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Effects of plants containing secondary metabolites on ruminal methanogenesis of sheep *in vitro*

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Abstract

The current study was carried out to determine the effect of secondary metabolites on *in vitro* rumen microbial fermentation and their potential to inhibit methane (CH_4) production involved in the global warming and greenhouse-gases (GHG). The obtained results showed that the addition of *Yucca schidigera* and *Quillaja saponaria* reduced significantly CH_4 production ($P < 0.05$). This reduction is proportional to the concentration and it becomes superior to 60 % beyond 8 mg / mL of saponins. CH_4 production and total volatile fatty acid (tVFA) concentration decreased linearly ($P<0.05$) with increasing dose of essential oils (EO). The effect was more pronounced with *Mentha pulegium*. The incorporation of *Acacia cyanophylla* tannins source to vetch-oat hay leads to a relative decrease in CH_4 in the gas pool. Supplementation with 60% and 30% of *Acacia cyanophylla* resulted in 37.5 % and 56.25 % lower CH4, respectively. Addition of *Acacia cyanophylla* reduced significantly tVFA. The proportion of 60% of *Acacia cyanophylla* induced the highest molar proportion in propionate. The results showed that plant secondary metabolites can be used as feed additives to reduce CH_4 production and to consequently mitigate greenhouse-gases emission.

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Keywords: Greenhouse-gases; methane, rumen fermentation; saponins, essential oils; tannins

1. Introduction

Nomenclature

CH_4 methane
CO_2 carbon dioxide
Greenhouse-gases (GHG)
tVFA total volatile fatty acids
CT condensed tannins

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EO essential oils
DM Dry matter

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With the growing concern about global warming and global climate change, researches become intensified in this domain, on which domestic ruminants has an important role because they are the major contributor to anthropogenic production of greenhouse-gases (GHG), like methane (CH_4). Methane is a strong GHG gas with a global warming potential 23 times higher than that of carbon dioxide (CO_2). As such, much research is currently conducted to minimize the CH_4 synthesis during rumen fermentation. Besides the contribution of CH_4 to GHG emission, CH_4 synthesis in the rumen also represents a loss of dietary energy. Much researches are focused on the addition of plant secondary metabolites to minimize CH_4 production [1]. Ideally, additives should reduce the CH_4 production, without interfering in the overall fermentation processes. The three main secondary plant compounds which appear effective in reducing CH_4 production *in vitro* are: saponins, essential oils (EO) and condensed tannins (CT). There is a need for identifying plant secondary metabolites with potential to modify rumen fermentation. The objective of this study was, therefore, to screen for adequate compound and optimal doses to decrease CH_4 production *in vitro* by testing several plants known for their richness in secondary metabolites on CH_4 production.

2. Material and methods

2.1. Experiment 1

The action of saponins on the *in vitro* gas production was determined according to the process of Menke et al. [2]. For every sample, three syringes were inoculated with 200 mg / DM of substrate. This solid phase were completed by a liquid phase constituted by 30 mL of culture media, made up of rumen content and buffer solution (1: 2) [2]. In the same conditions, three syringes containing only the culture media without substrate were simultaneously incubated as blank.
The tested saponins consist on gross extracts of shrubs collected from desert zones of Mexico and Chile: *Yucca schidigera* and *Quillaja saponaria*. *Yucca schidigera* extract was a powder, constituted exclusively of stems (NOR-FEED SUD Sarl). Its saponins content was about 44 g/kg DM. *Quillaja saponaria* extract was a powder, made up of selected parts of the plant (NOR-FEED SUD Sarl). Its saponins content was between 50-70g / kg DM. The added doses of extracts were: 0, 2, 4, 6, 8 mg / mL. Investigated substrate were dates by products and the vetch-oat. The follow-up of kinetic fermentation was realized by the measure of the total gas volume produced, in various intervals of time: 2, 4, 6, 8 and 24 hours. The specific volume of produced gases (CH_4) was determined by a soda treatment (NaOH 10N) [3].

2.2. Experiment 2

Essentials oils were obtained by steam distillation from *Juniperus phoenicea* and *Mentha pulegium* collected from Algeria. Incubation was done in 100 mL serum vials containing 0.4 g substrate (1:1 hay:concentrate) and 40 mL of rumen fluid-buffer mixture. Three wethers were used as donors of rumen fluid. Doses of EO tested were: 0.85, 1.67, 3.3 and 5 (µL/mL) for *Juniperus phoenicea* and 0.37, 0.75 and 1.5 (µL/mL) for *Mentha pulegium.* Methane production and fermentation parameters were measured. Methane production was determined by gas chromatography (Micro GC 3000A, Agilent Technologies, Les Ulis, France). Indiv idual gas standards were used for calibration. Total volatile fatty acids (tVFA) were analyzed by gas chromatography using crotonic acid as the internal standard on a CP 9002 Gas Chromatography (CP 9002 Chrompack, Middelburg, Germany) using a wall-coated open-tubular fused silica column (0.25 mm i.d. × 25 m) coated with CP-wax 58 (FFAP)-CB.