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ORIGINAL ARTICLE

Effect of increasing levels of seven tree species extracts added to a high concentrate diet on *in vitro* rumen gas output

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ABSTRACT

This study was conducted to investigate the effects of increasing levels of extracts of *Byrsonima crassifolia*, *Celtis pallida*, *Enterolobium cyclocarpum*, *Fraxinus excelsior*, *Ficus trigonata*, *Phoradendrom brevifolium* and *Prunus domestica* on *in vitro* gas production (GP) and ruminal fermentation of a high concentrate diet. Plant extracts were prepared at 1 g dry matter (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 of a high concentrate diet. *In vitro* GP was recorded at 2, 4, 6, 8, 10, 12, 24, 48 and 72 h of incubation. Increasing addition of extracts linearly increased ($P < 0.001$), the GP_{24}, GP_{48} and GP_{72} (mL/g DM), and linearly decreased ($P < 0.001$), the discrete GP lag time. Moreover, increasing extract doses linearly increased ($P < 0.001$) the asymptotic GP and decreased ($P < 0.001$) the rate of GP. GP_6 was not impacted by treatments and GP_{12} increased linearly ($P = 0.01$) with increasing addition of extracts. Rumen pH declined linearly ($P < 0.05$) with increasing doses of extracts added. As no interactions ($P > 0.05$) occurred between the extracts and doses, it could be conclude that all extracts positively modified rumen fermentation at doses of 1.2 to 1.8 mL extract/g diet DM.

Key words: in vitro *gas production, plant extracts, rumen fermentation.*

INTRODUCTION

There has been an increased interest in using natural feed additives such as plant secondary metabolites (PSM), generally recognized as safe for human consumption (Food and Drug Administration 2004) and to modify rumen microbial fermentation. Crude extracts of a variety of plants contain saponins, terpenoids, phenolics, phenolic glycosides, tannins, alkaloids and essential oils, that have the ability to alter rumen fermentation patterns (Salem *et al*. 2011, 2014). The effectiveness of plant extracts depends upon the source, type and level of PSM (Bodas *et al*. 2012). Oral administration of *Salix babylonica* and *Leucaena leucocephala* extracts to growing lambs as feed additives, in lower or moderate doses, have modified *in vitro* ruminal fermentation (Jiménez-Peralta *et al*. 2011), and increased digestibility and average daily gain of lambs (Salem *et al*. 2011). Some extracts have also improved animal growth and nutrient digestion (Salem *et al*. 2011, 2014) due to positive impacts of their PSM on ruminal microorganism activity (Jiménez-Peralta *et al*. 2011).

There is a constant relationship between PSM at relatively low and moderate concentrations, and gas production (GP). This depends on the nature, activity and concentration of its active principles (Jiménez-Peralta *et al*. 2011; Salem *et al*. 2014). Rumen microorganisms have the ability to degrade low concentrations of alkaloids (Wachenheim *et al*. 1992), saponins (Hart *et al*. 2008) and phenolics (Varel *et al*. 1991) and utilize them as an energy source, without negative effects on rumen fermentation. The result of stimulated effects on fermentation, due to

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presence of PSM should maintain more efficient nutrients utilization, and by increasing degradabilities of crude protein (CP) and cell-wall constituents, as well as increasing metabolizible protein (MP) due to improved synchronization between energy and protein release in the rumen. Fermentation of dietary carbohydrates to acetate, propionate and butyrate produce gases in the rumen which mainly constitutes hydrogen, carbon dioxide and methane. However, fermentability of protein produces relatively small GP compared to carbohydrate fermentation (Makkar *et al*. 1995). In contrast, consumption of large amounts of tannins or saponins may have a direct hemolytic effect and may even cause death (Athanasiadou & Kyriazakis 2004). Sarsaponins (*Yucca schidigera*; thymol, secondary metabolites of *Origanum vulgare*) have been reported to change the acetate and propionate proportions in rumen fluid (Singer *et al*. 2008).

Moreover, some other plant extracts with high flavonoid (Broudiscou *et al*. 2002) and tannin (Woodward *et al*. 2001) contents decreased methane production with more microbial biomass yield, which results in higher degradability and utilization of CP and cell-wall constituents. Jiménez-Peralta *et al*. (2011) found that addition individual extracts of *S. babylonica* and *L. leucocephala* increased gas volume after 24, 48 and 72 h; however, the combination of two extracts had lower values during the first 48 h of incubation. The highest two doses used (1.2 and 1.8 mL/g dry matter (DM) of diet) of individual extracts had more positive impacts than the 0 and 0.6 doses on *in vitro* fermentation. Salem (2012) evaluated if adaptation of lambs to ingestion of tree leaf extracts such as *S. babylonica* and *L. leucocephala* can modify *in vitro* GP of *Celtis ehrenbergiana, Ficus trigonata, Fraxinus excelsior* and *Prunus domestica*. They found that incubations of these tree species with rumen inoculum of lambs fed plant extracts for 60 days reduced ruminal *in vitro* GP and fermentation, and it has been demonstrated the orally administrated extracts mixture could modify rumen GP and fermentation of browse tree species, which may affect their utilization by growing lambs.

This experiment was conducted to evaluate the impact of different levels of seven tree species extracts (rich in PSM) added to a high concentrate diet, on *in vitro* rumen GP and fermentation kinetics.

MATERIALS AND METHODS

Preparation of extracts

Leaves from seven trees (*Byrsonima crassifolia*, *Celtis pallida*, *Enterolobium cyclocarpum*, *Fraxinus excelsior*, *Ficus trigonata*, *Phoradendrom brevifolium* and *Prunus domestica*) were collected randomly in triplicate from several young and mature trees during summer. Leaves were fresh chopped (1–2 cm) and immediately extracted at 1 g leaves/8 mL of solvent mixture. The mixture of solvents contained 10 mL methanol, 10 mL ethanol and 80 mL distilled water. Plant materials were individually soaked and incubated in solvent in the laboratory at 25–30°C for 48–72 h in closed flasks. Thereafter, all flasks were incubated in a water bath at 39°C for 1 h, and then immediately filtered, and the filtrates were collected and stored at 4°C for further use (Salem *et al*. 2011).

Treatments

The seven plant extracts were tested at four doses (0, 0.6, 1.2, 1.8 mL/g DM) of feed concentrate in three replicates for each treatment on the resultant *in vitro* fermentation kinetic profile of substrate. One kg diet (i.e. substrate) contained on a DM basis 220 g soya bean meal, 150 g alfalfa hay, 550 g sorghum grain, 35 g fishmeal, 25 g mineral-vitamin premix and 20 g salt. The composition of this substrate was (g/kg DM) 973 g organic matter (OM), 208 g CP, 12 g ether extract (EE), 364 g neutral detergent fiber (NDF), 41 g acid detergent fiber (ADF). The same substrate was fed to lambs used for the rumen fluid donor during incubations (Jiménez-Peralta *et al*. 2011).

In vitro incubations

Rumen fluid was collected by a stomach tube from eight growing lambs (Katahdin × Pelibuey, live weight 24 ± 0.3 kg) fed *ad libitum* the diet described in Table 1. Samples (1 g) of substrate were weighed into 120 mL serum bottles. Extract doses (0, 0.6, 1.2, 1.8 mL/g DM) of the seven tree species were applied directly onto the substrate inside the bottles immediately before adding buffer medium and rumen fluid.

Rumen fluid from each sheep was obtained shortly before the morning meal, flushed with CO_2, then mixed and strained through four layers of cheesecloth into a flask with an O_2-free headspace. Ten mL of particle-free rumen fluid was added to each bottle and 40 mL of the buffer solution of Goering and Van Soest (1970), without trypticase was immediately added in a proportion of 1:4 (v/v). The solution contained micro-minerals ($CaCl_2{\cdot}2H_2O$, $MnCl_2{\cdot}4H_2O$, $CoCl_2{\cdot}6H_2O$, $FeCl_3{\cdot}6H_2O$), macro-minerals (Na_2HPO_4 anhydrous, KH_2PO_4 anhydrous, $MgSO_4{\cdot}7H_2O$) and reducing solution (1N NaOH, $Na_2S{\cdot}9H_2O$), in addition to resazurin and buffer reagent of NH_4HCO_3 and $NaHCO_3$.

A total of 252 bottles, three bottles for each extract dose (0, 0.6, 1.2, 1.8 mL extract (g DM of substrate)) and seven trees of each individual extract sample (three of each) in three runs with three bottles as blanks (rumen fluid only), in each

Table 1 Secondary metabolite levels in extracts from leaves of seven tree species

Tree species	Secondary compounds, g/kg dry matter		
	Total phenolics	Saponins	Aqueous fraction
Byrsonima crassifolia	86.9	94.9	133.1
Celtis pallida	37.3	33.2	156.5
Enterolobium cyclocarpum	26.4	12.3	85.1
Fraxinus excelsior	58.9	12.4	116.8
Ficus trigonata	65.6	20.4	96.5
Phoradendron brevifolium	21.9	23.7	135.5
Prunus domestica	7.6	13.3	135.2

run, were incubated for 72 h. Once all the bottles were filled, they were immediately closed with rubber stoppers, shaken and placed in the incubator at 39°C. Volume of gas produced was recorded at incubation times of 2, 4, 6, 8, 10, 12, 24, 48 and 72 h after inoculation, using the reading pressure technique (RPT; Delta Ohm, Padua, Italy) described by Theodorou *et al*. (1994). At the end of the incubation period, bottles were uncapped, the pH was measured immediately with a pH meter (GLP 22, Crison Instruments, Barcelona, Spain), and the fermentation was stopped by swirling the bottles in ice. The contents of each bottle were then transferred to filtered fermentation residue for determination of DM degradability (DMD).

DMD

The DMD was determined at the end of the incubation period as previously described in Salem (2012). Briefly, after 72 h of incubation, the contents of each serum bottle were filtered through sintered glass crucibles under vacuum. Fermentation residues were dried at 105°C overnight to estimate potential DM disappearance. Weight loss after drying was taken as undegradable DM. The DMD (mg/g DM) at 72 h of incubation was calculated as the difference between substrate DM content and its undegradable DM.

Proximate analyses and secondary metabolites

Diet sample was analyzed for DM (#934.01), ash (#942.05), CP (#954.01) and EE (#920.39) according to Association of Official Analytical Chemists (1997). The NDF (Van Soest *et al*. 1991), ADF and lignin (Association of Official Analytical Chemists 1997; #973.18) were analyzed using an ANKOM 200 Fibre Analyser Unit (ANKOM Technology Corporation, Macedon, NY, USA). The NDF was assayed without use of α-amylase but with sodium sulphite in the NDF. Both NDF and ADF are expressed without residual ash.

The determination of PSM was previously descripted in Salem (2012). Briefly, 10 mL of extract were fractionated by funnel separation with a double volume of ethyl acetate to determine total phenolics by drying and quantifying the phenolics layer in the funnel. After phenolics separation, a double volume of n-butanol, was added to fractionate saponins. The remaining solution was considered to be the aqueous fraction that has the other PSM such as lectins, polypeptides and starch (Cowan 1999).

Calculations

Kinetic parameters of gas production (i.e. GP) were estimated by fitting GP results (mL/g DM) in the NLIN option of SAS (2001) according to the model described by France *et al*. (2000):

$$A = b \times (1 - e^{-c(t-L)})$$

where A is the volume of GP at time t; b is the asymptotic GP (mL/g DM); c is the rate of GP (/h) from the slowly fermentable feed fraction b; and L is the lag time prior to GP.

Metabolizable energy (ME, MJ/kg DM) and *in vitro* organic matter digestibility (OMD, %) were estimated according to Menke *et al*. (1979) as:

$$\text{ME (MJ/kg DM)} = 2.20 + 0.136\ \text{GP} + 0.057\ \text{CP}$$

$$\text{OMD (\%)} = 14.88 + 0.889\ \text{GP} + 0.45\ \text{CP} + 0.0651\ \text{XA}$$

where DM and CP in percent; XA, ash in percent; and GP, the net gas production in mL from 200 mg dry sample after 24 h of incubation.

The partitioning factor at 24 h of incubation ($PF_{24;}$ a measure of fermentation efficiency) was calculated as the ratio of DMD *in vitro* (mg/g DM) to the volume of gas (mL) produced at 24 h (i.e. DMD/total gas production (GP_{24}) according to Blümmel *et al*. (1997)).

Gas yields (GY_{24}) were calculated as the volume of gas produced after 24 h (mL gas/g DM) of incubation divided by the amount of DMD (g) as:

$$\text{Gas yields } (GY_{24}) = \text{mL gas per g DM/g DMD}$$

Short chain fatty acids (SCFA) were calculated (Getachew *et al*. 2002) as:

$$\text{SCFA (mmol/200 mg DM)} = 0.0222\ \text{GP} - 0.00425$$

where GP is 24 h net gas production (mL/200 mg DM).

Microbial biomass production (MP) was calculated as indicated by Blümmel *et al*. (1997):

$$\text{MP (mg/g DM)} = \text{mg DMD} - (\text{mL gas} \times 2.2\ \text{mg/mL})$$

where 2.2 mg/mL is a stoichiometric factor that expresses mg of C, H and O required for the SCFA gas associated with production of 1 mL of gas (Blümmel *et al*. 1997).

Statistical analyses

The experimental design for the *in vitro* ruminal GP and fermentation parameters analysis was a completely random design considering, as fixed factors, tree species (S) and extract doses (D) in the linear model (Steel & Torrie 1980). Data of each of the three runs within the same sample were averaged prior to statistical analysis. Mean values of each individual extract within each species (three samples of each) were used as the experimental unit. The statistical model was:

$$Y_{ijkl} = \mu + S_j + D_k + (S * D)_{jk} + E_{ijkl}$$

where Y_{ijk} = is every observation of the i_{th} tree species (S_i) when incubated in the j_{th} extract doses (D_j); μ is the general mean; S_i ($i = 1–4$) is the feed effect; D_j is the extract dose effect ($j = 1–4$); $(S*D)_{ij}$ is the interaction between tree species and extract dose; and E_{ijk} is experimental error. Linear and quadratic polynomial contrasts were used to examine responses of tree species extract to increasing addition of extract dose.

RESULTS

Total phenolics content varied widely; being particularly high in *B. crassifolia* and low in *P. domestica*. Saponins content ranged from 12.3 in *E. cyclocarpum* to 94.9 (g/kg DM) in *B. crassifolia*. The highest aqueous fraction content was for *C. pallida*, whereas the lowest was for *E. cyclocarpum* (Table 1).

No interactions were observed ($P > 0.05$) between extracts and doses on ruminal GP and *in vitro* ruminal fermentation parameters. However, relative to control, all extracts tended to linearly increase ($P < 0.05$) the asymptotic GP with decreased ($P = 0.012$, linear effect)

lag time with exception of *C. pallida*, which had no impact ($P > 0.05$). Both, *B. crassifolia* and *P. brevifolium* linearly decreased ($P < 0.05$) the *c* fraction of GP with no impact ($P > 0.05$) of extracts. Before 24 h of incubation, extracts did not alter ($P > 0.05$) GP; however, *C. pallida*, *E. cyclocarpum* and *P. domestica* started to linearly increase ($P < 0.05$) GP followed by *F. trigonata* and *P. brevifolium* after 48 h of incubation. At 72 h of incubation, all extracts tended to linearly increase ($P < 0.05$) GP. The asymptotic GP (mL/g DM) was linearly increased ($P < 0.01$) as the extract dose increased, whereas the rate of GP and the lag time before GP begins tended to linearly decrease ($P < 0.01$). Addition of all extracts except that of *E. cyclocarpum* at 0.6 and 1.2 mL extract/g DM linearly increased ($P < 0.05$) GP at GP_{24}, GP_{48} and GP_{72} (Table 2).

Extracts of *B. crassifolia*, *F. excelsior, F. trigonata* and *P. brevifolium* did not alter ($P > 0.05$) all *in vitro* ruminal fermentation profiles. However, *C. pallida*, *E. cyclocarpum* and *P. domestica* linearly increased ($P < 0.05$) DMD, OMD ME, PF_{24}, SCFA and MCP, with no effects ($P > 0.05$) on GY_{24}. The effective ($P < 0.05$, linear effect) doses for those parameters varied between different extracts (i.e. 1.2 and 1.8 for *C. pallida*, *F. excelsior*, *P. brevifolium* and *P. domestica*; 0.6 and 1.2 for *B. crassifolia* and *F. trigonata;* 0.6 and 1.8 for *E. cyclocarpum*) compared to other doses (Table 3).

DISCUSSION

Gas production

Many recent studies have shown that PSM have potentiality to improve rumen fermentation and GP favorably, at relatively low and moderate concentrations (Jiménez-Peralta *et al*. 2011; Salem *et al*. 2011, 2014; Bodas *et al*. 2012; Salem 2012; Abarghuei *et al*. 2013). In this study, PSM may suppress protozoal populations, increase bacterial and fungal populations, and microbial activity with decreasing methanogenesis which will be positively reflected in the form of increased amino acid flow to the duodenum (Mueller-Harvey 2006) and more muscle deposition and greater milk production (Abarghuei *et al*. 2013). The GP is generally a good indicator of digestibility, fermentability and microbial protein production. Higher gas values indicating a better nutrient availability for rumen microorganisms (Mahala & Fadel Elseed 2007). In our study, different responses on rumen fermentation were expected due to the different PSM contents of the different trees used.

Increased GP was paralleled with decreasing the initial delay before GP begins with individual extracts administration. The improvement in GP could be ascribed to the low or moderate amount of PSM in the trees studied, which had positive impacts on ruminal fermentation (Jiménez-Peralta *et al*. 2011; Salem *et al*. 2014), and possibly due to the ability of rumen microorganisms to degrade them in plant extract and utilize them as an energy source (Wachenheim *et al*. 1992; Hart *et al*. 2008). Degradation of some steroidal saponins by rumen microbes has been demonstrated (Gutierrez *et al*. 1959). *Butyrivibrio* and *Bacteroides* strains capable of degrading alfalfa saponins have been isolated from the rumen (Gutierrez *et al*. 1959). However, PSM at high doses are recognized as antimicrobial agents which act against bacteria, protozoa and fungi (Bodas *et al*. 2012). Phenolic compounds at high levels have the potential for intruding into the bacterial cell membrane to disintegrate membrane structures which causes ion leakage. However, the effects are modulated by the rumen pH, the diet in which they are included and by methods of preparation and extraction (Patra & Saxena 2009).

The varied response to individual extracts observed in the present study depended on the nature, activity and concentration of its active compounds (Bodas *et al*. 2012). However, differences in the activity of PSM of the individual plants in our study are not only due to their different chemical nature, activity and concentration of active compounds, but also to factors that can influence the concentration and activity of PSM within each plant species (Jiménez-Peralta *et al*. 2011). In addition, some variations among leaf extracts could be due to genotypic characteristics relative to the type of PSM activity on rumen digestibility (Patra & Saxena 2009). The increase in GP was expected and agrees with the results of previous studies (Jiménez-Peralta *et al*. 2011; Salem *et al*. 2011).

Degradation of OM in the rumen by the action of microorganisms produces gases, mainly hydrogen, carbon dioxide and methane. Methane production has negative effects on ruminants, causing energy loss and bloat as a result of its accumulation in the rumen. It is likely that the use of high doses of plant extracts with antimicrobial activity would decrease microbial activity and diet fermentability (Bodas *et al*. 2012).

In vitro rumen fermentation profile

Comparison between the fermentation patterns showed that only three extracts differed (*C. pallida*, *E. cyclocarpum* and *P. domestica*). It is very clear that the response of fermentation patterns depends on harmony between different PSM compounds in each extract. *C. pallida* had higher total phenolics and saponins, resulting in higher rumen fermentation patterns. *P. domestica* had intermediate content of saponins and the aqueous fraction was moderate. In contrast, *E. cyclocarpum* had the lowest saponins content and the aqueous fraction resulted in lower response.

Plant extracts with high content of active constituents, such as flavonoids, can decreased methane production and stimulate microbial metabolism causing higher degradability of CP and cell-wall constituents and the efficiency and yield of microbial biomass

Table 2 *In vitro* rumen gas kinetics and cumulative gas production after 72 h of incubation as affected by increasing levels of seven tree species extracts (mL/g dry matter (DM))

Tree species	Dose of extract	Gas production parameters†			*In vitro* gas production (mL/g DM)				
		b	*c*	*L*	GP_6	GP_{12}	GP_{24}	GP_{48}	GP_{72}
Control	0	231	0.059	3.14	71	119	176	216	226
Byrsonima crassifolia	0.6	315	0.047	2.04	78	137	214	282	304
	1.2	329	0.047	1.98	80	141	221	293	317
	1.8	395	0.030	1.69	64	117	199	296	345
	P								
	Linear	0.001	0.012	0.049	0.686	0.929	0.540	0.062	0.010
	Quadratic	0.599	0.792	0.440	0.403	0.358	0.300	0.283	0.340
Celtis pallida	0.6	269	0.081	2.52	103	167	230	263	268
	1.2	313	0.062	2.03	97	165	243	297	310
	1.8	363	0.050	1.99	95	165	255	331	353
	P								
	Linear	0.001	0.260	0.052	0.121	0.067	0.021	0.004	0.002
	Quadratic	0.538	0.318	0.297	0.303	0.310	0.337	0.416	0.481
Enterolobium cyclocarpum	0.6	255	0.079	2.37	96	156	217	249	254
	1.2	324	0.043	1.75	74	131	208	281	308
	1.8	373	0.046	1.89	89	157	247	330	358
	P								
	Linear	0.001	0.124	0.049	0.256	0.144	0.042	0.005	0.002
	Quadratic	0.488	0.307	0.233	0.717	0.779	0.916	0.818	0.644
Fraxinus excelsior	0.6	248	0.074	2.03	88	144	204	240	246
	1.2	294	0.053	1.63	80	138	211	270	287
	1.8	306	0.049	1.50	79	137	212	277	297
	P								
	Linear	0.027	0.226	0.009	0.600	0.449	0.238	0.079	0.042
	Quadratic	0.376	0.855	0.178	0.696	0.638	0.544	0.436	0.394
Ficus trigonata	0.6	294	0.071	2.43	102	169	241	285	292
	1.2	298	0.063	1.73	94	158	233	284	295
	1.8	319	0.050	1.30	82	143	222	289	310
	P								
	Linear	0.012	0.208	0.006	0.442	0.307	0.141	0.038	0.019
	Quadratic	0.410	0.223	0.352	0.205	0.213	0.238	0.307	0.362
Phoradendrom brevifolium	0.6	262	0.060	1.62	78	133	198	246	258
	1.2	330	0.049	1.70	83	145	226	297	319
	1.8	377	0.034	1.21	67	122	203	293	334
	P								
	Linear	0.001	0.013	0.007	0.819	0.912	0.406	0.040	0.006
	Quadratic	0.379	0.795	0.398	0.340	0.295	0.235	0.194	0.210
Prunus domestica	0.6	264	0.074	2.16	94	154	218	256	262
	1.2	300	0.060	1.90	91	154	229	283	296
	1.8	356	0.049	1.87	90	157	245	321	345
	P								
	Linear	0.001	0.206	0.031	0.197	0.110	0.034	0.006	0.002
	Quadratic	0.797	0.440	0.226	0.443	0.453	0.495	0.615	0.711
Pooled SEM		46.6	0.0126	0.851	22.9	36.1	47.3	49.7	48.0
P-values‡									
Tree species		0.142	0.001	0.696	0.189	0.304	0.600	0.818	0.582
Polynomial effects of extract doses:									
Linear		< 0.001	< 0.001	< 0.001	0.082	0.010	< 0.001	< 0.001	< 0.001
Quadratic		0.070	0.441	0.005	0.066	0.051	0.034	0.027	0.033

†*b* is the asymptotic gas production (mL/g DM); *c* is the rate of gas production (/h); *L* is the initial delay before gas production begins (h).
‡*P*-values of the overall interaction between tree species and extract doses were not significant (i.e. $P > 0.05$) for all the measured and estimated parameters.

(Broudiscou *et al*. 2002). Flavonoids produce phenolic acids, such as 3,4-dihydroxyphenylacetic acid from isoquercitrin and quercetin or phenylacetic acid from naringenin (Winter *et al*. 1989). Phenolic acids such as p-coumaric, ferulic, cinnamic and phloretic, and some monomeric phenolics, decrease methane, acetate and propionate production (Asiegbu *et al*. 1995). Some of the chemical compounds identified as responsible for

Table 3 *In vitro* rumen fermentation profile† of the high concentrate diet with increasing doses of seven tree species extracts (mL/g dry matter (DM))

Tree species	Dose of extract	pH	DMD	OMD	ME	PF_{24}	GY_{24}	SCFA	MCP
Control	0	6.3	591	556	8.2	3.9	5.8	604	173
Byrsonima crassifolia	0.6	6.3	747	625	9.2	4.7	5.4	676	186
	1.2	6.2	774	637	9.4	4.9	5.3	689	188
	1.8	6.0	684	597	8.8	4.4	5.5	647	183
	P								
	Linear	0.221	0.540	0.540	0.537	0.540	0.384	0.540	0.419
	Quadratic	0.672	0.300	0.300	0.300	0.299	0.353	0.300	0.337
Celtis pallida	0.6	6.4	811	653	9.6	5.1	5.3	706	190
	1.2	6.3	864	676	10.0	5.4	5.2	730	192
	1.8	6.2	914	698	10.3	5.6	5.2	753	194
	P								
	Linear	0.741	0.021	0.021	0.021	0.021	0.061	0.021	0.048
	Quadratic	0.723	0.337	0.337	0.335	0.335	0.360	0.337	0.353
Enterolobium cyclocarpum	0.6	6.3	759	630	9.3	4.8	5.4	681	187
	1.2	6.3	722	614	9.0	4.6	5.4	665	185
	1.8	6.2	883	684	10.1	5.5	5.2	738	193
	P								
	Linear	0.794	0.042	0.042	0.042	0.042	0.087	0.042	0.075
	Quadratic	0.785	0.916	0.916	0.916	0.919	0.801	0.916	0.863
Fraxinus excelsior									
	0.6	6.3	708	608	8.9	4.5	5.4	658	184
	1.2	6.3	736	620	9.1	4.7	5.4	671	186
	1.8	6.1	741	622	9.2	4.7	5.4	673	186
	P								
	Linear	0.322	0.238	0.238	0.237	0.235	0.195	0.238	0.206
	Quadratic	0.650	0.544	0.544	0.542	0.542	0.475	0.544	0.487
Ficus trigonata	0.6	6.2	857	673	9.9	5.3	5.2	727	192
	1.2	6.2	823	658	9.7	5.1	5.3	711	190
	1.8	6.1	779	639	9.4	4.9	5.3	691	188
	P								
	Linear	0.312	0.141	0.141	0.141	0.139	0.139	0.141	0.138
	Quadratic	0.869	0.238	0.238	0.238	0.239	0.313	0.238	0.294
Phoradendrom brevifolium	0.6	6.3	683	597	8.8	4.4	5.5	647	183
	1.2	6.3	796	646	9.5	5.0	5.3	699	189
	1.8	6.1	701	605	8.9	4.5	5.5	655	183
	P								
	Linear	0.383	0.406	0.405	0.406	0.405	0.302	0.405	0.331
	Quadratic	0.631	0.235	0.235	0.235	0.234	0.280	0.235	0.264
Prunus domestica	0.6	6.4	763	632	9.3	4.8	5.3	684	187
	1.2	6.3	808	651	9.6	5.1	5.3	704	190
	1.8	6.2	873	680	10.0	5.4	5.2	734	192
	P								
	Linear	0.606	0.034	0.034	0.034	0.034	0.075	0.034	0.063
	Quadratic	0.599	0.495	0.494	0.492	0.494	0.441	0.495	0.451
Pooled SEM		0.30	192.7	84.2	1.29	1.05	0.52	88.5	14.9
P-values‡									
Tree species		0.869	0.600	0.600	0.601	0.600	0.970	0.600	0.924
Polynomial effects of extract doses:									
Linear		0.039	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Quadratic		0.306	0.034	0.034	0.033	0.031	0.030	0.034	0.034

†DMD is dry matter degradability (mg/g DM incubated); OMD is *in vitro* organic matter digestibility (mg/g DM incubated); ME is metabolizable energy (MJ/kg DM); PF_{24} is the partitioning factor at 24 h of incubation (mg DMD: mL gas); GY_{24} is gas yield at 24 h (mL gas/g DMD); SCFA is short chain fatty acids (mmol/g DM); MCP is microbial CP production (mg/g DM).

‡*P*-values of the overall interaction between tree species and extract doses were not significant (i.e. $P > 0.05$) for all the measured and estimated parameters.

this effect were thymol, eugenol, carvacrol (Chiquette & Benchaar 2005), tannins (Hess *et al*. 2005) or tea saponins (Liu *et al*. 2005). Saponins and other phenolic compounds may increase total bacterial concentration as well as the efficiency of microbial synthesis changing rumen fermentation patterns (Hess *et al*. 2003). Moreover, the presence of some active chemical constituents of plant extracts can improve synchronization between energy and protein release in the rumen, resulting in higher microbial protein synthesis and PF_{24}. Some of these phenolic compounds may interact with biosynthesis of aromatic amino acids, as both biosynthesis pathways are linked through cinnamic acid (Salem *et al*. 2011). In addition, phenylpropanoic acid and phenylacetic acid were reported to enhance cellulose degradation and growth of several strains of *Ruminococcus albus* (Stack & Cotta 1986). The role of PSM in ruminal degradation and microbial protein synthesis is well documented. This should include protection of dietary proteins and reduction in microbial proteolysis, peptidolysis, deamination and degradation to ammonia, allowing their escape to the duodenum (Mueller-Harvey 2006). The mechanism in which PSM does this is based on the formation of a compound–protein complex which physically protects it from enzymatic attack by rumen microbial enzymes (Min *et al*. 2002). Another protective mechanism can be based on the reduced peptidolysis and deamination, likely due to direct inhibition of rumen microbial growth or inhibition of hyper-ammonia producing bacteria such as *Clostridium sticklandii, Peptostreptococcus anaerobius* and anaerobic fungi (McIntosh *et al*. 2003).

From these explanations we can expect that administration of plant extracts, rich in plant metabolite compounds, to ruminants will be reflected on improved animal performance in form of productive performance (milk, Abarghuei *et al*. 2013; average daily gain, Salem *et al*. 2011, 2014) in addition to reproduction (Patel *et al*. 2011).

Conclusions

Plant extracts used in the present study appear to have a potential to influence rumen fermentation for a more efficient utilization of dietary energy and protein. Administration of tree species extracts like *Celtis pallida, Enterolobium cyclocarpum* and *Prunus domestica* could potentially improve rumen fermentation kinetics at doses of 1.2 to 1.8 mL/g DM of diet. Since these extracts also suppressed ruminal feed degradability, various levels of the extracts should be tested to find out a suitable dose to get maximum returns without adversely affecting feed degradability. There is a need to study the adaptation of rumen microbes to the antimicrobial action of these PSM on prolonged feeding to ruminants. Moreover, these plant extracts should be tested *in vivo* to elucidate the suitable dose for improving ruminal fermentation with minimum adverse effects on animals, so that these can be practically explored for economic and ecologically friendly livestock production.

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