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Evaluation of Carob (*Ceratonia siliqua*) and Honey Locust (*Gleditsia triacanthos*) Pods as a Feed for Sheep

Research Article

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ABSTRACT

The nutritive value of *Ceratonia siliqua* and *Gleditsia triacanthos* pods was determined on the basis of their chemical composition, *in vitro* gas production and rumen fermentation end-products. *Medicago sativa* was used as a reference feed material. The studied samples showed differences in chemical composition and phenolic compounds. Crude protein (CP) content was particulary low (80 g/kg DM) in carob and higher in *Medicago sativa* and *G. triacanthos* pods with (159.79 and 121.56 g/kg DM, respectively). Inclusion of Polyethylene glycol (PEG) in fermentation medium results in a significant increase ($P<0.05$) of gas production in *Ceratonia siliqua* and *Gleditsia triacanthos* and no effect was observed with *M. sativa*. The highest values of gas production were observed for *C. siliqua* and *G. triacanthos*, whereas *Medicago sativa* had significantly low values. The highest asymptotic gas production was observed in *Ceratonia siliqua* and *Gleditsia triacanthos* (296.80 and 289.55 mL g^{-1} DM, respectively), whereas *Medicago sativa* recorded the lowest value (243.64 mL g^{-1} DM). The concentration of acetate differentiated two groups: *Medicago sativa* and *Gleditsia triacanthos* (86.58 and 66.32% respectively), while the fermentation of *Ceratonia siliqua* resulted in a lower acetate concentration (59.84%). Although there were noticeable differences among the three studied samples, *Ceratonia siliqua* and *Gleditsia triacanthos* pods showed better nutritional quality, indicating that they could be considered promising and interesting sources of feed for sheep during the dry season or as supplement to low quality diets.

KEY WORDS carob, digestibility, honey locust pods, *in vitro* gas production, tannins.

INTRODUCTION

The productivity of ruminant species in the most part of Algeria is limited by the low level energy and protein intake due to the lowest production of high quality forage especially during the summer season. Barley grain is the major source of supplemental feeding during the dry as well as reproductive seasons. However, prices of barley grain increased significantly due to substantial increase in international market price which put economical pressure on livestock owners. In addition, the area was under relatively long dry period which seriously impacted the production of range- land forge and increased the burden on livestock owners (Obeidata *et al*. 2011). Pods of several legume trees have been included in livestock diets in many parts of the world during critical periods of the year when quality and quantity of forages are restricted (Barakat *et al*. 2013). Among these, carob pods seem to be promising as a non-

conventional feed resource which can be used for small ruminants feeding (Guessous *et al*. 1989). Carob bean is the fruit of *Ceratonia siliqua*, which belongs to the Leguminosae family. The tree has been extensively cultivated in most countries of the Mediterranean for years and in many areas of North America. Carob pods are mostly used in the food industry for carob bean gum and locust bean gum (Battle and Tous 1997; El Hajaji *et al*. 2013). Some investigations explored carob pods as a readily available and inexpensive material for the production of bioethanol (Vourdoubas *et al*. 2002), and as a substrate for citric acid production (Roukas, 1998). Another promising feed for small ruminants in these regions, honey locust tree (*Gleditsia triacanthos*) is the one of introduced legume trees which were well adapted to almost parts of Algeria. This legume trees produce considerable amounts of pods every growing season. The pod yield can range from 12 to 27 kg per tree per year in young trees (Duke, 1983) to 87 kg per tree per year in adult trees (Papanastasis, 1996). Apparent seed digestibilities in sheep are reported to range between 75 to 90% (Small, 1983) and crude protein content of honey locust pods harvested in July and November ranged from 103 to 134 g kg^{-1} DM (Pereira, 2000).

Tannins are phenolic plant secondary compounds and are widely distributed through the plant kingdom, especially legumes and browses. Tannins are considered to have both adverse and beneficial effects, depending on chemical structure and concentration in diets (Piluzza *et al*. 2014).

Adverse effects include reduction of feed intake, digestibility of fibre and nitrogen, and animal performance (Waghorn, 2008). Polyethylene glycol (PEG) binds condensed tannins reducing their biological activity. It has been used in these experiments to evaluate the effects of tannins. According to Yisehak *et al*. (2014) supplementation with PEG increased nutrient intake and total tract crude protein (CP), neutral detergent fibre (NDF) and acid detergent fibre (ADF) digestibility.

The nutritive value of a ruminant feed is determined by the concentrations of its chemical components, as well as their rate and extent of digestion. Determining the digestibility of feeds *in vivo* is laborious, expensive and requires large quantities of feed. *In vitro* methods provide less expensive and more rapid alternatives. In addition, the gas production technique has been proved to be efficient in determining the nutritive value of feeds containing antinutritive factors (Kamalak *et al*. 2012).

Therefore, the objective of this study was to evaluate carob and honey locust pods obtained from an arid region in Algeria to: (1) quantify chemical compositions and level of condensed tannin contents and (2) assess the effect of tannin activity on feed digestibility and nutrient availability *in vitro* using a PEG tannin bio-assay.

MATERIALS AND METHODS

Study area and sampling

This experiment was conducted using plant samples collected from M'sila (N 35˚ 26' 07.9''-E 004˚ 20' 52.8'', 398 m altitude) (Figure 1). M'sila is in north central Algeria, in the Saharan Atlas region, at the northern edge of Saharan Desert between the Atlas Mountains and the el-Hodna depression and salt lake. According to Köppen classification, the climate of this region is *BWh* (dry desert climate), characterized by high temperatures ranging between 24 and 41 ˚C, and scarce and erratic annual precipitations for a total of 100 and 250 mm/year. Carob and honey locust pods were handly collected by from at least 10 different trees for each substrat. Samples were collected during the dry season, because this is the time of the year when these plants may be more important. Then, samples were immediately freeze-dried and milled in a hammer mill using a 1 mm sieve.

Chemical analysis

The oven dried samples were ground in a Willey Mill to pass through 1 mm sieve for the determination of chemical composition. Feed samples were analysed for dry matter (DM) following the method of AOAC (2000). Nitrogen was determined using the micro-Kjeldahl method (AOAC, 2000).

Crude protein (CP) was calculated as N × 6.25. The neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were analyzed according to Van Soest *et al*. (1991) using the ANKOM Fiber Analyzer (ANKOM Technology, Fairport, NY). Sodium sulphite was added to the solution for the NDF determination. Fibre fractions were expressed including residual ash. Samples were also analyzed for phenolic compounds following the procedures described by Makkar (2003). Total condensed tannins of dried pods were determined by butanol-HCl method as described by Makkar (2003).

The concentrations of phenols were expressed in g tannic acid equivalent/kg DM, whereas the concentration of condensed tannins was expressed in g quebracho equivalent/kg DM. All chemical analyses were performed in triplicate.

In vitro studies

Rumen fluid was obtained from four mature Merino sheep (body weight 49.04±4.23 kg) fitted with permanent rumen fistula (60 mm diameter) maintained in cages and fed *ad-libitum* lucerne hay (CP 167 g, NDF 502 g, ADF 355 g and ADL 71 g/kg DM) and had free access to water and a mineral/vitamin block. A sample of rumen contents was withdrawn prior to morning feeding, transferred into thermos flasks and taken immediately to the laboratory.

Rumen fluid from the four sheep was mixed, strained through various layers of cheesecloth and kept at 39 ˚C under a CO_2 atmosphere (Ammar *et al*. 2004). The rumen fluid was diluted (1:4 v/v) with a culture medium containing macro- and micro-mineral solutions, resazurin and a bicarbonate buffer solution and prepared as described by Menke and Steingass (1988). The medium was kept at 39 ˚C and saturated with CO_2. Oxygen in the medium was reduced by the addition of a solution containing cysteine hydrochloride and Na_2S as described by Van Soest *et al*. (1966). The method used for gas production measurements was as described by Theodorou *et al*. (1994).

About 500 mg of each sample were incubated in 50 mL of diluted rumen fluid (10 mL mixed rumen fluid+40 mL medium prepared under a CO_2 constant flow) in 120 mL serum bottles pre-warmed at 39 ˚C and flushed with CO_2. Six bottles containing only diluted rumen fluid were incubated as blanks and used to compensate for gas production in the absence of substrate. All the bottles were crimped with aluminium caps and placed in the incubator at 39 ˚C, being shaken at regular times. Volume of gas produced in each bottle was recorded at 3, 6, 9, 12, 16, 21, 26, 31, 36, 48, 60, 72, 96, 120 and 144 h after inoculation time, using a pressure transducer (Delta Ohm DTP704-2BGI, Herter Instruments SL, Barcelona). In order to estimate the fermentation kinetic parameters, gas production data were fitted using the exponential model proposed by France *et al*. (2000):

$$G= A\ [1-e^{-c(t-L)}]$$

Where:
G ($mL g^{-1}$): cumulative gas production at time *t*.
A ($mL g^{-1}$): asymptotic gas production.
c ($/h^{-1}$): fractional rate of gas production.
L (h): lag time.

The energy value and digestibility of feedstuffs were calculated from the amount of gas produced at 24 h of incubation with supplementary analyses of crude protein and ash, as follows (Menke and Steingass, 1988).

$$ME\ (MJ/kg\ DM)= 2.2 + 0.136 \times G24 + 0.057 \times CP + 0.029 \times CP^2$$
$$OMD\ (\%)= 14.88 + 0.889 \times G24 + 0.45 \times CP + 0.0651 \times Ash$$

Where:
ME: metabolizable energy.
G24: 24 h net gas production (mL/200 mg DM).
CP: crude protein (% of DM).
OMD: organic matter digestibility.
Ash: ash (% of DM).

The gas production technique described above was used for the effect of PEG effect. Incubations were carried out in serum bottles with or without the addition of 500 mg PEG. Ground samples (300 mg) were weighed out into serum bottles, kept at approximately 39 ˚C and flushed with CO_2 before use. Two bottles were used for each substrate with each inoculum source one for each treatment (with or without PEG). Bottles were tightly closed and placed in the incubator at 39 ˚C, being shaken at regular times. The volume of gas produced in each bottle was recorded at 6, 12, 24 and 48 h after inoculation time, using a pressure transducer. Gas production was corrected by subtracting the volume of gas produced from blank cultures. The gas production technique described above was also used for volatile fatty acids determination. After 24 h of incubation, bottles were swirled in ice to stop fermentation, and then opened. A sample of supernatant (0.8 ml) was added to 0.5 ml of deproteinizing solution (20 g metaphosphoric and 4 g crotonic acid/l 0.5N HCl) for volatile fatty acid (VFA, s) analysis. The VFA were determined by GC using a Perkin-Elmer Autosystem XL GC (Perkin-Elmer Inc., USA), equipped with a semicapillary TR-FFAP (30 m×0.53 mm×1 m) column (Supelco, USA), flame Ionization detector (FID) and an auto-sampler. Temperatures were 140 °C in the column and 250 ˚C both in the injector and the detector, and carrier gas (He) flux was 13 mL/min. Each sample was injected automatically with a split ratio of 1/3. Chromatograms were integrated using software Star Chromatography Workstation 6.2 (Varian Inc., USA).

Statistical analysis

One way analysis of variance (Steel and Torrie, 1997) was performed on chemical composition, gas production, fermentation kinetics parameters, metabolizable energy, organic matter digestibility and volatile fatty acids data. Tukey's test was used for the multiple comparison of means ($P<0.05$). Analysis of variance between different variables were performed using SAS software package (SAS, 2000).

RESULTS AND DISCUSSION

Chemical composition

The chemical composition and phenolics contents of *Gleditschia triacanthos* (*G. triacanthos*), *Ceratonia siliqua* (*C. siliqua*) pods and *Medicago sativa (M. sativa*) are in Table 1. Dry matter content of *C. siliqua* were higher ($P<0.05$) than those of *G. triacanthos* and *M. sativa* hay.

The ash content was lowest ($P<0.05$) in *G. triacanthos* and *C. siliqua* and highest in *M. sativa* ($P<0.05$). The highest crude protein and NDF contents were in *M. sativa.* Among the studied samples, NDF content was highest ($P<0.05$) in *M. sativa* followed by *G. triacanthos* and *C. siliqua* which did not differ.

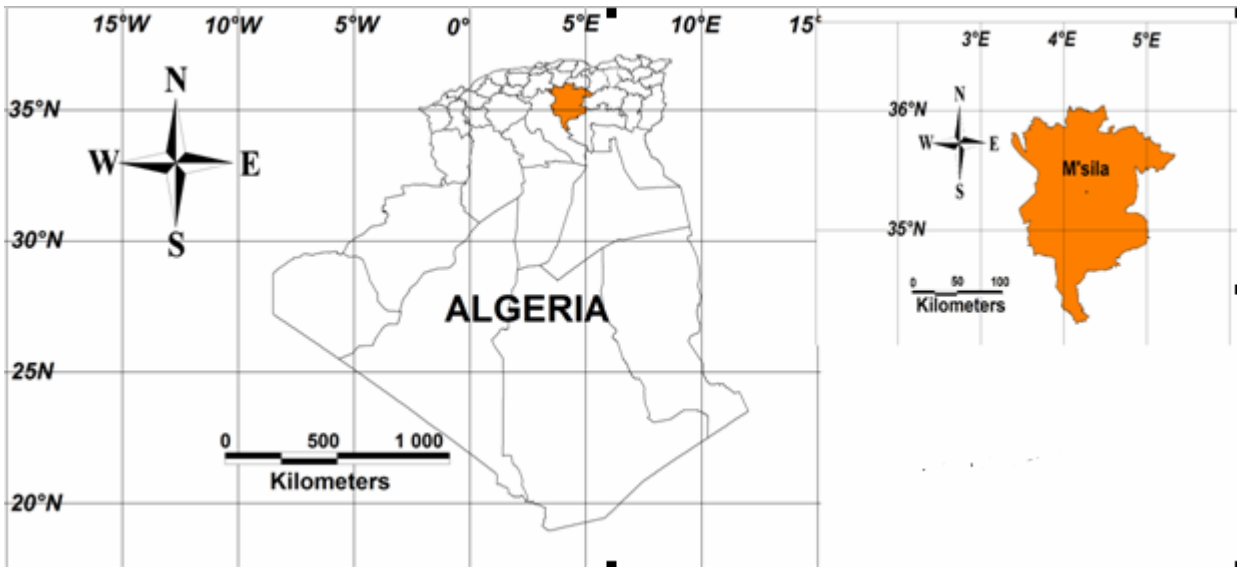

Figure 1 The study area

Table 1 Chemical composition (g kg⁻1 dry matter) of the three studied samples

Species	Dry matter	Ash	Crude protein	Neutral detergent fibre	Acid detergent fibre	Acid detergent lignin	Cellulose	Total extractable phenols	Total condensed tannins
Ceratonia siliqua	985.72^{a}	33.57^{c}	76.81^{c}	314.97^{b}	282.21^{b}	175.89^{a}	106.32^{c}	64.16^{b}	478.54^{a}
Gleditschia triacanthos	966.86^{b}	48.30^{b}	121.56^{b}	344.12^{b}	188.42^{c}	60.14^{c}	128.28^{b}	85.30^{a}	303.10^{b}
Medicago sativa	933.85^{c}	94.94^{a}	159.79^{a}	465.59^{a}	299.49^{a}	73.27^{b}	226.22^{a}	4.72^{c}	17.35^{c}
SEM	7.58	9.26	12.06	23.41	17.31	18.31	21.87	12.27	67.26
P-value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

M. sativa had more (P<0.05) ADF than the other studied samples. *C. siliqua* had the highest (P<0.05) ADL content while contents were lowest (P<0.05) in *M. sativa* and *G. triacanthos*, *C. siliqua* and *G. triacanthos* had the highest (P<0.05) total extractable phenols (TEP) and total condensed tannins (TCT) than those of *M. sativa.*

Natural grasslands associated with trees and / or shrubs have a considerable role in ruminant feeding in extensive Mediterranean production systems, such as the one in the semi-arid region in Algeria.

Legume trees or shrubs, such as *C. siliqua* and *G. triacanthos* can be used to supplement the available feedstuff during the periods of feed scarcity that are common in Mediterranean areas (Chassany and Flamant, 1996). In the present study, CP content was particulary low (80 g/kg DM) in carob pods in agreement with data reported by other authors (Silanikove *et al*. 1996; Silanikove *et al*. 2006). It is well known that carob pods, although rich in water soluble sugars (WSS), has a very low crude protein content, and contains hight levels of tannins, mainly of the condensed type, which minimize its nutritional value (Marakis *et al*. 1997).

As expected, the CP content was higher in the *M. sativa* and *G. triacanthos* pods. Legumenous shrubs and and trees have been used as feedstuffs for livestock in many regions of the world, mainly because of their high protein content (Ammar *et al*. 2004) throughout the year that can be attributed to the ability of these plants to fix atmospheric nitrogen (Ammar *et al*. 2005), suggesting the possibility that *G. triacanthos* pods may be used as a dry season feed supplement to low quality diets.

All of the samples studied herein contained high fibre (NDF, ADF) and ADL content. Similar results were reported for these samples and other Algerian and Mediterranean fodder shrubs (Frutos *et al*. 2002; Bruno-Soares and Abreu, 2003; Getachew *et al*. 2004; Boufennara *et al*. 2012). Consent with results for lignin content of carob pods (175.89 g/kg DM), Khazaal and Orskove, (1994) reported high values in samples harvested in different maturity stage (*Arbutus andrachronoids*, *Cistus incanus* and *Arbutus unedo*) with 182.8, 171.3 and 163.5 g/kg DM). According to Wilson and Kennedy, (1996), lignin is mainly found in the xylem in which the lignin concentrations reach levels which render cells copmpetely indigestible.

In contrast, lignin concentration in other tissues are low making them almost completely digestible. Concentration of phenolics varied widely among studied samples. The analysis of specific tannins is an indication of the presence of some anti-nutritive factors in feedstuff. It has been reported that plants with more than (60 g/kg DM) free condensed tannin are less palatable and digestible than forages with lower concentrations of this chemicals, although there is more protein to by-pass the rumen and higher nitrogen retention (Terrill *et al*. 1992). However, animals that regularly consume tannineferous feedstuffs adapt to minimize detrimental effects of tannins, due to extra mastication, large amounts of saliva and rumen fermentation (Salem *et al*. 2001).

Odenyo and Osuji, (1998) identified some tannins tolerant ruminal bacterial strains from enriched cultures of rumen microflora of goats to establish a medium containing high concentrations of crude tannins extract or tannic acid. A strain of the anerobe *Selenomonas ruminantium*, subspices ruminantium, capable growing on tannic acid or condensed tannin as a sole energy source, has been isolated from ruminal contents of feral goats browsing tannin-rich foliage (Skene and Brooker, 1995).

Effect of PEG on *in vitro* gas production

Inclusion of PEG in fermentation of the three studied samples (Table 2) results in a significant increase (P<0.0001) of gas production in *C. siliqua* and *G. triacanthos* and no effect was observed with *M. sativa*. In addition, the increase in gas production upon the addition of PEG, compared with that without PEG, for *C. siliqua* and *G. triacanthos* varied widely (P<0.05), being particularly high in *C. siliqua* (165.28 mL/g DM) and (133.06 mL/g DM) in *G. triacanthos*.

Inclusion of PEG in fermentation of studied samples (Table 2) results in a significant increase (P<0.0001) of gas production in *C. siliqua* and *G. triacanthos* species and no effect was recorded within *M. sativa*.

This may be due to the influence of PEG on the anti-nutritional factors contained in these samples such as condensed tannins. It is well established that the incorporation of PEG in the diet has beneficial effects, particularly for tanniniferous feeds having of 5-10 % contents of condensed tannins (Silanikove *et al*. 1996; Silanikove *et al*. 1997; Ben Salem *et al*. 2002).

The PEG inactivation of tannins increases voluntary feed untake, availability of nutrients and decreases microbial inhibition in degrading the tanniniferous feeds, which in turn increases the performance of animals (Bhat *et al*. 2013). The increased GP when samples were incubated with PEG were also reported for different forages by other authors.

Arhab *et al*. (2009) evaluated the influence of tannins present in arid zone forages from Algeria including *Aristida pulmosa*, *Astragalus gombiformis*, *Genista saharae* and vetch-oat hay on *in vitro* gas production (GP). They found that inclusion of PEG resulted in an overall increase in GP (20.2%). Moreover, the increase in GP when samples were incubated with PEG were also reported by others (Singh *et al*. 2005; Boufennara *et al*. 2013; Elahi *et al*. 2014). Bakhshizadeh and Taghizadeh (2013) determine the effect of PEG inclusion during *in vitro* incubation on GP they reported an increased GP at all incubation times than control; however, there was no significant increase in GP within levels of PEG. Therefore, tannins which bind strongly with dietary and endogenous protein would need to be counteracted with a competitive agent such as PEG. The addition of PEG, which has a relatively low cost, improves nutritional value of *C. siliqua* and *G. triacanthos* pods testified by an increased level of GP.

***In vitro* gas production kinetics**

Data of *in vitro* fermentation kinetics are shown in Table 3. The highest values of gas production, C and G_{24} were observed for *C. siliqua* and *G. triacanthos*, whereas *M. sativa* had significantly low values. Similar trends were observed for the *in vitro* fermentation kinetics estimated from the gas production curves.

Figure 2 shows the cumulative gas production profiles of the three different samples that were incubated in buffered rumen fluid. For the three samples studied heirin, fermentation started readily without lag time. The highest asymptotic gas production was observed in *C. siliqua* and *G. triacanthos* (296.80 and 289.55 mL g^{-1} DM, respectively), whereas *M. sativa* recorded the lowest value (243.64 mL g^{-1} DM).

The estimated metabolizable energy (ME), and organic matter digestibility (OMD) are presented in Table 3. The ME contents were particularly higher in *M. sativa*, while *C. siliqua* and *G. triacanthos* had significantly lower values of ME (13.35 and 11.48 MJ kg^{-1} DM; respectively). The OMD of *C. siliqua* was slightly higher than that of *G. triacanthos* and *M. sativa*.

The use of the *in vitro* gas production methodology to estimate digestion of feeds is based on the well established relationship between the feed digestibility and *in vitro* gas production, in combination with the feed chemical composition (Menke and Steingass, 1988; López, 2005). In the present study, the main value of this technique was to detect differences between fermentative activity in rumen fluid of sheep when carob and honey locust pods with different tannin contents were incubated. This technique is considered more sensitive to detect such differences than other *in vitro* gravimetric technique (Williams, 2000).

Table 2 *In vitro* gas production (mL/g DM) of the three studied samples, without (-) polyethylene glycol (PEG) or with (+) PEG

Table 2.

Species		Incubation time				
	PEG	6 h	12 h	24 h	48 h	Total
Ceratonia siliqua	-	38.74^{b}	36.18^{ab}	31.76^{a}	28.23^{ab}	134.91^{ab}
	+	52.64^{a}	42.52^{a}	37.20^{a}	32.92^{a}	165.28^{a}
Gleditschia triacanthos	-	30.63^{c}	29.31^{b}	24.88^{b}	21.36^{b}	106.19^{b}
	+	37.35^{b}	36.88^{ab}	31.56^{ab}	27.27^{ab}	133.06^{ab}
Medicago sativa	-	31.32^{c}	36.45^{ab}	32.02^{ab}	28.50^{ab}	128.30^{b}
	+	30.15^{c}	36.17^{ab}	30.85^{ab}	26.57^{ab}	123.75^{b}
SEM		1.94	1.17	1.16	1.17	4.90
P-value		< 0.0001	< 0.0223	< 0.0536	< 0.1032	< 0.0019

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

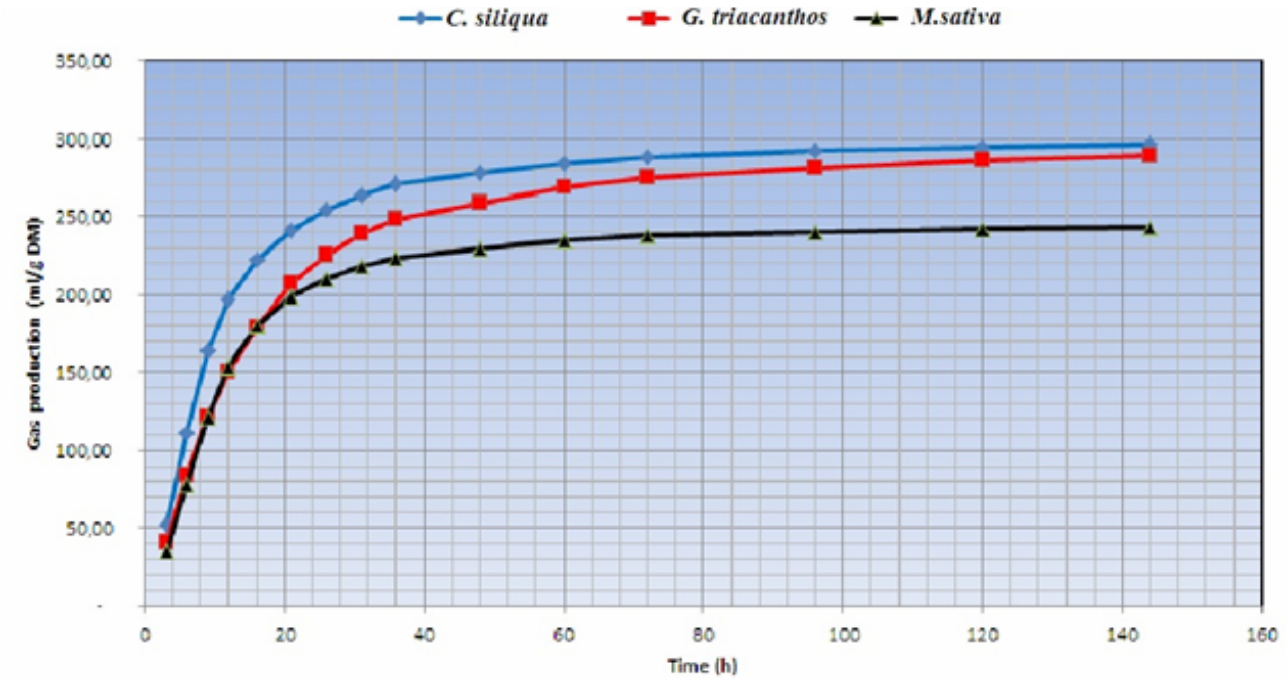

Figure 2 Cumulative gas production profiles

Table 3 *In vitro* fermentation kinetics of the three studied samples (estimated from gas production curves)

Species	A (mL/g)	c (h)	L (h)	G_{24} (mL/g)	ME (MJ kg^{-1} DM)	OMD (%)
Ceratonia siliqua	308.70^{a}	0.08055^{a}	0.5391	261.91^{a}	11.48^{c}	65.13^{a}
Gleditschia triacanthos	304.30^{a}	0.05867^{b}	0.4705	226.65^{b}	13.35^{b}	60.96^{b}
Medicago sativa	254.62^{b}	0.07774^{a}	0.8613	212.45^{b}	16.29^{a}	60.47^{b}
SEM	9.17	0.00398	0.1421	7.90	0.7098	0.8985
P-value	< 0.0011	< 0.0165	< 0.4221	< 0.0025	< 0.0001	< 0.0337

A: asymptotic gas production; c: fractional rate of gas production; L: lag time; G_{24}: 24 h net gas production; ME: metabolizable energy and OMD: organic matter digestibility.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Moreover, the gas production technique allows the monitoring of the kinitics of fermentation of the feed over long incubation period without the need to use a large number of tubes to terminate treatment after different incubation periods (Khazaal and Orskove, 1994). In the current experiment, the highest cumulative gas production after 144 h of incubation (Figure 2) was observed for carob pods (296.80 mL g^{-1} DM) and the lowest was obtained with *M. sativa* (234.64 mL g^{-1} DM). The high cell wall contents in *M. sativa* could have accounted for the limited substrate degradation and fermentation, and consequently for the low gas production observed. These results suggest that both carob and honey locust pods can be used as feeds for ruminants.

Since, carob pods were explored as a readily available and inexpensive material for the production of bioethanol (Vourdoubas *et al*. 2002), and as a substrate for citric acid

production (Roukas, 1998). The *C. siliqua* pods show a high level of total sugar (around 470 g kg^{-1} DM) and low values of CP and crude fibre (about 54 and 75 g kg^{-1} DM, respectively) (Tous and Battle, 1990). Similarly, according to Bruno-Soares and Abreu, (2003) in *G. triacanthos* pods, the major components in DM (about 600 g kg^{-1} DM) are neutral detergent fiber (310 g kg^{-1} DM) and total sugars (290 g kg^{-1} DM) where sucrose represents about 750 g kg^{-1} DM. Our results suggest that these samples would be also a highly fermentable feedstuff in the rumen that could represent a substantial supply of energy to the animal. Adequately combined with a source of degradable N, it can favor the synthesis of microbial protein in the rumen (Boufennra *et al*. 2016).

When feedstuffs are incubated *in vitro*, gas is produced mainly from the fermentation of carbohydrates (Blummel and Orskov, 1993), with a small contribution from the fermentation of protein or fat (Wolin, 1960). The slightly variation among plants samples in their fermentation of gas production, and ME and OMD mostly may be attributed to their variable nutrient and secondary compounds contents (Mbugua *et al*. 2008). Chemical composition and *in vitro* fermentation and digestibility are largely affected by plant species, plant morphological fraction, environmental factors, and maturity stage (Salem, 2005; Medjekal *et al*. 2015). Generally, as cell wall (fibre) increases, digestibility and energy content decrease (Van Soest, 1994). Our results were in line with those reported by Bruno-Suares and Abreu, (2003), Karabulut *et al*. (2006) and Obeidata *et al*. (2011), who observed higher digestibility of date pulp with lower cell-wall content. Hence, the supplementation of poor quality roughages with pods such carob and honey locust, which is commonly used by smallholder farmers, is likely to improve the performance of animals by supplying protein and soluble carbohydrates or energy (Nurfeta *et al*. 2008).

Fermentation end-products

There were differences (P<0.001) among the three studied samples in total and individual VFA concentration and acetate to propionate ratio after 24 h of incubation (Table 4). The lowest total VFA concentration was with *M. sativa* (68.98 mmol L^{-1}) and the highest with *C. siliqua* (89.10 mmol L^{-1}), whereas *G. triacanthos* showed an intermediate value (80.77mmol L^{-1}). The concentration of acetate (major fatty acid) differentiated two groups: *M. sativa and G. triacanthos* (86.58 and 66.32% respectively), while the fermentation of *C. siliqua* resulted in a lower acetate concentration (59.84%). On the other hand concentrations of propionate were higher in *C. siliqua* and *G. triacanthos* and lower *M. sativa*.

M. sativa produced the highest molar proportions of isovalerate and valerate, whereas the acetate/propionate ratios were significantly different between treatments (P<0.0024). Acetate to propionate ratios ranged from 2.06 in *C. siliqua* to 3.36 in *M. sativa*.

The variation in the *in vitro* gas production of studied samples was directly related to differences in other fermentation end-products (total VFA production). In the present experiment, the concentration of acetate (major fatty acid) differentiated two groups: *M. sativa* and *G. triacanthos* (86.58 and 66.32% respectively), while the fermentation of *C. siliqua* resulted in a lower acetate concentration (59.84%). The proportions of the dominant VFA produced in the rumen vary with diet, microbial growth rates, level of feeding, and rumen pH (Lopez *et al*. 2000). Degradation of fibrous or cellulosic materials is likely to produce a higher molar proportion of acetate and a lower proportion of propionate. However, feed with low fibre content would be expected to result in a reduction in the acetate: propionate ratio during rumen fermentation (Moss *et al*. 2000; Medjekal *et al*. 2016). Propionate is a useful end product of fermentation for ruminant because of it can be used for glucose synthesis (Leng *et al*. 1967) and is associated with higher efficiency of energy retention and utilisation in the rumen (Armstrong and Blaxter, 1957). Acetate is mostly unchanged by the liver and supplies the main source of energy by either being oxidized to ATP or stored in long chain fatty acids. Acetate and butyrate are the significant contributors to long chain fatty acids production for tissue deposition or secretion in milk (Madrid *et al*. 2002).

Consistent with our results, Bouazza *et al*. (2014) reported differences in VFA and methane production from the rumen fermentation of Algerian Acacia tree foliage. The most fermentable plant species (*Astragalus gombo* or *M. sativa*) led to higher production of both fermentation gas and VFA. Moreover, Medjekal *et al*. (2016) reported differences (P<0.05) in pH and VFA production at 24-hour incubations of browse species of Algerian arid and semi-arid areas, with pH ranging from 6.29 (straw) to 6.73 (*Hedysarum coronarium*), total VFA from 1.16 (*Stipa tenacissima*) to 3.59 (*Astragalus gombo*) mmol/g dry matter (DM) incubated and the acetate:propionate ratio from 3.06 (straw) to 5.81 (*Ononis natrix*).

According to Getachew *et al*. (2004), a high acetate:propionate ratio is an idication of fermentation of structural carbohydrates and thus of more fibrous feed. Furthermore, acetate to propionate ratio reduction in the rumen has been described as a common feature of several anti-methanogenic compounds, wich indicates a concurrent decrease of methane formation and shift in ruminal fermentation (Albengres Abecia *et al*. 2012).

Table 4 Total (mmol L^{-1}) production of volatile fatty acids, molar proportions (%), and acetate to propionate ratio (A:P) after 24 h of *in vitro* incubation

Species	Acetate	Propionate	Isobutyrate	Butyrate	Isovalerate	Valerate	Total	A:P
Ceratonia siliqua	59.66^{b}	29.84^{a}	0.51^{a}	9.02^{a}	0.28^{b}	0.75^{c}	89.10^{a}	2.06^{b}
Gleditschia triacanthos	66.32^{a}	27.19^{a}	0.19^{b}	6.23^{b}	0.40^{ab}	1.31^{b}	80.77^{ab}	2.49^{b}
Medicago Sativa	68.58^{a}	20.93^{b}	0.50^{b}	6.74^{b}	0.56^{a}	2.68^{a}	68.98^{b}	3.36^{a}
SEM	1.14	1.25	0.50	0.43	0.05	0.22	3.40	0.18
P-value	< 0.0004	< 0.0037	< 0.0095	< 0.0093	< 0.0572	< 0.0001	< 0.0408	< 0.0024

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

The acetate to propionate ratios observed with our samples are within the range of values reported in other *in vitro* studies (Brown *et al*. 2002; Albengres Abecia *et al*. 2012; Medjekal *et al*. 2016).

CONCLUSION

Chemical composition and *in vitro* gas production can be considered useful indicators for the preliminary evaluation of likely nutritive value of previously uninvestigated plants samples. Our study suggest the possibility that *C. siliqua* and *G. triacanthos* pods may be used as a dry season feed supplement to low quality diets. As reported by other authors, the addition of PEG inactivated the effects of tannins. Further studies are beaded to understand when the addition of PEG is useful *in vivo* to improve the nutritional value of both carob and honey locust pods.

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