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Effects of propyl propane thiosulfinate on nutrient utilization, ruminal fermentation, microbial population and methane emissions in goats

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ABSTRACT

The aim of this work was to investigate the effect of α-cyclodextrin-propyl propane thiosulfinate complex (CD-PTS) as antimethanogenic compound in ruminants using *in vivo* and *in vitro* approaches. The *in vivo* trial lasted 36 days and was designed to study the effect of CD-PTS on ruminal methane production, fermentation pattern, microbial abundances and nutrient utilization in goats. Twelve adult goats fed with alfalfa hay and concentrate (50:50), were split in two groups: treated with CD-PTS complex at 200 mg of active component/L rumen content per day or without any treatment (control). On days 7, 14, 21 and 28 methane emissions were recorded in chambers and rumen content samples were collected for pH, volatile fatty acid (VFA) and NH_3 analyses. On day 14 and 28 rumen samples were collected for quantification of bacterial, protozoal and archaeal numbers by quantitative real-time PCR (qPCR) and to study the archaeal population structure by denaturing gradient gel electrophoresis. In addition, on days 17 and 18 samples of alfalfa hay were placed into nylon bags and incubated in the rumen of each goat for 24 and 48 h to determine the ruminal degradation of dry matter (DM) and neutral detergent fiber (aNDFom). During the last 5 days of the trial, nutrient digestibility, N and energy balances and urinary purine derivative (PD) excretion were determined. Additionally, an *in vitro* experiment with pure cultures of methanogens was conducted to test propyl propane thiosulfinate (PTS) at a dose of 200 mg of active component/L culture medium and bromochloromethane (BCM) at a concentration of 10 mg/L culture medium against three different archaeal strains: *Methanobrevibacter ruminantium*, *Methanobrevibacter smithii* and *Methanobrevibacter millerae*. *In vivo*, no significant ($P \leq 0.17$) reduction on methane production was observed although a numerical decrease on day 28 was observed with CD-PTS. The dry matter intake (DMI), nutrient digestibility, N and energy balances, purine derivatives, creatinine and estimated microbial N flow were not affected by the treatment ($P \leq 0.18$). Likewise, total concentration of the analyzed microbial groups in the rumen, showed no difference ($P \leq 0.33$) between treated and non-treated goats. However, on day 28, the structure of the archaeal population in the rumen of goats treated with CD-PTS was different compared with that in control goats. The *in vitro* culture of methanogens showed a substantial reduction of methane production in all the strains by both BCM and PTS.

Abbreviations: ADFom, acid detergent fiber expressed exclusive of residual ash; aNDFom, neutral detergent fiber assayed with heat stable amylase and expressed exclusive of residual ash; BCM, bromochloromethane; BW, body weight; $BW^{0.75}$, metabolic weight; CD-PTS, α-cyclodextrin-propyl propane thiosulfinate complex; CP, crude protein; DGGE, denaturing gradient gel electrophoresis; DM, dry matter; DMI, dry matter intake; Lignin(sa), lignin measured by solubilization of cellulose with sulphuric acid; OM, organic matter; PD, purine derivative; PTS, propyl propane thiosulfinate; qPCR, quantitative polymerase-chain reaction; VFA, volatile fatty acid.

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1. Introduction

The enteric fermentation in ruminants yields methane, which represents a loss of around 2–12% of energy ingested by the animal (Johnson and Johnson, 1995) and significantly contributes to global anthropogenic methane emissions (Hristov et al., 2013). Because reduction in methane emissions could improve productivity in ruminants and decrease the environmental impact of animal production (McAllister and Newbold, 2008), significant efforts are being made to develop nutritional strategies, based on the use of compounds with antimicrobial activity, to reduce methane production in ruminants (Benchaar and Greathead, 2011). The majority of studies focus on identifying additives that specifically affect the activity of methanogenic archaea, responsible for the synthesis of methane in the rumen. Results obtained are mainly based on *in vitro* studies and have been in some cases contradictory (Hart et al., 2008; Patra and Saxena, 2010; Benchaar and Greathead, 2011). Few results obtained *in vivo* are variable and some cases do not confirm *in vitro* observations. This could be explained by different factors: the effective concentration of active compound in the rumen, the effect of the type of diet fed to animals and the time of treatment to allow adaptation of microbiota to the compound. The lack of direct comparative *in vitro–in vivo* studies is definitely limiting the understanding of the factors involved (Benchaar and Greathead, 2011).

The antimethanogenic effect of some garlic-derived compounds *in vitro* has been widely reported (Busquet et al., 2005b; Kamel et al., 2008; Mateos et al., 2013), but the few studies conducted *in vivo* not always confirm such potential (Patra and Saxena, 2010; Klevenhusen et al., 2011). Williams et al. (2009) suggested that ruminal methanogens take longer than 4 weeks to adapt to dietary changes, compared with only 10–15 days needed for bacterial community adaptation. Therefore, medium-term *in vivo* experiments would be necessary to state the effects of different compounds on methanogens and on methane emissions. The propyl propane thiosulfinate (PTS), a organosulphur garlic-derived compound, has been tested *in vitro* and short-term (9 days) *in vivo* trials in our group (Martínez-Fernández et al., 2013), exhibiting a substantial decrease (up to 33%) of methane production. However, the persistency of such an effect in a longer-term study needs to be confirmed.

Therefore, the present study was designed to investigate the effects of treating non-productive goats over a month with α-cyclodextrin-PTS complex (CD-PTS) on nutrient utilization, ruminal fermentation, methane emissions and rumen archaeal population. Additionally, an *in vitro* culture of three strains of methanogens was conducted to assess the effect of PTS on their growth.

2. Material and methods

2.1. Animals, diet and organosulphur compound

Twelve adult dry Murciano-granadina goats (37.8 ± 5.73) fitted with permanent rumen cannula were used. Animals had free access to water and were fed twice a day (09:00 h and 16:00 h) at approximately 1.1 times the energy requirements for maintenance level (Prieto et al., 1990) a diet that consisted of alfalfa hay chopped at 15–20 cm and concentrate in a 50:50 ratio and a mineral–vitamin supplement. The concentrate ingredients composition (g/kg) was wheat meal (350), barley (210), sunflower meal (150), corn meal (90), sorghum (80), soybean meal (50), soybean peel (40), NaCl (20.5) and a vitamin–mineral mixture (10.5). Chemical composition (g/kg) of alfalfa hay and concentrate, respectively, was: DM (923 and 914), OM (801 and 904), Neutral detergent fiber (475 and 338), acid detergent fiber (349 and 129), lignin (71.6 and 27.4), CP (217 and 185), ether extract (14.1 and 28.9) and gross energy (MJ/kg DM, 18.9 and 19.1). Animals were cared by trained personnel and managed in accordance with the Spanish guidelines for experimental animal protection (Royal Decree No. 1201, 2005) and the European Convention for the Protection of Vertebrates used for Experimental and other Scientific Purposes (European Directive 86/609). All the experimental procedures involved in this study were approved by the Animal Welfare Committee at the Institute of Animal Nutrition (CSIC, Spain). Chambers used for methane emissions measurement, temperature, humidity and air turn out were carefully monitored according to the animal welfare conditions. The CO_2 concentration was also continuously monitored to ensure a good air quality and renovation rate in the chambers. Animals did not show any stress-related behavior while they were allocated to chambers.

The PTS is an organosulphur compound derived from garlic as intermediate in the oxidation of thiols to sulfonic acids (Small et al., 1947) and it was provided by DMC Research Center SL (Granada, Spain). The obtained compound is liquid, not water-soluble and very volatile; therefore, in order to increase stability during handling and prior to supply to the animal, the PTS was entrapped in α-cyclodextrin matrix, that yielded a powder containing 90 g of PTS/kg DM as determined by GC–MS (Tung-His et al., 1989).

2.2. Experimental design and sampling

A 36 days trial was carried out. During the whole trial individual DMI was registered. Animals were randomly distributed into 2 experimental groups: control (without additive) and treated (0.208 g/kg of BW of CD-PTS mixture per day to achieve a concentration of 200 mg of active component/kg rumen content). The dose was chosen based on previous results from an *in vitro–in vivo* study in goats, in which PTS decreased CH_4 production by up to 33% without compromising rumen fermentation (Martínez-Fernández et al., 2013). Rumen volume in experimental animals was estimated as 11% of BW as determined in our group from slaughtering ten adult goats of the same breed (Abecia et al., 2012). Twice a day before feeding (9:00 and 15:00 h), the corresponding amounts of CD-PTS mixture plus 1 g of ground oats placed in a cellulose