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Oral administration of leaf extracts to rumen liquid donor lambs modifies *in vitro* gas production of other tree leaves☆

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ABSTRACT

This study was conducted to determine if adaptation of lambs to ingestion of tree leaf extracts of *Salix babylonica* and *Leucaena leucocephala* can modify *in vitro* gas production of *Celtis ehrenbergiana*, *Ficus trigonata*, *Fraxinus excelsior* and *Prunus domestica*. Samples of leaves were collected in triplicate (*i.e.*, three individual samples of each tree leaf). Rumen inoculum was collected from 8 growing lambs fed a total mixed ration *ad libitum* (control; *RC*). Incubations were repeated with the rumen fluid collected from another 8 growing lambs of the same breed fed the same ration, but fed a daily dose of 30 ml/d of *S. babylonica* and *L. leucocephala* extracts in a 1:1 (v:v) mixture (treatment; RX). Leaf samples were incubated with each rumen fluid (*i.e.*, RC and RX inoculums) in 3 runs on different weeks. Data of each of the three runs within sample replicate were averaged and used as the mean value of each individual sample within tree species for statistical analysis in a 4 (tree species) × 2 (rumen inoculum) factorial design. *In vitro* gas production was recorded at 2, 4, 6, 8, 10, 12, 24, 48 and 72 h of incubation. After 72 h, the incubation was stopped and supernatant pH was determined, and then filtered to determine apparent degraded substrate (ADS). Fermentation parameters, such as the 72 h partitioning factor (PF_{72}), 24 h gas yield (GY_{24}), *in vitro* organic matter digestibility (IVOMD), metabolizable energy (ME), short chain fatty acids concentration (SCFA), and microbial protein production (MP) were estimated. The crude protein content of the leaves ranged from 147 (*F. trigonata*) to 241 (*C. ehrenbergiana*) g/kg dry matter. The lowest fiber fraction values was in *P. domestica*, while *F. excelsior* had the highest, and *C. ehrenbergiana* and *F. trigonata* were intermediate. Secondary metabolites (*i.e.*, total phenolics, saponins, aqueous fraction) were lowest in *P. domestica* and highest in *F. trigonata*. Accumulated gas production was highest ($P<0.05$) in *F. excelsior* during the first 24 h of incubation. All fermentation parameters (*i.e.*, ADS, SCFA, GY_{24}, PF_{72}, IVOMD, ME, MP) varied ($P<0.01$) among tree leaves. The ADS, SCFA and MP were highest ($P<0.01$) in *F. excelsior*, lowest ($P<0.01$) in *F. trigonata*, and intermediate in *P. domestica* and *C. ehrenbergiana*. Incubation of tree leaves with RX inoculum did not affect gas production in the first 6 h, but it was lower ($P<0.05$) at 24–72 h, except for *F. trigonata*. *C. ehrenbergiana* had the highest ($P<0.05$) potential gas production, but rate of gas production and the discrete lag time did

Abbreviations: ADS, apparently degraded substrate; ADFom, acid detergent fiber; CP, crude protein; DM, dry matter; EE, ether extract; GY_{24}, gas yield at 24 h of incubation; IVOMD, *in vitro* organic matter digestibility; MP, microbial CP production; ME, metabolizable energy; NDFom, neutral detergent fiber; PF_{72}, partitioning factor at 72 h of incubation; SCFA, short chain fatty acids.

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not differ among leaves. As incubation of tree leaves with RX inoculum lowered (P<0.05) all fermentation parameters, oral administrated extracts of *S. babylonica* and *L. leucocephala* did not seem to adapt the rumen microbial population to better utilize these tree leaves.

1. Introduction

Browse trees and shrubs can be used as feed supplements in areas with long dry periods or harsh environmental conditions, such as northern and central Mexico, because they provide forage for grazing ruminants throughout the year, and/or at specific critical periods of the year, particularly after herbage senescence when the quantity and quality of herbaceous species is limited. Browse trees and shrubs can be an important component of goat and sheep diets (Papachristou and Nastis, 1996; Salem et al., 2006), and play an important role in nutrition of grazing ruminants in areas where few, or no, alternative feeds are available (Meuret et al., 1990). However use of tree and shrub leaves by ruminants may be restricted by negative effects on digestion of their generally high levels of secondary metabolites (Salem, 2005; Salem et al., 2006, 2007) and/or by their impacts on rumen microorganisms (McSweeney et al., 2001).

Many factors modify activities of rumen microorganisms which relate to fermentation of browse tree species and shrubs. These include animal species (Salem, 2005), diet composition (Getachew et al., 2005), shrub composition (Salem et al., 2006, 2007) and feed additives (Gado et al., 2009, 2011; Jiménez-Peralta et al., 2011; Salem et al., 2011a, 2012). Administration of leaf extracts to ruminants as feed additives have modified *in vitro* ruminal fermentation of high concentrate diets in lambs fed a daily dose of *Salix babylonica* and *Leucaena leucocephala* extract (Jiménez-Peralta et al., 2011), and improved *in vivo* digestibility and average daily gain of lambs (Salem et al., 2011a). Some plant extracts have also improved animal growth and nutrient digestion (Mapiye et al., 2010; Salem et al., 2011a) due to positive impacts of their secondary metabolites on activity of ruminal microorganisms (Jiménez-Peralta et al., 2011; Xu et al., 2010) and/or increased amino acid flow to the duodenum (Mueller-Harvey, 2006), which can result in more muscle deposition and, consequently, heavier carcasses (Gleghorn et al., 2004) and improved meat quality (Mapiye et al., 2010).

The aim was to determine if adaptation of lambs to ingestion of a tree leaf extract rich in plant secondary metabolites can modify or change *in vitro* digestion of some tree species leaves rich in secondary metabolites.

2. Materials and methods

2.1. Tree foliage species collection

Samples of leaves of the four species (*i.e.*, *Celtis ehrenbergiana*, *Ficus trigonata*, *Fraxinus excelsior* and *Prunus domestica*) were randomly and manually harvested from different parts of trees to obtain three individual samples of young and mature leaves from each tree species. Leaf samples were dried at 40 °C for 72 h in a forced air oven to constant weight, ground in a hammer mill to pass a 1 mm sieve and stored in plastic bags for subsequent determination of chemical components, secondary metabolites and *in vitro* gas production.

2.2. Animals as rumen inoculum donors

Rumen inoculum was collected by stomach tube from 8 growing Katahdin × Pelibuey lambs with a live weight of 24 ± 0.3 kg fed a total mixed ration (TMR) *ad libitum* based on soyabean meal, 220; alfalfa hay, 150; sorghum grain, 550; fishmeal, 35; mineral/vitamin premix, 25 and salt, 20 (g/kg dry matter (DM)) and used as the control inoculum (RC). Incubations were repeated with rumen inoculum collected from another 8 growing lambs of the same breed and fed the same TMR, but fed a daily 30 ml dose of extracts of *S. babylonica* and *L. leucocephala* in a 1:1 (v:v) mixture (RX). The RC and RX lambs were fed *ad libitum* a TMR formulated to meet all of their nutrient requirements (NRC, 1985). Extracts were orally administered daily to RX lambs before the 8:00 h feeding. Fresh water was always available.

2.3. Preparation of extracts for RX lambs

Tree leaves of *L. leucocephala* and *S. babylonica* were collected randomly from several young and mature trees during summer. Leaves were fresh chopped into 1–2 cm lengths and immediately extracted at 1 g leaf/8 ml of solvent which contained 10 ml methanol (99.8/100, analytical grade, Fermont®, Monterrey, Mexico), 10 ml ethanol (99/100, analytical grade, Fermont®, Monterrey, Mexico) and 80 ml of distilled water. Plant materials were individually soaked and incubated in solvent in a laboratory at room temperature (*i.e.*, 25–30 °C) for 48–72 h in closed 20 L jars. After incubation, jars were heated at 30 °C for 1 h, and then immediately filtered and individual filtrates (extract) were collected. Extract was prepared weekly (stock volume of 2 L each) by mixing the *S. babylonica* and *L. leucocephala* extracts (500:500, v:v). This mixture was stored at 4 °C prior to daily oral administration to the lambs (Salem et al., 2011a).