

Nutritional Quality of *Opuntia* ssp. at Different Phenological Stages: Implications for Forage Purposes

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Abstract

The study aimed to evaluate the chemical composition of forage cactus cladodes of the genus *Opuntia* spp. at different stages of phenological development. The experiment was conducted in a completely randomized design, with a 4×3 factorial scheme, with four species of cactus (*Opuntia stricta* Haw, *Opuntia undulata* Griffiths, *Opuntia atropes* Rose and *Opuntia larreri* FAC Weber) and three stages of cladode development (young, intermediate, and mature). The chemical composition of *Opuntia* cladodes varied according to species and phenological stage. Dry matter (DM), neutral detergent fiber (NDF) and acid detergent fiber (ADF) concentrations increased, and crude protein (CP) decreased with cladode maturation. The species *O. atropes* had the lowest levels of organic matter (OM), CP, total carbohydrates, and non-fibrous carbohydrates, as well as the highest values of mineral matter, NDF and ADF. Calcium and phosphorus contents increased with cladodium maturation. The concentrations of boron, copper and manganese were higher in the young and intermediate stages. The cladodes of *O. stricta*, *O. undulata* and *O. larreri* showed better nutritional quality for feeding domestic ruminants. Young and intermediate cladodes are more proteinaceous and less fibrous. These results can be useful in selecting the forage cactus species and in optimizing the use of cladodes for feeding different categories of ruminants in semiarid regions.

Keywords: animal feed, chemical composition, Erect Prickly Pear, mineral composition, V-19

1. Introduction

The lack of forage for animal feeding during the dry season is one of the main difficulties faced by domestic ruminant breeders in the Brazilian semiarid region. However, this barrier has been circumvented by the use of succulent stems (cladodes) of Cactaceae of the genera *Opuntia* and *Nopalea*, commonly called “forage cactus”, as a source of water and energy to supplement herds in the dry season (Oliveira et al., 2021).

The genus *Opuntia* has about 300 species, being one of the most used in Northeast Brazil for foraging purposes (Lucena et al., 2013). Its CAM photosynthetic metabolism (Crassulaceous Acid Metabolism) favors the efficient use of water (Ferraz et al., 2017), enabling cultivation in arid and semi-arid regions (Edvan et al., 2020).

Cladodes have a high concentration of non-fibrous carbohydrates (585.5 g.kg⁻¹ of DM) and total digestible nutrients (800.0 g.kg⁻¹ DM) (Silva & Sampaio, 2015). Although the contents of CP (41.0 - 63.0 g.kg⁻¹ DM), NDF (243.0 - 302.0 g.kg⁻¹ DM) and ADF (105.0 - 178.0 g.kg⁻¹ DM) show some limitations to ruminants feeding, an adequate balance of the diet with the addition of fiber and protein can overcome this disadvantage (Moraes et al., 2019; Silva et al., 2019; Siqueira et al., 2018).

Cladodes are also rich in minerals, mostly calcium (21.4 - 62.7 g.kg⁻¹ DM), potassium (4.6 - 42.0 g.kg⁻¹ DM), manganese (102.5 - 704.5 mg.kg⁻¹ DM), iron (59.3 to 208.2 mg.kg⁻¹ DM) and zinc (19.1 - 81.1 mg.kg⁻¹ DM) (Alves et al., 2017). However, mineral composition can be varied due to edaphoclimatic conditions, plant age, time of year, management practices, species, and genotypes (Dubeux Jr et al., 2021).

Forage cactus are an important resource for semiarid regions and has been widely studied from a nutritional point of view (Cordova-Torres et al., 2015; Vazquez-Mendoza et al., 2017; Pessoa et al., 2020). However, some aspects need a better understanding, such as nutritional quality, especially mineral composition of cladodes as a function of species/genotype and plant development stage. Thus, this study aimed to evaluate the chemical-bromatological and mineral composition of *Opuntia* species at different stages of phenological development, analyzing its implications for forage purposes.

2. Method

2.1 Obtaining Samples

Cladode samples of four species of *Opuntia* (Table 1) were collected in May 2018 at the experimental station of the *Instituto Agronômico de Pernambuco* (IPA) in *Arcoverde* (-8.43° S, -37.06° W; altitude 683.9m), state of *Pernambuco*, Brazil. Planting was established in December 2014 (spacing 1.0 x 0.5 m) and handled in an upland system with classic cultural treatments, including periodic weeding and organic fertilization (20 t.ha⁻¹ of cattle manure each two years). The local climate based on the Köppen-Geiger classification is BSh (hot and dry) with averages of annual temperature of 24.9 ± 1.97 °C, reactive humidity of 78.19 ± 8.24% and accumulated precipitation of 798.1 mm per year (INMET, 2019).

Table 1. Genotypes of species of the genus *Opuntia* spp. used in the study

Species	Access	Genotype
<i>Opuntia stricta</i> Haw	IPA-200016	Erect Prickly Pear (EPP)
<i>Opuntia undulata</i> Griffiths	IPA-200174	African Prickly Pear (APP)
<i>Opuntia atropes</i> Rose	IPA-200008	F-08
<i>Opuntia larreri</i> F.A.C. Weber	IPA-200149	V-19

The soil physicochemical characteristics in the cultivation area (0 to 20 cm deep) were analyzed according to the methodology proposed by Teixeira et al. (2017) (Table 2). The local climate data during the trial (2014 to 2018) were provided by the automatic meteorological station of the National Institute of Meteorology (INMET) (Figure 1).

 Table 2. Physical-chemical attributes of the soil of the cultivated area with species genotypes of the genus *Opuntia* spp.

pH	P	K ⁺	Na ⁺	H ⁺ +Al ³⁺	Al ³⁺	Ca ²⁺	Mg ²⁺	SB	CEC	OM
(1:2.5)	---mg/dm ³ ---	-----cmol _c /dm ³ -----				----- g/kg				
6.42	24.37	119.96	0.15	4.07	0.00	6.38	0.86	7.70	11.77	20.48
Sand			Silt		Clay		Textural classification			
-----g/kg-----										
717			180		103		Sandy-loam			

SB, sum of bases; CEC, cation exchange capacity; OM, organic matter.

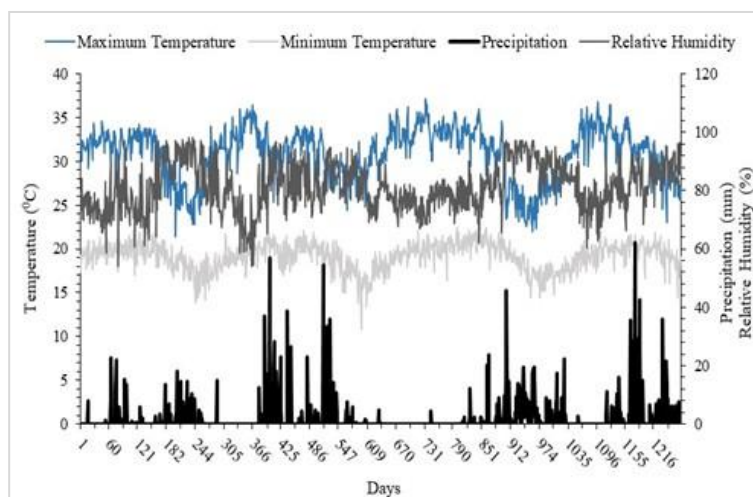


Figure 1. Precipitation (mm), temperature (°C) and relative humidity (%) in the cultivated area with species genotypes of the genus *Opuntia* spp.

Cladodes were collected from five plants of each genotype (n=5) at three phenological stages: young (expanding cladodes, with fragile structure and light green color, located at the distal or lateral ends of the plant), intermediate (cladodes with rigid structure and dark green color, located in the middle part of the plant) and mature (fully expanded cladodes, with rigid structure and whitish-green color, located in primary order, just above the base). After collection, the samples were cleaned, chopped, and dehydrated in a forced air oven at a temperature of 55°C for 72 hours. Then, crushed in a knife mill with a 1 mm sieve, identified, packed in plastic containers with airtight closure and stored at 25 °C.

2.2 Chemical Analysis and Nutritional

Dry matter (934.01), organic matter (942.05), crude protein (954.01), ether extract (EE) (920.39) and mineral matter (MM) (942.05) were performed according to the Association of Official Analytical Chemists (AOAC, 1990). Neutral and acid were determined using the methodology of Van Soest et al. (1991), adapted by Senger et al. (2008). Total carbohydrates (TC) were estimated using the equation described by Sniffen et al. (1992): $TC = 1000 - (CP + EE + MM)$, and non-fibrous carbohydrates (NFC) according to Van Soest et al. (1991): $NFC = 1000 - (CP + EE + MM + NDF)$.

Samples were mineralized in sulfuric acid in order to evaluate nitrogen (N); in a mixture of nitric-perchloric acid, in order to determine phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), copper (Cu), manganese (Mn), iron (Fe) and zinc (Zn); and incinerated to determine boron (B). N concentration was determined by the Kjeldahl method; P by molybdenum blue colorimetry; K by emission flame photometry; Ca, Mg, Cu, Mn, Fe and Zn through atomic absorption spectrophotometry; and S by barium sulfate turbidimetry, according to Malavolta et al. (1997). The concentration of B was determined by the azomethine-H colorimetry method (Silva, 2009).

2.3 Statistical Analysis

The experimental design used was completely randomized in a 4 x 3 factorial scheme

(genotypes of four species and three phenological stages of the cladode) and five replications. The data met the assumptions for parametric analysis and were subjected to analysis of variance at the 5% probability level ($P < 0.05$). Treatment means were compared by the Tukey test using the Statistical Analysis System (SAS®) software (Cody, 2015). The statistical model adopted was:

$$Y_{ijk} = \mu + G_i + P_j + GP_{ij} + E_{ijk}$$

Where: Y_{ijk} is the observed value for the analyzed variable, μ overall mean, G_i is the effect of the genotype of species i , P_j is the effect of the phenological stage j , GP_{ij} is the effect of the interaction between $G_i \times P_j$, and E_{ijk} the experimental error associated with Y_{ijk} .

Data were also submitted to multivariate analysis for exploratory purposes. In the cluster analysis, the Gower distance, and the hierarchical method UPGMA (Unweighted Peer Group with Arithmetic Mean) were used. In the Principal Component Analysis (PCA) treatments were plotted in relation to the first two components (PC1 and PC2). These evaluations were performed with the `prcomp` function in the statistical program R (R Core Team, 2018).

3. Results

3.1 Chemical Composition of Cladodes

There was interaction ($P < 0.05$) between the genotypes and phenological stages of the cladode in EE concentration, but for DM, OM, CP, and MM isolated effects of the factors were verified ($P < 0.05$) (Table 3). The EE content in EPP was higher in young cladodes and APP in the intermediate stage (22.68 g.kg^{-1} DM) compared to the young (19.56 g.kg^{-1} DM), but it presents similar content to that of mature cladodes (20.36 g.kg^{-1} DM). Concentrations in intermediate and mature cladodes were higher in APP and V-19 (Table 3).

The DM content differed between species ($P < 0.05$), being higher in EPP (67.93 g.kg^{-1} of natural matter - NM) when compared to V-19 (61.43 g.kg^{-1} NM), and both similar to APP and F-08. Regarding the developmental stages, mature cladodes (68.39 g.kg^{-1} MN) had a higher concentration of DM than young cladodes (62.46 g.kg^{-1} MN).

F-08 cladodes had the lowest OM and CP concentrations ($P < 0.05$) (62.07 g.kg^{-1} DM), and the highest MM concentration (181.83 g.kg^{-1} DM) (Table 3). The CP content was higher ($P < 0.05$) in young cladodes (71.72 g.kg^{-1} DM) compared to mature cladodes (64.88 g.kg^{-1} DM) and similar to intermediates (68.68 g.kg^{-1} DM).

Table 3. Chemical composition of species genotypes of the genus *Opuntia* spp. in different phenological stages

Phenological stages	Genotypes					SEM	P-value		
	EPP	APP	F-08	V-19	Average		G	P	G x P
	Dry Matter (DM, g.kg ⁻¹ Natural Matter)								
Young	66.88	62.24	62.14	58.60	62.46B	1.26			
Intermediate	66.04	65.32	67.15	60.43	64.74AB	1.09	0.009	0.002	>0.050
Mature	70.86	68.16	69.30	65.25	68.39A	1.26			
Average	67.93a	65.24ab	66.20ab	61.43b					
	Organic Matter (OM, g.kg ⁻¹ Dry Matter)								
Young	858.53	855.15	807.25	857.09	844.50	6.28			
Intermediate	856.54	843.15	810.42	840.64	837.69	5.83	<0.001	>0.050	0.140
Mature	840.59	851.22	836.84	853.32	845.49	4.31			
Average	851.89a	849.84a	818.17b	850.35a					
	Crude Protein (CP, g.kg ⁻¹ Dry Matter)								
Young	73.92	73.42	67.28	72.26	71.72A	1.53			
Intermediate	69.10	71.26	62.87	71.50	68.68AB	1.56	<0.001	0.004	>0.050
Mature	64.98	68.56	56.06	69.94	64.88B	1.59			
Average	69.33a	71.08a	62.07b	71.24a					
	Ether Extract (EE, g.kg ⁻¹ Dry Matter)								
Young	20.13aA	19.56aB	18.04aA	20.03aA	19.44	0.48			
Intermediate	15.59bB	22.68aA	16.14bA	20.25aA	18.66	0.73	<0.001	>0.050	<0.001
Mature	16.46cB	20.36aAB	17.43bA	22.40aA	19.16	0.64			
Average	17.39	20.87	17.20	20.89					
	Mineral Matter (MM, g.kg ⁻¹ Dry Matter)								
Young	141.47	144.85	192.75	142.91	155.50	6.28			
Intermediate	143.46	156.85	189.58	159.36	162.31	5.83	<0.001	>0.050	0.140
Mature	159.41	148.78	163.16	146.68	154.51	4.31			
Average	148.11b	150.16b	181.83a	149.65b					

EPP (*Opuntia stricta*); APP (*Opuntia undulata*); F-08 (*Opuntia atropes*); V-19 (*Opuntia larreri*). SEM, standard error of mean; G, genotypes; P, phenological stages; G x P, interaction of genotypes and phenological stages. Means followed by lowercase letters differ in the rows and capital letters in columns (P <0.05).

NDF and ADF contents differed (P <0.05) between cladode species and developmental stages (Table 4). The F-08 presented lower NDF values in the intermediate (277.35 g.kg⁻¹ DM) and young (282.36 g.kg⁻¹ DM) cladodes, while in the mature stage it was higher (357.63 g.kg⁻¹ DM) than in the other species. ADF concentrations in APP (168.44 g.kg⁻¹ DM) and F-08 (213.23 g.kg⁻¹ DM) were higher in mature cladodes.

TC and NFC concentrations were not influenced (P >0.05) by the interaction between the factors and there was no effect (P >0.05) of developmental stages on these variables either (Table 4). Regarding the genotypes, it was found that the concentrations of TC (738.9 g.kg⁻¹DM) and NFC (433.12 g.kg⁻¹ DM) in F-08 were lower (P <0.05) than the others.

Table 4. Fiber compounds of species genotypes of the genus *Opuntia* spp. in different phenological stages

Phenological stages	Genotypes					SEM	P-value		
	EPP	APP	F-08	V-19	Average		G	P	G x P
Neutral Detergent Fiber (NDF, g.kg ⁻¹ Dry Matter)									
Young	284.55aA	267.87aA	282.36aB	254.85aA	272.41	4.79			
Intermediate	285.98aA	264.81aA	277.35aB	255.86aA	271.00	5.76	<0.001	0.001	0.001
Mature	269.89bA	277.26bA	357.63aA	283.67bA	297.11	9.14			
Average	280.14	269.98	305.78	264.80					
Acid Detergent Fiber (ADF, g.kg ⁻¹ Dry Matter)									
Young	148.83aA	147.79aB	162.59aB	150.72aA	152.48	3.38			
Intermediate	153.40aA	148.58aB	159.57aB	144.51aA	151.51	2.75	<0.001	<0.001	0.001
Mature	155.13bA	168.44bA	213.23aA	158.46bA	173.82	5.90			
Average	152.45	154.94	178.46	151.23					
Total Carbohydrates (TC, g.kg ⁻¹ Dry Matter)									
Young	764.47	762.17	721.94	764.80	753.34	6.11			
Intermediate	771.85	749.20	731.42	748.89	750.34	5.74	0.001	0.255	0.126
Mature	759.15	762.30	763.35	760.98	761.44	4.15			
Average	765.16a	757.89ab	738.90b	758.22ab					
Non-Fibrous Carbohydrates (NFC, g.kg ⁻¹ Dry Matter)									
Young	479.92	494.30	439.57	509.95	480.94	9.30			
Intermediate	485.88	484.39	454.07	493.03	479.34	8.72	<0.001	0.250	>0.050
Mature	489.26	485.03	405.72	477.31	464.33	9.75			
Average	485.02a	487.91a	433.12b	493.43a					

EPP (*Opuntia stricta*); APP (*Opuntia undulata*); F-08 (*Opuntia atropes*); V-19 (*Opuntia larreri*). SEM, standard error of mean; G, genotypes; P, phenological stages; G x P, interaction of genotypes and phenological stages. Means followed by lowercase letters differ in the rows and capital letters in columns (P < 0.05).

3.2 Mineral Composition of Cladodes

There was no effect of the factors (P > 0.05) on the contents of minerals N (11.0 to 13.94 g.kg⁻¹ DM), K (24.22 to 39.18 g.kg⁻¹ DM), Mg (6.89 to 7.39 g.kg⁻¹ DM), S (3.34 to 4.72 g.kg⁻¹ DM), Fe (93.4 to 128.24 mg.kg⁻¹ DM) and Zn (52.04 to 135.89 mg.kg⁻¹ DM) (Figure 2 and Figure 3).

The P concentration in EPP was higher (P < 0.05) in mature cladodes, similar to V-19, but V-19 exhibited a similar value in the intermediate stage (Figure 2b). In the APP genotype, the lowest concentration occurred in young cladodes. The P content in young and intermediate cladodes was higher in APP and F-08, but the highest concentration in mature cladodes was found in APP.

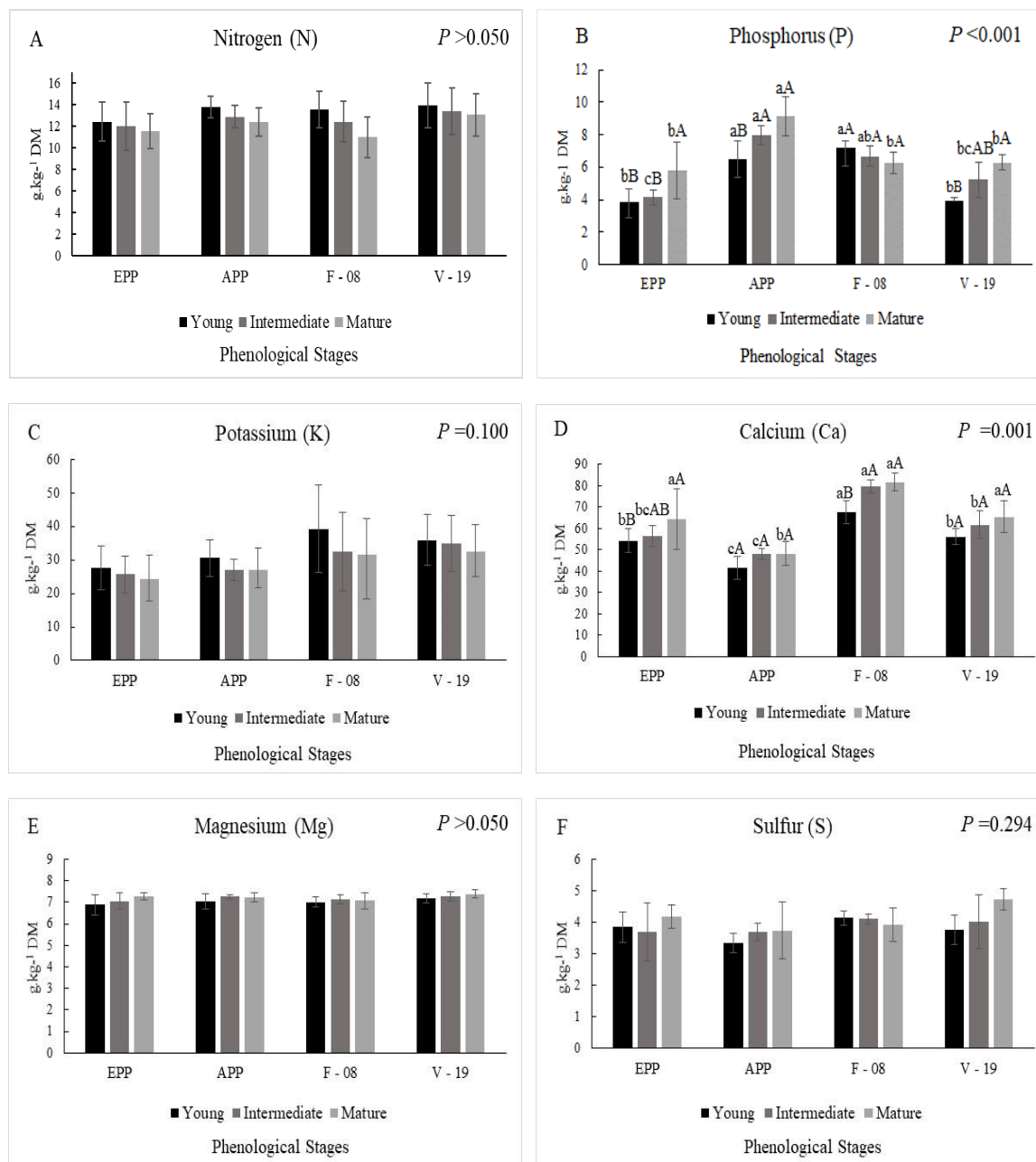


Figure 2. Macrominerals composition of species genotypes of the genus *Opuntia* spp. in different phenological stages. Lowercase letters compare the genotypes and capital letters compare the phenological stages ($P < 0.05$). *EPP* (*Opuntia stricta*); *APP* (*Opuntia undulata*); *F-08* (*Opuntia atropes*); *V-19* (*Opuntia larreri*)

Ca contents in EPP and F-08 genotypes were higher ($P < 0.05$) in mature and intermediate cladodes. The lowest concentrations of Ca occurred at all stages of APP development (Figure 2d). Mineral B content was higher ($P < 0.05$) in young and intermediate cladodes in EPP, APP and F-08 species. The lowest concentrations of B in the mature stage were found in F-08, and in the young and intermediate cladodes in V-19 (Figure 3a).

The APP and F-08 genotypes had the highest ($P < 0.05$) Cu contents in the young and intermediate cladodes. The highest Cu concentration in young cladodes was observed in APP

and in the intermediate and mature stages in EPP and APP (Figure 3b).

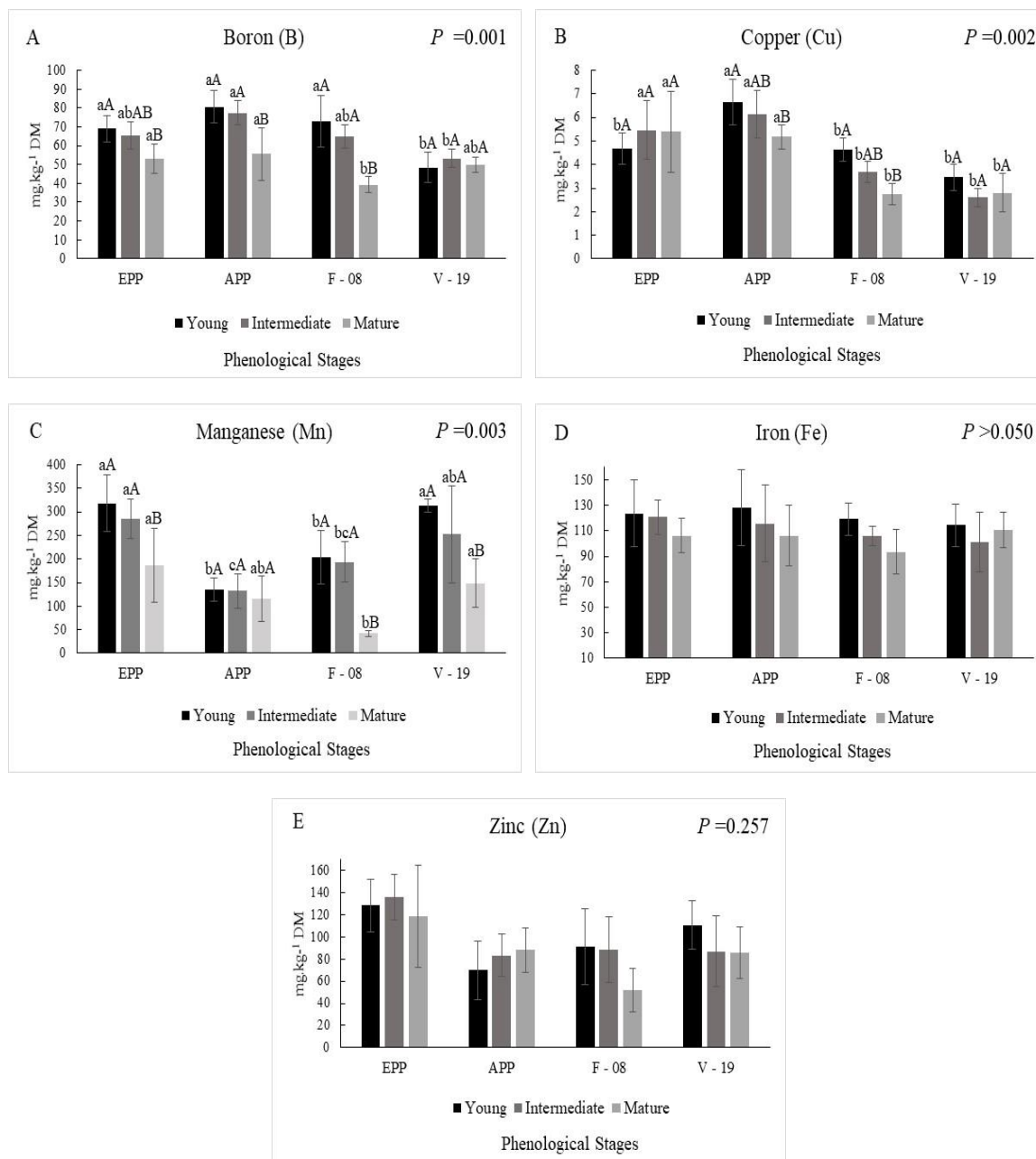


Figure 3. Microminerals composition of species genotypes of the genus *Opuntia* spp. in different phenological stages. Lowercase letters compare the genotypes and capital letters compare the phenological stages ($P < 0.05$). *EPP* (*Opuntia stricta*); *APP* (*Opuntia undulata*); *F-08* (*Opuntia atropes*); *V-19* (*Opuntia larreri*)

The Mn highest levels in EPP, F-08 and V-19 occurred in the young and intermediate cladodes. Values differed at developmental stages ($P < 0.05$), mature cladodes at F-08 had lower Mn concentration, while higher concentrations at young and intermediate stages were observed at EPP and V-19 (Figure 3c).

3.3 Multivariate Data Analysis

In the exploratory data analysis using PCA (Figure 4), the first two components (PC1 and PC2) explained 56.5% of the total data variation using all variables (Figure 4a), but by selecting the characteristics with the greatest potential for discrimination (11 variables) the contribution of these same components increased to 81.5% (Figure 4f).

When all variables were used, the factor loadings CP, NFC, ADF, Fe, NDF and Ca showed a greater contribution for PC1 (Figure 4b), and TC, K, N, OM, MM and DM for PC2 (Figure 4c). High magnitude correlations occurred between TC x OM ($r = 0.93$), Zn x Mn ($r = 0.82$), Ca x MM ($r = 0.82$), MM x OM ($r = -1.00$), MM x TC ($r = -0.92$) and NFC x NDF ($r = -0.86$) (Figure 4d).

By observing the dispersion of treatments in PC1 and PC2, five groups can be clearly distinguished (Figure 4e). Group 1 consisted of young and intermediate F-08 cladodes; group 2 formed by V-19 in the intermediate and mature stages; group 3 consisting of young cladodes of EPP, APP and V-19 genotypes, and intermediates of EPP and APP; group 4 was formed by the mature stage of EPP and APP; and group 5 with the isolated mature F-08 cladodes.

The factor loadings of the selected variables NFC, CP, Ca, OM, MM and ADF had a greater contribution for PC1 (Figure 4g) and TC, N, K, NDF, OM and MM for PC2 (Figure 4h). High magnitude correlations occurred between TC x OM ($r = 0.92$), CP x NFC ($r = 0.82$), CP x Fe ($r = 0.72$), MM x TC ($r = -0.92$), CP x ADF ($r = -0.86$) and Ca x OM ($r = -0.82$) (Figure 4i). In the dispersion of treatments in PC1 and PC2, three groups were formed (Figure 4j): group 1 constituted by young and intermediate cladodes of F-08, group 2 constituted by cladodes of EPP, APP and V-19 in the three stages of development, and group 3 formed by the mature F-08 cladodes.

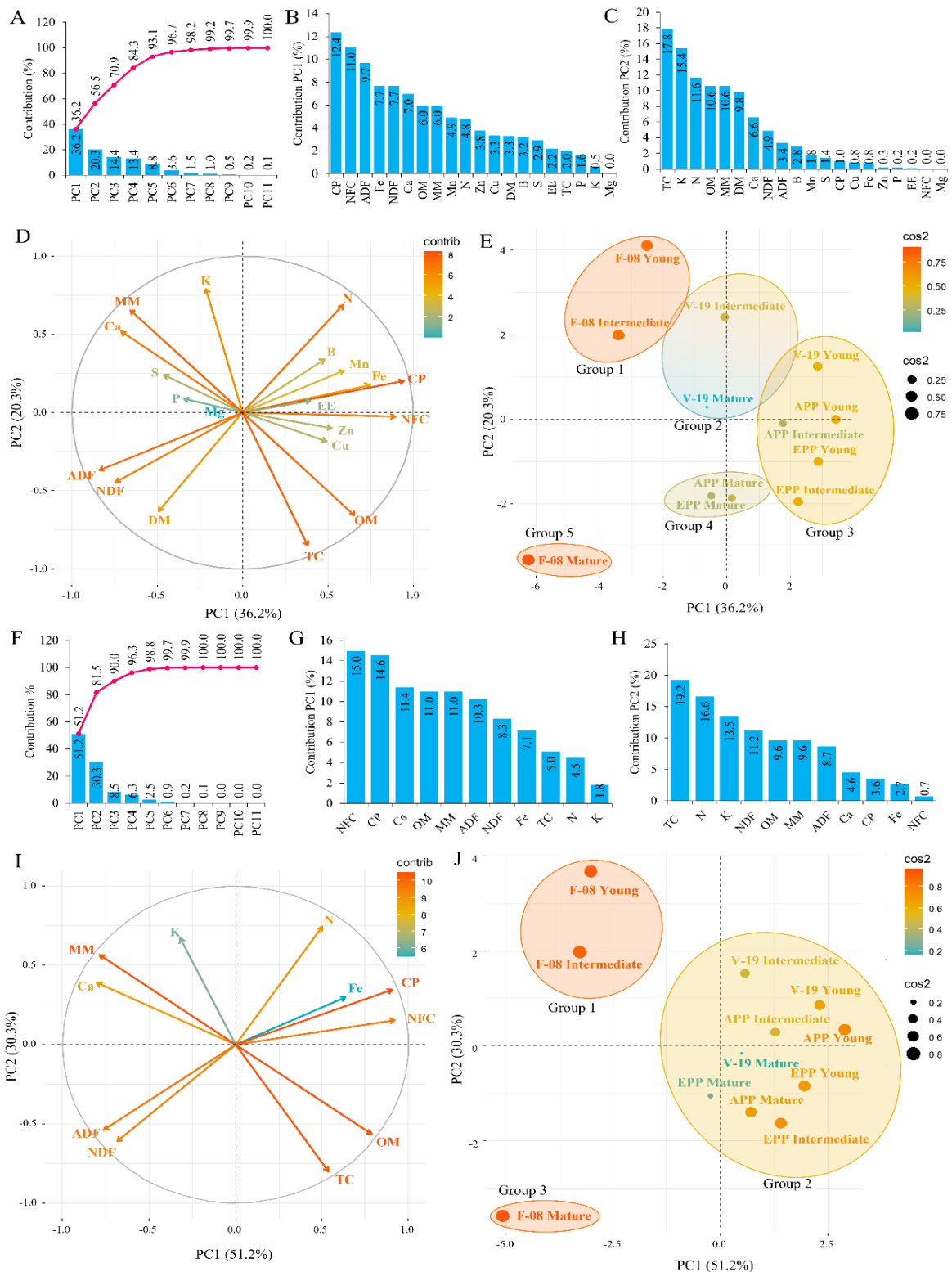


Figure 4. Analysis of principal components for chemical-bromatological and mineral composition of genotypes of species of the genus *Opuntia spp.* at different phenological stages. A and F individual and cumulative contribution of the main components as a function of all characteristics (A) and selected characteristics (F), B and G contribution of the first

main component as a function of all characteristics (B) and selected characteristics (G), C and H contribution of the second principal component as a function of all characteristics (C) and selected characteristics (H), D and I circle correlation with the contribution of characteristics for the first two components with all characteristics (D) and selected characteristics (I). The arrows represent the direction of the feature, and the color gradient represents each one's contribution to the components. From blue to red, from low to high contributions. E J distribution of treatments as a function of the first two components for all characteristics (E) and selected characteristics (J). The color gradient indicates the contribution of the first two components to explain the variation of treatments, also represented by the size of the circles that varies from 0.25 to 0.75 (E) and 0.20 to 0.80 (J). EPP (*Opuntia stricta*), APP (*Opuntia undulata*), F-08 (*Opuntia atropes*), V-19 (*Opuntia larreri*).

4. Discussion

Opuntia ssp. are succulent plants and have a low DM content compared to other traditional forage plants. The values found (58.6 to 70.86 g.kg⁻¹ DM) were lower compared to other studies with forage cactus (90.7 to 112.0 g.kg⁻¹ DM) performed by Inácio et al. (2020) and Barros et al. (2018). This may be related to the higher cladodes water content in this study since they were collected in the rainy season. The lower concentration of DM in mature cladodes is probably due to increase in the proportion of supporting tissues that make possible supporting the expansion of new structures, in addition to the thickening and lignification of the cell wall (Chapman et al., 2014).

Due to the low content of DM in cladodes, the nutritional requirements of ruminants, fed exclusively on palm, may not be achieved, and it is necessary to complement diets based on *Opuntia* with other sources of roughage to optimize consumption. On the other hand, its inclusion in the diet reduces voluntary water intake by animals (Costa et al., 2012; Gouveia et al., 2019) since it supplies part of their water needs.

The CP contents observed in this research (56.06 to 73.92 g.kg⁻¹ DM) were higher than those reported by Siqueira et al. (2018) (41.0 g.kg⁻¹ DM) and Conceição et al. (2018) (55.5 g.kg⁻¹ DM). These variations are attributed to soil moisture and fertility (Vazquez-Mendoza et al., 2017), and are directly related to the N content in cladodes (Figure 2a), as this element is one of the components of the protein. This means that an adequate supply of N for the plant is crucial to increase the concentration of CP in the cladodes.

The difference in CP content between mature and young cladodes can be explained by the cuticle thickening and the nitrogen components translocation to the developing structures. Mature cladodes have thicker cuticles due to expansion of the water storage parenchyma (very slowly degradable) at the expense of cell content (Astello-García et al., 2015). In this study, the CP in APP cladodes (71.08 g.kg⁻¹ DM) and V-19 (71.24 g.kg⁻¹ DM) are above the minimum necessary (70.0 g.kg⁻¹ DM) for ruminal microorganisms adequate growth and development, responsible for the degradation of nutrients in the fibrous fraction (Silva et al., 2011).

Opuntia cladodes showed high concentrations of MM, similar to those described by Donato et al. (2014) (141.0 g.kg⁻¹ DM) and Souza et al. (2010) (152.0 g.kg⁻¹ DM). However, the F-08 species stood out in relation to the others, indicating that the accumulation of minerals differs within the genus. The increase in EE concentrations with cladode maturation, evidenced mainly in APP, acts as a plant energy reserve for carrying out biochemical and physiological processes under water stress conditions (Alves et al. 2016).

The increase in NDF and ADF contents with cladodium development (Table 4) was similar to what occurs in other forage plants. This fact was previously verified by Pinos-Rodríguez et al. (2010), by demonstrating that there is a linear relationship between the maturation of *Opuntia* and the increase in fibrous fractions. This happens because as the physiological age of the plant advances, the cell wall becomes thicker with an increase in the concentrations of cellulose, hemicellulose, and lignin, implying an increase in fibrous proportions. However, it seems to have a negative impact on the content of nitrogenous compounds, since with the maturation of the cladodes the CP is lower (64.88 g.kg⁻¹ DM).

The higher ADF concentration in mature F-08 cladodes may indicate that the DM digestibility in this species tends to be lower compared to the others, as these characteristics are inversely proportional. On the other hand, the NDF of the studied species (254.8 to 357.6 g.kg⁻¹ DM) are below that recommended in the ruminant diet (350.0 to 450.0 g.kg⁻¹ DM) and can reduce the total chewing time and saliva secretion, essential to keep the rumen environment stable and maximize production (Vilela et al., 2010). Therefore, *Opuntia* cladodes must be provided in association with a physically effective fiber source, such as silage, hay, crop residues and sugarcane bagasse, in order to avoid possible metabolic disturbances.

TC were the most abundant DM components, and the genotypes have a high concentration of NFC, which was not altered by cladode maturation. NFC contents are higher than most bulky feeds commonly used in rations, suggesting that *Opuntia* cladodes have the potential to replace other sources of energy concentrate in the diet (Costa et al., 2012; Felix et al., 2016). Furthermore, the rapid fermentation of these carbohydrates favors ruminal microorganisms and contributes to microbial protein synthesis (McDonald et al., 2011).

As minerals are directly related to nutrient absorption, physiological status, DM intake and performance of ruminants, the nutritional requirements of each animal category must be considered when confronting with the amount of minerals present in *Opuntia* cladodes. Mayer and Cushman (2019) found higher concentrations of K (47.8 to 73.6 g.kg⁻¹ DM) and Mg (14.2 to 21.2 g.kg⁻¹ DM), and when compared with the maximum values allowed for beef cattle during the pregnancy and lactation, found that it was above tolerable levels. The mineral concentrations in the studied cactus forage species meet the nutritional requirements of cattle in these categories, but the tolerance of Ca is exceeded in all genotypes and K tolerance is exceeded in F-08 and V-19.

The P concentration in mature cladodes (Figure 2b) is certainly related to the participation of this mineral in the photosynthesis and respiration processes of plants with CAM metabolism, since more developed organs produce a greater amount of photosynthates (Queiroz et al.,

2015). In animal nutrition, cladodes with higher P contents may contribute to supplement part of the deficiency often found in goats raised extensively in semiarid regions (Barbosa et al., 2018).

Ca was the most abundant macromineral in this study (41.72 to 81.8 g.kg⁻¹ DM) and EPP and F-08 contents increased with cladode maturation. According to Hernandez-Urbiola et al. (2011), the concentration of this mineral in *O. ficus* increased with the plant aging, corroborating the results of this research. Ca accumulation in cladodes may be related to heat stress, where it is able to mitigate the effects by improving stomatal function and other cellular processes (Contreras-Padilla et al., 2011).

Higher Ca than P contents, result in a high Ca:P ratio. The unbalance of these minerals in the forage can cause the appearance of kidney stones and affect the performance (Santos et al., 2009). However, the main form of Ca in cladodes is calcium oxalate (McConn & Nakata 2004; Tovar-Puente et al., 2007), which makes this mineral unavailable and inhibits poisoning in animals. From a nutritional point of view, both minerals are interesting for livestock feed, essential for bone formation, ionic balance, and signal transmission within cells (Agabriel 2010).

The concentration of B, Cu and Mn in young and intermediate cladodes suggests that they are required in greater amounts by developing tissues. This nutrient redistribution process is essential to the plant life cycle, as some minerals increase in the area part, others decrease, resulting in translocation (Lima et al., 2011). Thus, the cladodes supply in young and intermediate stages, located in the extremities or median portion of the plant, can be aimed at animal categories with greater demand for these minerals.

Among the microminerals, Mn was the most abundant in *Opuntia* species (41.6 to 317.7 mg.kg⁻¹ DM), showing typical behavior of a nutrient synthesized in larger amounts in young structures (Figure 3c). Alves et al. (2017) observed differences in Mn concentration between secondary and tertiary order cladodes, despite not defining a pattern of accumulation in the plant, ranging from 228.5 to 512.5 mg.kg⁻¹ DM in *O. stricta*, from 102.5 to 139.47 mg.kg⁻¹ DM for *O. atropes*, and from 323.5 to 523.9 mg.kg⁻¹ DM for *O. larreri*.

In the exploratory analysis using PCA, the variables EE, P, Mg, S, B, Cu, Zn and Mn had a minor contribution to the divergence between treatments (Figure 4b; 4c). When disregarding them, the accumulated value of the first principal components was greater than 80%, which according to Cruz et al. (2012) is sufficient to explain the variability of the data. In the formation of groups, F-08 presented lower nutritional quality compared to the other species, using all characteristics and selected characters (Figure 4e; 4j), explained by the strong contribution of the variables ADF and MM in mature cladodes, while Ca, K, and MM explain in the young and intermediate stages of development.

5. Conclusions

The cladodes of the species *O. stricta*, *O. undulata* and *O. larreri* showed a better nutritional quality for ruminant feeding. Young and intermediate cladodes are more proteinaceous and less fibrous. These results can be useful in selecting the forage cactus species with superior

nutritional quality and in optimizing the use of cladodes for feeding ruminants of different categories in semiarid regions.

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