

An antimethanogenic index for meadow plants consumed by ruminants

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HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers. L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés. Take home Message An antimethanogenic index could complete information on the multiple services provided by a meadow and is an opportunity to re-introduce plants rejected in borders as a consequence of grassland management in the last decades.

Introduction Permanent meadows, especially in mountainous areas, have numerous advantages over temporary meadows in terms of ecosystem services, as they help protect biodiversity, confer resilience to climate stresses (e.g. drought), and meet rising societal demand (e.g. high-quality meat and cheese). However, past decades have seen big change in grassland management (e.g. unbalanced cutting frequencies, fertilizer doses) to achieve higher productivity, but with a concomitant decline in botanical composition and biodiversity (Pierik *et al.* 2017). Here we develop a new antimethanogenic index (AMI) that could characterize the plants growing not just in the meadows but also in neighbouring environments like hedges, borders, under-brush, and ditches. The purpose of the AMI is to evaluate the potential of a plant species to reduce methane emissions by grazing ruminants, while taking into account its energy value. The AMI could add new information on the multiple services provided by pasture, and thus rehabilitate some plants that had become marginalized.

Material and methods The AMI was built from *in vitro* rumen fermentation data. A set of 212 plants was collected in the French Massif Central area , at flowering stage in the vast majority of cases. Samples were frozen in liquid nitrogen, freezedried, ground, and kept out of light. The fermentation profiles (gas and volatile fatty acids (VFA) produced) were determined by incubating 600-mg pure plant substrates in 40-mL rumen mixed bacteria cultures (buffer solution:rumen fluid at 2:1, v/v) for 24h at 39°C. All incubations were repeated 3 times. Each run period included 4 incubations of perennial ryegrass (PRG) used as control for a total of 48 runs. Methane and VFA productions were normalized and expressed as a ratio of mean PRG values for each period to eliminate inter-period drift. A plant was declared antimethanogenic (p<0.01) when its methane production was lower than the value fitted to the methane=f(VFA) linear regression, minus 2.58 times the standard deviation (s.d.) of PRG (s.d.), with s.d. assumed as uniform on the experimental domain. The index was calculated as: AMI=(Af-Am)/Amax, where Af is the CH₄ value fitted to the CH₄ =f(VFA) linear regression minus 2.58 times the PRG s.d., Am is measured CH₄ value, and Amax is maximum (Af-Am) value observed among the 212 plant samples.

Results & Discussion Outliers, namely 16 very particular plants that had a big effect on methane (1 methanogenic activator; 15 antimethanogenic) were discarded to calculate the regression (CH4=1.06(+-0.04)VFA-0.12(+-0.03), R²=0.80, RSE=0.08). The plants were then classified into three groups: 44 plants that activated methanogenesis (AMI < -0.20, over the grey zone, figure 1), 104 plants with normal stoichiometry (Demeyer 1981) of fermentation (-0.20<= AMI <= 0, grey zone), and 64 plants with antimethanogenic effect (AMI > 0) below the grey zone. The strongest effect was observed with *Bidens tripartita* (AMI=1), thereby confirming our previous observations (Macheboeuf *et al.* 2014), but numerous other plants had a promising positive AMI, including *Origanum vulgare* (0.60), *Scrophularia nodosa* (0.55), *Serratula tinctoria* (0.49), *Succisa pratensis* (0.19), *Polygonum bistorta* (0.17), *Hypericum perforatum* (0.16)...





Conclusion AMI was able to classify plants according to their antimethanogenic potential while taking into account their VFA production. AMI findings also highlight the importance of keeping certain species in the meadow. Once a meadow's botanical composition is known, the AMI index could be included in the criteria used to evaluate its environmental potential.

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