Short communication

In vitro gas production and *in situ* degradation of Mesquite leaves and pods in Arabian camels in Iran

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Abstract

This experiment was conducted to study the use of mesquite tree (*Prosopis juliflora*) leaves and pods in the diet of Arabian camel (*Camelus dromedarius*) in Khuzestan, Iran. Fresh leaves and pods were collected, air dried and ground. The gas production parameters of samples were determined by gas test and parameters of foregut degradability by *in situ* technique. The data were analysed in a completely randomized design with four replicates.

The gas production potential of the *Prosopis juliflora* pods was higher ($P<0.05$) than that of its leaves (42.16 vs 16.66 mL), but the production rates were similar ($P>0.05$). The values of the partitioning factor, microbial biomass, biomass efficiency and truly digested organic matter were higher for leaves than for pods ($P<0.05$). The rapidly degradable fraction and effective degradability of dry matter of *P. juliflora* pods were 0.48 and 0.67, respectively, higher values ($P<0.05$) than those of leaves (0.34 and 0.56, respectively). However, the slowly degradable fraction of leaves was significantly higher ($P<0.05$) than that of pods (0.51 vs 0.25, respectively). The extent to which leaves, and pods were fermented and their *in situ* degradation rate suggest their usefulness as dietary ingredients for camels and possibly for other foregut fermenters.

Keywords: Rumen degradability, fermentation, mesquite, dromedary camel

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Introduction

Mesquite (*Prosopis juliflora*) is a genus of trees and shrubs in the legume family (*Fabaceae*) and one of the rangeland trees that can grow in a wide range of soil and climatic conditions. It belongs to the genus Prosopis. The tree is native to the arid and semi-arid regions of South America, Central America and the Caribbean (Sawal *et al.*, 2001). *Prosopis juliflora* trees in Brazil and Kenya generally commence fruiting in the second year after planting (Batista *et al.*, 2002). The annual pod yield varies, as some old trees in India produce up to 100 kg of pods per tree per year, while the average for young trees is considered to be about 40 kg per tree per year. The pods of *P. juliflora* have high crude protein (CP), mineral and amino acid contents (Sawal *et al.*, 2001). Because of the high tolerance of this plant to drought, and the long hot summer season in

Iran's arid regions, it is found in major parts of southern Iran, especially the Khuzestan, Bushehr and Hormozgan provinces (Moslehi, 2002).

The results of feeding trials indicated that feeding *Prosopis* pods at 60% diets for goats, sheep, beef and dairy cattle resulted in good weight gains and milk production (Osuga *et al.*, 2008). The use of *P. juliflora* pods provides a readily available and affordable source of energy throughout the year and will increase livestock output and profitability (Abdulrazak *et al.*, 2000; Mwangi and Swallow, 2005).

The leaves of *P. juliflora* have high CP (about 20%) and a low level of fibres (23.4%), but they are generally unpalatable due to the presence of tannins, flavonoids and polyphenols. However, livestock are known to occasionally browse the dry foliage of this tree due to the denaturation or removal of unpalatable compounds during the drying process (Batista *et al.*, 2002). Talpada and Shukla (1990) reported that the amount of tannin in the seeds and pods of *P. juliflora* were 1.9% and 1.5% (% DM basis), respectively.

Due to the weather conditions in the south of Iran and the abundance of *P. juliflora* along with a shortage of grasslands and food sources in the area, it seems that *P. juliflora* can be used to provide part of the nutritional demands of livestock in the region. The aim of this trial was to investigate the *in vitro* fermentability and *in situ* degradability of *P. juliflora* leaves and pods in the Arabian camel (*Camelus dromedarius*).

Materials and methods

About 5 kg leaves and pods of *P. juliflora* from 8 trees were collected from the Khuzestan province in Iran at the end of spring, then air-dried and ground to pass a 1 mm mesh sieve. Gas production was measured following the incubation of 300 mg of ground samples of leaves and pods with 35 mL buffered foregut fluid under anaerobic conditions with continuous CO_2 supply in 100 mL glass vials. Produced gas was recorded for 2, 4, 6, 8, 10, 12, 16, 24, 48, 72 and 96 h, in a water bath at 39 °C (Menke and Steingass, 1988). The foregut fluid was collected before morning feeding from two camels (400±12 Kg, BW) fitted with a permanent foregut fistula. The camels had been on a roughage- based diet (60% straw and 40% alfalfa) for 1-month a. After 96 h of incubation, the concentration of ammonia-N (NH_3-N) in each vial was measured using the distillation method (Kjeltec 2300 Autoanalyzer, Foss Tecator AB, Hoganas, Sweden). Cumulative gas production data were fitted to the exponential equation $Y=B\ (1-e^{-Ct})$ (Menke and Steingass, 1988), where Y is the volume of gas produced at time t, B is the gas production (mL) from the fermentable fraction, C is the rate constant of gas production (mL/h) and t is the incubation time (h). The partitioning factor, microbial biomass and truly digested organic matter were calculated according to Makkar and Becker (1996).

Dry matter degradability was measured by *in situ* technique using 2 male one-humped camels fitted with a foregut fistula (400±12 Kg, BW). Five g of each milled sample (2.0 mm mesh sieve) were transferred into a polyester bag (10×20 cm, 52 µm pore size) and incubated in the foregut for 2, 4, 6, 8, 16, 24, 4 8, 72 and 96 hours (4 replicates per each time). At the end of each incubation time, the bags were immediately hand-rinsed under cold tap water until clear and dried in a forced air draft oven at 60 °C for 48 hours. The bags without incubation (0 h) were washed to estimate the wash-out fraction at initial time. The degradability of DM was calculated using the equation: $P = a + b\ (1- e^{-ct})$ (Orskov and McDonald, 1979), where, P= fraction degraded in the time t, a= soluble fraction, b= potentially degradable fraction, c= degradation rate and t= incubation time.

The effective degradability (ED) (k=0.03, 0.05 and 0.08/h) was calculated using the following equation:

ED = a+(bc/(c+k), where k is the estimated rate of outflow from the rumen (Orskov and McDonald, 1979).

The data were subjected to analysis of variance of the completely randomized design using the General Linear Model (GLM) procedure of the SAS. The Duncan's multiple range test was used to compare the means difference at $P < 0.05$.

Results and discussion

The result of the gas production is presented in Table 2. The gas production potential of *P. juliflora* pods was higher ($P<0.05$) than that of leaves (42.16 vs 16.66 mL, respectively), but the gas production rate of leaves and pods were similar ($P>0.05$). The significant increase in gas production potential of *P. juliflora* pods can be attributed to its high content of soluble carbohydrates, which act as a source of energy for the foregut microorganisms, prepare favourable conditions for the growth of microorganisms, and increase gas production and digestibility (Alemzadeh, 2006).

According to studies, 69% DM of *P. juliflora* pods are carbohydrates. Gross energy value and ME of pods are 15.3 and 12.8 MJ/Kg DM respectively, which can be comparable to maize (13.0 MJ/Kg DM). It has been reported that pods can be replaced with maize in livestock feed formulations (Odero *et al.*, 2010).

Batista *et al.* (2002) reported that total carbohydrates, non-structural carbohydrates and soluble proteins of *P. juliflora* pods were 79.8, 52.4, and 57.2 % DM, and that the acid lignin content of pods was lower than that of leaves, which influences fermentation and digestion. In addition, Prosopis pods are a rich source of protein and energy, and contain 20% soluble sugars such as sucrose, which leads to an increase in palatability, feed intake and digestion (Talpada *et al.*, 2003). The chemical composition of *P. juliflora* leaves and pods is given in Table 1.

Table 1. Chemical composition (% DM basis) of *Prosopis juliflora* leaves and pods.

	DM	CP	Ash	CF	EE	Ca	P	NDF	ADF	ADL	NFE
Leaves	92.5	10.4	9.1	23.7	1.6	0.9	0.3	48.4	35.4	13.1	59.6
Pods	95.2	11.7	4.5	30.5	1.7	0.24	1.2	26.5	17.5	2.4	48.3

The increase of gas production in the current study can be attributed to the higher activity of camels' foregut cellulolytic bacteria and enzyme production, which plays a crucial role in increase the digestion of fiber via the growth of colonies on fibrous materials (Galdwell and Brayant, 1966).

All reported digestive studies in camelids show that they have a special ability to use low quality forages. The foregut cellulolytic activity in camelids is high because microorganisms have sufficient enzymatic activity to hydrolyse the cell wall carbohydrates and ferment the hydrolysed oligosaccharides (Jouany *et al.*, 1992). Kayoli *et al.* (1991) reported that dromedaries were able to digest low quality forage with higher yields than sheep, and that, for straw, the activity of cellulolytic bacteria in camels was 20% higher than in other ruminants, like sheep.

Gas production reduction in *P. juliflora* leaves can be related to the presence of tannins in the leaves, as tannins connect to nutrients and keep them away from the microorganisms, thus

preventing microorganism's growth and reducing methanogens population and their enzymatic activity (Frutos *et al.,* 2002; McSweeney *et al.*, 2001; Beauchemin *et al.*, 2007).

In agreement with current results, Rubanzal *et al.* (2001) concluded that the protein and dry matter digestion of Prosopis branches is reduced due to the presence of anti-nutritional factors such as tannins in the leaves. Prosopis leaves contain 8-10% tannin, as 9% condensed tannin and 0.3% hydrolyzable tannin (Bhatta *et al.*, 2004).

Carula *et al.* (2005) reported that methanogens were inhibited by tannin because it reduces fiber degradation and limits the hydrogen production from acetate synthesis. According to the research, in plant species that contain tannins, the relationship between tannins and gas production is negative (Khazaal *et al.*, 1994).

The amount of neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin in mesquite leaves were reported at 49.4, 38.4, and 14%, which may be linked to a reduction in the potential of gas production (Soltan, 2012). Increasing the content of lignin in the plant cell wall has also been found to cause lower fermentation, gas production and degradability (Sallam *et al.*, 2010).

The concentration of NH_3-N in a medium containing *P. juliflora* pods and leaves was 16.38 and 14.15 mg/100 mL, respectively. It seems that the presence of tannin in the leaves decreases ammonia nitrogen concentration in the rumen. Tannins bind to protein and reduce the growth rate and, ultimately, fibrolytic bacteria and protozoa count in the rumen; consequently, protein degradation and ammonia nitrogen production in the rumen decrease (McSweeney *et al.*, 2001). It is reported that Prosopis leaves contain 2.5 % tannic acid, which significantly decreases CP digestibility (Bhandari *et al.*, 1979). In agreement with the current result, Soltan *et al.* (2012) concluded that *in vitro* ammonia nitrogen in a treatment containing mesquite leaves was lower than in other treatments (9.30 mg/100 mL).

The *P. juliflora* leaves had the highest partitioning factor (Pf), microbial biomass and microbial biomass efficiency (Table 3). The highest Pf in the leaves probably relates to lower gas production, and, therefore more energy being available for microbial protein synthesis (Blummel *et al.*, 2003). Kennedy (2002) reported that legume foliage increased microbial production and degradation of low-quality feed.

Table 2. *In vitro* gas parameters and NH_3-N content in a medium containing *P. juliflora* pods and leaves incubated with camel foregut fluid for 96 h.

Treatment	Potential of gas production (mL/300 mg)	Gas production rate (mL h^{-1})	Ammonia nitrogen (mg/100 mL)
Pod	42.16 [a]	0.05	16.38 [a]
Leaves	16.66 [b]	0.02	14.15 [b]
SEM	0.75	0.1	0.05
P-value	0.0001	0.1	0.003

SEM: Standard error of the mean, [a, b]: Means with common letter (s) within each column do not differ significantly (P > 0.05).

Table 3. The parameters of gas production following *in vitro* fermentation of *P. juliflora* pods and leaves incubated in foregut fluids of Arabian camels for 96 h.

Treatment	Partitioning factor (mg mL^{-1})	Microbial biomass (mg)	Efficiency of microbial biomass (%)	Truly digested organic matter (mg)	Cell wall degradability (%)
Pod	2.55 [b]	11.60 [b]	13.00 [b]	83.10 [b]	0.64
Leaves	4.03[a]	64.05 [a]	45.00 [a]	141.05[a]	0.24
SEM	0.05	1.87	0.01	2.64	0.70
P-value	0.002	0.002	0.006	0.004	0.20

SEM: Standard error of the means, [a, b]: Means with common letter (s) within each column do not differ significantly (P > 0.05).

The dry matter degradability parameters of *P. juliflora* pods and leaves at different hours of incubation are given in Table 4. The rapidly degradable fraction and effective degradability values of *P. juliflora* were higher (P<0.05) in pods than leaves, while the slowly degradable fraction of dry matter of leaves was higher (P<0.05) than pods. This difference may be due to more polyphenolic compounds being present in the leaves of *P. juliflora*. Tannins are able to combine with nutrients such as carbohydrates, proteins, polysaccharides, minerals, also cell membrane of bacteria and digestive enzymes and form complexes which reduce the degradability parameters (Danesh Mesgaran, 2009).

The phenols in Prosopis leaves are 5.2 to 5.6 times higher than those in alfalfa; this reduces digestive enzymes and available nutrients for microorganisms, followed by a reduction of degradability parameters (Cameron et al., 1988). Paricard *et al.* (1992) reported that the use of high-tannin Mulga forage caused sulphur and nitrogen to decrease, which in turn decreased feed consumption and digestibility in sheep.

The increase of rapidly degradable fraction, potential degradability and effective degradability's of *P. juliflora* pods was due to the availability of higher soluble carbohydrates and proteins (Alemzadeh *et al.*, 2009).

Non-structural carbohydrates, soluble proteins and soluble sugars were high in the Prosopis pods, which positively influences degradability (Talpada *et al.*, 2003). Providing nutrients (energy, protein and minerals) for microorganisms at the same time causes an increase in the activity of the proteolytic bacteria. The equilibrium in the entry of energy and nitrogen in the rumen improves the level of microbial activity and its growth and regeneration, and nitrogen enters the microbial protein synthesis in the rumen. This balance improves the production of volatile fatty acids and increases the digestive activity of cellulose (Danesh Mesgaran, 2009).

Alemzadeh *et al.* (2007) reported that the rapidly degradable fraction, slowly degradable fraction, potential of degradability and effective degradability of *P. juliflora* pods in sheep were 36.83, 36.70, 0.08, 73.53 and 73.60 %, respectively. These estimates were lower than estimates obtained in the present study in camels for the slowly degradable fraction, and potential of degradation of leaves, and the rapidly degradable fraction, rate

constant of degradation and potential of degradation of pods. Also, Batista *et al.* (2002) reported estimates for the digestibility of dry matter, crude protein and NDF of mesquite pods in the rumen of steers being 64.7%, 65.5% and 7.8%, respectively.

Table 4. Parameters of *in situ* dry matter rumen degradability of *P. juliflora* leaves and pods in the foregut of the Arabian camel (*Camelus dromedarius*).

treat	Rapidly degradable fraction	Slowly degradable fraction	Rate constant of degradation	Potential of degradability	Effective degradability
Pod	0.48 [a]	0.25 [b]	0.09	0.85	0.67 [a]
Leaves	0.34 [b]	0.51 [a]	0.03	0.74	0.56 [b]
SEM	0.006	0.06	0.017	0.06	0.007
P-value	0.0001	0.04	0.06	0.3	0.0007

SEM: Standard error of the means, [a, b]: Means with common letter (s) within each column do not differ significantly (P > 0.05).

Conclusion

Results of the *in situ* degradation and *in vitro* fermentation measurements of *P. juliflora* pods and leaves with camel foregut fluid suggest potential value in using both parts as dietary ingredients in the diet of the Arabian camels (*Camelus dromedarius*). Further feeding trials are needed to assess the nutritional value of mesquite pods and leaves in the diets of camels, especially in areas of Iran where mesquite trees are abundant and there is a shortage of conventional feed sources.

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