Comparative analysis of the *in vitro* fermentation of wasted cladodes (*Opuntia* spp.), lucerne and oat hays

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Abstract

Wasted cladodes from the cactus pear (*Opuntia ficus* spp.) are an environmental nuisance, and alternative measures are required to utilize this product, such as utilizing it as a ruminant feedstuff. By simulating ruminal fermentation through *in vitro* gas production, the fermentation characteristics of dried cladodes were compared with that of lucerne hay and oat hay. A completely randomized design with six replications was used to assess the kinetic parameters of gas production (lag time, rate of gas production and maximum volume of gas (V-max)), *in vitro* dry matter digestibility (IVDMD), and volatile fatty acid (VFA) and ammonia nitrogen ($N-NH_3$) production during an incubation period of 96 h. Cladodes were collected from containers for organic wastes at large food stores in Mexico. The Vmax was similar between the substrates but the rate of gas production and IVDMD was higher for dried cladodes than for lucerne and oat hays, though the lag time of the cladodes was longer (9.5 vs. 6.5 and 6.7 h, respectively). Acetic and total VFA concentrations were similar during fermentation of dried cladodes and lucerne hay. These results suggested that ruminal microorganism fermented dried cladodes as well as when lucerne hay or oat hay were used as substrates in an *in vitro* fermentation system.

Introduction

The commercialized cultivation of cactus pear (*Opuntia* spp.) shows plenty of potential, as these plants survive under limited soil water conditions. Cactus pear contains adequate energy and has high vitamin A and moisture levels (Andrade-Montemayor *et al.*, 2011). Tender cladodes (nopales) from the cactus pear require frequent pruning and can produce more than 150 t of green matter/ha/year, a large proportion of which is typically discarded (Ortíz-Heredia *et al.*, 2013), though could be used as a ruminant feedstuff. In Mexico, 70% of the produced tender cladodes is marketed through large food stores to supermarkets, street markets and establishments (Callejas-Juárez *et al.*, 2009), where a large quantity of waste is generated daily as unsold products (Arias *et al.*, 2003). The recycling of this waste product could be a source of non-conventional feed for animals (EPA, 2012), thus reducing the emission of greenhouse gases and alleviating the problem of the filling up of landfills (EPA, 2014). In only a few studies the nutrient contents in wasted cladodes and their potential in the feeding of ruminants have been evaluated (Abidi *et al.*, 2009; Pinos-Rodriguez *et al.*, 2010). Therefore, the objective of the present study was to compare the kinetics of gas production, *in vitro* dry matter digestibility (IVDMD) and volatile fatty acid (VFA) synthesis between dried wasted cladodes, and oat hay and lucerne hays.

Materials and Methods

This study was conducted at the Laboratory of Socio-environmental Resources and Sustainability at Universidad Autónoma Metropolitana-Iztapalapa (UAM-I), Mexico City, and at the Laboratory of Livestock Microbiology, Universidad Autónoma Chapingo (UACh), Chapingo, Mexico State. Broken cladodes or those that did not look appealing to consumers compared with fresh fruit were obtained from organic waste

containers in the Central de Abasto (supply centre) in Mexico State. The lucerne and oat hays were obtained from an experimental farm at UACh. The wasted cladodes were cut into small portions and dehydrated for 96 h at 55 ºC in a drying oven (Riossa) equipped with forced air. The samples were subsequently ground to pass through a 1-mm sieve (Mill Thomas Wiley Mill, Philadelphia, Pa, USA) and analysed for dry matter (DM), organic matter (OM), crude protein (CP) and ash (AOAC, 1990). For acid detergent lignin (ADL), neutral detergent fibre (NDF) and acid detergent fibre (ADF), a fibre analyser (ANKOM[200], NY, USA) was used after alpha amylase and sodium sulphite were added in the NDF procedure.

In vitro gas production was performed according to Theodorou *et al.* (1994). The procedures performed on the animals were approved by the ethics committee of UACh in compliance with the guidelines of the law protecting animals in Mexico State. The kinetics of gas production (maximum volume of gas (Vmax), production rate of gas (S) and lag time (L)), *in vitro* dry matter digestibility (IVDMD) and concentration of VFAs and ammonia nitrogen (N-NH$_3$) were evaluated. A total of 0.5 g DM from each substrate was deposited in amber glass bottles (125 mL) and 90 mL reduced mineral solution (50 mL 80 g Na$_2$CO$_3$/L, 75 mL 6 g K$_2$HPO$_4$/L, 75 mL 0.6 g KH$_2$PO$_4$/L, 6 g (NH$_4$)$_2$SO$_4$/L, 0.9 g NaCl/L, 0.18 g MgSO$_4$/L, and 0.12 g CaCl.H$_2$O/L; 780 mL distilled H$_2$O; 20 mL 25 g L-cysteine/L and 25 g Na$_2$S/L; and 0.4 mL 1 g resazurin/L) was mixed with ruminal inoculum in a 9 : 1 (v/v) ratio under a continuous flow of CO$_2$. The ruminal inoculum was extracted from two rumen-cannulated sheep (57 ± 2.3 kg bodyweight), that were fed maize silage and 0.5 kg/d of a commercial feed (140 g CP and 40 g ether extract/kg). The inoculum was filtered through four layers of gauze. The bottles were hermetically sealed with a hand crimper (Wheapon, USA), rubber stopper and aluminium arum, and incubated at 39 ºC. The gas pressure inside each bottle was measured at 0, 1, 2, 3, 5, 7, 9, 11, 13, 16, 20, 24, 30, 36, 46, 58, 70, 82 and 96 h of incubation with a manometer (Metrón, México) over a range of 0 - 1 kg/cm^2 connecting a three-phase key and hypodermic needle (20G x 1½"). The gas was released after each reading, and the pressure was transformed to a gas volume using the equation:

$$P = -0.0159 + 0.0163V$$

obtained through linear regression (R^2 = 0.996) between the volume and pressure of the gas. The IVDMD was obtained after triplicate filtering (Whatman #541 vacuum pump, 500 mm Hg and 36.8 L/min, Felisa) the contents of these bottles and drying the residues for 48 h at 55 ºC.

The concentrations of VFAs and N-NH$_3$ were measured after incubation for 24 h. Four mL liquid medium from each treatment was extracted from the two bottles and mixed with 1 mL 25% metaphosphoric acid (w/v). Two millilitres of each mixture was centrifuged at 9000 x *g* for 15 min, and 1 µL of supernatant was injected into a gas chromatograph (Perkin Elmer, Claurus 500). Hydrogen was used as carrier gas with a 20 mL/min flow rate, injector and detector temperature of 250 ºC, oven temperature 80 ºC for 1 min with increments of 20 ºC/min until 140 ºC and a run time of 8 min. The concentration of N-NH$_3$ was calculated according to McCollough (1967).

The design was completely randomized with six replicates for each substrate (treatment). The statistical model was:

$$y_{ij} = \mu + \beta_i + \varepsilon_{ij} \qquad\qquad i : 1,2,3$$

where: y_{ij} = response variable, μ = overall mean, β_i = effect of substrate and ε_{ij} = random error.

The gas production variables were obtained using the logistic model (Schofield *et al.*, 1994 and an NLIN procedure (SAS, 2003)):

$$F(t) = v/1 + e^{2-4r(t-L)}$$

The data were analysed using a MIXED procedure (SAS, 2003), and the means were compared using the Tukey's test ($P \le 0.05$).

Results and Discussion

The moisture content (96%) in the wasted cladodes (Table 1) was similar to values reported by Arias *et al.* (2003) and Pinos-Rodríguez *et al.* (2010), who obtained ranges of 93% to 95%. This high moisture content is important during the dry season in arid and semi-arid regions and where water is a limiting factor for livestock production (Tegegne *et al.*, 2007). However, the high moisture content of the waste cladodes might promote rapid decomposition, which is common in moist forages (EPA, 2014). The CP level in the cladodes (156 g/kg DM) was 40% higher than that of oat hay, and 40% below that of lucerne hay. The CP level in cladodes varies according to species (Andrade-Montemayor *et al.*, 2011) and age, consistent with

the results of Pinos-Rodriguez *et al.* (2010) in harvested cladodes at 37 and 45 days old, containing 156 and 153 g CP/kg DM, respectively. Tegegne (2003) reported that the CP was between 106 and 150 g/kg in young cladodes grown in orchards, consistent with the results of the present study, as the unsold cladodes were obtained from orchards. The NDF, ADF and ash (Table 1) values were similar to those of Cordova Torres *et al.* (2009), who recorded 450 - 540 g NDF, 180 - 280 g ADF and 165 - 250 g ash/kg DM in five Mexican varieties of *Opuntia*. This reflects a low lignin concentration, which is advantageous to ruminal microorganisms to ferment wasted cladodes properly, compared with lucerne and oat hays. This contributes to the use of these materials in ruminant feed since the sixteenth century (Anaya-Pérez & Bautista-Zane, 2008).

Table 1 Chemical composition (g/kg) of wasted cladodes, and lucerne hay and oat hays

Substrate	Moisture	DM	OM	Ash	CP	NDF	ADF	Hem	Lignin
Lucerne hay	112	888	891	109	219	437	292	145	59
Oat hay	135	865	911	90	94	601	335	267	34
Cladodes	961	39	756	245	156	495	153	342	21

DM: dry matter; OM: organic matter; CP: crude protein; NDF: neutral detergent fibre; ADF: acid detergent fibre; Hem: hemicellulose.

The Vmax was similar among substrates (Figure 1 and Table 2), suggesting that the availability of carbohydrates for fermentation was similar. This finding indicates that the species of cactus in Mexico had similar availability of carbohydrates to fermentation of lucerne and oat hays. The rate of gas production (S) for cladodes was highest ($P \leq 0.05$) compared with lucerne and oat hays (Table 2), indicating that hydrolysis after microbial colonization was rapid, as shown in the accumulated gas volume (Figure 1) and the fractional volume of gas (Figure 2) at 12 h and 36 h after incubation.
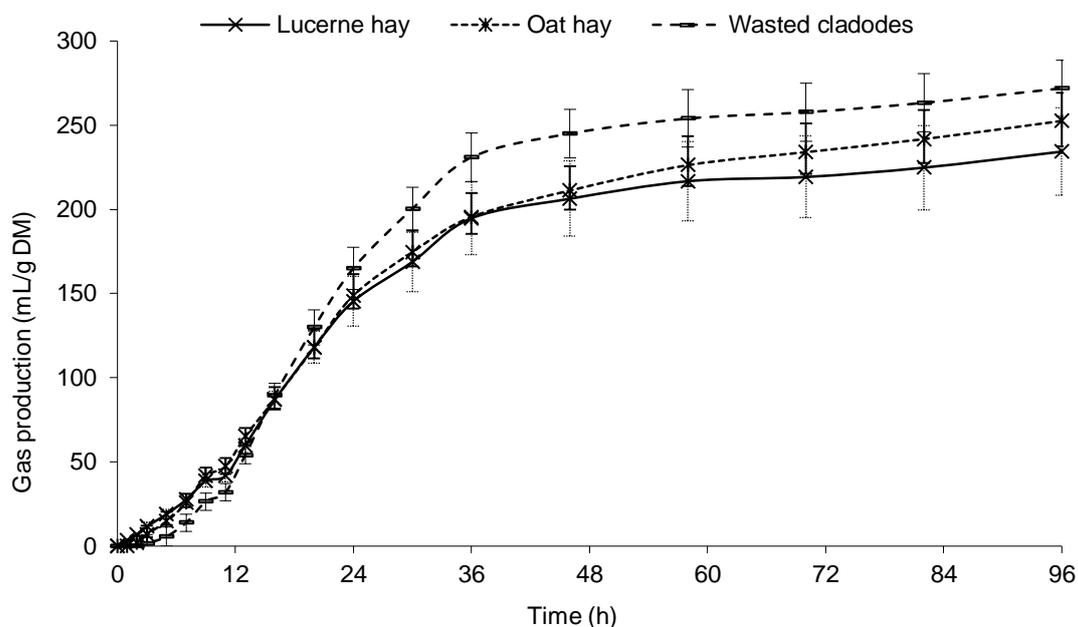
Figure 1 Cumulative gas volume from *in vitro* fermentation of wasted cladodes and lucerne and oat hays.

The rate of gas production (0.04/h) in dried cladodes was similar to that reported by Abidi *et al.* (2009) for spineless cactus harvested in the spring, suggesting that the harvest season partially affects the chemical composition and rate of gas production. The long lag time (9.5 h) for cladodes indicates that rumen

microorganisms required more time to adapt than with lucerne and oat hays (6.5 and 6.7 h, respectively). Cactus pear contains a thick serous layer on the cuticle of the epidermis (Ben Salem-Fnayou *et al.*, 2014). This cuticle is a protective layer that prevents the ingress of microorganisms into the dermal tissue and is considered a structural barrier to microbial degradation (Akin, 1989). The presence of this layer might reflect the prolonged time lag observed for cladodes compared with lucerne and oat hays. The IVDMD of cladodes (73.6%, Table 1) was higher (P <0.05) than that for lucerne and oat hays, despite a similar gas production. Shoop *et al.* (1977) reported that the IVDMD of cactus pear (63.8%) was similar to that of lucerne (63.7%), but the *in vivo* DM digestibility of cactus pear after incubation for 16 h was higher (18%) than for lucerne.

Table 2 Kinetics of gas production, *in vitro* dry matter digestibility (IVDMD) and concentration of volatile fatty acids

Variable	Lucerne hay	Oat hay	Cladodes	SEM
Vmax, mL	219.6[a]	232.3[a]	256.0[a]	9.3
S, /h	3.8-E2[a]	3.6-E2[a]	4.4-E2[b]	7.1 E4
L, h	6.5[a]	6.7[a]	9.5[b]	0.2
IVDMD, g	62.1[a]	52.8[b]	73.7[c]	0.2
Acetic, mM/L	16.7[a]	8.4[b]	15.1[a]	0.4
Propionic, mM/L	3.7[a]	1.9[b]	3.0[ab]	0.2
Butyric, mM/L	1.1[a]	0.9[a]	1.1[a]	0.1
Total, mmol	21.5[a]	11.2[b]	19.2[a]	0.6
N-NH3, mg/dL	15.1[a]	12.7[a]	13.8[a]	1.2
Acetic, %	77.7[a]	74.6[a]	78.7[a]	1.1
Propionic, %	17.0[a]	17.2[a]	15.5[a]	0.9
Butyric, %	5.2[a]	8.2[b]	5.8[a]	0.2

[abc] within rows, mean values with different superscripts are significantly different (P <0.05);
Vmax: maximum volume of gas;
S: rate of gas production; L: lag time; SEM: standard error of mean.
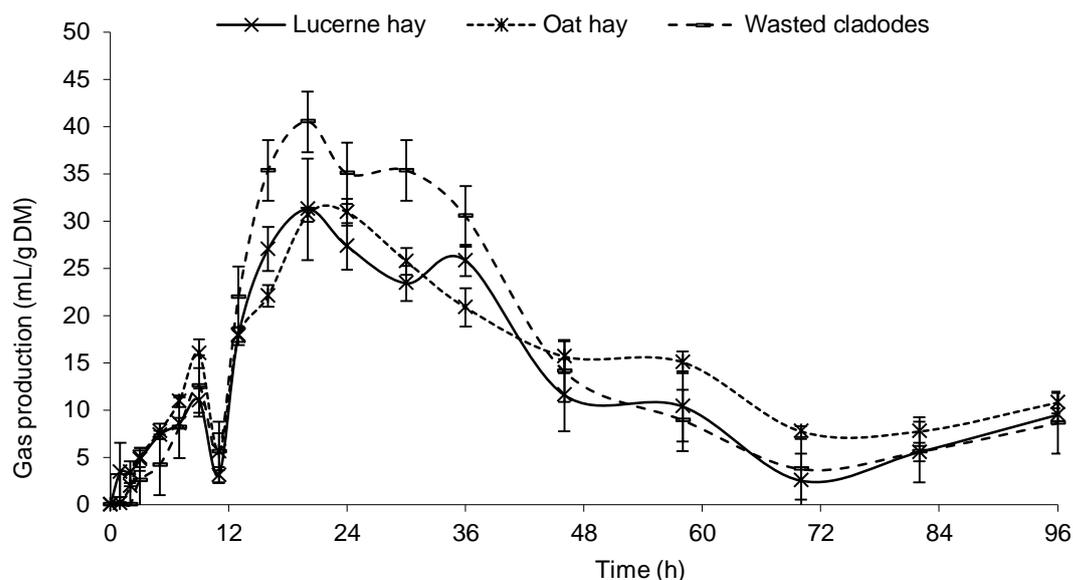
Figure 2 Fractional volume of gas production derived from *in vitro* fermentation of wasted cladodes and lucerne and oat hays.

Pinos-Rodríguez *et al.* (2010) reported that the IVDMD of cladodes at 45 - 60 days old was 75% and 73%, respectively, similar to the results of the present study, while Batista *et al.* (2003) reported that the IVDMD for 10 varieties of cactus was 60% - 70% after incubation for 72 h. This finding further justified the use of cactus in ruminant feed, consistent with the results of Tegegne *et al.* (2007).

The concentration of VFAs that originated from the *in vitro* fermentation of cladodes was similar (*P* >0.05) to that obtained from lucerne hay and higher (*P* <0.05) than that obtained for oat hay (Table 2). VFAs are the main source of energy in ruminants (Sutton *et al.*, 1985), and the total production and type of VFA affect nutrient use, weight gain and volume and milk composition (BBSRC, 1998), suggesting that, based on the synthesis of VFAs, cactus could replace oat hay completely and replace lucerne hay partially in ruminant diets as long as DM intake was not affected.

The synthesis of VFAs from the *in vitro* fermentation of cladodes led primarily to the production of acetic acid, consistent with the reports of Ben Salem *et al.* (1996) and Abidi *et al.* (2009). The proportion of VFAs from cladodes did not differ (*P* >0.05) from that of lucerne hay (Table 1). According to the BBSRC (1998), the proportion of VFAs is the most important factor in the balance distribution of nutrients, thus the incorporation of cladodes in the diet for ruminants compared with oat and lucerne hays does not affect the performance and quality of production. The concentration of N-NH$_3$ was similar (*P* >0.05) between treatments and sufficient for adequate synthesis of microbial protein, according to Satter & Slyter (1974). Ben Salem *et al.* (1996) reported a similar concentration (10.8 mg N-NH$_3$/dL) in the ruminal fluid from sheep receiving 600 g DM/d of cactus, indicating that replacing oat or lucerne hay with cactus cladodes does not affect the production of microbial protein.

Conclusions

Volatile fatty acid concentration and gas production volume derived from *in vitro* fermentation of wasted cladodes were similar to those obtained for lucerne and oat hays. However, the lag for cladodes was longer. The results suggest that cladodes could be fed to ruminants in the place of lucerne and oat hays without compromising the performance of the animals. Hence, feeding cladode wastes to ruminants would enhance environmentally friendly management of this waste product.

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