Nutritional Composition, *in vitro* Degradability and Gas Production of *Opuntia ficus indica* and Four Other Wild Cacti Species

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Abstract: This study was conducted to evaluate the potential use of spineless cacti specie (*Opuntia ficus indica* var. copena [OFI]) and four native Opuntía species with spines (*O. megacantha* [OMA]; *O. streptacantha* [OSA]*; O. hyptiacantha* [OHA], and *O. robusta* [ORA]). As alternative feed supplements for ruminants, considering their chemical composition, *in vitro* degradation behavior and gas production. Dry matter (DM) concentration of cacti species varied from 78 to 138 (g/kg of DM). Organic matter (OM), ash and crude protein (CP) concentrations differed ($P < 0.001$) between species. Neutral detergent fiber (NDF) concentration was highest ($P=0.008$) for ORA (542 g/kg of DM) and lowest for OFI (459g/kg of DM), whereas acid detergent fiber (ADF) was highest ($P < 0.001$) for OFI (287 g/kg of DM) and the lowest for OMA (184 g/kg of DM). Ash concentrations varied between species, OFI containing the highest ($P < 0.001$) (251 g/kg of DM), of which a high proportion was calcium (52.6 g/kg of DM). Selenium levels were very low in all species, in some cases not being detected. The highest ($P < 0.05$) potential DM degradation was recorded for OFI with 768 g/kg of DM, having a soluble fraction of 452 g/kg of DM, a potentially degradable fraction of 317 g/kg of DM and a fractional rate of degradation of 0.01/h. Of the cacti species with spines, the lowest ($P < 0.001$) DM degradation values were registered for OSA (399 g/kg). *In vitro* CP potential degradation differed ($P < 0.001$) between species; the highest value being for OFI (989 g/kg) and the lowest for OHA (578 g/kg). The highest ($P < 0.05$) NDF potential degradation was found for OMA (989 g/kg) and the lowest for OSA (376 g/kg). Only OFI and OMA were evaluated to determine total gas, CO_2 and CH_4 production. Total gas production was higher ($P < 0.001$) for OMA (53.5 mL /g DM) than for OFI (43.8 mL/g DM). Although total VFA (Volatil fatty acids) (µmol/g DM) production ($P =0.87$) and concentrations of butyric acid and acetic acid did not differ ($P=0.347$ and $P= 0.448$, respectively) between both species, the concentration of propionic acid was higher in OFI ($P= 0.055$). Total gas (mL gas/kg DM) and methane production (% of gas production) were higher ($P=0.008$ and $P=0.05$, repectively) for OMA. In conclusion, water and ash concentratons were high in all varieties, and the high DM solubility and degradability may have been related to both ash and protein solubility. The specie OMA had the best qualitative characteristics among the tested cacti species with spines to be considered as an alternative feed supplement for goats. Mechanical processes to eliminate the spines of the wild cacti species can simplify feed management and prevent damage to the animal mouth and digestive tract.

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1. Introduction

In the semiarid regions of the world, cacti (*Opuntia spp.*) are one of the most widely used low cost alternative feed, primarily during the dry season (Flores-Valdez and Aranda-Osorio, 1997), because it can easily adapt to different environmental conditions (Stintzing and Carle, 2005). Cacti grows throughout the American continent and their distribution extend from Canada to Patagonia, although they have also been grown in large areas of the mediterranean region, Africa and Australia (Snyman, 2006). In Mexico, cacti species are found naturally in 4 million hectares, mainly in the northern states (Flores-Valdez and Aranda-Osorio, 1997). In arid and semi-arid regions of México, cactus is used as a forage source because it provides water and feed but the cladodes of some spineless varieties are grown for human consumption and their fruits and stems are used for medical and

cosmetic purposes (Flores-Valdez and Aranda-Osorio, 1997).

Cactus is commonly used as an emergency feed supplement in semi-arid regions of countries of the American (Brazil, Chile and Mexico) and African (Morroco, South Africa and Tunisia) continents (Ben Salem and Smith, 2008; Kawas *et al*., 2010). It is low in dry matter (DM; 70 g/kg) and high in fiber (up to 580 g of Neutral Detergent Fiber NDF/kg of DM) and ash (up to 250 g/kg of DM), primarily due to its calcium concentration. Both fiber and ash may reduce energy intake (Dávila-Gutierrez, 1996; Ben Salem and Nefzaoui., 2003; Felker *et al*., 2006).

Spineless cactus (*O. ficus-indica* f. *inermis*) may contain less than 50 g Crude Protein/kg of Dry Matter (CPg/kg of DM) (Ben Salem *et al*., 2002), and a significant amount of the protein,, may be associated with the less degradable fraction of the plant cell wall, determined as acid detergent insoluble nitrogen (N-ADF) (Dávila-Gutierrez, 1996). This level of CP may provide less N for ruminal bacteria than the 70 g CP/kg of DM needed in the diet for normal function of the rumen (NRC, 1987). The high *in vitro* DM digestibility of *O. ficus-indica*, approximately 750 g/kg of DM, has been related to its low lignin concentration of less than 50 to 80 g/kg (Ramírez *et al*., 2000; Ramírez *et al*., 2001; Cerrillo and Juárez, 2004; Salem *et al*., 2006; Salem *et al*., 2012).

Among many other varieties, the most widely used cacti species are *O. streptacantha, O. robusta, O. rastrera, O. engelmannii* and *O. megacantha*. As most studies have been conducted with cactus *O. ficus-indica*, this study will compare the nutritional characteristics and the degradation kinetics of this specie (var. Copena) with various wild *Opuntia* species (*O. megacantha*, *O. streptacantha*, *O. robusta*, O. *hyptiacantha*), with the objective of determining their possible use as alternative feed supplements for goats in the semiarid regions of Mexico.

2. Materials and Methods

2.1 Sampling and chemical composition

A spineless variety of cactus, *O. ficus-indica* (var. Copena) was compared to various wild cacti species (*O. megacantha*, *O. streptacantha*, *O. robusta*, O. *hyptiacantha*) in terms of their nutritional characteristics and their degradation kinetics. Opuntias sampling were conducted during the spring of 2010 in the rangeland of the experimental center of Amazcala campus of the Autonomous University of Querétaro, which consists of 100 hectares located in El Marques municipality in the state of Querétaro, México. Sampling was performed by longitudinal transects and species having a higher abundance were considered for the study; taking representative samples of each species at random.

Cacti species were characterized using the botanical identification cards provided by the Department of Natural Sciences of the Autonomous University of Querétaro.

Ten samples of each cacti species were oven-dried at 60°C for 48 h and ground through a 1mm screen in a Wiley mill before being analyzed. Analyses were conducted in triplicate for determination of DM, N and organic matter (OM) (AOAC, 2000. Methods 934.01, 976.05 and 967.05, respectively). Ash was obtained after combustion of cactus samples at 600°C to calculate Organic Matter (OM) concentration (OM g/kg = 1000 – ash). Neutral detergent fiber with heat stable amylase (NDF), acid detergent fiber (ADF) and Lignin determined by solubilization of cellulose with sulphuric acid, were determined according to procedures reported by Van Soest *et al*. (1991) using filtration bags (F57) and an Ankom® fiber analyzer model A200. Neutral detergent CP (NDCP; CP associated with NDF, as percent DM) and acid detergent CP (ADCP; CP associated with ADF, as percent DM) were also determined (Licitra *et al*., 1996). Hemicellulose was calculated as the difference between NDF and ADF. Macrominerals (Ca, P, Na, K and Mg) and trace mineral (Fe, Mn, Cu, Zn, Co, Se and Mo) concentrations were determined using a Perkin Elmer Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) (Kawas and Pérez, 2008).

2.2 In vitro degradability

In vitro DM, NDF and CP degradability were determined in *O. ficus- indica* (var. Copena) and in the four native *Opuntia* species. Ruminal fluid was collected from three ruminally-fistulated cattle that were maintained under grazing conditions (native grass and Opuntias) but also fed concentrate (16% CP and 1.8 Mcal ENl/kg) 3 kg/cattle. The *in vitro* degradation technique was that recommended by Mehrez and Ørskov (1977), modified for the *in vitro* conditions established for an Ankom Daisy II apparatus using *in vitro* true digestibility Ankom technology (ANKOM Technology®, Method 3, 2013). Triplicate samples (0.5 g each) from each cacti species and a blank sample were introduced in marked Ankom bags. Samples were incubated and removed at 0, 2, 4, 6, 8, 12, 18, 24, 36, 48 and 72 hours. This study was repeated in 3 runs, making a total of 9 samples for each incubation time. The degradation kinetics of DM, NDF and CP were estimated according to the equation suggested by Ørskov and McDonald (1979). The following degradation model was used:

$$Dg = a + b\,(1 - e^{-ct})$$

Where:

Dg = nutrient degradation in the incubation time t (g/kg)

a = soluble fraction (g/kg)

b = potentially degradable fraction (g/kg)

c = speed or fractional rate of degradation (fractional rate/h)

t = time (h)

The estimated potentially degradable fraction includes both the soluble fraction and the potentially degradable fraction (a + b), and the effective degradability with the equation: ED= a+b (c/(c+kp)

Where:

ED = Effective degradability of nutrient fraction (DM, CP or NDF).

a = Soluble fraction (g/kg)

b = potentially degradable fraction (g/kg)

c = speed or rate of fraction degradation (fractional rate/h)

kp= speed or fractional rate of passage (/h)

Using three kp estimation of ED= 0.02/h; 0.04/h and 0.08/h.

2.3. Gas production

Gas production (mL/g of DM) was determined using an automated equipment (Ankom technology®, Gas Production Measurement, 2013) as recommended by Williams (2000). One gram sample, buffer solutions (buffer A, 124.5 mL; and buffer B, 25.5 mL) and 37.5 mL of ruminal liquor as inoculum were used as recommended by Kansas State University (Ankom technology®, Method 3, 2013). Incubated samples were kept under anaerobic conditions with CO_2 in a water bath at 39°C. Other parameters used in the equipment were a 48 h test time, 30 seconds for live interval, 15 minutes for sample interval, and 250 miliseconds (ms) for time to open the valve. This study was repeated in 3 runs with 3 replicates and one blank sample.

2.4. In vitro fermentation products and calculation of metabolizable energy

Total Volatil Fatty Acids (VFA´s) production and molar VFA´s concentrations were determined using a Perkin Elmer® gas chromatograph (Jaroslav *et al*., 2000). Concentrations of CO_2 and CH_4 were estimated using the stoichiometric method recommended by Van Soest (1994).

The estimated kinetic parameters of GP were fitted using a nonlinear model from Statgraphic centurión XVI® versión 16.1.11 (2008), according to France *et al*. (2000) without the soluble fraction (a) as follows:

$GP = b \times (1-e^{-c\,(t-lt)})$

Where:

GP is gas production at time t; b is the asymptotic GP (ml); c is the rate of GP (/h), and lt is the discrete lag time prior to gas production.

2.3. Experimental design and statistical analyses.

Chemical components and *in vitro* degradation kinetic variables were analyzed as a completely randomized design using SPSS® (version 16.0 for Windows®). Differences between cacti species in chemical composition were determined by the Duncan's multiple comparison procedure (Steel *et al*., 1997). For *in vitro* degradation kinetics data, a 5 × 11 factorial arrangement of treatments (5 cacti species × 11 sampling times) was used, considering the effects of cacti species, sample time, and species × sampling time interaction, with cacti species considered the experimental unit. Run as a source of variation was not statistically significant, therefore it was removed from the model.

The statistical model was:

$Y_{ijkl} = \mu + CE_i + T_j + (CExT)_{ixj} + E_{ijk}$

Where:

Y_{ijkl}: is every observation of cactus specie (CE_j) in *k*th incubation time; μ is the general mean; *CEi* is *i*th cactus especie effect; T_j is *j*th incubation time; $(CE xT)_{i\ x\ j}$ is the interaction between cactus especie and incubation time; and E_{ijk} is the experimental error. Duncan's multiple comparison procedure (Steel *et al*., 1997) was used to evaluate the difference between species. The differences between degradation kinetic parameters were evaluated by the T student method (Steel *et al*., 1997).

3. Results

3.1. Chemical composition

Dry matter concentrations of OFI and the other wild cacti species ranged from approximately 78 to 138 g/kg of DM, being lower (P = 0.03) for OFI and similar among wild Opuntias (Table 1).

Organic matter, CP, NDF, ADF and ash concentrations varied between species (P < 0.001). Ash levels ranged from 164 to 251 g/kg of DM. The NDF concentrations ranged from 459 g/kg of DM (OFI) to 542 g/kg of DM (ORA), ADF ranged from 184 (OSA) to 287g/kg of DM (OFI) and hemicellulose averaged 265 g/kg of DM. No differences (P > 0.05) in Lignin, NDCP and ADCP concentration were observed between species.Crude protein ranged from 45 to 72 g/kg of DM and varied between species (P<0.001) (Table 1). Concentrations of NDCP and ADCP averaged 43 and 17 g/kg of DM intake, respectively. As a percent of total CP, NDCP and ADCP averaged 76.2% and 27.3%, respectively. Neither CP bound to NDF (NDCP) nor ADF (ADCP) differed (P = 0.08 and 0.7, respectively) between species.

3.2. Minerals

Mineral concentrations varied between cacti species (Table 2). Calcium ranged from 27.7 to 52.6 g/kg (P < 0.001), with ORA and OMA being low in Ca and OFI containing the highest Ca level. All cacti species had a low P concentration ranging from 2.2 to 3.0 g/kg (P = 0.022).

The species ORA and OHA were higher in Mg (P= 0.05) than other cacti species. Magnesium levels ranged from 12.7 to 17.1 g/kg (P= 0.05). Sodium and potassium concentrations did not differ between species (P= 0.199 and P= 0.384, respectively). Sodium concentrations ranged from 0.4 to 0.5 g/kg of DM and K from 12.0 to 25.1 g/kg of DM.

Iron concentrations ranged from 43.3 to 70.1 mg/kg (P= 0.002), whereas Mn concentrations ranged from 103 to 471 mg/kg (P < 0.001). Manganese concentrations were higher than 400 mg/kg for OMA and OSA. Copper ranged (P=0.004) from 4.3 mg/kg (OHA and ORA) to 8.3 mg/kg (OFI). In general, Se concentrations were low or negligible in OHA and highest (P < 0.001) in OMA and OFI. Cobalt concentration was higher (P < 0.001) in OMA (103 µg/kg) and OSA (95.2 µg/kg) and undetectable in OHA.

Table 1. Chemical composition of five cacti species.

	Cacti species[2]							
Component	OMA	OHA	ORA	OSA	OFI		SEM[3]	P[4]
Dry matter, (g/kg)	121^{a}	138^{a}	126^{a}	109^{ab}	78^{b}		16.3	0.03
DM basis, (g/kg)[1]								
Ash (g/kg)	164^{e}	197^{b}	177^{c}	167^{d}	251^{a}		12	<0.001
OM (g/kg)	835^{a}	803^{d}	822^{c}	833^{b}	749^{e}		11	<0.001
NDF (g/kg)	477^{bc}	511^{ab}	542^{a}	483^{c}	459^{c}		15.9	0.008
ADF (g/kg)	184^{c}	230^{b}	203^{bc}	187^{c}	287^{a}		15.5	<0.001
CP (g/kg)	45^{e}	59^{c}	72^{a}	48^{d}	69^{b}		11	<0.001
Lignin (g/kg)	13	20	21	14	25		5.5	0.06
NDCP	42	43	53	42	39		9.7	0.08
ADCP	18	16	18	0.6	30		12.7	0.7

[1]Dry matter, DM; organic matter, OM; neutral detergent fiber with heat stable amylase, NDF; acid detergent fiber, ADF; crude protein, CP; Lignin determined by solubilization of cellulose with sulphuric acid,Lignin ; ADCP, acid detergent CP, % DM ; NDCP, neutral detergent CP, % DM.
[2] OMA, *O. megacantha*, OHA, *O. hyptiacantha*; ORA, *O. robusta;* OSA, *O. streptacantha*, OFI, *O. ficus indica.*
[3]SEM, Standard error of the mean.
[4] P= Probability.
Means in the same row with different superscripts are significantly different (P<0.05).

Table 2. Chemical composition of five cactus species.

	Cactus species[1]							
Element	OMA	OHA	ORA	OSA	OFI		SEM[2]	P[3]
Calcium, %	2.92^{d}	4.78^{b}	2.77^{d}	3.45^{c}	5.26^{a}		0.10	***
Phosphorus, %	0.22^{b}	0.30^{a}	0.26^{ab}	0.23^{b}	0.21^{b}		0.01	*
Magnesium, %	1.43^{b}	1.65^{a}	1.71^{a}	1.52^{ab}	1.27^{c}		0.05	**
Sodium, %	0.05^{a}	0.04^{b}	0.04^{b}	0.04^{b}	0.05^{a}		0.003	**
Potassium, %	1.87^{a}	1.63^{a}	2.51^{ab}	1.20^{ac}	1.90^{a}		0.25	*
Iron, ppm	70.1^{a}	62.8^{b}	47.6^{c}	62.5^{b}	43.3^{c}		1.47	***
Manganese, ppm	471^{a}	103^{c}	131^{c}	469^{a}	295^{b}		11.8	***
Zinc, ppm	16.2^{c}	9.7^{c}	15.2^{b}	18.9^{b}	25.7^{a}		1.24	***
Copper, ppm	5.1^{b}	4.3^{b}	4.3^{b}	5.0^{b}	8.3^{a}		0.25	***
Molybdenum, ppm	15.6^{a}	14.9^{b}	14.9^{b}	15.3^{ab}	15.5^{a}		0.15	*
Selenium, ppb	86.5^{b}	ND	41.0^{d}	51.5^{c}	107.8^{a}		1.79	***
Cobalt, ppb	103.0^{a}	ND	42.6^{d}	95.2^{b}	55.0^{c}		2.21	***

[1] OMA, *O. megacantha,;* OHA, *O. hyptiacantha,;* ORA, *O. robusta;* OSA, *O. streptacantha;* OFI, *O. ficus indica;* ND, *Not detected*.
[2]SEM, Standard error of the mean.
[3]NS* P < 0.05; ** P < 0.01; *** P < 0.001); NS, not significant.

3.3. In vitro DM degradation values

Initial DM degradation at time 0 was different (P < 0.05) between species, showing a fast initial degradation until h-6, with similar degradation values for all species at h-72, except for OSA which had the lowest (P < 0.001) degradation (Fig. 1). *In vitro* DM

degradation at time 0 ranged from 298 g/kg for ORA to 441 g/kg for OFI. At h-72, *in vitro* degradations ranged from 396 g/kg for OSA to 518 g/kg for OMA.

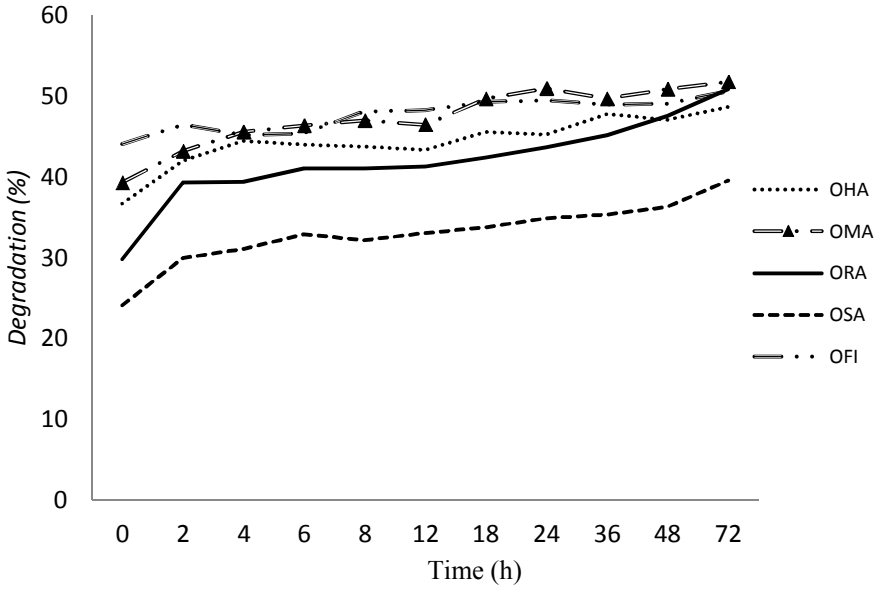

Fig 1. *In vitro* Dry Matter degradation of Opuntia *ficus indica* (OFI) and four wild spineless Opuntias.

3.4. In vitro DM degradation kinetics

Important differences in *in vitro* DM degradation kinetics of the five cacti species were observed in this study (Fig. 2). Results of *in vitro* DM degradation kinetics showed that OSA had a minimal DM degradation rate, whereas OFI degraded slowly but steadily up to h-72.

An average soluble fraction of 339 g/kg ± 22.7 was obtained, varying ($P < 0.001$) between cacti species (Table 3). Both the soluble (a) and the slowly degradable (b) fractions were lowest for OSA and highest for OFI. The fractional rate of passage (c/h) ($P<0.001$) varied from 0.01 for OSA to 0.17 for OHA. The estimated potentially degradable fraction, comprised as the sum of the soluble and slowly degradable factions, averaged 549 g/kg, being lower for OSA (399 g/kg) and higher for OFI (768 g/kg) ($P<0.01$). The effective DM degradation to differents kp (0.02, 0.04 and 0.08) were higher ($P<0.001$) in OFI and lower in OSA.

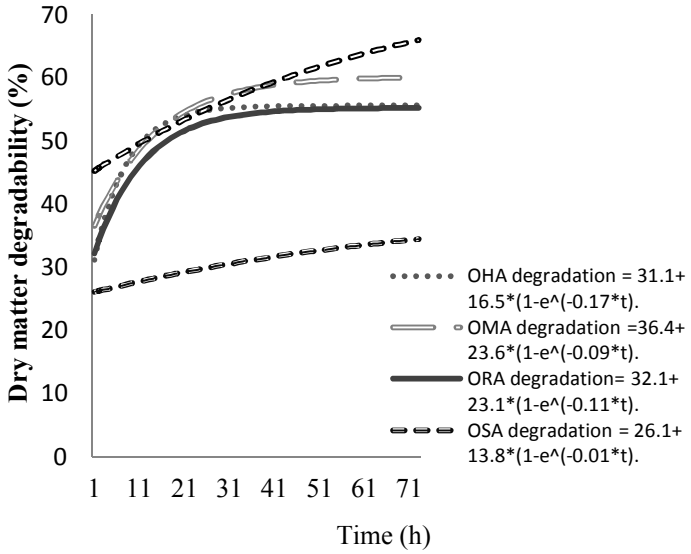

Fig 2. *In vitro* Dry Matter degradation kinetics of Opuntia *ficus indica* (OFI) and four wild spineless Opuntias

Table 3. *In vitro* DM degradation kinetics of five cactus species.

	Fraction (%)[2]			PD[3]	Effective degradation[4]					
Species[1]	a	b	c	a + b	Kp = 0.02	Kp = 0.04	Kp = 0.06	Kp = 0.08	R^2	SEM[5]
OHA	31.1^{d}	16.5^{b}	0.17^{a}	47.6^{c}	53.0^{c}	51.0^{c}	49.3^{c}	47.9^{c}	62.3	5.1
OMA	36.4^{b}	23.6^{b}	0.09^{b}	60.1^{b}	55.9^{b}	53.0^{b}	50.8^{b}	49.2^{b}	65.7	3.4
ORA	32.1^{c}	23.0^{bc}	0.11^{b}	55.2^{d}	51.8^{d}	49.4^{d}	47.4^{d}	45.9^{d}	85.8	2.1
OSA	26.0^{e}	13.8^{c}	0.01^{c}	39.9^{e}	32.3^{e}	30.1^{e}	29.0^{e}	28.4^{e}	83.6	1.5
OFI	45.2^{a}	31.7^{a}	0.01^{c}	76.8^{a}	60.6^{a}	55.4^{a}	52.8^{a}	51.2^{a}	73.1	1.1
Mean	33.9	21.4	0.10	54.9	49.4	46.9	45.3	44.0	77.4	2.5
SEM[5]	2.2	2.3	0.06	4.4	3.4	3.18	3.0	2.8	3.8	1.3
P[6]	**	**	***	***	***	***	***	***		

[1] OHA, *O. hyptiacantha,;* OMA, *O. megacantha,;* ORA, *O. robusta,;* OSA, *O. streptacantha,;* OFI, *O. ficus indica.*
[2] a = soluble degradation fraction (%); b = potentially degradable fraction; c = fraction rate of degradation.
[3] PD, potentially degradable fraction.
[4] Effective degradation = a + b (c/c + kp); kp = fractional rate of passage.
[5] SEM, standard error of the mean.
[6] NS, not significant (P > 0.05); ** P < 0.01; *** P < 0.001.

3.5. In vitro CP degradation kinetics

Whereas OFI had the highest CP soluble fraction and a fast degradation rate, ORA degraded slowly but steadily and had a high *in vitro* CP degradation at 72 h (Fig. 3). The CP soluble fraction varied (P < 0.001) between cacti species with an average of 671 g/kg CP (Table 4). The CP soluble fraction was lower for OHA (319 g/kg CP), it was highest for OFI (982 g/kg CP). The CP slowly degradable fraction averaged 165g/kg CP, being lower for OFI (7g/kg CP) and higher for ORA (377 g/kg CP). The average CP degradation rate was 0.247/h, being lowest for ORA (0.057/h) and highest for OMA (0.8/h). The potential degradability averaged 836 g/kg CP, varying (P < 0.001) between cacti species.

The potentially degradable CP fraction was highest for ORA (984 g/kg CP) and lowest for OHA (578 g/kg CP). To estimate the effective CP degradation of (EDCP), four fractional rates of passage (kp) were used. Fractional degradation rates of 0.02/h, 0.04/h, 0.06/h and 0.08/h resulted in average effective degradations of 812, 797, 785 and 776 g/kg CP, respectively.

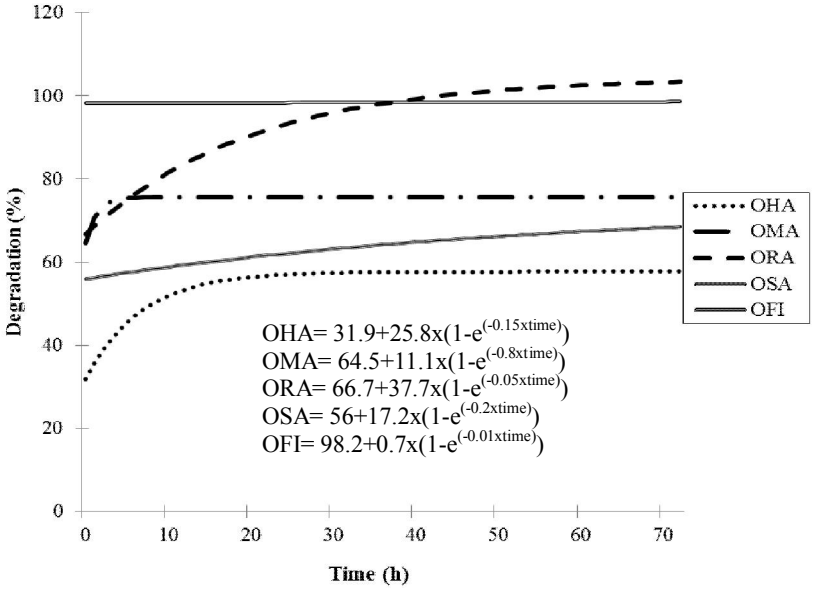

Fig. 3. Crude protein degradation kinetic of five cactus species.

Table 4. *In vitro* crude protein degradation kinetics of five cacti species[1].

				Potential degradability[3]	Effective degradation[4] (g/kg)				
Item[2]	a[b](g/kg)	b[b](g/kg)	c/h	a + b	Kp = 0.02/h	Kp = 0.04/h	Kp = 0.06/h	Kp = 0.08/h	R^2
OHA	319[c]	258[c]	0.1[cf]	578[c]	547[c]	523[c]	504[c]	488[c]	83.6
OMA	645[d]	111[d]	0.8[d]	755[d]	753[d]	750[d]	747[d]	745[d]	51.7
ORA	607[e]	377[e]	0.057[e]	984[e]	946[e]	888[e]	851[e]	824[e]	73.6
OSA	560[f]	172[f]	0.18[f]	732[f]	715[f]	701[f]	689[f]	680[f]	85.7
OFI	982[g]	7[g]	0.1[ce]	989[g]	988[g]	987[g]	986[g]	986[g]	27.5
Mean	671	165	0.247	836	812	797	785	776	61.9
SEM[5]	77	45	0.01	61	57	57	58	60	
P[6]	<0.001	<0.001	<0.001	<0.001		<0.001	<0.001	<0.001	<0.001

[1] OHA, *O. hyptiacantha;* OMA, *O. megacantha;* ORA, *O. robusta,;* OSA, *O. streptacantha,;* OFI, *O. ficus indica.*
[2] a = soluble degradation fraction ; b = potentially degradable fraction; c/h = fractional rate of degradation/hour.
[3] Potential Degradability, potentially degradable fraction.
[4] Effective degradation = a + b (c/c + kp); kp = fractional rate of passage/h
[5] SEM: standard error of the mean.
[6] P= Probability

3.6. In vitro NDF degradation kinetics

An average NDF potential degradation of 610 g/kg NDF was obtained for the five cacti species, with notable differences (P < 0.001) between them (Fig. 4). Whereas the potential degradation was lowest for OSA (376 g/kg NDF), it was highest for OMA (989 g/kg NDF). The soluble fraction varied (P < 0.001) between cacti species (Table 5), being lowest for OSA (278 g/kg NDF) and highest for OFI (495 g/kg NDF). The NDF slowly degradable fraction averaged 245 g/kg NDF, with the lowest (P < 0.001) values obtained for OSA and OFI with 97 and 100 g/kg NDF, respectively, and was highest for OMA (663 g/kg NDF). The average NDF fractional rate of degradation was 0.094/h, varying greatly between cacti species (0.2, 0.13, 0.06, 0.04, and 0.04/h for OFI, OSA, OHA, OMA and ORA, respectively).

To estimate the effective degradation (a+b (c/c+kp)), four fractional rates of passage (kp) were used (0.02, 0.04, 0.06 and 0.08 kp/h), resulting in an overall mean of 554, 523, 501 and 487 g/kg NDF, respectively. In this case, the variability of the fractional rate of degradation influenced the level of effective NDF degradation. Whereas OSA and OFI had a higher NDF degradation rate (0.13 and 0.17/h, respectively), OMA had a steady and high rate of NDF degradation up to 72 h.

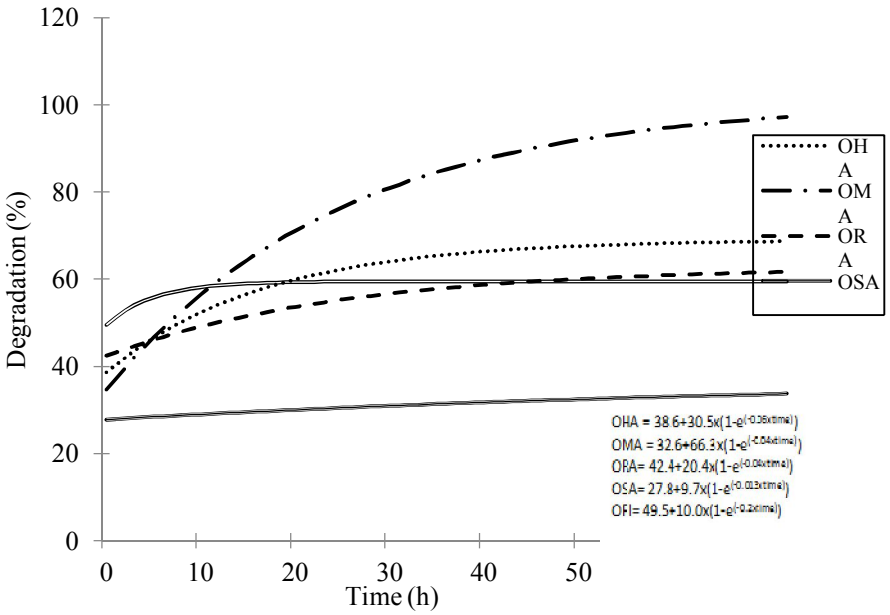

Fig. 4. Neutral detergent fiber degradation kinetics of five cacti species.

Table 5. *In vitro* degradation kinetics of neutral detergent fiber of five cacti species[1].

				Potencial Degradability[3] (g/kg)	Effective degradation[4](g/kg)				
Item[2]	a[b](g/kg)	b[b](g/kg)	c/h	a + b	Kp = 0.02/h	Kp = 0.04/h	Kp = 0.06/h	Kp = 0.08/h	R^2
OHA	386[a]	305[c]	0.06[a]	691[c]	615[c]	569[c]	539[c]	517[b]	49.1
OMA	326[b]	663[d]	0.04[a]	989[d]	808[d]	692[d]	623[d]	577[c]	68.6
ORA	424[c]	204[abc]	0.04[a]	627[e]	559[e]	525[e]	505[e]	491[d]	46.3
OSA	278[d]	97[a]	0.13[b]	376[f]	374[f]	373[f]	371[f]	369[e]	42.4
OFI	495[e]	100[a]	0.17[c]	595[g]	587[g]	579[g]	574[g]	569[f]	53.9
Mean	366	245	0.09	610	554	523	501	487	41.2
SEM[5]	28	80	0.2	82	53	39	32	28	
P[6]	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	

[1] OHA, *O. hyptiacantha,;* OMA, *O. megacantha,;* ORA, *O. robusta,;* OSA, *O. streptacantha,;*. OFI, *O. ficus indica.*
[2] a = soluble degradation fraction; b = potentially degradable fraction; c/h = fractional rate of degradation/hour.
[3] PD, potentially degradable fraction.
[4] Effective degradation = a + b (c/c + kp); kp = fractional rate of passage/h
[5]SEM: standard error of the mean from the model
[6] P= Probability.

3.7. In vitro fermentation products

In this experiment, two cacti species were used: one without spines (OFI) and an abundant wild Mexican species with spines (OMA) (Table 6). No differences (P > 0.05) were observed in the concentrations of total VFA, CO_2 or CH_4 between cacti species; neither were molar percent acetic or propionic acids.

No differences (P > 0.05) were observed for *in vitro* total VFA production, molar percent of butyric and acetic acids, CH_4 or CO_2 (Table 6). Total VFA production averaged 111 mmol/g DM, whereas mean concentrations of acetic and butyric acids were 74.6 µmol/g DM and 10.96 µmol/g DM, respectively. Propionic acid concentration was lower (P < 0.05) for OMA (29.04 µmol/g DM) than for OFI (30.19 µmol/g DM).

In vitro gas production was greater (P =0.008) for OMA (53.5 mL/kg DM) than for OFI (43.8 vs. mL/g DM). Gas production fractional rate was similar (P > 0.05) between the two cacti species (0.02/h). Conversely, gas production lag time (Fig. 5) was lower (P < 0.001) for OMA than for OFI (8.36 vs. 26.8 h). No differences (P > 0.05) in the production of CO_2 µmol/g of DM or CH_4 µmol/g of DM were observed in this study but CH_4 as a percent of total gas production was lower in OFI (P<0.05).

Table 6. In vitro volatile fatty acids and gas production of *Opuntia ficus indica* and *Opuntia megacantha*.

	OFI[1]	OMA[1]	SEM[2]	P[3]
Volatile fatty acids VFA (µmol/g DM)	114.8	107.2	27.3	0.878
Acetic acid (µmol/g DM)	73.4	75.8	3.44	0.448
Propionic acid (µmol/g DM)	30.2	18.7	3.04	0.055
Butyric acid (µmol/g DM)	10.7	11.25	0.75	0.374
Gas production (GP) GP (ml gas/g DM)	43.8	53.5	0.03	0.008
Carbon dioxide (µmol)	0.52	0.55	0.01	0.785
Methane (µmol)	0.30	0.35	0.02	0.513
Carbon dioxide (%)	63.6	60.9	1.36	0.181
Methane (%)	36.4	39.1	1.36	0.050
Metabolizable Energy (MJ/kg DM)[4]	9.11	8.043		

[1] OFI, Opuntia ficus indicus; OMA, Opuntia Megacantha.
[2] SEM= Standard error of the mean.
[3] P= Probability
[4] Calculate from equation of Menke *et al*. (1988).
Means in the same raw with different superscripts are significantly different (P<0.05).

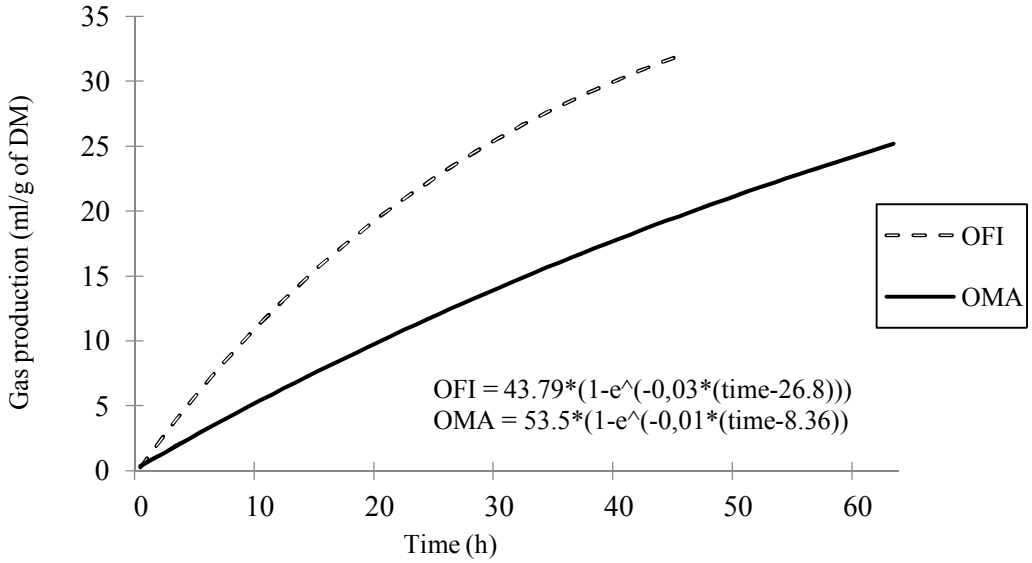

Fig. 5. *In vitro* gas production for *Opuntia-ficus Indica* and *Opuntia Megacantha*.

4. Discussion

4.1. Nutritional value

Cacti water concentration is high (850 to 900 g/kg DM) and can provide most of the animals drinking requirements during drought periods (Tegenge *et al*., 2006; Felker *et al*., 2006). Ben Salem *et al*. (2002) reported an ash concentration of 238 g/kg DM. Some cacti species are high in fiber (up to 580 g/kg NDF) and ash (up to 250 g/kg DM), which may reduce energy intake (Dávila-Gutiérrez, 1996). It is important to emphasize that OFI, the cacti species with the highest ash concentration (251g/kg DM), also had the highest ($P < 0.001$) Ca concentration (526 g/kg DM). As indicated by Ramírez *et al*. (2000, 2001), Cerrillo and Juarez (2004), and Cerrillo *et al*. (2006), high fiber digestibility may be expected when lignin levels are low. The values reported herein are similar to those reported by other authors who have investigated wild species of cacti (Mondragon-Jacobo *et al*., 2003, Fuentes, 2003; Guevara *et al*., 2004).

Ben Salem *et al*. (2002) reported the composition of OFI, with a CP concentration of 50 g/kg DM. This level of CP may provide less N for ruminal bacteria than the 70 g/kg DM of CP needed in the diet for normal ruminal function (NRC, 1987). This may further be exacerbated by the fact that some of the CP is associated to the cell wall fraction.

4.2. Minerals

Mineral requirement of mineral the diet of goats vary with growth performance, reproductive status (maintenance, breeding, gestation or lactation) and breed. (NRC, 2007).

Magnesium requirement for growing kids and lactating does varies from 0.5 to 2 g/kg DM (NRC, 2007). In goats, high Mg intake may cause urolithiasis (NRC, 1980; NRC, 2007). In cattle, reduced diet digestibility or diarrhea have been observed only with diets that contain more than 11.5 g of Mg/kg DM. Nevertheless, a maximum tolerable Mg concentration of 6 g/kg DM in the diet of ruminants has been established (Underwood, 1985; NRC, 2005).

Sodium level ranged from 0.4 to 0.5 g/kg DM, which may come short of satisfying the minimum nutritional requirement of 0.5 to 1 g/kg DM in goat and sheep diets (NRC, 2007). Potassium ranged from 18 to 25 g/kg DM. High water, Mg and K concentrations of cacti may in combination cause a laxative effect.

The NRC (1980) reports 1000 mg/kg DM as a maximum Mn level in the diet without any adverse effects. Iron and Mn concentrations were greater than those required in small ruminant diets (NRC, 2007). The copper requirements for lactating does, mature goats and bucks, and growing goats, have been recommended (Solaiman *et al*., 2006), taking into consideration normal Mo and S intakes (NRC, 2007), being these concentrations lower than those required in the diet of goats (NRC, 2007). Concentrations obtained for Se and Co are insufficient to satisfy the daily requirement for goats of 0.16 mg/kg DM, established by the NRC (2007).

4.3. In vitro DM degradation and degradation kinetics

In general, cactus degradation rates (c/h) were fast initially, plateauing at 6 h, with a slow degradation until 72 h. These degradation values are

greater than those reported by Villegas-Díaz *et al*. (2009) and Fuentes (2003). The greater DM degradation for OMA may be related to lower ADF and lignin concentrations (Andrade-Montemayor, 2005), and more energy being available to the ruminal environment. These degradation rates vary depending on the solubility of chemical components such as carbohydrate, protein and lignin (Kozloski, 2009; Khattab *et al*., 2013). The potential DM degradation values were similar to those found by Teixeira *et al*. (1999) and Carvalho *et al*. (2006), for *in situ* degradation of OFI with goats. Using the gas production technique, Cerrillo and Juarez (2004) observed a potential DM degradation for *Opuntia* spp. of 448 g/kg DM which is lower than those found in this study for OFI. The effective degradation values obtained in this study were greater than those reported by Teixeira *et al*. (1999) who utilized more mature cacti in their study, but similar to Villegas-Díaz (2009).

4.4. In vitro CP degradation kinetics

Cacti have low CP concentrations, 60% or more being soluble, and a fast rate of degradation, and therefore, the effect of rate of passage on CP degradation is not an important issue (Conklin-Bruttain *et al*., 1999; Carvalho *et al* 2006). To attain maximum microbial protein synthesis and minimize urinary N loss, carbohydrate supplementation may be needed for ruminants fed cactus (Tamminga *et al*., 1994; Tamminga, 1996).

4.5. In vitro NDF degradation kinetics

Degradation fractional rates for NDF vary depending on the solubility of carbohydrate and lignin components (Van Soest, 1991; Kozloski, 2009). The NDF degradability was highly variable between cacti species. With the exception of OSA, all other cacti species had a soluble fraction greater than the potentially degradable fraction, thus significantly influencing the effective degradability and the fractional rate of degradation (Andrade-Montemayor, 2005). The potential degradation as well as the effective degradation depended on characteristics and chemical composition of forages, and age and degree of lignification of NDF (Van Soest *et al*. 1982). In several studies with cacti species, degradability decreased with advancing herbage maturity (Teixeira *et al*. 1999; Villegas- Díaz, 2009). A lower NDF concentration and high degradability can contribute to greater forage consumption (Ramirez *et al* 2000). In the present study, the NDF degradability of cacti was comparable to that of alfalfa (Ramirez *et al*., 2000).

4.5. In vitro fermentation products and gas production

Gas production is an indicator of degradability and the energy density of feed (Menke and Steingass, 1988; Salem, 2005; Salem *et al*., 2007; Elghandour *et al*., 2014). Gas production depend of fiber an protein content (Elghandour *et al*. 2014) Gas production was lower than values observed by Cerrillo (2004) in *Opuntias* and other plants consumed by goats. *Opuntias* have variable tannin concentrations which can reduce total gas and methane production, thus reducing energy loss (Ben Salem *et al*., 2002; Anuraga *et al*., 2009., Salem *et al*., 2014 a., Salem *et al*.,2014b;Togtokhbayar *et al*., 2015). The higher gas production of OMA was by the lower soluble and fermentable content however has a lower rate of gas production and lower lag time, however OFI has a higher lag time, with a rapid gas production by the higher soluble content but lower gas production (Salem et a., 2007; Elghandour *et al*. 2014).

Total VFA production and VFA ratios are directly related to methane and carbon dioxide production with a greater amount of acetic acid synthesis increasing methane production (Naga and Harmeyer, 1975;Van Soest, 1994). In the current study, the low variability observed in VFA concentrations between *Opuntias* samples allowed us to detect statistical differences in gas production.

Determining gas production is a tool for predicting the energy concentration of forage plants (Menke and Steingass, 1988; Salem, 2005, Salem *et al*., 2007; Togtokhbayar *et al*., 2015). The equation of Menke and Steingass (1988) is commonly used to estimate metabolizable energy. In this equation, gas production and CP concentrations are associated with the energy level of the forage, resulting in a lower energy density of OMA due to its low CP concentration, although gas production was higher.

5. Conclusions

Some cactis species contain high ash and fiber levels which reduce energy availability of forage DM. The high DM solubility of certain cacti species may be related to a high concentration of soluble protein and minerals. Considering that the CP concentration is generally lower than 70 g/kg DM, and that a high proportion is associated with the less degradable plant cell walls in some cacti species, protein supplementation may be needed for normal fermentation and ruminal function. One favorable characteristic of OFI was its high degradation and metabolizable energy values, and lower gas production. While *O. streptacantha* had the lowest degradation values, other species such as *O. megacantha, O. hyptiacantha* and *O. robusta* had similar degradation responses. Although some cacti species have thorns, these can be mechanically removed before being fed to small ruminants.

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