Nutrient Content of *Opuntia* Forage Clones in the Mendoza Plain, Argentina♦

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ABSTRACT

Variations in organic matter (OM), *in vitro* organic matter digestibility (IVOMD), crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), and dry matter (DM) with cladode age were examined for seven *Opuntia* forage clones. The nutritional parameters were measured for three age classes. Data were analysed using ANOVA, linear regression analysis and principal components analysis. For all age classes combined, clones showed high OM (81.6% to 86.8% DM), and IVOMD (69.5% to 82.1%) and low protein content (3.2% to 5.0% DM). They had from 22.7% to 27.1% NDF, and from 12.0% to 16.0% ADF (dry-weight basis); DM ranged from 7.3% to 11.5%. A significant ($p < 0.05$) or close to be significant ($p = 0.08$) linear relationship between each nutritional parameter and age classes was found for all clones, except for OM that showed a significant ($p < 0.05$) linear relationship only for two clones. Each clone was associated with a high content of one or two particular nutritional parameters. The clone SJ of *O. ficus-indica* that had greater average percent CP than the other clones and a high IVOMD value appears to be the most outstanding clone.

Keywords: *Opuntia* clones; forage; cladode age; organic matter; in vitro organic matter digestibility; neutral detergent fibre; acid detergent fibre; crude protein; dry matter

INTRODUCTION

Nutrient content of *Opunti*a spp. depends on the genetic characteristics of the species or clones, the cladode's age, the cladode sampling location, the pad harvesting season and the growing conditions, such as soil fertility and climate (Monjauze and Le Houérou, 1965; Boza et al., 1995; Nefzaoui and Ben Salem, 2001; Gugliuzza et al., 2002). Typical range in values for nutritional parameters of *Opuntia* spp. has been reported by Flachowsky and Yami (1985), Silva (1987), Robles Cruz and Boza López (1993), Felker (1995), Le Houérou (1996, 2002), Mohamed-Yasseen et al. (1996), Fuentes-Rodríguez (1997), Azócar (2001), López-García et al. (2001), Nefzaoui and Ben Salem (2001) and Arias et al. (2003) among others. Several studies have been conducted to assess the nutrient content of *Opuntia* species or clones as a function of cladode age (Monjauze and Le Houérou, 1965; Gregory and Felker, 1992; Boza et al., 1995; Nefzaoui and Ben Salem, 2001). Our previous study (Guevara et al., 2000) reported data on crude protein content of the terminal cladodes (about one-year old) for four *Opuntia* clones.

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Variations in some relevant nutritional parameters of seven *Opuntia* forage clones as a function of cladode age were assessed in the present study.

MATERIAL AND METHODS

The nutrient content was determined for seven *Opuntia* clones chosen to represent diverse origins and growth forms. They were: three accessions of *Opuntia ficus-indica* L. f. *inermis* (Web.) Le Houér.*:* 'Cuenca' (C), proceeding from plants obtained from Mexico; 'San Juan' (SJ), collected in Barreal, San Juan, Argentina; and 'San Rafael' (SR), collected in San Rafael, Mendoza; one accession of *O. robusta* Wend. (R), obtained from Judith Ochoa's collection in Santiago del Estero, Argentina; two accessions of *O. paraguayensis* K. Sch., collected in La Consulta (LC) and La Remonta (LR), Mendoza; and one accession of *O. spinulifera* Salm-Dyck f. *nacuniana* Le Houér., f. *nov.* (S), introduced from Mexico.

The collection was established on El Divisadero Cattle and Range Experiment Station, located (33º 45' S, 67º 41' W, elev. 520 m) in the north-central Mendoza plain. Rainfall and temperatures in the study site have been reported by Guevara et al. (2000). Soils are Torripsamments with greater silt content in interdunal depressions. Some major soil characteristics include: pH, 6.4-7.6; organic matter, 0.09% to 0.22%; total N, 360 ppm to 420 ppm; extractable P, 9 ppm to 20 ppm; extractable K, 990 ppm to 1420 ppm; and EC of soil saturation extract, 0.17 dS m^{-1} to 0.38 dS m^{-1} (Masotta and Berra, 1994).

Clones were planted in a sand dune (10% to 15% slope) with north exposure using single cladodes on 1 October 1996. The field was laid out in a randomized complete-block design with three replicates. Each replicate consisted of a row of 25 cladodes spaced 1 m apart with 3 m between rows.

The nutritional parameters were measured for three age classes. Age class 1 corresponded to one growing season cladodes (about 1-year old) and was represented by the terminal cladode; age classes 2 and 3 corresponded to two- and three- growing-season cladodes, i.e., about 2 and 3 years old, respectively.

Core samples (2.1-cm diameter) were taken from the cladodes using a sharpened bronze pipe that was held against a wooden backboard, essentially as described by Huffman and Jacoby (1985). Four to five cores per cladode, depending on the cladode size, were taken following a gradient throughout the maximum length of the pads on October 16-19, 2001. One sample for each age class from each of the three replicates was collected. Each sample was a composite from a variable number of randomly selected individuals (8-15) of each clone. Core samples were oven-dried at 70ºC until no further change in weight was observed. Crude protein (CP; N X 6.25) was determined by the Kjeldahl method (Müller, 1961). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined according to Van Soest et al. (1991). *In vitro* organic matter digestibility (IVDMD) was measured using the procedure of Tilley and Terry (1963). Analysis of organic matter (OM) followed AAOC (1980). Samples were analysed in duplicate and the results averaged.

Analysis of variance procedure was used to test for differences in percent OM, IVOMD, CP, NDF, ADF, and dry matter (DM) among clones and age classes. Means were separated with DGC' test ($p<0.05$) (Di Rienzo et al., 2002). On the other hand, age classes were regressed separately on each nutritional parameter using data from the three replicates ($n = 9$). Least squares method was used for obtaining estimates of parameters in linear regression models. As a complement of ANOVA procedure, principal components analyses were performed using OM, IVOMD, NDF, ADF, CP, and DM as variables and clone or age class as classification criterion. The biplot display proposed by Gabriel (1971) was used. Data were analysed using the InfoStat statistical software (InfoStat, 2004).

RESULTS AND DISCUSSION
Analysis of Variance and Linear Regression Models

Organic matter (OM)

The ANOVA for OM content, among clones and age classes, showed that there were significant differences among clones, whereas the differences in percent OM among age classes were not significant. The clone by age interaction was significant (Table 1). This significant interaction precluded mean separation tests for clones when all age classes were combined and for age classes when all clones were pooled.

Organic matter content for all clones and age classes pooled averaged 84.4% (S.D. = 2.1) (Table 2). Our *O. ficus-indica* clones (C, SJ, and SR) were found to have OM content (84.5% to 86.8%) in the range of values measured for the same species by Robles Cruz and Boza López (1993) and López-García et al. (2001): 84.0% and 86.9%, respectively. In contrast, slightly higher OM content (about 90%) was reported by Azócar and Rojo (1991) and Fuentes-Rodríguez (1997) and lower OM concentrations were measured by other authors: 76.9% (Silva, 1987), 80.0% (Nefzaoui et al., 2000), 74.6% (Ben Salem et al., 2002a), and 76.2% (Ben Salem et al., 2004). On the other hand, OM content of our *O. robusta* clone (R) was very similar to those found by Fuentes-Rodríguez (1997) and López-García et al. (2001). Both studies reported 81.4% OM content.

Comparisons of means among clones for each age class are shown in Table 2. Clones SR and C were found to have higher OM content than all of the other clones for the three age classes.

There was a significant linear relationship ($p < 0.05$) between percent OM and age class only for clones LR and S but the relationship was opposite in these clones (Table 2).

***In vitro* Organic Matter Digestibility (IVOMD)**

The ANOVA for IVOMD, among clones and age classes, indicated that there were significant differences among clones and among age classes. The clone by age interaction was not significant for IVOMD (Table 1). When all age classes were combined (Table 3), clone SR exhibited lower IVOMD than those for all of the other clones. When all clones were pooled, there were significant differences in IVOMD among all age classes.

In vitro OMD averaged 78.9% (S.D. = 5.7) for all clones and age classes combined. For all age classes pooled, our *O. ficus-indica* clones had IVOMD ranges from 69.5% (clone SR) to 81.8% (clone SJ) (Table 3), values comparable with those reported for the same species in other studies: 70.9% (Flachowsky and Yami, 1985) and 82.3% (Azócar, 2001). In contrast, percent IVOMD higher than those found in our study was measured for *O. ficus-indica*: 85.7% (Silva, 1987), 89.7% (Robles Cruz and Boza López, 1993), 85.7% (Boza et al., 1995), and 89.4% (Arias et al., 2003).

Clones LR and SJ were found to have higher IVOMD than all of the other clones for all age classes (Table 3).

There was a significant negative linear relationship ($p < 0.05$) between percent IVOMD and age class for each clone, except for clone S. (Table 3). The annual decrease rate in percent IVOMD ranged from -4.88 (clone SJ) to -1.77 (clone LC).

Crude Protein (CP)

The ANOVA for CP content, among clones and age classes, indicated that there were significant differences among clones and among age classes. The clone by age interaction was also significant (Table 1). There was a significant block differences in the ANOVA for CP. It probably means that cladode N is very sensitive to changes in soil N and that reports of cladode N must take this very clearly into consideration.

Crude protein content for all clones and age classes combined averaged 4.0% (S.D. = 0.9) (Table 4). Our *O. ficus-indica* clones were found to have protein concentrations (4.1% to 5.0%) consistent with those reported for the same species by other authors: 4.4% to 5.5% (Flachowsky and Yami, 1985), 4.3% to 4.9% (Boza et al., 1995), 3.8% to 4.8% (Nefzaoui and Ben Salem, 2001), 5.0% (Ben Salem et al., 2002a), and 4.6% (Ben Salem et al., 2004). Considerably higher protein content (8.5%) was measured by Gregory and Felker (1992) for the Mexican cultivar *O. ficus-indica* 1279 when four age classes (from less than 6-months old to about 30- to 36-months old) were combined. The trial of these authors was from plants that were fertilized (158 g of triple superphosphate and about 2 kg of dry cow manure per plant). On the other hand, the protein content of our *O. robusta* clone (R) (4.3%) was in agreement with that found (4.4%) by Fuentes-Rodríguez (1997) for Mexican cultivars. In contrast, the *O. robusta* cv. Monterrey from South Africa was found to have 8.4% protein when all age classes were combined (Gregory and Felker, 1992).

The percent CP of the studied clones was below the requirements of maintenance of both 40-kg goats (7.7%; National Research Council, 1981) and dry pregnant mature cows (5.9%; National Research Council, 1994). The low N content can be increased by fertilization. Gonzalez (1989) found that the mean CP of N-fertilized prickly-pear cactus was significantly higher than that of prickly pear not fertilized with N during the three-year period when fertilizer was applied. Thus, CP content of not fertilized cactus was about 5.5% and CP levels were increased to 8.6%, 9.7%, and 9.9% by the application of 67, 135, and 224 kg N ha^{-1}, respectively. On the other hand, the application of N fertilizer maintained percent CP higher even four years after fertilizer was applied. Cactus from plots fertilized with 135 and 224 kg N ha^{-1} had CP levels adequate for lactating cows (9.2%; National Research Council, 1994) and all N treatments increased CP contents sufficiently to meet dry cow requirements. The author pointed out that CP levels were readily increased by the application of N fertilizer and that it should be applied at least every two years. Another alternative for increasing the CP content of the livestock diet is the combination of spineless cacti with N-rich shrubs such as *Atriplex nummularia* L. The good complementary between cacti and Old man saltbush, in terms of feeding value, has been addressed by Nefzaoui (2000), Ben Salem et al. (2002a,b; 2004), among other authors.

Crude protein of the clones was considerably lower than the mean of the seven most common warm-season perennial grasses of the central Mendoza plain (6.8%, S.D. = 0.8; Guevara et al., 1991) and also lower than the mean CP content of the five most important browse species of the same area (11.5%, S.D. = 1.8; Van den Bosch et al., 1997). For two of the perennial grasses studied in the central Mendoza plain: *Trichoris crinita* (Lag.) Parodi and *Digitaria californica* (Benth.) Hern., CP content was also higher than those for *Opuntia* clones in the Calden forest of Argentina (mean annual rainfall: 400 mm to 600 mm); it ranged from 7% to 9% (Cerqueira et al., 2004). Crude protein content of weeping lovegrass [*Eragrostis curvula* (Shrad.) Nees], an introduced warm-season perennial grass in arid and semiarid zones of Argentina, was 9.2% in spring-summer and 3.9% in fall-winter (Gargano and Adúriz, 2000). The higher CP content of the native vegetation than that for *Opuntia* is not of concern because cacti have been planted on a large scale in various arid lands of the world over several million hectares not for the purpose of replacing forage from rangelands but as a standing buffer feed for drought periods, i.e., as a part of drought-evading strategy or 'drought insurance'.

Comparisons of means among clones for each age class are shown in Table 4. Clone C exhibited the greatest CP content for age class 1, while clone SJ was found to have a significant higher protein than all of the other clones for age classes 2 and 3.

There was a significant negative linear relationship between protein content and age class ($p < 0.05$) for each clone (Table 4). The greatest annual decrease rate in protein content with age class was exhibited by clone C (-1.67), while clones LC and SJ were found to have the lowest decrease rates (-0.36 and -0.31, respectively). Thus, for clone C, the amount of protein for age class 1 was more than twice (2.4) that of age class 3. In contrast, for clone SJ the amount of protein for age class 1 was only 13% higher than that for age class 3. Coincident with our results, the studies of Monjauze and Le Houérou (1965), Gregory and Felker (1992) and Nefzaoui and Ben Salem (2001) found that protein content decreases with cladode age although the decreased rate with age varied according to the particular clone. Thus, for Tunisian clones of *O. ficus-indica*, the amount of protein for age class 1 was more than twice that of the age class 3 (Monjauze and Le Houérou, 1965). In contrast, for the *O. ficus-indica* clone studied by Nefzaoui and Ben Salem (2001), the amount of protein for age class 1 was only 40% higher than that for age class 3.

Gugliuzza et al. (2002) found that N cladode content changed with the sample location in the cladode. During bloom, N content decreased significantly moving from the basal to the apical area of the cladode. At fruit harvest, the apical part of the cladode had still the lowest N content, while no differences appeared in the other parts of the cladode. Because, in our study, samples were taken from the basal to the apical part of the cladodes, the cladode sampling location could not have affected the N content of the various clones.

Neutral Detergent Fibre (NDF)

The insoluble portion of the forage (NDF) contains the cellulose, hemicellulose, lignin, and silica and is commonly referred to as the cell-wall fraction. Neutral detergent fibre has been shown to be negatively correlated with dry-matter intake. Thus, as the NDF in forages increases, animals will be able to consume less forage (Holland and Kesar, 1990).

There were significant differences among clones and among age classes for NDF content. The clone-by-age class interaction was also significant (Table 1).

The overall cell wall content averaged 23.8% (S.D. = 1.8) (Table 5). Neutral detergent fibre in our *O. ficus-indica* clones ranged from 23.6% (clone SJ) to 27.1% (clone C). These values were similar to those found for *O. ficus-indica* clones from Spain: 21.8% (Silva, 1987), 26.4% (Robles Cruz and Boza López, 1993), and 21.4% (Boza et al., 1995) and that for clones cultivated in Central Tunisia: 25.5% (Ben Salem et al., 2002a). In contrast, Ben Salem et al. (2004) reported NDF content of 33.8% for current-year pads of *O. ficus indica*.

The clones had considerably lower NDF than those found by Silva Colomer et al. (1989) for the deferred forage of *Trichloris crinita*: 72.2% and 69.2% in stems and leaves, respectively. On the other hand, the studied clones were found to have lower NDF than those of the browse species (59.0%, S.D. = 2.6; Van den Bosch et al., 1997).

Clone C was found to have higher NDF concentration than all of the other clones for all age classes (Table 5).

A significant positive linear relationship ($p < 0.05$) between NDF content and age class was found for each clone, except for clone LC in which the relationship was close to being significant (Table 5). The clone S was found to have the highest NDF increase rate with age (2.28), while clone LC showed the lowest rate (0.39). There were no available studies on the effect of cladode age on the NDF content. However, several studies showed that crude fibre (CF) content, a poor indicator of feed-fibre status, increases with pad age (Monjauze and Le Houérou, 1965; Boza et al., 1995; Nefzaoui and Ben Salem, 2001). The crude-fibre system was criticized for often underestimating good-quality forages and over estimating poor-quality forages (Holland and Kesar, 1990).

Acid Detergent Fibre (ADF)
Acid detergent fibre includes the cellulose, lignin, and silica. Acid detergent fibre may be the most important determination of the forage analysis because it has been shown to be negatively correlated with how digestible a forage may be when fed. As the ADF increases, the forage becomes less digestible (Holland and Kesar, 1990).

The ANOVA for ADF concentration, among clones and age classes, showed that there were significant differences among clones and among age classes. On the other hand, the clone by age class interaction was significant (Table 1) for percent ADF.

Acid detergent fibre concentration for all clones and age classes pooled averaged 14.7% (S.D. = 1.8) (Table 6). The *O. ficus-indica* clones were found to have ADF contents (14.3% to 16.0%) in the range of values reported for O. *ficus- indica* clones from Spain (Boza et al., 1995) and Tunisia (Ben Salem et al., 2004): 14.7% and 16.8%, respectively. In contrast, our *O. ficus-indica* clones exhibited percent ADF higher than those measured in other studies: 12.7% (Silva, 1987), and 11.7% (Robles Cruz and Boza López, 1993).

The ADF content of the *Opuntia* clones was considerable lower than those found by Silva Colomer et al. (1989) for *Trichloris crinita*: 41.6% and 33.0% for stems and leaves, respectively. Browse species of the central Mendoza plain were found to have considerably higher mean ADF content (42.1%, S.D. = 2.9; Van den Bosch et al., 1997) than those of the *Opuntia* clones.

The clones SJ and S had significantly higher ADF content than all of the other clones at age class 1; the first clone exhibited higher ADF than all of the other clones at age class 2 and the second one at age class 3 (Table 6).

The ADF content showed a significant positive linear relationship ($p < 0.05$) with age class for each clone (Table 6). The clone LC was found to have the greatest ADF increase rate with cladode age (2.10), while clone C showed the lowest rate (0.66).

The Van Soest analyses indicated that cactus is a readily digestible forage (Shoop et al., 1977). According to Nefzaoui and Ben Salem (2001), a rapid rate of digestion means a faster passage of this forage through the digestive tract. This also means that cactus dry matter remains in the gastrointestinal tract only for a short time, leaving more volume available for further intake. In other words, the gut fill of cactus is low, explaining why an increase of cactus volume in the diet does not reduce the intake of other components of the ration. This is very important for arid zones where livestock is fed mainly with coarse feeds that have low intakes, which lead to poor animal performance.

Dry Matter (DM)
There were significant differences in DM content among clones and among age classes. There was a significant clone-by-age interaction for DM content (Table 1).

Dry matter content for all clones and age classes combined was 9.1% (S.D. = 1.8) (Table 7). It is known that DM content depends on the pad harvesting season. Thus, DM content of pads aged 1 to 3 years ranged from 10% to 15% in the rainy season to 15% to 25% in the dry season (Le Houérou, 1996, 2002). This fact complicates direct comparisons of our result with those from other studies.

Clones LC and LR showed significantly greater DM content than those for all of the other clones at age classes 1 and 2 (Table 7). The latter two clones and clone C exhibited the highest DM content for age class 3. The ranking of clones according to DM content for age class 1 was similar to that found in our previous study (Guevara et al., 2000).

A significant linear positive relationship ($p < 0.05$) between DM content and age class was found for each clone, except for clone R in which the relationship was close to being significant (Table 7). Clone C had the greatest DM increase rate (2.39), while clone R showed the lowest rate (0.49). The studies of Monjauze and Le Houérou (1965), Boza et al. (1995) and Nefzaoui and Ben Salem (2001) also showed that *O. ficus-indica* DM content increases with cladode age.

Principal Component Analysis

The analysis of the first two principal components using clones as classification criterion is shown in Figure 1(a). The variables DM, ADF, and CP showed the highest factor loading: 0.62, -0.56, and -0.51, respectively, for explaining 36% of the variability among clones through the first component (PC1). Clones SJ, S, and R could be characterised by high CP and ADF contents, while clones LC and LR were associated with high DM content.

Differences among clones in OM (factor loading = 0.70), NDF (0.57), and IVOMD (-0.40) can be explained through the second principal component (PC2). This component accounted for 32% of the total variability. The second component separated clones SR and C from clones R and LR. The first two clones showed high OM and NDF contents while clones R and LR were associated with high IVOMD values.

For a deeper exploration of the previous relationships, the third component (PC3) was analysed. The variables IVOMD and NDF showed the highest factor loading: 0.80 and 0.53, respectively, for explaining 17% of the variability among clones through the third component The biplot display [Figure 1(b)] corroborated that clone C was associated with high NDF; it also had high IVOMD value but the display indicated that clone SR was mainly characterised by a low IVOMD.

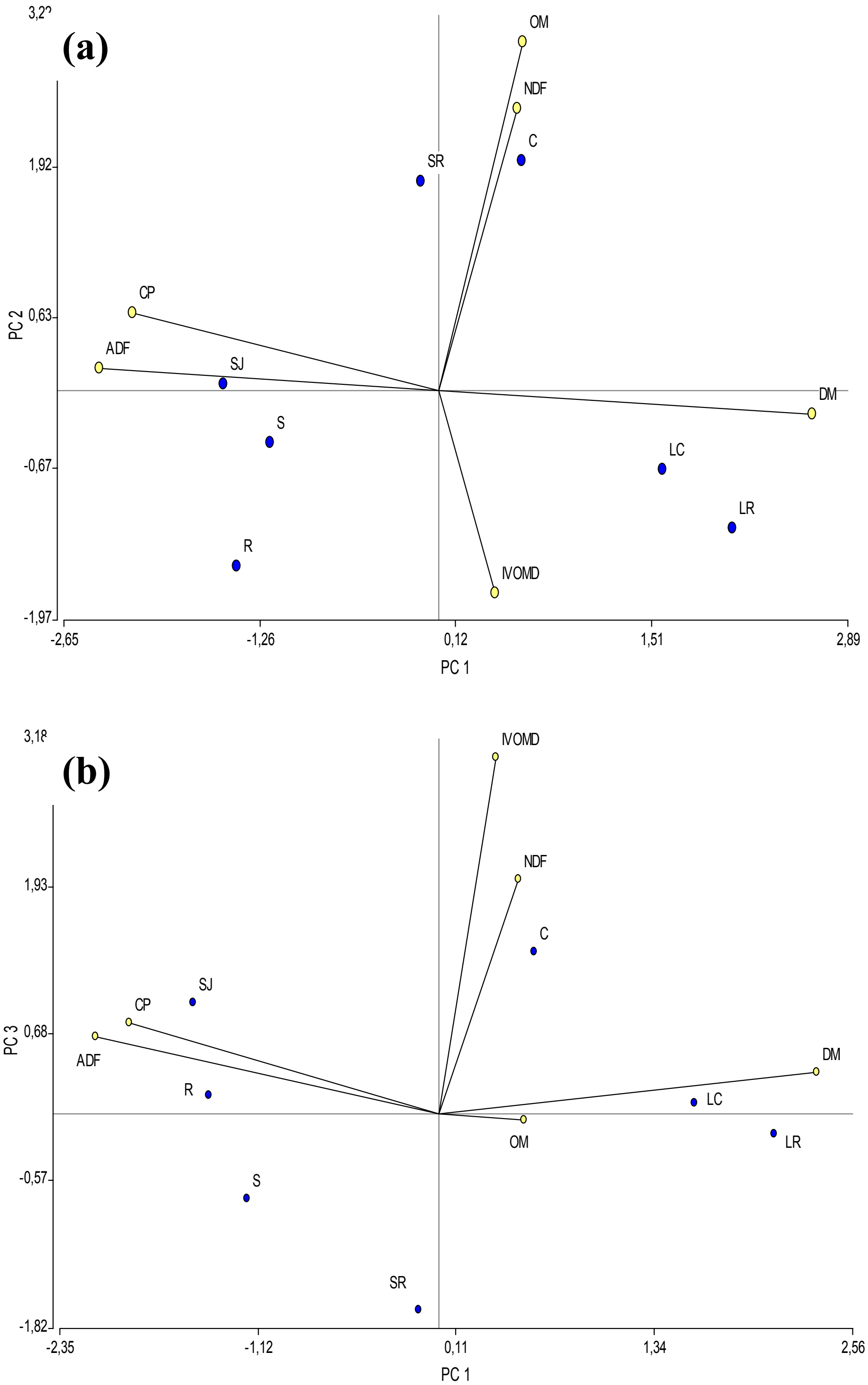

Figure 1. Biplot Displays Derived from Principal Components Analysis
Using Clone as Classification Criterion

Principal components analysis for age classes (Figure 2) suggested a high negative correlation between both CP and IVOMD and each of the variables located on the left of PC1 (DM, NDF, and ADF). The first component accounted for 88% of total variability among age classes. All the variables, except OM, showed similar factor loading: 0.43 (CP and IVOMD), -0.42 (DM), and -0.43 (NDF and ADF). Age class 1 cladodes showed high CP and IVOMD values, while age class 3 pads could be characterised by high DM, ADF, and NDF contents. Age class 2 cladodes showed intermediate values for all of these variables. The variable OM showed the highest factor loading (0.93) for explaining 12% of the variability among age classes through the second component (PC2). Age class 2 cladodes showed slightly lower OM content than those of the other two age classes. It was not necessary to analyse the third component because the first two components accounted for 100% of total variability among age classes.

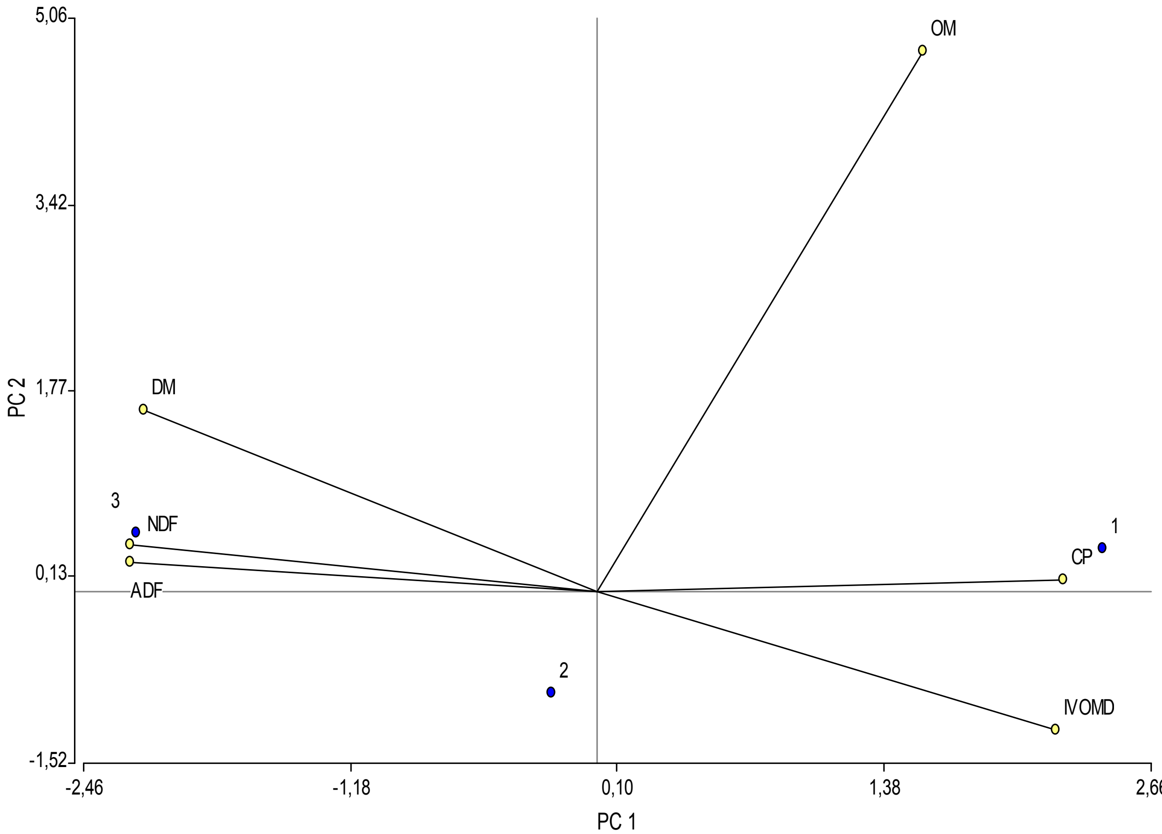

Figure 2. Biplot Display Derived from Principal Components Analysis Using Age Class as Classification Criterion

In conclusion, most of the studied *Opuntia* clones showed a high percent IVOMD, even in the older age class cladodes. The percent protein of the clones was low. Each clone was associated with high content of one or two particular nutritional parameters. The clone SJ of *O. ficus-indica* that had the greatest average percent CP, the lowest CP decrease rate with age class, and exhibited a high IVOMD value appears to be the most outstanding clone. Our previous study (Guevara et al., 2000) showed that this clone also exhibited a high biomass production and a considerably high frost resistance (3-year-old-plants established in a dune suffered about 16% damage to above ground biomass after freezes of -16ºC and -17ºC).

Table 1. F-Ratios and Significance Levels from ANOVAS for Nutrient Contents of *Opuntia* Clones

Source	d.f.	Organic matter		In vitro organic matter digestibility		Crude protein		Neutral detergent fibre		Acid detergent fibre		Dry matter	
		F	p	F	p	F	p	F	p	F	p	F	p
Model	22	10.38	<0.0001	5.89	<0.0001	45.99	<0.0001	375.13	<0.0001	469.72	<0.0001	62.71	<0.0001
Clone	6	31.32	<0.0001	15.39	<0.0001	62.05	<0.0001	903.53	<0.0001	873.81	<0.0001	158.29	<0.0001
Age	2	0.34	0.714	15.19	<0.0001	239.54	<0.0001	945.05	<0.0001	2,145.0	<0.0001	141.06	<0.0001
Block	2	1.47	0.242	0.25	0.780	17.41	<0.0001	0.37	0.693	0.28	0.757	3.11	0.055
Clone x Age	12	3.13	0.003	0.53	0.885	10.46	<0.0001	78.40	<0.0001	66.70	<0.0001	11.79	<0.0001
Error	40												
Total	62												

Table 2. Mean Organic-Matter Content (%) of *Opuntia* Clones for All Age Classes Combined and Each Age Class, and Parameters And Data Statistics of Linear-Regression Equations of OM on Cladode Age Classes for Each *Opuntia* Clone

Clone	Age class				Parameter		Data statistics	
	All combined	1	2	3	Intercept	Slope	Adjusted R2	p-value
SR	86.8	86.7 a	86.0 a	87.6 a	85.89	0.43	0.10	0.2078
C	86.8	86.5 a	86.8 a	87.3 a	86.04	0.40	0.22	0.1140
S	83.5	84.5 b	84.5 b	81.5 c	86.43	-1.47	0.46	0.0267
SJ	84.5	84.4 b	84.5 b	84.7 b	84.12	0.23	0.01	0.4487
LC	83.7	83.5 b	84.0 b	83.6 b	83.59	0.05	0.01	0.8331
R	81.6	83.1 b	80.1 c	81.7 c	83.73	-0.72	0.20	0.1452
LR	83.7	83.0 b	84.0 b	84.1 b	82.59	0.57	0.60	0.0085
Mean	84.4	84.5	84.3	84.4				

Means in a column followed by same letter are not significantly different at $p < 0.05$ using DGC's test.
Regression equation: y=a+b*x, where y=%IVOMD, a=intercept, b=slope, and x=age class in years.

Table 3. Mean *in vitro* Organic Mater Digestibility (%) of *Opuntia* Clones for All Age Classes Combined and Each Age Class, and Parameters and Data Statistics of Linear-Regression Equations of IVOMD on Cladode Age Classes for Each *Opuntia* Clone

Clone	Age class				Parameter		Data statistics	
	All combined	1	2	3	Intercept	Slope	Adjusted R2	p-value
LR	82.1 a	83.2 a	82.9 a	80.0 a	88.26	-2.72	0.53	0.0240
SJ	81.8 a	85.8 a	83.5 a	76.1 b	91.53	-4.88	0.65	0.0332
R	81.4 a	83.8 a	82.0 a	78.3 b	86.82	-2.73	0.43	0.0333
C	80.5 a	83.0 a	80.4 a	78.2 b	85.37	-2.42	0.59	0.0096
LC	80.2 a	81.4 a	81.4 a	77.9 b	83.78	-1.77	0.51	0.0185
S	76.7 b	80.0 a	76.5 b	80.4 a	83.02	-3.15	0.06	0.2559
SR	69.5 c	73.9 b	68.3 c	66.2 c	77.20	-3.85	0.53	0.0155
Mean	78.9	81.6	79.3	76.7				

Means in a column followed by same letter are not significantly different at $p < 0.05$ using DGC's test.
Regression equation: y=a+b*x, where y=%IVOMD, a=intercept, b=slope, and x=age class in years.

Table 4. Mean Crude-Protein Content (%DM) of *Opuntia* Clones for All Age Classes Combined and Each Age Class and Parameters and Data Statistics of Linear-Regression Equations of CP on Cladode Age Classes for Each *Opuntia* Clone

Clone	Age class				Parameter		Data statistics	
	All combined	1	2	3	Intercept	Slope	Adjusted R2	p-value
C	4.1	6.0 a	3.7 c	2.6 c	7.40	-1.67	0.92	0.0001
R	4.3	5.3 b	4.1 b	3.4 b	6.20	-0.96	0.96	<0.0001
SJ	5.0	5.2 b	5.1 a	4.6 a	5.61	-0.31	0.66	0.0047
SR	4.2	4.8 c	4.3 b	3.5 b	5.42	-0.61	0.72	0.0024
S	3.7	4.7 c	3.5 c	3.1 c	5.34	-0.81	0.90	0.0001
LR	3.6	4.4 c	3.5 c	2.8 c	5.18	-0.79	0.84	0.0003
LC	3.2	3.5 d	3.1 d	2.8 c	3.87	-0.36	0.52	0.0172
Mean	4.0	4. 8	3.9	3.3				

Means in a column followed by same letter are not significantly different at $p < 0.05$ using DGC's test.
Regression equation: y=a+b*x, where y=%CP, a=intercept, b=slope, and x=age class in years.

Table 5. Mean Neutral-Detergent Fibre Content (%DM) of *Opuntia* Clones for All Age Classes Combined and Each Age Class and Parameters and Data Statistics of Linear-Regression Equations of NDF on Cladode Age Classes for Each *Opuntia* Clone

Clone	Age class				Parameter		Data statistics	
	All combined	1	2	3	Intercept	Slope	Adjusted R2	p-value
C	27.1	26.3 a	26.9 a	28.1 a	25.28	0.89	0.88	0.0033
LC	23.8	23.3 b	23.9 b	24.1 c	22.99	0.39	0.49	0.0736
SJ	23.6	23.0 c	23.8 b	24.0 c	22.55	0.52	0.80	0.0101
SR	23.8	22.9 c	23.9 b	24.7 b	21.97	0.92	0.95	0.0007
LR	22.7	21.9 d	22.2 c	24.0 c	20.62	1.04	0.78	0.0125
R	22.7	21.9 d	22.1 c	24.1 c	20.46	1.11	0.77	0.0141
S	22.9	20.1 e	23.9 b	24.6 b	18.31	2.28	0.83	0.0074
Mean	23.8	22.8	23.8	24.8				

Means in a column followed by same letter are not significantly different at $p < 0.05$ using DGC's test.
Regression equation: y=a+b*x, where y=%NDF, a=intercept, b=slope, and x=age class in years.

Table 6. Mean Acid-Detergent Fibre Content (%DM) of *Opuntia* Clones for All Age Classes Combined and Each Age Class and Parameters and Data Statistics of Linear-Regression Equations of ADF on Cladode Age Classes for Each *Opuntia* Clone

Clone	Age class				Parameter		Data statistics	
	All combined	1	2	3	Intercept	Slope	Adjusted R2	p-value
SJ	16.0	15.0 a	16.0 a	17.1 b	13.84	1.09	0.97	0.0002
S	16.0	14.9 a	15.2 b	17.9 a	13.03	1.48	0.77	0.0136
C	14.6	14.0 b	14.4 d	15.3 e	13.27	0.66	0.93	0.0012
R	15.3	13.5 c	16.0 a	16.4 c	12.46	1.42	0.79	0.0118
LC	14.8	12.8 d	14.4 d	17.0 b	10.55	2.10	0.97	0.0002
SR	14.3	12.5 e	14.8 c	15.5 d	11.24	1.51	0.87	0.0044
LR	12.0	10.3 f	12.5 e	13.3 f	9.05	1.49	0.88	0.0035
Mean	14.7	13.3	14.8	16.1				

Means in a column followed by same letter are not significantly different at $p < 0.05$ using DGC's test.
Regression equation: y=a+b*x, where y=%ADF, a=intercept, b=slope, and x=age class in years.

Table 7. Mean Dry-Matter Content (%) Of *Opuntia* Clones for All Age Classes Combined and Each Age Class and Parameters and Data Statistics of Linear-Regression Equations of DM on Cladode Age Classes for Each *Opuntia* Clone

Clone	Age class				Parameter		Data statistics	
	All combined	1	2	3	Intercept	Slope	Adjusted R2	p-value
LC	11.5	10.6 a	11.9 a	12.0 a	10.15	0.67	0.75	0.0016
LR	11.0	10.1 b	11.2 b	11.7 a	9.35	0.81	0.80	0.0007
SR	8.9	8.6 c	8.1 d	10.0 b	7.47	0.71	0.39	0.0427
SJ	8.7	7.7 d	8.6 c	10.0 b	6.42	1.15	0.86	0.0002
R	7.3	6.8 e	7.2 e	7.8 d	6.29	0.49	0.33	0.0793
C	9.1	6.9 e	8.9 c	11.7 a	4.35	2.39	0.97	<0.0001
S	7.4	6.8 e	6.9 e	8.4 c	5.74	0.82	0.64	0.0058
Mean	9.1	8.2	9.0	10.2				

Means in a column followed by same letter are not significantly different at $p < 0.05$ using DGC's test.
Regression equation: y=a+b*x, where y=%DM, a=intercept, b=slope, and x=age class in years.

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