



Influence of polyethylene glycol on *in vitro* gas production profiles and microbial protein synthesis of some shrub species[☆]

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ABSTRACT

The aim was to determine effects of addition of polyethylene glycol (PEG) on *in vitro* gas production and microbial protein synthesis of the shrub species *Acacia constricta*, *Acacia shaffneri*, *Calliandra eriophylla*, *Condalia lycioides*, *Cordia parvifolia*, *Larrea tridentata* and *Mimosa biuncifera*, which are commonly consumed by grazing small ruminants in semi-arid regions of Mexico despite the abundance of tannins in their foliage. Three individual samples (a minimum of 10 plants of each) of each shrub species were collected from different areas and prepared for chemical and secondary metabolite analysis. *In vitro* evaluations for each sample of each shrub species were completed in three incubation runs in different weeks using calibrated glass syringes in a 7 × 2 factorial design (i.e., 7 shrub species × 2 treatments with or without PEG). *In vitro* gas production after 24 and 96 h, partitioning factor (PF), metabolizable energy (ME) content, efficiency of microbial protein synthesis (EMPS), and volatile fatty acids (VFA) were determined. A shrub species × PEG interaction ($P < 0.05$) only occurred for some VFA, such as C3, C4, C4i and C5i, concentrations. Relative to control, addition of PEG increased ($P < 0.05$) the concentrations of individual VFA in *A. shaffneri*, *C. lycioides* and *C. parvifolia*. Interactions between shrub and PEG occurred ($P < 0.001$) for all gas production parameters (i.e., *b*, *k*, IVGP₂₄, IVGP₄₈). The fractional rate of gas production and IVGP_{24h} were highest in *A. constricta* and lowest ($P < 0.001$) in *A. shaffneri*. *L. tridentata* had the highest PF and *C. lycioides* the lowest. *A. constricta* had the highest ME content. Purine content and EMPS differed (shrubs × PEG; $P < 0.001$) among shrubs. Microbial protein synthesis was highest in *M. biuncifera* and lowest in *C. eriophylla*, while total VFA were highest in *C. eriophylla*. Incorporation of PEG increased ($P < 0.001$) *in vitro* fermentation parameters and ME content with *L. tridentata* and *C. lycioides* being the highest. PEG also promoted

Abbreviations: ADFom, acid detergent fiber expressed exclusive of residual ash; CP, crude protein; CT, condensed tannins; DM, dry matter; EE, ether extract; EMPS, efficiency of microbial protein synthesis; ME, metabolizable energy; NDFom, neutral detergent fiber assayed without α -amylase and expressed exclusive of residual ash; PEG, polyethylene glycol; PF, partitioning factor; VFA, volatile fatty acids.

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reductions in the EMPS and PF values. *In vitro* fermentation variables such as fractional rate of gas production, VFA concentrations and EMPS support the potential of *A. constricta*, *C. parvifolia* and *M. biuncifera* as protein and energy sources for small ruminants in semiarid regions of North Mexico. Increments in gas production in these shrub species confirmed the affinity of PEG to bind condensed tannins and reduce EMPS.

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1. Introduction

Browse constitutes an essential component of the diet of range ruminants in dry areas with prolonged periods of drought when available herbaceous species senesce and have insufficient quality and/or quantity to meet their maintenance nutrient requirements (Parissi et al., 2005; Salem et al., 2007). High crude protein (CP) content, digestible nutrients, minerals and a substantial contribution to the diet selected by small grazing ruminants, up to 820 g/kg, support the relevance of browse to ruminant nutrition (Ramirez, 1999; Sanon et al., 2008).

Many native browse species have been undervalued because of insufficient knowledge of their feeding value and for their often high content of tannins, which may be detrimental to animal performance (Min et al., 2003). The nutritive value of feeds utilized in ruminant nutrition are commonly estimated by the concentrations of chemical components, and the rates and extents of nutrient digestion (Hamid et al., 2007). The *in vitro* gas production procedure has become a useful tool to study potential rumen degradation of ruminant feeds (Getachew et al., 2002; Salem et al., 2007). This method allows estimation of how much substrate is used to produce volatile fatty acids (VFA) and the energetic value of browse, as well as to determine the amount of substrate truly fermented which is converted into microbial protein (Blümmel et al., 2003; Salem et al., 2007). Furthermore, when investigating plants containing secondary compounds, it is crucial to consider effects of such vegetative species on rumen microbial fermentation, making the *in vitro* gas production technique valuable when assessing the potential of tannin rich browse in ruminant nutrition (Salem et al., 2007; Norman et al., 2010).

Polyethylene glycol (PEG), a tannin complexing agent, has the potential to reduce phenolic related anti-nutritive effects in browse plants by forming tannin–PEG complexes (Khazaal et al., 1996), and has been used to mitigate adverse effects of secondary compounds on rumen fermentation. Addition of PEG to high tannin browse species increased *in vitro* gas production, ammonia N concentration and short chain fatty acid production, although microbial N production and efficiency of microbial protein synthesis decreased (Getachew et al., 2001; Salem et al., 2007).

Studies related to use of gas procedures to estimate rumen fermentation of individual native browses from semiarid regions of North Mexico are scarce. Thus, our objective was to evaluate effects of PEG on *in vitro* fermentation profiles and partitioning of fermentation products of native browses selected by goats in semiarid regions.

2. Materials and methods

2.1. Site and sampling procedures

The study was completed at the Facultad de Medicina Veterinaria y Zootecnia of the Universidad Juárez del Estado de Durango, in Durango (México). Foliage samples from *Acacia constricta*, *Acacia shaffneri*, *Calliandra eriophylla*, *Condalia lycioides*, *Cordia parvifolia*, *Larrea tridentata* and *Mimosa biuncifera* were collected from Cuencamé county of the state of Durango located at 24°55'38" LN and 103°48' 21" LO. The site has a dry climate with total annual rainfall of 450 mm, annual mean temperature of 21 °C and an altitude of 1580 m above sea level. The main soils types in the study area are regosol, vertisol, rendzina, xerosol and litosol (INEGI, 2006).

Three samples of at least 10 plants of each within each shrub species were collected from different areas and prepared for chemical and secondary metabolite analysis. Leaves, petioles and thin twigs were manually harvested from different parts of the plant at the optimum stage of vegetation, which was April and May of 2006 and 2007. Samples were dried at 55 °C for 48 h, ground to pass a 1 mm screen in a Wiley mill and stored in sealed dark containers for later analyses.

2.2. Chemical composition

The N (#954.01), ether extract (EE; #929.29) and ash (#938.08) contents were determined as described by AOAC (1997). The neutral detergent fiber (NDFom), acid detergent fiber (ADFom), and lignin(sa) determinations were completed following Van Soest et al. (1991). Condensed tannin (CT) contents were determined using the butanol–HCl procedure and results are as leucocyanidin equivalents (Makkar, 2003a,b).

2.3. *In vitro* gas production

Three incubation runs were conducted for each sample within each shrub species. The rate and extent of *in vitro* gas production from shrub foliages were obtained from a 96 h incubation (Menke and Steingass, 1988) in which triplicate 500 mg samples were incubated in 100 ml calibrated glass syringes. Effects of PEG on *in vitro* gas production was determined

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