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YIELD AND CHEMICAL QUALITY OF CHEPIL (*Crotalaria longirostrata* Hook. & Arn) FORAGE AT DIFFERENT SEEDING DENSITIES AND CUTTING FREQUENCY

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ABSTRACT

Ruminant grazing in the tropics requires supplementation given the nutritional characteristics of pastures to cover protein needs. The objective of this research was to evaluate the yield and chemical quality of chepil (*Crotalaria longirostrata* Hook. & Arn) forage at different seeding densities and cutting frequencies to obtain the optimum harvest time. The experiment was set up in a completely randomized design with three replications. The treatments were planting densities T1: 200 000; T2: 100 000; and T3: 50 000 plants ha^{-1}. The variables evaluated were: dry matter yield, morphological composition, growth rate, leaf:stem ratio, intercepted radiation, plant height, crude protein, neutral detergent fiber, acid detergent fiber, dry matter, and ash. For the 64-day cutting frequency, the three densities presented the highest yield with 3406, 3500, and 4200 kg MS ha^{-1}, for 200 000, 100 000 and 50 000 plants ha^{-1}, respectively ($p < 0.05$). PC decreased ($p < 0.05$) in T2 and T3 (23 %) in comparison to T1 (24 %). The planting density with the highest percentage of crude protein was 200 000 plants ha^{-1} with 24 %. The densities of 100 000 and 50 000 plants ha^{-1} presented a lower crude protein percentage, with 23 % ($p < 0.05$). It is concluded that the optimum cutting time is at 36 days of regrowth, when the chepil is planted at a density of 100 000 plants ha^{-1}, and harvested at the frequency of 36 days of regrowth, since 95 % of intercepted radiation and better quality between yield and chemical characteristics, mainly crude protein, are obtained.

Keywords: Fabaceae, fodder legumes, chipilin, thresher.

INTRODUCTION

In Mexico, the search for different unconventional protein sources is necessary (Galindo *et al*., 2005; Sosa-Pérez *et al*., 2017). In underdeveloped countries, tropical fabaceae are an accessible and economically viable alternative for human and animal feed (Renté-Martí *et al*., 2020; Pincay-Ganchozo *et al*., 2021). Legumes in animal feed contain nutritional compounds that supplement the needs of ruminants and non-ruminants at different times of the year; they are also used as mulch, for phytoremediation, and as agroecological transition in sustainable agriculture (Voisin *et al*., 2013; Ruiz *et al*., 2015; Almeida-Santos *et al*., 2019).

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In the tropics and subtropics of the Americas, approximately 89 species of the genus *Crotalaria* have been reported, most of which are important for food, bioenergy, medicine, and ornamentals (Avendaño, 2011). Some of these species are characterized by rapid biomass production (Parenti *et al*., 2021), being drought-tolerant, resistant to nematodes and other pests, and high germination rates, which in turn influence the yield and quality of dry matter produced (Wang *et al*., 2002; Kamireddy *et al*., 2013). Studies conducted on *C. juncea* show that, between 40 and 60 days of development, it presents 7 Mg ha^{-1} of dry matter per cutting, contributing up to 176.37 kg N ha^{-1} (Ríos-Hilario *et al*., 2022); in addition, the leaf contains 27 % protein (Almeida-Santos *et al*., 2019).

Chepil, chipil, chipile, or chipilín (*Crotalaria longirostrata* Hook. & Arn.) is endemic to Central America, where it is consumed as a green leafy vegetable (Camarillo-Castillo and Mangan, 2020). In southern Mexico, it is used in traditional cooking (Chávez-Quiñones *et al*., 2009). It is an herbaceous species that develops annually during the rainy season and is collected wild; sometimes it is associated with the milpa or is semi-cultivated as a shrub. With agronomic management, it can be biannual or perennial. Chepil seeds have low germination rates ranging from 12.3 to 80 % (Rojas-García *et al*., 2021). Its leaves have a high protein content ranging from 30.6 to 38.3 % (Arias *et al*., 2003; Jiménez-Aguilar and Grusak, 2015), making it as high in quality as alfalfa; however, there are few studies on dry matter yield, physical and chemical quality (Pérez-Cornelio *et al*., 2016).

The chepil could be considered the alfalfa of the tropics, and it could be used in animal feed, added mainly in the diet in the form of meal, green, or silage. It is drought tolerant, provides organic matter in poor soils, and its seeds are resistant to fire (Rojas-García *et al*., 2021), which makes it an alternative species for the dry season. The objective of this research was to evaluate the yield and chemical quality of chepil (*Crotalaria longirostrata* Hook. & Arn) at different planting densities and cutting frequencies to obtain the optimum harvest time.

MATERIALS AND METHODS

The research was carried out in experimental plots and in the Animal Nutrition laboratory of the Faculty of Veterinary Medicine No. 2 of the Autonomous University of Guerrero, located in Cuajinicuilapa, Guerrero, Mexico (16° 28′ 28′′ N, 98° 25′ 11.27′′ W, at 46 m altitude). The climate of the region is classified as Aw and is known as dry tropic (García, 2004). Temperature and precipitation data were obtained from the Conagua agro-meteorological station, which was located 2 km from the experimental plots. During the study period, the average annual temperature was 27.5 °C, and the total precipitation was 668 mm.

Mature chepil (*C. longirostrata*) pods were collected from wild plants from January to May 2020. Planting took place on June 1st of the same year in 3 L polystyrene (unicel) trays. A 50:50 v/v mixture of composted manure and river soil was used as substrate; 2 kg of substrate were placed in each tray, then 60 g of seeds were sprinkled, covered approximately 0.2 cm with the same substrate, and irrigated to field capacity, repeating the irrigations every two days. A total of 10 trays were sown. Thirty days after planting, when the plants were 15 to 20 cm tall, they were transplanted into the field. On June 29, the experimental plots were delimited and transplanted, with the treatments being: 200 000, 100 000, and 50 000 plants ha^{-1}.

Each plot measured 3 x 3 m, and one plot was established for each week of evaluation (10 plots), in a completely randomized design with three replications. To obtain each density, the spacing between plants was 10, 20, and 30 cm, and 50 cm between rows. Weed control was done with the help of a hoe whenever the crop required it. No fertilizers or agrochemicals were used, and drip irrigation was used every third day. A uniformity cut was made 50 days after transplanting, leaving a height of 40 cm. The variables were evaluated from 15 days after the uniformity cut, with intervals of one week until week 10 or the beginning of flowering, with sampling for forage yield at 15, 22, 29, 36, 43, 50, 57, 64, 71, and 79 days after cutting; while for bromatological composition, sampling was carried out every two weeks: 15, 29, 43, 57, and 71 days after cutting.

Variables evaluated

Total dry matter, leaf and stem yields

Two random destructive samplings were carried out in one linear meter. For this purpose, the forage was cut, leaving a remaining height of 40 cm in each experimental plot. Of the total yield sample, 20 % was obtained by separating its morphological components (leaf and stem). These were placed in paper bags, labeled, and placed in an electric oven (Faithfull, WGLL-230BE, Cangzhou, China) at a temperature of 55 °C, until constant weight.

Plant height

In each experimental plot, twenty heights were measured with a ruler graduated in centimeters, using the highest morphological component as a reference.

Intercepted radiation

Five measurements of intercepted radiation were taken randomly one day before each cutting, using the wood ruler method, by placing a graduated ruler (100 cm) on the ground in the middle of the experimental plots, from furrow to furrow, below the plant canopy (Maldonado-Peralta *et al.*, 2019).

Growth rate

It was calculated using the data obtained in the total dry matter yield variable for each of the treatments evaluated, using the following formula:

$$TC = R / T$$

where TC = seasonal average growth rate (kg DM ha^{-1} d^{-1}); R = seasonal yield (kg DM ha^{-1}), and T = days elapsed from one cutting to another.

Leaf:stem ratio

It was obtained from the morphological composition, by dividing the dry leaf component by the stem.

Chemical composition

It was determined using the AOAC (2005) methods: MS (method 930.15), ash (method 942.05), and crude protein (PC; method 984.13). Neutral detergent fiber (FDN) and acid detergent fiber (FDA) were evaluated with the ANKOM Technology methodology according to van Soest *et al.* (1991).

Statistical analysis

The experimental design was completely randomized with three replications. Data were analyzed using PROC GLM (SAS, 2011), with the effects of planting density and cutting frequency held constant. Treatments means were compared using Tukey's test ($\alpha = p < 0.05$).

RESULTS AND DISCUSSION

Chepil total dry matter yield showed statistical differences between planting density and cutting stages ($p < 0.05$) (Figure 1). For the 64-day cutting frequency, the three densities produced the highest yield, with 3406, 3500, and 4200 kg MS ha^{-1} for 200 000, 100 000, and 50 000 plants ha^{-1}, respectively ($p < 0.05$). Subsequently, due to the loss of the lower leaves of the plant canopy caused by senescence or the onset of flowering, they tended to decrease. Another trend in yield behavior is that chepil develops stem bifurcation on day 34 after planting, and there is inter-specific competition for water, light, and nutrient resources.

Alonzo-Griffith and Paniagua-Alcaraz (2010) reported lower averages of 1401 kg MS ha^{-1} at 128 days after sowing in alfalfa when evaluating different calcareous doses. In turn, Godoy-Espinoza *et al.* (2012) obtained higher values than those of this research in *Arachis pintoi*, with 7084 and 12 480 kg MS ha^{-1} at 60 and 75 days of regrowth age, respectively. Other researchers, such as Romero *et al*. (2013), report lower values for the legume clitoria (*Clitoria ternatea*) with 1800 kg MS ha^{-1}. Rojas-García *et al.* (2017) reported higher yields of alfalfa (*Medicago sativa L.*) varieties in the summer season, with annual average of 6246 kg MS ha^{-1}. Lagunes-Rivera *et al*. (2019) report an average value similar to this research, with 2668 kg MS ha^{-1} in different legumes.

For the leaf component yield, it was observed that the density with the highest percentage of leaves was 50 000 plants ha^{-1} with 38.13 %, followed by 100 000 and 200 000 plants ha^{-1} with 37.76 and 37.19 %. From days 15 to 36, the three densities had the highest leaf percentage, with values ranging between 60 and 40 % ($p < 0.05$). The opposite occurred in the stem component, with higher yields after a cutting frequency

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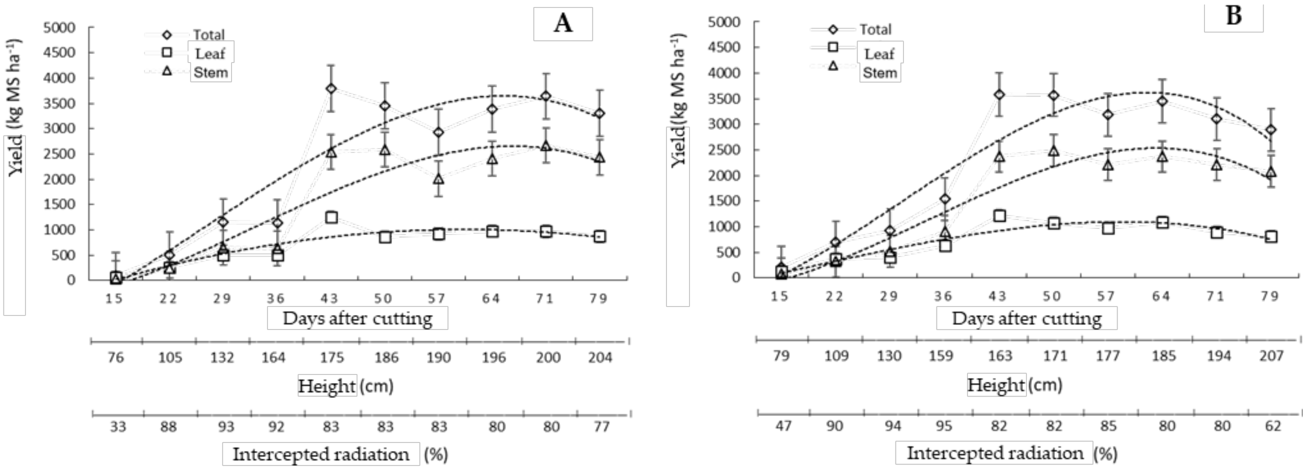

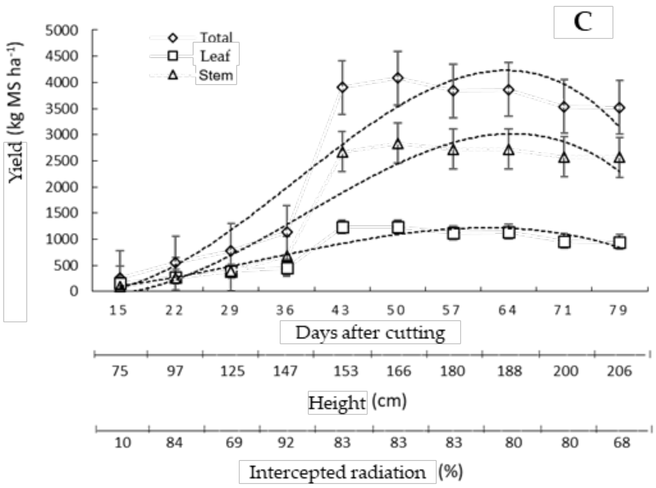

Figure 1. Total, leaf, and stem yield (kg MS ha^{-1}), plant height (cm), and intercepted radiation (%) of chepil. A: sown at 200 000 plants ha^{-1}; B: sown at 100 000 plants ha^{-1}, C: sown at 50 000 plants ha^{-1}.

of 64 days, with an average of 73 % stem in the three planting densities ($p < 0.05$). In turn, Rojas-García *et al.* (2017) reported alfalfa (*Medicago sativa* L.) yield and obtained an average of 45 % leaves at the 29-day cutting frequency, which is comparable to the values obtained in this study.

Regarding the height of the chepil grassland at different sowing densities and cutting stages (Figure 1), at the 43 to the 79-day cutting frequencies, the plants presented greater height (163 to 203 cm) independently of the sowing density ($p < 0.05$); however, at a density of 200 000 plants ha^{-1}, it was observed that the greatest average height was 163 cm, while at a sowing density of 50 000 plants ha^{-1}, the lowest was 154 cm ($p < 0.05$). The higher plant growth at these densities can be attributed to interspecific competition for light, which increased plant height at the densest planting density. On the other hand, Pincay-Ganchozo *et al.* (2021) used different scarification methods on *Clitoria ternatea* and reported plants with a height of 79 cm when cut at 75 days. Sosa-

Rubio *et al*. (2008) reported, in tropical legumes, a relationship between plant height and dry matter yield as observed in this trial.

The intercepted radiation is observed as plant density and cutting stages vary (Figure 1). The planting density of 100 000 plants ha^{-1} intercepted 95 % of incident radiation at the 36-day mowing frequency and subsequently decreased to 62 % at the 79-day mowing frequency (p < 0.05). The planting densities with the highest average intercepted radiation were 100 000 and 200 000 plants ha^{-1}, with 80 and 79 %, respectively. The density of 50 000 plants ha^{-1} was the one with the lowest average intercepted radiation percentage, with a value of 73 %. This behavior is possibly due to the loss of lower leaves from the plant canopy, plant maturity, and competition with other plants (Maldonado-Peralta *et al*., 2019). Several researchers report an optimum intercepted radiation of 95 %, indicating that this is when the best structural characteristics of legume grassland are found (Rojas-García *et al*., 2017). On the other hand, Alonzo-Griffith and Paniagua-Alcaraz (2010) report similar values of legume intercepted radiation, with an average of 90 %.

Chepil growth rates vary depending on planting density and cutting stage (Table 1). Statistical differences were found in plant densities (p < 0.05). The density of 50 000 plants ha^{-1} produced 65 kg MS ha^{-1} d^{-1}, while the densities of 200 000 and 100 000 plants ha^{-1} showed a lower growth rate with 59 kg MS ha^{-1} d^{-1}. From the 15 to the 36-day mowing frequencies, all three planting densities showed slow growth, which increased at the 43-day mowing frequency and then decreased as the experimental period continued. This behavior is possibly due to the recovery process when performing the uniformization cutting, which leaves a remaining plant height of

Table 1. Growth rate (kg MS ha^{-1} d^{-1}) in chepil (*Crotalaria longirostrata*), grown at different planting densities and cutting frequencies.

	Planting density (plants ha^{-1})			
Age of plants	T1: 200 000	T2: 100 000	T3: 50 000	Average
15	6 F c	14 E b	17 F a	12 F
22	29 E b	32 DE a	25 E c	29 E
29	40 CD a	32 DE b	27 E c	33 DE
36	32 DE a	43 D a	31 E a	35 D
43	88 A ab	83 A b	91 A a	87 A
50	69 B b	71 B b	82 B a	74 B
57	51 C b	56 C b	67 C a	58 C
64	53 C b	54 C b	60 C a	56 C
71	52 C a	44 D b	50 D a	49 CD
79	42 D ab	37 DE b	45 D a	41 D
Average	59 b	59 b	65a	

ABC: Means with the same uppercase literal in the same column are not statistically different (p > 0.05); abc: means with the same lowercase literal in the same row are not statistically different (p > 0.05).

40 cm. On the other hand, the highest growth rate was observed at the 43-day cutting frequency at a planting density of 50 000 plants ha^{-1} with 91 kg MS ha^{-1} (p < 0.05). Sánchez-Santillán *et al*. (2019) reported a growth rate of 25 kg MS ha^{-1} d^{-1} in winter and 70 kg MS ha^{-1} d^{-1} in summer in alfalfa at 45 days of regrowth; which is similar to the results reported in this study.

Regarding the leaf:stem ratio of chepil when varying planting density and cutting frequency (Table 2), statistical differences were found between cutting frequency and planting density (p < 0.05). The leaf:stem ratio was higher in 50 000 and 100 000 plants ha^{-1} with a value of 0.70; the density of 200 000 plants ha^{-1} had the lowest percentage, with a value of 0.68 (p < 0.05). For both the 15 and 22-day cutting frequencies, the three densities presented the highest leaf:stem ratios, with 1.61 and 1.18, respectively, while the density of 50 000 plants ha^{-1} at frequency 15 had the highest leaf:stem ratio, with 1.67 (p < 0.05), and the 71 and 79-day cutting stages presented the lowest leaf:stem ratios. This is possibly due to the loss of lower leaves from the plant canopy caused by senescence or the onset of flowering. Rojas-García *et al.* (2017) reported similar values for the leaf:stem ratio of alfalfa varieties in the summer and autumn seasons (0.92 and 1.46, respectively). Other researchers, such as Lagunes-Rivera *et al*. (2019), observed values of 1.36 leaf:stem ratio at the 15 and 22-day cutting stages.

Table 2. Leaf:stem ratio in cultivated chepil (*Crotalaria longirostrata*) at different planting densities and cutting frequencies.

	Planting density (plants ha^{-1})			
Age of plants	T1: 200 000	T2: 100 000	T3: 50 000	Average
15	1.58 A b	1.57 A b	1.67 A a	1.61 A
22	1.17 A b	1.20 A a	1.16 A b	1.18 B
29	0.79 B b	0.82 B b	1.04 B a	0.88 C
36	0.80 B a	0.69 C b	0.67 C b	0.72 C
43	0.49 C b	0.52 C a	0.47 D b	0.49 D
50	0.34 C b	0.44 D a	0.44 D a	0.41 D
57	0.46 C a	0.45 E a	0.41 D b	0.44 D
64	0.41 C b	0.46 E a	0.42 D b	0.43 D
71	0.37 C b	0.41 E a	0.38 E b	0.39 E
79	0.36 C c	0.39 F a	0.37 E b	0.37 E
Average	0.68 b	0.70a	0.70a	

ABC: Means with the same uppercase literal in the same column are not statistically different (p > 0.05); abc: means with the same lowercase literal in the same row are not statistically different (p > 0.05).

For the percentage of crude protein, acid detergent fiber, and neutral detergent fiber (Table 3), the planting density with the highest average crude protein was 200 000 plants ha^{-1}, with 24 %, while the densities of 100 000 and 50 000 plants ha^{-1} had the lowest percentage, with 23 % (p < 0.05). Regardless of plant density, crude protein

Table 3. Percentage of crude protein, neutral detergent fiber, and acid detergent fiber of chepil (*Crotalaria longirostrata*) grown at different planting densities and cutting frequencies.

Age of plant	Planting density (plants ha^{-1}) Crude protein (%) T1: 200 000	T2: 100 000	T3: 50 000	Average
15	30 A a	29 A a	29 A a	29 A
29	28 A a	28 A a	26 AB b	27AB
43	25 B a	21 B b	20 B b	22 B
57	20 C a	19 B b	20 B a	20 B
71	19 C a	19 B a	19 B a	19 B
Average	24 a	23 b	23 b	
	Neutral detergent fiber (%)			
15	41 B b	42 B a	41 B b	41 C
29	48 B a	42 B b	48 B a	46 B
43	53 AB b	59 A a	62 A a	58 A
57	57 A ab	61 A a	62 A a	60 A
71	60 A a	60 A a	60 A a	60 A
Average	52 b	53 b	55 a	
	Acid detergent fiber (%)			
15	35 B a	36 AB ab	35 B a	35 B
29	38AB a	30 B b	29 C a	32 C
43	33 B a	37 AB a	41 AB a	37 AB
57	38 AB a	40 A a	42 A a	40 ª
71	40 A a	39 A a	44 A a	41 ª
Average	37 b	37 b	38 a	

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was highest at the youngest cutting frequencies of 15 and 22 days, averaging 29 to 27 %, respectively, while at the 71-day cutting frequency, the lowest percentage was observed at 17 % ($p < 0.05$). This could be due to the loss of lower canopy leaves and the increase of stems, which have a higher percentage of cellulose, hemicellulose, and lignin, reducing the quality of the plant. Several researchers (Alonzo-Griffith and Paniagua-Alcaraz, 2010; Godoy-Espinoza *et al*., 2012; Romero *et al*., 2013; Portillo-López *et al*., 2019; Lagunes-Rivera *et al*., 2019) reported similar values in legumes, with protein percentages ranging from 28 to 14 % in the cutting stages from 30 to 75 days of age, respectively, with a behavior similar to that of this research; however, Balseca *et al*. (2015) reported an average of 8.26 % PC in legumes, a lower result than this study. On the other hand, the planting density with the highest percentage of neutral detergent fiber (Table 3) was 50 000 plants ha^{-1} with 55 % ($p < 0.05$); the density with the lowest percentage was 200 000 plants ha^{-1} with 52 %. Regarding cutting stages, days 43, 57, and 71 presented the highest percentage of FDN, with 58, 60, and 60, respectively ($p < 0.05$). Romero *et al.* (2013) and Portillo-López *et al.* (2019) conducted research on *Clitoria ternatea* and found lower percentages than this research, but with the same behavior of increasing as the time of FDN regrowth passes, of 25 and 40 %, at 30 and 60 days from sowing, respectively. Valles-de la Mora *et al.* (2014), in a study on *Cratylia argentea*, found an average value of 57.48 % FDN, while Lagunes-Rivera *et al.* (2019) reported 64.25 % FDN; however, Balseca *et al.* (2015) obtained a higher average of 71 %.

Similar behavior was observed in the acid detergent fiber (Table 3), where the planting density with the highest percentage was 50 000 plants ha^{-1}, with 38 % ($p < 0.05$), and the densities of 100 000 and 200 000 plants ha^{-1} had the lowest percentage, with an average of 37 %. The cutting stages with the highest and lowest percentages of acid detergent fiber were at 71 and 15 days, with 41 and 35 %, respectively. Romero *et al.* (2013) conducted research on *Clitoria ternatea* and found lower FDA percentages of 18, 25, and 25 % at 30, 60, and 75 days of plant age, respectively, which is similar to the behavior observed in this study. Valles-de la Mora *et al.* (2014), in their research on *Cratylia argentea* conducted during rainy season, found an average value of 37.35 % FDA, similar to this research. Portillo-López *et al.* (2019) found similar percentages of FDA, ranging from 30.1 to 38.2 % in white clover (*Trifolium repens* L.), red clover (*Trifolium pratense* L.), and vicia (*Vicia sativa* L.) during the low rainfall season; however, Lagunes-Rivera *et al.* (2019) reported higher average values of 42.25 %.

For the percentage of dry matter and ash (Table 4), in general, the planting density of 100 000 plants ha^{-1} obtained the highest average percentage of dry matter, with

Table 4. Percentage dry matter and ash of chepil (*Crotalaria longirostrata*) grown at different planting densities and cutting frequencies.

	Plant density (plants ha^{-1}) Dry matter (%)			
Age of plant	T1: 200 000	T2: 100 000	T3: 50 000	Average
15	17 B	18 B	17C	17 B
29	18 B	18 B	19 B	18 B
43	22 AB	24 A	23 A	23 A
57	22 AB	24 A	21 AB	22 AB
71	24 A	25 A	24 A	24 A
Average	21 b	22 a	21 b	
		Ash (%)		
15	6 AB b	6 AB a	6 A a	6 AB
29	7 A a	7 A a	6 A b	7ª
43	4 B b	7 A a	6 AB b	6 B
57	7 A a	5 B c	5 B b	6 B
71	5 AB a	5 B b	5 AB b	5 C
Average	6 a	6 a	6 a	

ABC: Means with the same uppercase literal in the same column are not statistically different ($p > 0.05$); abc: means with the same lowercase literal in the same row are not statistically different ($p > 0.05$).

22 %; while the densities of 200 000 and 50 000 plants ha^{-1} obtained lower values, with 21 % ($p < 0.05$). The highest percentage of MS was obtained at the 71-day cut-off frequency, while the 15-day cut-off frequency had the lowest value with 24 and 17 %, respectively ($p < 0.05$). In terms of ash content, statistical differences were found in cutting frequencies. A decrease was observed as cutting frequency increased, with the highest percentage on day 29 (7 %) and the lowest on day 71, with 5 %; however, no statistical differences were found in planting densities, with an average of 6 % ash ($p < 0.05$). Similar averages were reported by Fernández-Valeriano and Sánchez-Chávez (2017) in beans, with a value of 8 %.

CONCLUSIONS

The optimum cutting time for forage is when chepil is sown at a density of 100 000 plants ha^{-1} and harvested at a frequency of 36 days after the uniformity cut, with 95 % intercepted radiation. At this stage it presents better quality, yield, and protein characteristics. It is recommended that further research be conducted using different remaining heights, evaluating them at different times of the year and with more harvesting time, as well as analyzing weight gain in domestic animals.

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