Influence of rumen protozoa on methane emissions in ruminants: A meta-analysis approach

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Introduction Methane (CH_4) produced by ruminants is the most important greenhouse gas coming from livestock breeding (Steinfeld *et al.*, 2006). In the rumen, CH_4 is produced by methanogenic archaea, mainly from carbon dioxide (CO_2) and hydrogen (H_2) released during fermentation of feeds by other microbes. Protozoa are implicated in methanogenesis through the production of large quantities of H_2 and through their close interaction with archaea, which are the main users of H_2 (Morgavi *et al.*, 2010). However, the relationship between protozoal concentration and the amount of CH_4 emissions is not well quantified. In this study we made a quantitative analysis of the literature to assess this relationship.

Material and methods A database was built from 59 publications reporting data from 76 experiments and 270 treatments. Only *in vivo* experiments giving measured data on both CH_4 production and rumen protozoal concentration on a same group of animals were included in the database. An experiment consisted in one control treatment and at least one experimental treatment testing a CH_4 mitigation strategy on the same basal diet. Quantitative parameters (chemical composition of the diet, intake, total tract digestibility, rumen fermentation and microbial ecosystem) and qualitative parameters (animal species, diet composition, methods of CH_4 and protozoa determination) were considered. Experiments were encoded according to 3 classes of CH_4 mitigation strategies: biotechnological additives (experimental defaunation, probiotics, prebiotics, enzymes), additives (plant extracts, chemical compounds, organic acids) or feed components (forages, concentrates, lipids). Treatments testing associations of two or more strategies were not considered. Within each class, the quantity, source and form of the additive was encoded. Protozoal concentrations were expressed in $\log_{10}$ cells/mL to get a normal distribution of data. Daily CH_4 emissions were expressed as a function of dry matter intake (DMI) to allow interspecies comparisons. The relationship between CH_4 emissions and protozoal concentration was studied with a variance-covariance model allowing dissociation between intra- and inter-experiment variability, with experiment as a fixed effect (Sauvant *et al.*, 2008). Experiments included in the model had a within-experiment variation of protozoal concentration higher than 5.3 $\log_{10}$ cells/mL (2.2 x 10^5/mL) corresponding to the mean s.e.m. of the database for this variable. The influence of potential qualitative and quantitative secondary factors on parameters of the model (slopes, LSMeans, residuals) was tested. Relevant significant factors were finally tested in the model. All statistical analyses were carried out using the GLM model (Minitab, version 16, State College, PA).

Results CH_4 emissions were similar (P=0.365) between animal species and averaged 18.9 ± 5.6 g/kg DMI for cattle, sheep and goat (n_{exp}=67). A significant reduction of both CH_4 emissions and protozoal concentration was observed in 20% of experiments, most of them using lipids. A significant reduction of CH_4 without variation of protozoal concentration was reported in 43% of experiments, most of them using chemical components or essential oil. No variation of CH_4 and protozoal concentration was observed in 28% of experiments, most of them testing the effect of different forage sources. No variation of CH_4 when protozoa decreased was reported in 9% of experiments, most of them testing experimental defaunation. In the model using experiments with a reliable within-experiment variation of protozoal concentration, the average protozoal concentration was 5.9 ± 0.4 $\log_{10}$ cells/mL (10.7 ± 9.4 ×10^5/mL). Within a protozoal concentration ranging between 4.6 and 6.8 $\log_{10}$ cells/mL (0.4 to 63.1 × 10^5/mL), the response law between CH_4 emission and protozoal concentration was linear: CH_4 (g/kg DMI) = -16.6 (s.e. 9.07) + 5.77 (s.e. 1.53; P<0.001) × protozoa ($\log_{10}$ cells/mL) with n_{exp}=25, n_t=75, r.m.s.e.=2.97 and r^2_{adj}=0.75. The model was not influenced by animal species, CH_4 mitigation strategy and CH_4 method of measurement. However, butyrate molar proportion of the rumen volatile fatty acids was significantly correlated with CH_4 LSMeans (P=0.012) and residuals (P=0.016). This shows that for a given level of protozoa, butyrate could partly explain differences in CH_4 between experiments. This factor was thus significant when included in the model instead of experiment effect, leading to the equation: CH_4 (g/kg DMI) = -27.4 (s.e. 10.04; P<0.001) + 6.26 (s.e. 1.77; P<0.001) × protozoa ($\log_{10}$ cells/mL) + 0.778 (s.e. 0.305; P<0.05) × butyrate (mol/100mol) with n_t=64, r.m.s.e.=5.24 and r^2_{adj}=0.27. This meta-analysis indicates that CH_4 emissions are regulated by both protozoal concentration and rumen butyrate which is preferentially produced by protozoa (Brossard *et al.*, 2004).

Conclusions In this database, a reduction in protozoal concentration by lipids or plant extracts always leads to a reduction in CH_4 emissions. The meta-analysis also revealed that for a same change in protozoal concentration, CH_4 emissions are lower when butyrate proportion in the rumen decreases. Nevertheless, protozoal concentration is not the only explanatory factor of CH_4 emissions, as shown by experiments testing chemical components.

Acknowledgements J. Guyader is the recipient of an INRA- Région Auvergne PhD scholarship

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