Agroforest Syst (2020) 94:1433–1443
https://doi.org/10.1007/s10457-019-00408-1

In vitro screening of Algerian steppe browse plants for digestibility, rumen fermentation profile and methane mitigation

Lyas Bouazza · Souhil Boufennara · Mustapha Bensaada · Azzeddine Zeraib · Khalid Rahal · Cristina Saro · María José Ranilla · Secundino López

Received: 11 June 2018 / Accepted: 5 June 2019 / Published online: 10 June 2019

Abstract The aim of this study was to screen the nutritive value and the effects of anti-nutritional secondary compounds (condensed tannins) on in vitro rumen fermentation and methane mitigation of Algerian steppe browse species: *Albizia julibrissin* (pods), *Acacia nilotica* (pods), *Punica granatum* (leaves and pericarp), *Vicia faba* (leaves), *Artemisia herba-alba* (aerial part), *Attriplex halimus* (leaves) and *Calligonum azel* (bark). Chemical composition, and in vitro digestibility, and rumen fermentation kinetics and end-products accumulation in batch cultures were determined. Polyethylene glycol (PEG), a tannin binding agent was used to measure the biological activity of tannins. Protein content was high for *A. julibrissin* and *V. faba* and low for the pericarp of *P. granatum* and bark of *C. azel*. The highest concentrations of total extractable phenols and tannins were observed in *P. granatum*, whereas *A. halimus* showed the lowest concentrations. *A. nilotica, C. azel* and *A. julibrissin* showed the highest and *A. halimus* and *A. herba-alba* the lowest total condensed tannin contents. *Vicia faba* was the most digestible forage. All the browse species used in the current study, with the exception of *C. azel* bark, can be used as alternative feedstuffs for ruminant nutrition. The most promising forage in terms of reduced methane emissions is *Atriplex halimus* foliage, because the decreased methane production is not associated to a reduced rumen degradation and fermentation of this forage in the rumen. However, in vivo studies are warranted to confirm its potential to be included in ruminant diets.

Keywords Nutritive value · Rumen · Roughage · Tannin · In vitro fermentation · Methane

Introduction

Algerian steppe (30 Mha of land) constitutes a transition area between the Sahara Desert and the green belt in the North of the country. The steppe is used mainly for sheep production, farming local breeds well adapted to the extreme environmental conditions and with a particular productive performance. Droughts occur frequently and have a critical influence on vegetation, and thus on rangelands. Currently, steppe rangelands are in a process of degradation due to the fragility of the physical environment, intensified by changes in the pastoral methods (Aidoud 1994).

L. Bouazza (✉) · S. Boufennara · M. Bensaada · A. Zeraib · K. Rahal
Département de biologie moléculaire et cellulaire, Faculté des sciences de la nature et de la vie, Université Abbès Laghrour, 40000 Khenchela, Algeria
e-mail: ilbouazza@yahoo.fr

C. Saro · M. J. Ranilla · S. López
Instituto de Ganadería de Montaña (CSIC-Universidad de León), Departamento de Producción Animal, Universidad de León, 24007 León, Spain

Perennial acacia and shrubby plants from Saharan areas can be used to mitigate desertification, enhancing soil fixation, restoration of the vegetation and rehabilitation of rangelands. Trees, especially those of the *Acacia* genus, are adapted to arid environments. Acacia leaves and pods are a potential source of protein for herbivores, especially when herbaceous vegetation becomes withered during droughts (Osuji and Odenyo 1997). However, their content in anti-nutritional factors (secondary compounds), such as condensed tannins, limits their use as forage (Waghorn et al. 2002). Methane emission from ruminants contributes to the greenhouse gas effect and global warming (UNFCCC 2005). The digestion of feed by rumen microbes (archaea, bacteria, protozoa and fungi) under anaerobic conditions results in the production of volatile fatty acids (VFA), ammonia, CO_2 and methane (Martin et al. 2010). Rumen methane represents a loss of 2–12% of the feed energy (Boadi et al. 2004). Thus, reducing ruminal CH_4 will improve the efficiency of nutrient utilization and contribute to protect the environment. To mitigate enteric CH_4 emissions from ruminants several dietary strategies have been suggested (Beauchemin et al. 2008; Patra et al. 2017). Condensed tannins affect rumen fermentation, and tannin-rich plants can be used to reduce methane production by ruminants (Jayanegara et al. 2009; Goel and Makkar 2012; Naumann et al. 2018). The addition of polyethylene glycol (PEG) has been used to block the tannin activity in plants (Getachew et al. 2000a). The objectives of this study were to screen the nutritive value of Algerian-steppe browse plants, and to assess the effects of their tannins on in vitro rumen fermentation and methanogenesis.

Materials and methods

Plant species

Substrates used in this study were deseeded pods from *Acacia nilotica* and *Albizia julibrissin, Punica granatum* fruits and leaves, aerial part of *Artemisia herba-alba*, and leaves from *Atriplex halimus* and *Vicia faba*, all collected from the steppe area of Djelfa province (34°40′N 3°15′E). Additionally, bark of *Calligonum azel* was collected from the Saharan area of Nakhla (El Oued province, 33°16′N 6°57′E). Samples from different specimens were pooled and oven-dried at 55 °C.

Chemical analysis

Dry matter (DM), ash and crude protein (CP) contents were determined by the methods 934.01, 942.05 and 990.03 of AOAC (2002), respectively. Neutral and acid detergent fibre and acid detergent lignin were determined using the ANKOM fibre analyzer following the procedures described by Van Soest et al. (1991). Extraction of phenolic compounds and colorimetric analyses of phenolics and tannins were performed according to the procedures described by Makkar (2003).

Animals and collection of rumen fluid

Three cannulated mature Merino sheep (49.4 ± 4.23 kg body weight) fed with lucerne hay ad libitum (167 g CP and 502 g NDF per kg DM) with free access to fresh water and mineral/vitamin blocks were used. Rumen digesta was collected before morning feeding, transferred into thermos flasks and taken to laboratory to be filtered through cheesecloth to obtain rumen fluid for the incubations.

In vitro incubations

The bioassay for the assessment of the activity of tannins has been described in detail by Ammar et al. (2004), using the in vitro gas production technique. Samples of forages were weighted in serum bottles that were sealed and incubated at 39 °C, either with or without the addition of PEG (500 mg). The volume of gas produced was recorded using a pressure transducer and a calibrated syringe at 6, 12, 24 and 48 h after inoculation. Gas production was corrected with the values of the blank bottles (without substrate). The increase in gas when PEG was added is a measure of tannin activity (Makkar et al. 1995).

In vitro gas production technique was adapted from that described by Theodorou et al. (1994) to estimate fermentation kinetics. Samples (500 mg) were incubated with 50 mL of buffered rumen fluid in serum bottles. Blanks (bottles with only rumen fluid) were included in each incubation batch. Bottles were closed with rubber stoppers and sealed with aluminium caps and placed in an incubator at 39 °C. The volume of gas produced was recorded at 3, 6, 9, 12, 16, 21, 26, 31, 36,

48, 72, 96, 120 and 144 h using a pressure transducer and a calibrated syringe.

In vitro fermentation end-products were assessed in 24-h batch cultures. Samples (400 mg) of each substrate were incubated in serum bottles filled with 40 mL of buffered rumen fluid at 39 °C. After 24 h of incubation, the volume of gas produced was measured as described above and a sample of gas was taken for the analysis of methane. Bottles were opened, pH was measured and a sample (0.8 mL) was added to a deproteinising solution (0.5 mL) for volatile fatty acids analysis. Methane in fermentation gas was determined by gas chromatography (GC) using a Shimadzu GC-14 B GC (Shimadzu, Japan) equipped with CarboxenTM 1000, 45/60, 2 m × 3.2 mm column (Supelco, USA) and flame ionization detector as described by Garcia-González et al. (2008a). Volatile fatty acids were analysed by gas chromatography using crotonic acid as internal standard (Garcia-González et al. 2008b).

In vitro DM digestibility was determined using the ANKOM-DAISY procedure (Ammar et al. 1999). The procedure followed general conditions described in the standard in vitro digestibility method (Goering and Van Soest 1970). Samples of each substrate (400 mg) were weighted into fibre bags (#57 bags; ANKOM Technology Corporation, Fairport, NY, USA). Bags were sealed and incubated in buffered rumen fluid for 48 h at 39 °C. Bags were washed in cold water and subsequently extracted with boiling neutral detergent for 1 h to determine the undigested residue and to estimate in vitro digestibility (Ammar et al. 1999).

Calculations and statistical analysis

Gas production data were fitted using the exponential model proposed by France et al. (2000):

$$G = A\left[1-\mathrm{e}^{(-c\,(t-L))}\right] \text{ for } t \geq L$$

where G (mL/g) denotes the cumulative gas production at time t; A (mL/g) is the asymptotic gas production; c (h^{-1}) is the fractional fermentation rate and L (h) is the lag time. Extent of DM degradation in the rumen (dg, %) was calculated as proposed by France et al. (2000).

Data were subjected to analysis of variance performed using the GLM procedure of SAS, with browse species and PEG addition as fixed effects and with source of inoculum as a blocking factor (random effect). The Tukey test was used for the multiple comparison of means.

Results and discussion

Chemical composition of the plant material collected from the different species is shown in Table 1. The CP content of the plant species varied widely, being particularly high for deseeded pods of *A. julibrissin* and leaves of *V. faba* and low for the pericarp of *P. granatum* and barks of *C. azel*. The fibre contents ranged from 179 to 513 g NDF/kg DM, and from 113 to 407 g ADF/kg DM. The highest value of ADL was observed for bark of *C. azel* (223 g/kg DM). CP and NDF concentrations influenced the extent of ruminal fermentation of organic matter (OM) and thus the amount of end-products (in particular VFA) released (Njidda and Nasiru 2010).

Tannin composition of the plant species is shown in Table 2. The highest concentrations of total extractable phenols and tannins were observed in *P. granatum* leaves and pericarp, whereas *A. halimus* showed the lowest concentrations. *A. nilotica, C. azel* and *A. julibrissin* showed the highest, and *A. halimus* and *A. herba-alba* the lowest total condensed tannin contents.

For screening plants for potential antimethanogenic activity, a proposed approach is to perform a tannin bioassay jointly with phenolic and tannin chemical analyses (Ammar et al. 2004; Jayanegara et al. 2009). Table 3 shows the effect of adding PEG to batch cultures on fermentation gas production after 6, 12, 24 and 48 h of in vitro incubation of the plant samples in buffered rumen fluid. The response to PEG treatment (represented by the increase over the value without PEG addition) varied with the incubation time. The greatest response to PEG was recorded with *C. azel* barks at 24 h and *A. nilotica* pods at 6 h of incubation. The response to PEG treatment declined with incubation time, except for the pericarp of *P. granatum* and *C. azel*. Screening plants for the biological activity of tannins using a bioassay (gas production with and without PEG) seemed to be a better alternative than chemical analyses (Mlambo et al. 2009). Condensed tannins limit in vitro fermentation, thus the increase in gas production following inclusion of PEG provides a measure of potential effects of tannins on nutrient

Table 1 Chemical composition of the browse plants

Plant species	Dry matter (g/kg)	Dry matter (g/kg)				
		Organic matter	Neutral detergent fibre	Acid detergent fibre	Acid detergent lignin	Crude protein
Albizia julibrissin	904	927	365	278	85	242
Punica granatum (pericarp)	919	956	277	169	50	34
Punica granatum (leaves)	915	911	222	155	95	109
Vicia faba	888	857	179	121	40	194
Atriplex halimus	871	815	253	113	47	157
Acacia nilotica	909	929	323	227	94	167
Artemisia herba-alba	901	920	359	273	115	123
Calligonum azel	916	891	513	407	223	57

Table 2 Concentrations of phenolic compounds (g standard equivalent*/kg DM) in browse species

Plant species	Total extractable phenols	Total extractable tannins	Bound condensed tannin	Free condensed tannin	Total condensed tannin
Albizia julibrissin	77	62	110	545	655
Punica granatum (pericarp)	286	281	37	188	224
Punica granatum (leaves)	312	306	68	11	79
Vicia faba	65	48	157	21	178
Atriplex halimus	26	22	46	3	49
Acacia nilotica	114	111	148	703	851
Artemisia herba-alba	41	37	47	25	72
Calligonum azel	146	137	148	570	719

*Total phenols and tannins were expressed as tannic acid equivalent and condensed tannins as leucocyanidin equivalent

degradability (Makkar 1988). The highest gas production value ($P < 0.05$) was observed in *V. faba* (Table 3). This can be attributed to its lower condensed tannins and lignin but highest CP content (Tables 1 and 2). Comparable results were obtained with pods and leaves of acacia species (Alam et al. 2007; Mlambo et al. 2008; Bouazza et al. 2012). Biological activity of tannins has been reported to vary among forage species due to the chemical structure and nature of tannins (Rubanza et al. 2005) and degree of polymerization (Schofield et al. 2001). In vitro studies show that some tannins are more active than others (Aerts et al. 1999; Osborne and McNeill 2001; Bueno et al. 2008). The PEG-based in vitro tannin bioassay complements the chemical methods because it specifically highlights the presence of potential active tannins, whereas tannin concentration is reported not to be always a good predictor of their biological activity (Mlambo et al. 2009; Vitti et al. 2005). Condensed tannins build complexes with different cell plant constituents and digestive enzymes thus decreasing digestibility (Waghorn 2008). The factor limiting rumen fermentation and digestion is the interaction between tannins and feed constituents such as structural (cellulose, hemicellulose, pectins) and non-structural carbohydrates and all proteins (McSweeney et al. 2001; Jayanegara et al. 2012, 2015), so that PEG binds tannins preventing

Table 3 Gas production (mL/g dry matter incubated) of the browse plant species

Incubation time (h)		*Albizia julibrissin*	*Punica granatum* pericarp	*Punica granatum* leaves	*Vicia faba*	*Atriplex halimus*	*Acacia nilotica*	*Artemisia herba-alba*	*Calligonum azel*	SEM	*P* value		
											Plant species	PEG	Plant × PEG
6	w/o PEG	17.2^{d}	43.6^{b}	30.2^{c}	80.3^{a}	9.4^{de}	4.0^{ef}	17.9^{d}	0.0^{g}	5.2	< 0.001	< 0.001	< 0.001
	w/ PEG	37.6^{c}	55.9^{b}	34.7^{c}	79.8^{a}	8.1^{e}	36.1^{c}	20.8^{d}	0.0^{f}	4.9			
12	w/o PEG	56.6^{d}	97.6^{b}	75.7^{c}	150^{a}	50.1^{d}	23.7^{e}	54.8^{d}	0.0^{f}	8.9	< 0.001	< 0.001	< 0.001
	w/ PEG	96.3^{c}	110^{b}	84.8^{c}	149^{a}	46.8^{d}	89.7^{c}	59.5^{d}	13.0^{e}	8.1			
24	w/o PEG	109^{c}	136^{b}	132^{b}	192^{a}	109^{c}	63.9^{d}	108^{c}	1.5^{e}	10.9	< 0.001	< 0.001	< 0.001
	w/ PEG	145^{c}	163^{b}	138^{c}	193^{a}	108^{d}	145^{c}	114^{d}	28.0^{e}	8.6			
48	w/o PEG	131^{c}	149^{bc}	161^{b}	208^{a}	149^{bc}	84.3^{d}	134^{bc}	8.9^{e}	11.7	< 0.001	< 0.001	< 0.001
	w/ PEG	173^{bc}	197^{ba}	171^{bc}	225^{a}	150^{cd}	180^{bc}	135^{d}	44.6^{e}	10.7			

w/o PEG or w/PEG: incubated either without or with polyethylene glycol, respectively

SEM Standard error of the mean

^{a,b,c,d}means in the same row with different superscripts are significantly different ($P < 0.05$)

its binding to feed constituents thus suppressing their effects on digestion. The main interaction between tannins and feed molecules is via hydrogen bonds (Mueller-Harvey 2006).

Table 4 shows that the addition of PEG affects ($P < 0.05$) the asymptotic gas production (A) and fractional rate of fermentation (c) to a lesser extent than gas production measurements at certain incubation times. The lowest ($P < 0.05$) A, c and dg values were for *C. azel* in absence and presence of PEG, showing that this is a rather undegradable material. In contrast, *Vicia faba* foliage was the most degradable substrate, with the highest A, c and dg values. Guimarães-Beelen et al. (2006) suggested that when the rate of gas production is reduced, the bacteria proliferation is restricted. Elahi et al. (2014) explained that tannin complexes limit the attachment of bacteria to the feed components.

Despite the increase in gas production upon the addition of PEG, in vitro digestibility was not significantly affected ($P < 0.05$) by the addition of PEG (Table 4). Similar observations have been reported elsewhere (Makkar et al. 1995; Getachew et al. 2000b; Osuga et al. 2008; Bouazza et al. 2014). This could be due mainly to the tannin-PEG complexes, which become insoluble in neutral detergent solution, thus distorting the weight of the incubation residue (Osuga et al. 2008). However, the chemical structure, concentration and biological effects of tannins in forages, and their nutritional value, show large variability (McSweeney et al. 2001).

Methane production was lowest when *C. azel* bark or *A. halimus* foliage were fermented in vitro (Table 5). *C. azel* bark is a low degradable substrate, and less methane is produced just because this material is fermented only to a minor extent. When compared the values with or without the addition of PEG, it seems that tannin has a depressing activity on methane production when either *A. julibrissin* or *A. nilotica* are fermented. Other acacia species seem to be effective reducing methane in the rumen (Grainger et al. 2009). The inhibitory effects of condensed tannins on methanogenesis have been attributed to their direct effects on rumen methanogenic archaea and protozoa (Patra and Saxena 2009), indirectly leading to a depression of fibre degradation (Tiemann et al. 2008; Patra and Saxena 2011). Any reduction in fibre degradation is likely to reduce methane by limiting the availability of H_2 as a substrate for methanogenesis (Moss et al. 2000; Jayanegara et al. 2011). Tannins are known to decrease protozoa (Bhatta et al. 2009) which are in close association with methanogens (Morgavi et al. 2010). The magnitude of decrease due to condensed tannins in the present study was relatively higher than in other studies using tannins extracted and purified from plant leaves (Jayanegara et al. 2015). Different forms of tannins (whole plants or extracted tannins) may influence the CH_4 emissions and rumen fermentations parameters differently, most probably affecting the magnitude of the effects (Jayanegara et al. 2011). The reduced methane production when *A. halimus* was incubated is in agreement with results reported by other authors (Soltan et al. 2012; Medjekal et al. 2018). In this particular case, the effect cannot be attributed to phenolic compounds or tannins, as these were very low in *Atriplex*. In the whole plant, rumen methanogenesis can be affected not only by tannins, but also by other components such as fibre (Beauchemin et al. 2008), lipids (Machmüller et al. 2000), saponins (Hess et al. 2003) or essential oils (Benchaar et al. 2008). Furthermore, *Atriplex* foliage was a highly degradable material (based on gas production kinetics and in vitro digestibility coefficients), suggesting that the observed low methane production could be due to a specific effect on methanogenesis rather to a broad inhibitory effect on ruminal fermentation. If this effect could be further confirmed, *A. halimus* could be a promising highly digestible roughage limiting the methane emissions by ruminants.

Ruminal VFA concentrations mainly indicate the degradation patterns of carbohydrates by microbes. Based on total VFA, acetate and propionate concentrations, *V. faba* leaves seemed to be the most fermentable substrate (Table 5). The highest acetate to propionate ratio was observed with *C. azel* bark. High acetate to propionate ratios will indicate a more acetogenic fermentation, due to the activity of fibrolytic bacteria degrading substrates rich in structural carbohydrates (Getachew et al. 2004). The depressing effects of tannins on ruminal fermentation of *A. nilotica* pods are revealed from the substantial increase in total and individual VFA concentrations in response to the addition of PEG. This finding was consistent with other researchers using tannin-rich species in their studies (Getachew et al. 2008; Singh et al. 2012; Goel and Makkar 2012), in which total VFA production was limited when tannin-rich tropical

Table 4 In vitro gas production kinetics and digestibility of the browse plant species

Parameter		*Albizia julibrissin*	*Punica granatum* pericarp	*Punica granatum* leaves	*Vicia faba*	*Atriplex halimus*	*Acacia nilotica*	*Artemisia herba-alba*	*Calligonum azel*	SEM	P value		
											Plant species	PEG	Plant × PEG
A (mL/g)	w/o PEG	180^{bc}	178^{bc}	203^{cd}	223^{d}	194^{c}	152^{b}	178^{bc}	66.9^{a}	5.5	< 0.001	0.318	0.823
	w/PEG	177^{bc}	189^{bc}	203^{cd}	231^{d}	192^{bc}	159^{b}	187^{bc}	62.3^{a}	6.7			
c (h^{-1})	w/o PEG	0.052^{c}	0.067^{d}	0.043^{c}	0.097^{e}	0.042^{c}	0.028^{b}	0.053^{c}	0.013^{a}	0.0026	< 0.001	< 0.001	0.100
	w/PEG	0.050^{d}	0.052^{d}	0.043^{cd}	0.086^{e}	0.035^{bc}	0.024^{ab}	0.046^{cd}	0.012^{a}	0.0023			
L (h)	w/o PEG	0.96^{ab}	0.00^{a}	0.00^{a}	0.19^{a}	3.30^{b}	1.00^{ab}	1.19^{ab}	0.92^{ab}	0.548	< 0.001	0.534	0.904
	w/PEG	1.20^{a}	0.00^{a}	0.10^{a}	0.33^{a}	2.61^{a}	1.42^{a}	0.40^{a}	0.00^{a}	0.623a			
dg (%)	w/o PEG	44.1^{c}	54.5^{d}	52.4^{d}	69.7^{e}	45.7^{c}	29.7^{b}	47.4^{c}	12.9^{a}	0.85	< 0.001	< 0.001	0.247
	w/PEG	42.2^{c}	49.5^{de}	52.0^{e}	67.2^{f}	42.6^{c}	26.7^{b}	45.2^{cd}	12.8^{a}	0.83			
AIVD (%)	w/o PEG	68.8^{ef}	59.6^{de}	53.3^{cd}	72.0^{f}	66.3^{ef}	37.5^{b}	45.6^{bc}	27.1^{a}	3.89	< 0.001	0.106	0.410
	w/PEG	70.7^{d}	59.2^{c}	59.0^{c}	72.5^{d}	69.1^{d}	39.8^{b}	48.0^{b}	24.0^{a}	4.13			
TIVD (%)	w/o PEG	73.9^{d}	74.4^{d}	80.0^{e}	87.4^{f}	83.6^{ef}	51.1^{b}	60.4^{c}	44.3^{f}	3.80	< 0.001	0.015	0.330
	w/PEG	75.7^{c}	76.0^{c}	82.2^{e}	86.8^{e}	85.6^{e}	54.3^{b}	60.4^{c}	43.8^{a}	3.84			

A asymptotic gas production, *c* fractional rate of fermentation, *L* Lag time, *dg* rumen degradability, *AIVD* apparent in vitro digestibility, *TIVD* true in vitro digestibility

w/o PEG or w/PEG, incubated either without or with polyethylene glycol, respectively

SEM standard error of the mean

^{a,b,c,d,e,f}means in the same row with different superscripts are significantly different ($P < 0.05$)

Table 5 Fermentation end-products (methane in mmol/g dry matter incubated and volatile fatty acid (VFA) concentrations in mmol/L) when browse plant species were incubated in vitro in buffered rumen fluid for 24 h

		Albizia julibrissin	*Punica granatum* pericarp	*Punica granatum* leaves	*Vicia faba*	*Atriplex halimus*	*Acacia nilotica*	*Artemisia herba-alba*	*Calligonum azel*	SEM	*P* value Plant species	*P* value PEG	*P* value Plant × PEG
Methane	w/o PEG	2.55^{b}	2.63^{b}	1.12^{cd}	4.92^{a}	0.93^{d}	1.72^{c}	1.63^{c}	0.13^{e}	0.094	< 0.001	< 0.001	< 0.001
	w/PEG	3.22^{c}	2.45^{d}	0.88^{e}	4.67^{a}	2.00^{d}	3.81^{b}	1.04^{e}	0.01^{f}	0.080			
Acetate	w/o PEG	31.5^{b}	23.0^{de}	21.4^{e}	38.5^{a}	26.9^{c}	21.2^{e}	24.4^{d}	13.6^{f}	0.31	< 0.001	< 0.001	< 0.001
	w/PEG	32.3^{b}	27.1^{d}	19.6^{f}	38.6^{a}	27.6^{cd}	28.5^{c}	22.2^{e}	16.7^{g}	0.14			
Propionate	w/o PEG	11.8^{a}	6.93^{b}	6.12^{c}	12.2^{a}	7.35^{b}	7.49^{b}	6.24^{c}	3.35^{d}	0.092	< 0.001	< 0.001	< 0.001
	w/PEG	12.2^{a}	8.37^{c}	5.17^{e}	12.1^{a}	7.56^{d}	9.45^{b}	5.39^{e}	3.97^{f}	0.095			
Isobutyrate	w/o PEG	0.75^{ab}	0.38^{c}	0.46^{c}	0.94^{a}	0.57^{bc}	0.54^{cd}	0.75^{ab}	0.49^{c}	0.028	< 0.001	< 0.001	0.002
	w/PEG	0.88^{a}	0.57^{c}	0.49^{c}	0.89^{a}	0.62^{bc}	0.80^{ab}	0.76^{ab}	0.55^{c}	0.026			
Butyrate	w/o PEG	2.64^{d}	3.66^{b}	2.83^{d}	4.69^{a}	3.23^{c}	1.69^{e}	2.69^{d}	1.67^{e}	0.045	< 0.001	< 0.001	< 0.001
	w/PEG	3.34^{d}	4.33^{b}	2.80^{e}	4.67^{a}	3.38^{d}	3.67^{c}	2.51^{f}	2.20^{g}	0.021			
Isovalerate	w/o PEG	0.94^{c}	0.52^{e}	0.68^{d}	1.30^{a}	0.93^{c}	0.76^{d}	1.18^{b}	0.68^{d}	0.018	< 0.001	< 0.001	< 0.001
	w/PEG	1.23^{ab}	0.68^{d}	0.73^{d}	1.34^{a}	0.88^{c}	1.22^{ab}	1.14^{b}	0.99^{c}	0.021			
Valerate	w/o PEG	0.54^{b}	0.33^{d}	0.36^{cd}	1.36^{a}	0.53^{b}	0.47^{bcd}	0.50^{bc}	0.33^{d}	0.021	< 0.001	< 0.001	< 0.001
	w/PEG	0.71^{bc}	0.43^{d}	0.39^{d}	1.39^{a}	0.49^{cd}	0.78^{b}	0.50^{cd}	0.38^{d}	0.034			
Total VFA	w/o PEG	48.2^{b}	34.8^{d}	31.8^{e}	58.9^{a}	39.5^{c}	32.2^{e}	35.8^{d}	20.2^{f}	0.25	< 0.001	< 0.001	< 0.001
	w/PEG	50.6^{b}	41.4^{d}	29.2^{f}	59.0^{a}	40.5^{d}	44.4^{c}	32.5^{e}	24.8^{g}	0.15			
Acetate to propionate ratio	w/o PEG	2.68^{d}	3.32^{bc}	3.50^{ab}	3.16^{bcd}	3.66^{ab}	2.84^{cd}	3.92^{a}	4.08^{a}	0.085	< 0.001	0.020	0.158
	w/PEG	2.64^{e}	3.24^{d}	3.78^{bc}	3.18^{d}	3.65^{c}	3.02^{d}	4.12^{ab}	4.20^{a}	0.049			

w/o PEG or w/PEG, incubated either without or with polyethylene glycol, respectively

SEM standard error of the mean

[a,b,c,d]means in the same row with different superscripts are significantly different ($P < 0.05$)

species are fermented. For most of the forages tested, the results are in agreement with studies in sheep (Priolo et al. 2000) and goats (Silanikove et al. 2006) where VFA concentrations were not affected when animals received the same diets but supplemented with PEG concentrations. Effects on VFA concentration could be also due to the presence of other secondary metabolites that can affect ruminal fermentation negatively (Rira et al. 2015).

Conclusions

In conclusion, all the browse species used in the current study, with the exception of *Calligonum azel* bark, can be used as alternative feedstuffs for ruminant nutrition. *Vicia faba* was the most digestible forage. *Albizia julibrissin* and *Acacia nilotica* are also digestible roughages rich in protein, although their use in ruminant diets can be restrained by their high tannin contents. Methane production with *Calligonum azel* bark is very low, but because this is a rather indigestible material. The most promising forage in terms of reduced methane emissions is *Atriplex halimus* forage, because the decreased methane production is not associated to a reduced rumen degradation and fermentation of this forage in the rumen.

Compliance with Ethical Standards

Human and animal rights Animals were handled and cared in accordance with the Spanish guidelines for experimental animal protection (Royal Decree 1201/2005) and experimental protocols were approved by the Institutional Animal Care and Use Committee of the University of León.

References

Aerts RJ, McNabb WC, Molan A, Brand A, Barry TN, Peters JS (1999) Condensed tannins from *Lotus corniculatus* and *Lotus pedunculatus* exert different effects on in the in vitro rumen degradation of ribulose-1,5(bisphosphate carboxylase/oxygenase (Rubisco) protein. J Sci Food Agric 78: 79–85. https://doi.org/10.1002/(SICI)1097-0010(199901)79:1%3c79:AID-JSFA187%3e3.0.CO;2-K

Aidoud A (1994) Pâturage et desertification des steppes arides en Algérie. Cas de la steppe (*Stipa tenacissima* L.). Paralelo 37(16):33–42 **(GéoProdig, portail d'information géographique)**

Alam MR, Amin MR, Kabir AKMA, Moniruzzaman M, McNeil DM (2007) Effect of tannin in *Acacia nilotica*, *Albizia procera* and *Sesbania acculeata* foliage determined in vitro, in sacco and in vivo. Asian-Australas J Anim Sci 20:220–228. https://doi.org/10.5713/ajas.2007.220

Ammar H, López S, Bochi O, Garcia R, Ranilla MJ (1999) Composition and in vitro digestibility of leaves and stems of grasses and legumes harvested from permanent mountain meadows at different maturity stages. J Anim Feed Sci 8:599–610. https://doi.org/10.22358/jafs/69184/1999

Ammar H, López S, González JS, Ranilla MJ (2004) Comparison between analytical methods and biological assays for the assessment of tannin-related antinutritive effects in some Spanish browse species. J Sci Food Agric 84: 1349–1356. https://doi.org/10.1002/jsfa.1766

Association of Official Analytical Chemists (AOAC) (2002) Official methods of analysis, 17th edn. AOAC International, Arlington

Beauchemin KA, Kreuzer M, O'Mara F, McAllister TA (2008) Nutritional management for enteric methane abatement: a review. Aust J Exp Agric 48:21–27. https://doi.org/10.1071/EA07199

Benchaar C, McAllister TA, Chouinard P (2008) Digestion, ruminal fermentation, ciliate protozoal populations, and milk production from dairy cows fed cinnamaldehyde, quebracho condensed tannin, or *Yucca schidigera* saponin extracts. J Dairy Sci 91:4765–4777. https://doi.org/10.3168/jds.2008-1338

Bhatta R, Uyeno Y, Tajima K, Takenaka A, Yabumoto Y, Nonaka I, Enishi O, Kurihara M (2009) Difference in the nature of tannins on in vitro ruminal methane and volatile fatty acid production and methanogenic archaea and protozoal populations. J Dairy Sci 92:5512–5522. https://doi.org/10.3168/jds.2008-1441

Boadi D, Benchaar C, Chiquette J, Massé D (2004) Mitigation strategies to reduce enteric methane emissions from dairy cows: Update review. Can J Anim Sci 84:319–335. https://doi.org/10.4141/A03-109

Bouazza L, Bodas R, Boufennara S, Bousseboua H, López S (2012) Nutritive evaluation of foliage from fodder trees and shrubs characteristic of Algerian arid and semi-arid areas. J Anim Feed Sci 21:521–536. https://doi.org/10.22358/jafs/66126/2012

Bouazza L, Boufennara S, Bousseboua H, Tejido ML, Ammar H, Bodas R, López S (2014) Methane production from the rumen fermentation of Algerian Acacia tree foliage. Forage resources and ecosystem services provided by mountain and Mediterranean grasslands and rangelands. Opt Mediterr A 109:797–800

Bueno ICS, Vitti DMSS, Louvandini H, Abdalla AL (2008) A new approach for in vitro bioassay to measure tannin biological effects based on a gas production technique. Anim Feed Sci Technol 141:153–170. https://doi.org/10.1016/j.anifeedsci.2007.04.011

Elahi MY, Nia MM, Salem AZM, Mansouri H, Olivares-Pérez J, Cerrillo-Soto MA, Kholif AE (2014) Effect of polyethylene glicol on in vitro gas production kinetics of *Prosopis cineraria* leaves at different growth stages. Ital J Anim Sci 13:2. https://doi.org/10.4081/ijas.2014.3175

France J, Dijkstra J, Dhanoa MS, López S, Bannink A (2000) Estimating the extent of degradation of ruminant feeds from a description of their gas production profiles observed in vitro: derivation of models and ther mathematical

considerations. Br J Nutr 83:143–150. https://doi.org/10.1017/S0007114500000180

García-González R, López S, Fernández M, González JS (2008a) Dose–response effects of *Rheum officinale* root and *Frangula alnus* bark on ruminal methane production in vitro. Anim Feed Sci Technol 145:319–334. https://doi.org/10.1016/j.anifeedsci.2007.05.040

García-González R, López S, Fernández M, Bodas R, González JS (2008b) Screening the activity of plants and spices for decreasing ruminal methane production in vitro. Anim Feed Sci Technol 147:36–52. https://doi.org/10.1016/j.anifeedsci.2007.09.008

Getachew G, Makkar HPS, Becker K (2000a) Tannins in tropical browses: effects on in vitro microbial fermentation and microbial protein synthesis in media containing different amount of nitrogen. J Agric Food Chem 48:3581–3588. https://doi.org/10.1021/jf990740v

Getachew G, Makkar HPS, Becker K (2000b) Effet of polyethylene glycol on in vitro degradability of nitrogen and microbial protein synthesis from tannin rich browse and herbaceous legumes. Br J Nutr 84:73–83. https://doi.org/10.1017/S0007114500001252

Getachew G, Robinson PH, DePeters EJ, Taylor SJ (2004) Relationships between chemical composition, dry matter degradation and in vitro gas production of several ruminant feeds. Anim Feed Sci Technol 111:57–71. https://doi.org/10.1016/S0377-8401(03)00217-7

Getachew G, Pittroff W, Putnam DH, Dandekar A, Goyal S, DePeters EJ (2008) The influence of addition of gallic acid, tannic acid, or quebracho tannins to alfalfa hay on in vitro rumen fermentation and microbial protein synthesis. Anim Feed Sci Technol 140:444–461. https://doi.org/10.1016/j.anifeedsci.2007.03.011

Goel G, Makkar HPS (2012) Methane mitigation from ruminants using tannins and saponins. Trop Anim Health Prod 44:729–739. https://doi.org/10.1007/s11250-011-9966-2

Goering HK, Van Soest PJ (1970) Forage fibre analyses (apparatus, reagents, procedures, and some applications). Agric Handb No 379. ARS-USDA, Washington DC

Grainger C, Clarke T, Auldist MJ, Beauchemin KA, McGinn SM, Waghorn GC, Eckard RJ (2009) Potential use of *Acacia mearnsii* condensed tannins to reduce methane emissions and nitrogen excretion from grazing dairy cows. Can J Anim Sci 89:241–251. https://doi.org/10.4141/CJAS08110

Guimarães-Beelen PM, Berchielli TT, Beelen R, Medeiros AN (2006) Influence of condensed tannins from Brazilian semi-arid legumes on ruminal degradability, microbial colonization and ruminal enzymatic activity in Saanen goats. Small Rumin Res 61:35–44. https://doi.org/10.1016/j.smallrumres.2005.01.007

Hess HD, Monsalve LM, Lascano CE, Carulla JE, Díaz TE, Kreuzer M (2003) Supplementation of a tropical grass diet with forage legumes and *Sapindus saponaria* fruits: effects on in vitro ruminal nitrogen turnover and methanogenesis. Aust J Agric Res 54:703–713. https://doi.org/10.1071/AR02241

Jayanegara A, Togtokhbayar N, Makkar HPS, Becker K (2009) Tannins determined by various methods as predictors of methane production reduction potential of plants by an in vitro rumen fermentation system. Anim Feed Sci Technol 150:230–237. https://doi.org/10.1016/j.anifeedsci.2008.10.011

Jayanegara A, Wina E, Soliva CR, Marquardt S, Kreuzer M, Leiber F (2011) Dependence of forage quality and methanogenic potential of tropical plants on their phenolic fractions as determined by principal component analysis. Anim Feed Sci Technol 163:231–243. https://doi.org/10.1016/j.anifeedsci.2010.11.009

Jayanegara A, Leiber F, Kreuzer M (2012) Meta-analysis of the relationship between dietary tannin level and methane formation in ruminants from in vivo and in vitro experiments. J Anim Physiol Anim Nutr 96:365–375. https://doi.org/10.1111/j.1439-0396.2011.01172.x

Jayanegara A, Goel G, Makkar HPS, Becker K (2015) Divergence between purified hydrolysable and condensed tannin effects on methane emission, rumen fermentation and microbial population in vitro. Anim Feed Sci Technol 209:60–68. https://doi.org/10.1016/j.anifeedsci.2015.08.002

Machmüller A, Ossowski DA, Kreuzer M (2000) Comparative evaluation of the effects of coconut oil, oilseeds and crystalline fat on methane release, digestion and energy balance in lambs. Anim Feed Sci Technol 85:41–60. https://doi.org/10.1016/S0377-8401(00)00126-7

Makkar HPS (2003) Quantification of tannins in tree and shrub foliage: a laboratory manual. Springer, Dordrecht

Makkar HPS, Dawra RK, Singh B (1988) Changes in tannin content, polymerisation and protein precipitation capacity in oak (Quercus incana) leaves with maturity. J Sci Food Agric 44:301–307. https://doi.org/10.1002/jsfa.2740440403

Makkar HPS, Blümmel M, Becker K (1995) Formation of complexes between polyvinyl pyrrolidones or polyethylene glycols and tannins, and their implication in gas production and true digestibility in in vitro techniques. Br J Nutr 73:897–913. https://doi.org/10.1079/BJN19950095

Martin C, Morgavi DP, Doreau M (2010) Methane mitigation in ruminants: from microbe to the farm scale. Animal 4:351–365. https://doi.org/10.1017/S1751731109990620

McSweeney CS, Palmer B, McNeill DM, Krause DO (2001) Microbial interactions with tannins: nutritional consequences for ruminants. Anim Feed Sci Technol 91:83–93. https://doi.org/10.1016/S0377-8401(01)00232-2

Medjekal S, Ghadbane M, Benderradji L, Bodas R, Bousseboua H, Lopez S (2018) Methane production from browse species of Algerian arid areas. In: Kallel A, Ksibi M, Ben Dhia H, Khélifi N (eds) Recent advances in environmental science from the Euro-Mediterranean and surrounding regions. Springer, Cham, pp 1301–1303. https://doi.org/10.1007/978-3-319-70548-4_382

Mlambo V, Mould FL, Sikosana JLN, Smith T, Owen E, Mueller-Harvey I (2008) Chemical composition and in vitro fermentation of tannin-rich tree fruits. Anim Feed Sci Technol 140:402–417. https://doi.org/10.1016/j.anifeedsci.2007.03.001

Mlambo V, Mould FL, Smith T, Owen E, Sikosana JLN, Mueller-Harvey I (2009) In vitro biological activity of tannins from *Acacia* and other tree fruits: correlations with colorimetric and gravimetric phenolic assays. S Afr J Anim Sci 39:131–143. https://doi.org/10.4314/sajas.v39i2.44387

Morgavi DP, Forano E, Martin C, Newbold CJ (2010) Microbial ecosystem and methanogenesis in ruminants. Animal

4:1024–1036. https://doi.org/10.1017/S1751731110000546

Moss AR, Jouany J, Newbold J (2000) Methane production by ruminants: its contribution to global warming. Ann Zootech 49:231–253. https://doi.org/10.1051/animres:2000119

Mueller-Harvey I (2006) Unravelling the conundrum of tannins in animal nutrition and health. J Sci Food Agric 86:2010–2037. https://doi.org/10.1002/jsfa.2577

Naumann H, Sepela R, Rezaire A, Masih SE, Reinhardt LA, Robe JT, Sullivan ML, Zeller WE, Hagerman AE (2018) Relationships between structures of condensed tannins from Texas legumes and methane production during in vitro rumen digestion. Molecules 23(9):2123. https://doi.org/10.3390/molecules23092123

Njidda AA, Nasiru A (2010) In vitro gas production and dry matter digestibility of tannin-containing forages of semi-arid region of north-eastern Nigeria. Pak J Nutr 9:60–66. https://doi.org/10.3923/pjn.2010.60.66

Osborne NJT, McNeill DM (2001) Characterisation of *Leucaena* condensed tannins by size and protein precipitation capacity. J Sci Food Agric 81:1113–1119. https://doi.org/10.1002/jsfa.920

Osuga IM, Wambui CC, Abdulrazak SA, Ichinohe T, Fujihara T (2008) Evaluation of nutritive value and palatability by goats and sheep of selected browse foliages from semiarid area of Kenya. Anim Sci J 79:582–589. https://doi.org/10.1111/j.1740-0929.2008.00567.x

Osuji PO, Odenyo AA (1997) The role of legume forages as supplements to low quality roughages-ILRI experience. Anim Feed Sci Technol 69:27–38. https://doi.org/10.1016/S0377-8401(97)81620-3

Patra AK, Saxena J (2009) Dietary phytochemicals as rumen modifiers: a review of the effects on microbial populations. Antonie Van Leeuwenhoek 96:363–375. https://doi.org/10.1007/s10482-009-9364-1

Patra AK, Saxena J (2011) Exploitation of dietary tannins to improve rumen metabolism and ruminant nutrition. J Sci Food Agric 91:24–37. https://doi.org/10.1002/jsfa.4152

Patra A, Park T, Kim M, Yu Z (2017) Rumen methanogens and mitigation of methane emission by anti-methanogenic compounds and substances. J Anim Sci Biotechnol 8:3. https://doi.org/10.1186/s40104-017-0145-9

Priolo A, Waghorn G, Lanza M, Biondi L, Pennisi P (2000) Polyethylene glycol as a means for reducing the impact of condensed tannins in carob pulp: effects on lamb growth performance and meat quality. J Anim Sci 78:810–816. https://doi.org/10.2527/2000.784810x

Rira M, Morgavi DP, Archimède H, Marie-Magdeleine C, Popova M, Bousseboua H, Doreau M (2015) Potential of tannin-rich plants for modulating ruminal microbes and ruminal fermentation in sheep. J Anim Sci 93:334–347. https://doi.org/10.2527/jas.2014-7961

Rubanza CDK, Shem MN, Otsyina R, Bakengesa SS, Ichinohe T, Fujihara T (2005) Polyphenolics and tannins effect on in vitro digestibility of selected *Acacia* species leaves. Anim Feed Sci Technol 119:129–142. https://doi.org/10.1016/j.anifeedsci.2004.12.004

Schofield P, Mbugua DM, Pell AN (2001) Analysis of condensed tannins: a review. Anim Feed Sci Technol 91:21–40. https://doi.org/10.1016/S0377-8401(01)00228-0

Silanikove N, Landau S, Or D, Kababya D, Bruckental I, Nitsan Z (2006) Analytical approach and effects of condensed tannins in carob pods (*Ceratonia siliqua*) on feed intake, digestive and metabolic responses of kids. Livest Sci 99:29–38. https://doi.org/10.1016/j.livprodsci.2005.05.018

Singh S, Kushwaha BP, Nag SK, Mishra AK, Singh A, Anele UY (2012) In vitro ruminal fermentation, protein and carbohydrate fractionation, methane production and prediction of twelve commonly used Indian green forages. Anim Feed Sci Technol 178:2–11. https://doi.org/10.1016/j.anifeedsci.2012.08.019

Soltan YA, Morsy AS, Sallam SMA, Louvandini H, Abdalla AL (2012) Comparative in vitro evaluation of forage legumes (prosopis, acacia, atriplex, and leucaena) on ruminal fermentation and methanogenesis. J Anim Feed Sci 21:759–772. https://doi.org/10.22358/jafs/66148/2012

Theodorou MK, Williams BA, Dhanoa MS, McAllan AB, France J (1994) A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. Anim Feed Sci Technol 48:185–197. https://doi.org/10.1016/0377-8401(94)90171-6

Tiemann TT, Avila P, Ramírez G, Lascano C, Kreuzer M, Hess HD (2008) In vitro ruminal fermentation of tanniniferous tropical plants: plant-specific tannin effects and counteracting efficiency of PEG. Anim Feed Sci Technol 146:222–241. https://doi.org/10.1016/j.anifeedsci.2007.12.009

United Nations Framework Convention on Climate Change (UNFCCC) (2005) Key GHG data. UNFCCC, Bonn

Van Soest PJ, Robertson JB, Lewis BA (1991) Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J Dairy Sci 74:3583–3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2

Vitti DMSS, Abdalla AL, Bueno ICS, Silva Filho JC, Costa C, Bueno MS, Nozella EF, Longo C, Vieira EQ, Cabral Filho SLS, Godoy PB, Mueller-Harvey I (2005) Do all tannins have similar nutritional effects? A comparison of three Brazilian fodder legumes. Anim Feed Sci Technol 119:345–361. https://doi.org/10.1016/j.anifeedsci.2004.06.004

Waghorn GC (2008) Beneficial and detrimental effects of dietary condensed tannins for sustainable sheep and goat production - progress and challenges. Anim Feed Sci Technol 147:116–139. https://doi.org/10.1016/j.anifeedsci.2007.09.013

Waghorn GC, Tavendale MH, Woodfield DR (2002) Methanogenesis from forages fed to sheep. Proc NZ Grassl Assoc 64:167–171