













# Effect of using hydrogel polymers on morphometric, forage production, and nutritional characteristics of cactus pear

Pablo Ramon Costa<sup>1</sup>, Emerson Moreira de Aguiar<sup>1</sup>, Antonio Leandro Chaves Gurgel<sup>2</sup>, Patrick Bezerra Fernandes<sup>3</sup>, João Virgínio Emerenciano Neto<sup>1</sup>, Murilo dos Santos Ferreira<sup>1</sup>, Williana da Silva Melo<sup>1</sup>, Gelson dos Santos Difante<sup>4</sup>, Luis Carlos Vinhas Itavo<sup>4</sup>, Tairon Pannunzio Dias-Silva<sup>2</sup>, Marcos Jácome de Araújo<sup>2</sup>, Larissa de Oliveira Fontes<sup>2</sup>, Maíra Vieira Ataíde<sup>5</sup>, José Henrique da Silva Taveira<sup>6</sup>

<sup>1</sup>Universidade Federal do Rio Grande do Norte, Unidade Acadêmica Especializada em Ciências Agrárias, Macaíba/RN, Brasil.

<sup>2</sup>Universidade Federal do Piauí, *Campus* Professora Cinobelina Elvas, Bom Jesus/PI, Brasil.

<sup>3</sup>Instituto Federal de Educação Ciência e Tecnologia Goiano, Campus Rio Verde, Rio Verde/GO, Brasil.

<sup>4</sup>Universidade Federal de Mato Grosso do Sul, Faculdade de Medicina Veterinária e Zootecnia, Campo Grande/MS, Brasil.

<sup>5</sup>Universidade Estadual de Goiás, *Campus* Oeste, São Luís de Montes Belos/GO, Brasil.

<sup>6</sup>Universidade Estadual de Goiás, *Campus* Quirinópolis, Quirinópolis/GO, Brasil.

† *in memoriam*

\*Corresponding author: E-mail: antonio.gurgel@ufpi.edu.br

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**Abstract.** The use of cactus pear has shown poor performance in some areas of semi-arid climate regions, as the species needs a wide gradient between temperatures during the day and night to develop, which does not occur in such zones. To lessen this negative occurrence, moisture availability provides better performance for the species, such as using hydrogel instead of irrigation. The objective of this study was to evaluate the effect of using hydrogel polymers on the production of cactus pear (*Opuntia stricta* cv. Mexican Elephant Ear). Three treatments with six replications were used, totalling 18 experimental units. The treatments were, (1) Conventional fertilization using fertilizer in granulated format; (2) Foliar fertilization with polymer using a dilution dose of 6 grams of polymer L<sup>-1</sup> of water associated with the fertilizer in liquid form through the foliar route; (3) Foliar fertilization without polymer using only foliar fertilization in the same formulation as the treatment with polymer. There was no effect of fertilization strategy on plant height, cladode length, cladode width, cladode thickness, cladode area, and biomass. There was an effect of the fertilization strategy on dry matter, in which higher estimates were measured for conventional fertilization and foliar fertilization without polymer. On the other hand, foliar fertilization with polymer promoted increases of 13% in mineral matter and 42% in crude protein in relation to conventional fertilization. The fertilization strategy did not change the other chemical attributes of cactus pear. The use of the hydrogel did not influence the morphometric development of the Mexican Elephant Ear cultivar but promoted increments in the chemical composition.

**Keywords:** Fertilization, forage, Mexican elephant ear, polymers, Semi-arid.

## Introduction

The forage cactus plants are widely used in arid and semi-arid regions, since, due to their phenotypic plasticity, they are more efficient in water use in scenarios with low precipitation rates (Silva *et al.*, 2021; Pereira *et al.*, 2021; Lima *et al.*, 2023). Furthermore, according to Edvan *et al.* (2020) plants of *Opuntia stricta* cv. Mexican elephant ear provides high nutritional values compared to other palm species,

which proves the potential of this plant for feeding ruminants.

The cactus pear can be inserted into the diet of ruminants as a source of energy or in association with other foods. Thus, when feeding dairy cows, Sánchez *et al.* (2022) verified that the partial replacement of sugarcane (*Saccharum officinarum*) by palm cladodes could increase milk production. According to Pereira *et al.* (2021), cactus pear can completely replace corn in the diet of dairy goats, as it does not change dry matter intake or milk production, influencing the reduction of feed costs. Likewise, Silva *et al.* (2021) verified that the association of leguminous hays with cactus pear in feedlot lambs provided carcasses with desirable degrees of muscularity and adiposity.

Although *O. stricta* plants are adapted to regions with a semi-arid climate, the Mexican elephant ear cultivar exhibits the highest rates of carbon (CO<sub>2</sub>) absorption during the rainy season (Souza *et al.*, 2022). Moreover, in low-altitude regions characterized by low humidity, high nighttime temperatures, and annual precipitation below 400 mm, there is a risk of excessive water loss, leading to severe wilting of cladodes and even plant death (Nascimento *et al.*, 2020). This is a critical consideration to ensure the maximum potential for forage production. Therefore, to enhance agronomic performance and prolong the lifespan of palm groves, it is crucial to prevent severe water restrictions from the early stages of their development. The plant demonstrates water use efficiency, with values ranging from 1.63 to 1.82 kg m<sup>-3</sup> (Souza *et al.*, 2023).

The use of hydrogel ensures moisture retention in the soil, increasing water availability for plants (Saha *et al.*, 2020; Sousa *et al.*, 2023; Edvan *et al.*, 2023), being a promising strategy to be used in the production of plants of agronomic interest in arid regions, as it enhances the germination process and initial development of crops (Ai *et al.*, 2020). In sandy textured soil under saline conditions, Costa *et al.* (2022) found that the use of hydrogel polymer reduced water loss by 58%. In the production of *Pinus pinea*, El-Asmar *et al.* (2017) observed that the hydrogel promoted increases of 25% in biomass, and, at the same time, prolonged the survival time of the seedlings. However, there is a lack of information about the use of hydrogel in cactus pear culture.

In view of this, the hypothesis was that the use of hydrogel polymers will promote better morphometric development and enhance the production and nutritional value of the cactus pear Mexican elephant ear cultivar. Therefore, the objective of this study was to evaluate the effect of using hydrogel polymers on the production of cactus pear, *O. stricta* cv. Mexican elephant ear, cultivated in northeastern Brazil.

## Material and Methods

### Study area

The experiment was carried out in the area of the Academic Unit Specialized in Agricultural Sciences, located at the Agricultural School of Jundiá, belonging to the Federal University of Rio Grande do Norte, campus Macaíba/RN, Brazil (Latitude 5°53'32.76"S and longitude 35° 21'41.70"W and altitude of 50 m), being implemented in January 2017, and conducted until January 2020 (Figure 1). The climate of the region, according to the Thornthwaite (1948) climate classification is dry subhumid, with water surplus from May to August. The average annual precipitation is 1048 mm and the annual average accumulated potential evapotranspiration is 1472 mm.



**Figure 1.** Panoramic view of the experimental area, illustrating the arrangement of plants in their respective plots.

### **Experimental design and treatments**

A completely randomized experimental design was employed, involving three treatments, each with six replications, totaling 18 experimental units (Figure 1). The treatments were, (1) Conventional fertilization using fertilizer in granulated format; (2) Foliar fertilization with polymer using a dilution dose of 6 grams of polymer L<sup>-1</sup> of water associated with the fertilizer in liquid form through the foliar route; (3) Foliar fertilization without polymer using only foliar fertilization in the same formulation as the treatment with polymer.

### **Soil analysis**

Before the implementation of the experiment, the chemical and physical characterization of the soil was carried out in the 0-20 cm layer, collecting 20 simple samples at equidistant points. Subsequently, a composite sample was taken and sent to the laboratory for analysis according to Teixeira *et al.* (2017). The soil was classified as Neosol Quartzareno type (Santos *et al.*, 2018), with a sandy loam texture (964 g kg<sup>-1</sup> of sand). Regarding the chemical parameters, the results were, active acidity (pH in water) of 5.27, 0.46, 0.30 and 1.98 cmol<sub>c</sub> dm<sup>-3</sup>, respectively of calcium (Ca), magnesium (Mg), potential acidity (H+Al); 27.33 and 52.00 mg dm<sup>-3</sup> of potassium (K) and phosphorus (P) (Mehlich I); cation exchange capacity (CEC) of 2.91, 24.0 g dm<sup>-3</sup> of organic matter (OM) and base saturation of 31.74%.

### **Land preparation**

For the experiment, cactus pear (*Opuntia stricta* cv. Mexican Elephant Ear). The planting area was previously prepared by means of leveling harrowing, followed by liming, 60 days before planting, and leveling harrowing again, in order to incorporate the applied corrective. The applied rate of limestone was 1.7 t ha<sup>-1</sup> according to soil analysis recommendations and using the base saturation method. Subsequently furrows were made in the area.

### **Plant density**

The cladodes were planted in January in the double row system, with compaction in the format of a playing card, spacing 1.0 m x 0.5 m x 0.15 m, totaling 133,333 plants ha<sup>-1</sup>, the intended size of the area for each plot was 56.25 m<sup>2</sup>, which corresponds to 7.5 x 7.5 m. The standardization cut was performed at 24 months.

### **Fertilization**

In the beginning, 100 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub> was added, in all treatments, in the source of simple superphosphate, based on the results of the soil analysis and the recommendation of fertilization for the crop, being deposited at the bottom of the furrow prior to planting the cladodes. In the treatment using foliar fertilization with polymer, was necessary to hydrate the polymer in water, using a water tank with a capacity of 1000 L, in which 2.7 kg of polymer was deposited, and the volume of water used for hydration, which was 450 L, that is, 6 grams of polymer per liter of water. The polymer remained in contact with water for 30 minutes, for complete hydration, until it reached a gelatinous appearance. The hydrogel polymer (Potassium Polyacrylic Copolymer Polyacrylamide) has a cation exchange capacity of 532.26 mmol<sub>c</sub> dm<sup>-3</sup> and a water retention capacity of 1,526.69%.

The application of the hydrated polymer occurred at a dose of 6.0 g m<sup>-1</sup> or 1.0 L m<sup>-1</sup> of furrow. To apply the product, a watering can was placed in the furrow, promoting direct contact with part of the plant. After the application of the polymer, the grooves were covered with soil, in approximately 2/3 of the cladode.

The fertilizer used was composed exclusively of chemical fertilizers, with the doses used for N (nitrogen), P, and K in the three treatments respectively, 391, 100, and 542 kg ha<sup>-1</sup>, with the difference in relation to the form of application (Blanco-Macías *et al.*, 2010), so that in conventional fertilization was applied via soil, and in foliar fertilization with and without polymer via foliar, with the exception of P, applied via soil in all treatments.

The preparation of the liquid fertilizer formulation for the foliar fertilization treatments with and without polymer comprised 15 kg of soluble potassium chloride (61% of K<sub>2</sub>O) and 15 kg of urea (44% of N), both added in a 40 L container of water, this mixture was decanted for 24 hours. For spraying, a knapsack-type sprayer was used, with a 20 L capacity, to which the potassium chloride (KCl) solution associated with urea was added, and the solution was filtered at the time of packaging in the sprayer, in order to avoid bottom bodies and impurities within it.

The application of foliar fertilization was carried out at night, due to the physiological aspects of the cactus, that is, particularities related to the stomatal opening that always occurs after this time, therefore, foliar fertilization tends to be more efficient if applied after this time. The liquid fertilizer solution was distributed in the foliar fertilization treatments with and without polymer via the foliar route, in which 586.66 L ha<sup>-1</sup> of the solution was applied.

Fertilizer dosages (N and K) were applied every 90 days apart. The fertilizers applied via granules in the conventional fertilization treatment followed the same dose application interval proportions, with four annual applications, spaced every 90 days. Phosphorus was applied annually in all treatments, in a single-dose format.

### **Pest control**

During the experimental period, cochineal control (*Dactylopius coccus*) was carried out using a mixture of mineral oil and 2% neutral detergent. The fungal, viral, and bacterial diseases were not observed in cactus pear plants. The control of invasive plants was carried out through manual cleaning at periodic intervals.

### **Variables measured**

The morphometric data were analyzed at the time of harvest, being obtained from 10 cladodes selected at random from the useful area of each plot, in order to estimate the morphometric data of the plants, with the aid of a ruler to measure the width (cm) and the length (cm) of the cladode and its respective area and using a caliper the thickness (mm) was obtained. The cladode area (AC, cm<sup>2</sup>) was determined using the following equation,  $AC = L \times C \times 0.75$  (Lima *et al.*, 2020). The plant height was obtained using a 1.50 m ruler, which was measured from the base to the end of the last cladode.

At the time of harvesting, the area of each plot was measured, preserving an area called the useful area, where the material for analysis of the variables was obtained. The useful area covered the three central double rows, measuring 4x3 m, eliminating the two double rows on both sides.

The total harvest of cladodes from the usable area was carried out by cutting them at the joint between plants and after collecting morphometric data. The mother cladode and two primary cladodes, as described earlier, were preserved for each plant. All harvested cladodes were placed in jute bags, properly identified, and weighed. Subsequently, the dry matter content (DM) was determined to estimate forage production (t ha<sup>-1</sup>).

After determining the DM, the samples were sent to the Multiuser Laboratory of Animal Nutrition at UFRN and crushed in a Willey knife mill. Subsequently, the concentrations of mineral matter (MM) were determined by the methodology Silva and Queiroz (2002). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined by the methodology of Van Soest *et al.* (1969); ether extract (EE), lignin, crude protein (CP), neutral detergent-insoluble protein and detergent-insoluble protein were determined according to procedures described by Silva and Queiroz (2002).

The Total Carbohydrates (CHO) content was obtained by the equation,  $\%CHO = 100 - (\%CP + \%EE + MM)$  and for the non-fiber Carbohydrates (NFC) content the equation was used:  $\%NFC = 100 - (\%NDF + \%PB + \%EE + \%MM)$ , both formulas described by Sniffen *et al.* (1992). The content of total digestible nutrients (TDN) by the equation proposed by the NRC (2001).

### **Statistical analysis**

The data were analyzed following a completely randomized model ( $Y_{ij} = \mu + F_i + \epsilon_{ij}$ ),  $Y_{ij}$ : observed value;  $\mu$  = general constant;  $F_i$ : effect of fertilization strategy ( $i$  = conventional fertilization, foliar fertilization with polymer, foliar fertilization without polymer);  $\epsilon_{ij}$ : random error, associated with each observed value. After this procedure, the Tukey mean test was applied at 5% probability.

Correlations ( $r$ ) were obtained by Pearson's correlation analysis using the t-test, considering significance at 5%. The criterion for classifying the correlation coefficient was  $r \geq 70\%$  meaning strong association and  $50\% < r < 70\%$  indicating moderate correlation. All statistical procedures were performed using R software version 4.2.1.

## **Results**

### **Morphometric characteristics and biomass**

No effect of fertilization strategy was observed on plant height, cladode length, cladode width, cladode thickness, cladode area, and biomass (Table 1).

**Table 1.** Morphometric characteristics and biomass production of cactus pear in different fertilization strategies.

	Fertilization strategy			P-value	CV (%)
	Conventional fertilization (kg ha <sup>-1</sup> )	Foliar fertilization with polymer (L ha <sup>-1</sup> )	Foliar fertilization without polymer (L ha <sup>-1</sup> )		
Plant height (cm)	55.86 <sup>a</sup>	54.41 <sup>a</sup>	54.73 <sup>a</sup>	0.760	6.42
Cladode length (cm)	2408 <sup>a</sup>	23.67 <sup>a</sup>	23.34 <sup>a</sup>	0.587	5.13
Cladode width (cm)	17.50 <sup>a</sup>	17.32 <sup>a</sup>	16.98 <sup>a</sup>	0.450	4.12
Thickness of the cladode (mm)	10.39 <sup>a</sup>	10.32 <sup>a</sup>	10.16 <sup>a</sup>	0.214	2.14
Area of the cladode (cm <sup>2</sup> )	317.00 <sup>a</sup>	307.50 <sup>a</sup>	297.16 <sup>a</sup>	0.399	8.01
Biomass (t ha <sup>-1</sup> )	9.23 <sup>a</sup>	8.56 <sup>a</sup>	10.65 <sup>a</sup>	0.057	14.80

Means followed by lowercase letters, the same on the lines do not differ from each other by Tukey's test at 5%. CV: coefficient of variation.

### Chemical composition

An effect of the fertilization strategy on dry matter was observed, in which higher estimates were measured for conventional fertilization and foliar fertilization without polymer. On the other hand, foliar fertilization with polymer promoted increases of 13% in mineral matter and 42% in crude protein in relation to conventional fertilization (Table 2).

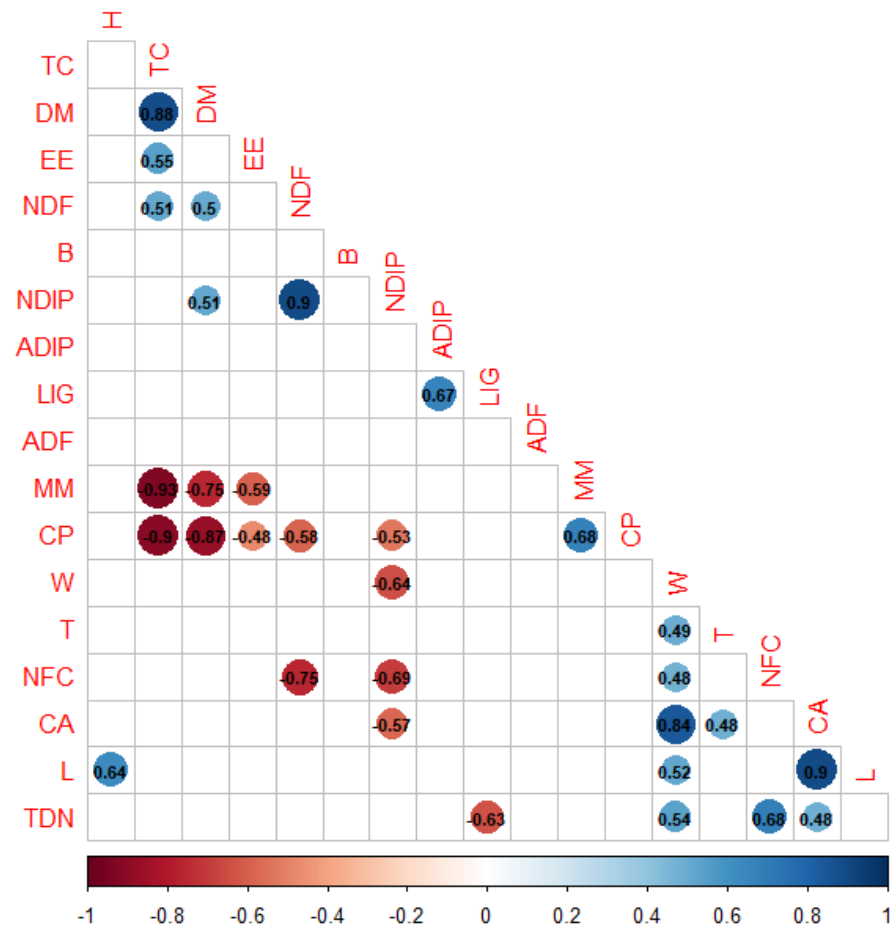
No effect of fertilization strategy was observed for insoluble protein in neutral detergent, insoluble protein in acid detergent, neutral detergent fiber, acid detergent fiber, lignin, total carbohydrates, non-fiber carbohydrates, ether extract, and total digestible nutrients (Table 2).

**Table 2.** Chemical composition of cactus pear in different fertilization strategies.

	Fertilization strategy			P-value	CV (%)
	Conventional fertilization (kg ha <sup>-1</sup> )	Foliar fertilization with polymer (L ha <sup>-1</sup> )	Foliar fertilization without polymer (L ha <sup>-1</sup> )		
DM*	8.91 <sup>a</sup>	6.34 <sup>b</sup>	7.85 <sup>a</sup>	<0.001	10.06
% of DM					
MM	11.26 <sup>b</sup>	14.14 <sup>a</sup>	12.53 <sup>ab</sup>	0.006	10.48
CP	3.68 <sup>b</sup>	6.30 <sup>a</sup>	5.43 <sup>a</sup>	0.002	19.98
NDIP	0.28 <sup>a</sup>	0.24 <sup>a</sup>	0.28 <sup>a</sup>	0.218	14.97
ADIP	0.09 <sup>a</sup>	0.091 <sup>a</sup>	0.10 <sup>a</sup>	0.718	21.47
NDF	40.14 <sup>a</sup>	35.45 <sup>a</sup>	39.69 <sup>a</sup>	0.105	102.00
ADF	13.54 <sup>a</sup>	14.89 <sup>a</sup>	15.35 <sup>a</sup>	0.482	18.05
Lignin	3.03 <sup>a</sup>	3.44 <sup>a</sup>	3.25 <sup>a</sup>	0.547	19.72
TC	83.98 <sup>a</sup>	78.60 <sup>b</sup>	81.02 <sup>b</sup>	<0.001	2.27
NFC	43.83 <sup>a</sup>	43.15 <sup>a</sup>	41.32 <sup>a</sup>	0.511	8.88
EE	1.07 <sup>a</sup>	0.943 <sup>a</sup>	1.01 <sup>a</sup>	0.455	16.92
TDN	62.73 <sup>a</sup>	61.18 <sup>a</sup>	61.26 <sup>a</sup>	2.77	0.243

\*% of fresh matter; DM: Dry matter; MM: Mineral matter; CP: Crude protein; NDIP: neutral detergent insoluble protein; ADIP: Acid detergent insoluble protein; NDF: Neutral detergent fiber; FDA: Acid detergent fiber; TC: Total carbohydrates; NFC: non-fibrous carbohydrates; EE: Ethereal extract; TDN: Total digestible nutrients. Means followed by lowercase letters the same on the lines do not differ from each other by Tukey's test at 5%. CV: coefficient of variation.

There was a positive and strong correlation between dry matter and total carbohydrates, insoluble protein in neutral detergent and fiber in acid detergent, width and length of the cladode with the area of the cladode. Mineral matter and crude protein exhibited a high negative correlation with mineral matter, dry matter, and total carbohydrates. A strong and negative correlation was observed between non-fiber carbohydrates and neutral detergent fiber (Figure 2).



**Figure 2.** Correlation matrix of morphometric characteristics and chemical composition of cactus pear in different fertilization strategies. Blank cells in the correlation matrix indicate no significant effect occurred at 5%. FC: Conventional fertilization; FFH: Foliar fertilization with polymer; FF: Foliar fertilization without polymer. PH: Plant height; CL: Cladode length; WC: Width of the cladode; CL: cladode length; ThC: Thickness of the cladode; CA: Cladode area; BIO: Biomass; DM: Dry matter; MM: Mineral matter; CP: Crude protein; NDIP: neutral detergent insoluble protein; ADIP: Acid detergent insoluble protein; NDF: Neutral detergent fiber; FDA: Acid detergent fiber; TC: Total carbohydrates; NFC: non-fibrous carbohydrates; EE: Etheral extract; TDN: Total digestible nutrients.

## Discussion

The fertilization strategies did not influence the morphometric characteristics and forage production of the Mexican Elephant Ear cultivar. Possibly the density used had an impact on the generation of plants of lower height, according to Pereira *et al.* (2022) for the Mexican Elephant Ear cultivar, the use of values greater than 100,000 plants ha<sup>-1</sup> can generate a plant height of less than 70 cm. Increased density stimulates competition between plants, slowing down the emission of new cladodes.

The effects of conventional fertilization on palm forage production will show greater effects in the long term. Because the use of chemical fertilizers in granulometric form, leads to an increase in dry mass yield in the third cycle of palm harvest (Lédo *et al.*, 2019).

Regarding the cladode biometry data, is possible to verify that there is a strong correlation between the variables (Figure 2) showing that the width and length are important measures to make predictions of the total area of the cladode (Lucena *et al.*, 2019; Lucena *et al.*, 2020). The use of the two variables can also be applied to obtain the leaf area in other plants with foraging aptitude (*Arachis pintoi*, *Stylosanthes spp.*, *Calopogonium mucunoides*, *Neonotonia wightii*, *Megathyrus maximus* cv. BRS Zuri), generating high precision estimates, as it was observed by Homem *et al.* (2017), and Fernandes *et al.* (2020).

Conventional fertilization and foliar fertilization without the hydrogel polymer impacted increases in dry matter, indicating that cladodes produced under these management conditions exhibited higher specific gravity. According to Dubeux Jr *et al.* (2021) when there is an adequate supply of N-P-K in palm groves cultivated under high density, it is possible to promote increases in the dry matter concentration and induce an improvement in the nutritive value of the cladode.

However, conventional fertilization provided lower crude protein values than the other strategies (Table 2); even with the use of the recommended doses of nutrients for the maximum performance of the plant, in soils with sandy texture due to their respective granulometry, there is little retention of K, therefore, high losses by leaching occur, associated with this, in the semi-arid region at high temperatures cause losses by volatilization; occurring when using urea as a N-source (Duarte *et al.*, 2013; Perin *et al.*, 2020).

Foliar fertilization represents an attractive supplementary strategy, as it avoids adverse impacts on the soil. However, its implementation often faces challenges such as limited penetration through foliar barriers, leaf damage, and a restricted capacity for nutrient translocation (Husted *et al.*, 2022). Similarly, the use of hydrogel in agriculture emerges as a promising strategy, as hydrogel polymers ensure water retention and availability for plants, enhancing accessibility to essential nutrients necessary for organ development and expansion (Zhang *et al.*, 2022). Additionally, this approach contributes to reducing the water footprint in food production.

The plants will express greater nutritional value with the maximum potential for nutrient utilization. In agricultural scenarios, cultivating plants with forage potential, when there is no compromise in the supply of N, higher values of crude protein will be measured (Leite *et al.*, 2021; Hughes *et al.*, 2022). Leite *et al.* (2018) verified that the increasing offer of N does not influence the morphology of cactus pear plants, however, when the supply occurs without the interference of abiotic factors, it generates positive effects on the concentration of crude protein in the cladode. Subsequently, the increase in crude protein concentration causes a reduction in total carbohydrate contents (Figure 2). On the other hand, the values obtained in the three fertilization strategies are satisfactory. In the genus *Opuntia spp.*, values of crude protein and total carbohydrates are measured in the proportions of 5.20% and 84.07%, respectively (Pessoa *et al.*, 2020).

Due to the negative correlation between non-fibrous carbohydrates and neutral detergent fiber, it is possible to infer that less than 50% of the proportion of carbohydrates is formed by fibrous carbohydrate structures (Table 2 and Figure 2). Magalhães *et al.* (2021) verified that forage cactus



genotypes provide adequate values of total digestible nutrients, non-fiber carbohydrates, and total fraction of unavailable nitrogen, indicating that they can be used in the diet of ruminants. However, supplementation is required to increase fiber content.

Although the use of hydrogel has not enhanced the production of biomass, it is important to mention that its use should not be disregarded, since the climate perspectives for the coming decades indicate that it will be necessary to use strategies that allow the efficient use of water, thus, it will be possible to promote agricultural sustainability (Liu *et al.*, 2022). Furthermore, it is of utmost importance that future research includes a cost analysis of applying hydrogel in palm cultivation. Since this product is not widely adopted in production systems, the economic feasibility of its use will depend on an increase in demand, potentially leading to a reduction in product costs.

### **Conclusions**

The use of the hydrogel did not influence the morphometric development, or biomass production in the *Opuntia stricta* cv. Mexican Elephant Ear. On the other hand, the polymer promotes increments in the chemical composition of the plant, increasing the levels of crude protein in conventional fertilization.

### **ETHICS STATEMENT**

Not applicable.

### **CONSENT FOR PUBLICATION**

Not applicable.

### **AVAILABILITY OF SUPPORTING DATA**

All data generated or analyzed during this study are included in this published article.

### **COMPETING INTERESTS**

The authors declare that they have no competing interests.

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### **AUTHOR CONTRIBUTIONS**

Conceptualization, P.R.C., E.M.d.A., A.L.C.G., J.V.E.N., M.d.S.F. and W.d.S.M.; methodology, P.R.C., E.M.d.A., M.d.S.F. and W.d.S.M.; software, A.L.C.G., P.B.F. and L.C.V.I.; validation, E.M.d.A., A.L.C.G., J.V.E.N., G.d.S.D., T.P.D.S., M.J.d.A., L.d.O.F. and J.H.d.S.T.; formal analysis, A.L.C.G. and P.B.F.; investigation, P.R.C., E.M.d.A., M.d.S.F., W.d.S.M. and M.V.A.; resources, E.M.d.A.; data curation, P.R.C., E.M.d.A., A.L.C.G. and P.B.F.; writing—original draft preparation, P.R.C., A.L.C.G., P.B.F. and M.V.A.; writing—review and editing, A.L.C.G.; visualization, P.B.F., J.V.E.N., G.d.S.D., L.C.V.I., T.P.D.S., M.J.d.A., L.d.O.F. supervision, E.M.d.A.; project administration, P.R.C. and E.M.d.A.; funding acquisition, P.R.C. and E.M.d.A.

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