally while acetate production (µmol/flask) and $^{13}CO_2$-incorporation into acetate (µmol ^{13}C) were stimulated by BES from 211 and 42 µmol to 326 and 219 µmol respectively. These data suggest a shift of metabolic hydrogen to acetate. However, as similar effects were observed with or without excess of H_2, it would seem that H_2 is not an extracellular precursor for reduction of CO_2 to acetate.

Conclusion

Reductive acetogenesis, which does not occur in the rumen, is an important hydrogen acceptor reaction in the hindgut which competes with methanogenesis for metabolic hydrogen.

References

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Sparling R, Daniels L (1987) *Can J Microbiol* 33, 1132-1136