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Influence of individual and mixed extracts of two tree species on *in vitro* gas production kinetics of a high concentrate diet fed to growing lambs

F.S. Jiménez-Peralta [a], A.Z.M. Salem [a,b,*], P. Mejia-Hernández [a], M. González-Ronquillo [c], B. Albarrán-Portillo [a], R. Rojo-Rubio [a], J.L. Tinoco-Jaramillo [a]

[a] Centro Universitario UAEM-Temascaltepec, Universidad Autónoma del Estado de México, km 67.5 Carr. Toluca-Tejupilco, Estado de México, C.P. 51300, México
[b] Department of Animal Production, Faculty of Agriculture, El-Shatby, Alexandria University, Egypt
[c] Universidad Autónoma del Estado de México, Departamento de Nutrición Animal, Facultad de Medicina Veterinaria y Zootecnia. Instituto Literario 100. Toluca, Edo. de México. CP. 50000, México

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ABSTRACT

This study was conducted to investigate effects of different doses of *Leucaena leucocephala* (LL) and *Salix babylonica* (SB) extracts, rich in secondary metabolites, and their mixture (LLSB, 1:1, v/v) on *in vitro* gas production and some ruminal fermentation patterns such as truly degraded substrate (TDS), short chain fatty acids (SCFA), and microbial protein production (MP) of a high concentrate diet (HCD) fed to growing lambs. The HCD contained (g/kg DM): crude protein (CP), 208; ether extract (EE), 12; neutral detergent fibre (NDFom), 364; acid detergent fibre (aADFom), 41. Plant extracts were prepared at 1 g DM/8 ml of solvent mixture (methanol:ethanol:water, 1:1:8) and added at levels of 0, 0.6, 1.2 and 1.8 ml/g DM. Rumen liquor was collected from 8 growing lambs (Katahdin × Pelibuey, LW 24 ± 0.3 kg) fed the same HCD. *In vitro* gas production (GP) was recorded at 2, 4, 6, 8, 10, 12, 24, 48 and 72 h of incubation. After 72 h, the incubation was stopped and the inoculants pH was determined and filtered to determine TDS. Ruminal fermentation parameters such as 24 h partitioning factor (PF_{24}), gas yield (GY_{24}), *in vitro* organic matter digestibility (IVOMD), metabolizable energy (ME), SCFA, and MP were also estimated. Tree species × extract level interaction ($P<0.05$) only occurred for gas production at 24 (GP_{24}), 48 (GP_{48}) and 72 h (GP_{72}) of incubation, but there were no interactions before 24 h of incubation. Relative to control, addition of extracts increased ($P<0.05$) gas volume GP_{24}, GP_{48} and GP_{72}, except LLSB extract which had lower ($P<0.05$) values during the first 48 h of incubation *versus* control (*i.e.*, 0 ml/g DM). There was no significant impact of extracts on gas production parameters (*i.e.*, *b*; asymptotic gas production, *c*; rate of gas production and *L*; discrete lag time prior to gas production), while the *L* tended to decrease ($P=0.073$) with increasing extract dose only in LL and SB extracts. Addition of either dose of supplemental LL and SB extracts increased ($P<0.05$) gas production and this increase was higher ($P<0.05$) for 1.2 ml and 1.8 ml than 0.6 ml extract/g DM for GP_{24}, GP_{48} and GP_{72}. In general, gas productions were higher ($P<0.05$) in SB than LL extract. There were no interaction in final ruminal pH, PF_{24} and GY_{24}, while some fermentation parameters (*i.e.*, TDS, IVOMD, ME, SCFA, and MP) were higher ($P<0.05$) in LL and SB extract doses *versus* control. The highest two extract doses (*i.e.*, 1.2 and 1.8 ml/g DM) increased ($P<0.05$) TDS, IVOMD, ME, SCFA and MP values *versus* 0.6 ml/g DM. The SB extract also had higher positive impacts ($P<0.05$) on rumen fermentation parameters *versus* LL extract. It is suggested that the

Abbreviations: aADFom, acid detergent fibre; CP, crude protein; DM, dry matter; EE, ether extract; GY_{24}, gas yield at 24 h of incubation; HCD, high concentrate diet; IVOMD, *in vitro* organic matter digestibility; MP, microbial protein production; ME, metabolizable energy; NDFom, neutral detergent fibre; PF_{24}, partitioning factor at 24 h of incubation; PSM, plant secondary metabolites; SCFA, short chain fatty acids; SP, saponins; TDS, truly degraded substrate; TP, total phenolics.

* Corresponding author. Centro Universitario UAEM-Temascaltepec, Universidad Autónoma del Estado de México, km 67.5 Carr. Toluca-Tejupilco, Estado de México, C.P. 51300, México. Tel./fax: +52 1 521 7162695171.
E-mail address: asalem70@yahoo.com (A.Z.M. Salem).

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individual extracts of LL and SB, but not the mixture, could positively modify rumen gas production and fermentation, which may improve nutrient utilization in growing lambs.

1. Introduction

Many chemical feed additives such as antibiotics, ionophores, methane inhibitors and defaunating agents have been introduced into ruminant feeding to improve rumen fermentation with the aim to enhance efficiency of ruminant production (Hutjens, 1992). However, most of these additives are not used routinely because of toxicity problems to the host animals and potential rumen microbial adaptation. The risk of the presence of residues of these chemicals in milk and meat; and their potential effects on human health, led to its prohibition for use in animal feeds in the European Union in 2006 (Official Journal of the European Union, 2003). Moreover, increased potential resistance to antibiotics as a result of increased use in feeds, and the contribution of methane released by ruminants to greenhouse effects, have compelled ruminant nutritionists and microbiologists to explore natural alternatives to these chemical feed additives for eco-friendly animal production. A group of natural products known as phytogenics have been considered as possible alternatives in recent years (Makkar and Becker, 1997; Busquet et al., 2006; Patra et al., 2006). Phytochemicals, or plant secondary metabolites (PSM), are a part of herbivore diets but are not primarily involved in biochemical processes such as plant growth, development and reproduction. However these bioactive compounds with rumen modifying capability may be of interest in ruminant nutrition (Wallace et al., 2002).

PSM in plants produce a line of defense which ensures survival of plant structure and reproductive elements by protecting against insect predation and/or deterring herbivory. Previously, these PSM were considered as anti-nutritional factors in animal nutrition because of their antibacterial properties and adverse effects on nutrient utilization.

Some bacteria species are capable of metabolizing phenolic compounds (Akin, 1980; Krumholz and Bryant, 1985, 1986a,b; Chen et al., 1988) and may act as catalysts for fibre degradation by increasing access of fibrolytic bacteria to the cell-wall polysaccharides of the diet. Therefore, PSM have beneficial impacts to the rumen function on the basis of their stimulating effect on fermentation, and increasing degradabilities of CP and cell-wall constituents, as well as increasing microbial protein production (MP). However, numerous recent studies have attempted to exploit these PSM as natural feed additives in order to improve the efficiency of rumen fermentation by, for example, enhancing protein metabolism, decreasing methane production, reducing nutritional stress such as bloat and/or improving animal health and productivity (McIntosh et al., 2003; Patra et al., 2006; Benchaar et al., 2007).

Many reviews have recently been published on the potential of plant extracts and PSM, such as saponins, tannins and essential oils (EO), as rumen modifiers (Kamel, 2001; Wallace et al., 2002; Calsamiglia et al., 2007; Hart et al., 2008; Kamra et al., 2008), but they have been primarily focused on changes of rumen fermentation. Sarsaponins are PSM of yucca (*Yucca schidigera*) that have been reported to decrease ammonia N concentration and alter the acetate and propionate proportions in ruminal fluid (Grobner et al., 1982; Ryan et al., 1997; Singer et al., 2008). However, other authors found no effect of yucca extract on ammonia N concentration (Wang et al., 1997; Hristov et al., 1999). Thymol, a PSM present in oregano (*Origanum vulgare*), decreased acetate and propionate concentrations and increased the acetate: propionate ratio in *in vitro* mixed ruminal fluid incubations (Evans and Martin, 2000). Although many other plant extracts have been shown to affect microbial activity (Cowan, 1999), few have been tested for their effects on ruminal microbial fermentation. Some plant extracts having a high content of flavonoids decrease methane production and stimulate microbial metabolism which increases both degradability of crude protein and cell-wall constituents and the efficiency and yield of microbial biomass (Broudiscou et al., 2002). Tannins have also been found to reduce methane production (Woodward et al., 2001). However, the effectiveness of plant extracts having high content of saponins, flavonoids and tannins varies depending upon the source, type and level of secondary metabolite in it.

This experiment was conducted to evaluate impacts of natural plant extracts of *Leucaena leucocephala* (LL) and *Salix babylonica* (SB) extracts, rich in secondary metabolites, and their 1:1 mixture at four doses on *in vitro* gas production and ruminal fermentation of growing lambs fed a high concentrate fed.

2. Materials and methods

2.1. Preparation of extracts

Tree plant leaves (*L. leucocephala* (LL) and *S. babylonica* (SB)) were collected randomly from several young and mature trees during summer. Leaves were fresh chopped (1–2 cm) and immediately extracted at 1 g leaf/8 ml of solvent mixture. The mixture of solvents contained 10 ml methanol (99.8/100, analytical grade, Fermont®, Monterrey, Mexico), 10 ethanol ml (99/100, analytical grade, Fermont®, Monterrey, Mexico) and 80 ml distilled water. Plant materials were individually soaked and incubated in solvent at in the laboratory at 25–30 °C for 48–72 h in closed flasks. After incubation, all flasks were incubated in a water bath at 39 °C for one hour, and then immediately filtered and the filtrates were collected and stored at 4 °C for further use.

2.2. Treatments

Two plants extract (*i.e.*, LL and SB) and their 1:1 (v/v) mixture (*i.e.*, LLSB) were examined at four doses (*i.e.*, 0, 0.6, 1.2, 1.8 ml/g DM of high concentrate diet (HCD)) in three replicates for each treatment on the resultant *in vitro* fermentation kinetic profile of HCD. The HCD contained in g/kg on a DM basis: organic matter (OM), 973; crude protein (CP), 208; ether extract (EE), 12; neutral detergent fibre (NDFom), 364; acid detergent fibre (aADFom), 41, Table 1), which was also used to feed the rumen fluid donor lambs.