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RESEARCH ARTICLE

Nutritional Yield and Composition of Spiny and Spineless Varieties of Safflower (*Carthamus tinctorius* L.) Forage Harvested at Four Phenological Stages

Xochilt Militza Ochoa-Espinoza^{1,2}, David Guadalupe Reta-Sánchez^{3,*}, Pedro Cano-Ríos², Juan Isidro Sánchez-Duarte⁴, Esmeralda Ochoa-Martínez⁴, José Eduardo García-Martínez⁵ and Arturo Reyes-González⁴

¹Campo Experimental Norman E. Borlaug, Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias. Cd. Obregón, Sonora, México

²Universidad Autónoma Agraria Antonio Narro-Unidad Laguna. Torreón, Coahuila, México

³Campo Experimental Delicias, Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias. Delicias, Chihuahua, México

⁴Campo Experimental La Laguna, Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias. Matamoros, Coahuila, México

⁵Universidad Autónoma Agraria Antonio Narro. Saltillo, Coahuila, México

Abstract:

Background:

Delaying harvesting in spineless safflower (*Carthamus tinctorius* L.) can increase dry matter (DM) yield, maintaining an acceptable nutritional composition.

Objective:

The objective of this study was to compare the forage potential of spineless safflower cultivars with that of spiny cultivars harvested in four phenological stages.

Methods:

The research was carried out during the 2017-2018 and 2018-2019 cycles in Matamoros, Coahuila, Mexico. Two spineless (CD868 and Selkino) and two spiny (Gila and Guayalejo) cultivars were evaluated. The phenological stages were: beginning of capitulum formation (E50), capitulum clearly separated from the younger leaves (E55), distinguishable medium and intermediate external bracts (E59), and beginning of flowering (E61). A randomized complete blocks design was used with four replications in a 4 × 4 factorial arrangement of treatments.

Results:

No interactions were found between phenological stages and cultivars. The spineless cultivars showed better or equal nutritional composition when compared to spiny cultivars but with better forage in E50. Yields of DM and nutrients increased when harvesting was delayed from E50 to E61, maintaining an acceptable nutritional composition. The highest yields of DM (10816 kg ha⁻¹), crude protein (CP) (2071 kg ha⁻¹), net energy for lactation (NE_L) (52978 MJ ha⁻¹ DM), and digestible DM (6350 kg ha⁻¹) occurred in E61.

Conclusion:

Spineless cultivars harvested at stage E61 increased the forage potential with regards to the spiny cultivars harvested in E55, which did not have fully developed spines, due to their higher yields of DM (58%), CP (29%), NE_L (39%), and digestible DM (41%).

Keywords: Nutrient concentration, Dry matter, Forage species, Energy, Digestibility, Spineless safflower.

Article History

Received: July 24, 2021

Revised: November 29, 2021

Accepted: December 14, 2021

1. INTRODUCTION

Safflower (*Carthamus tinctorius* L.) is one of humanity's oldest crops. It is cultivated mainly in India for the production

of oil from its seeds and a reddish dye from its flowers [1, 2]. Safflower seeds and paste can also be used for feeding animals [3, 4]. Safflower forage may be grazed directly by the cattle, or it may be stored as hay or silage for feeding ruminants [5, 6]. It may become very appealing for these types of animals [4]. However, since the safflower produces spines on the leaves and inflorescences, its consumption by animals may be restricted [7]. This problem of forage consumption may worsen as the plant incrementally grows and matures.

* Address correspondence to this author at the Campo Experimental Delicias, Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, km. 2 Carretera Delicias-Rosales. C.P. 33000. Centro, Cd. Delicias, Chihuahua, Mexico; Tel: 8000882222; E-mail: dretasan@yahoo.com, reta.david@inifap.gob.mx

A practical strategy to avoid the presence of spines on the safflower forage is to harvest the plant during the budding stage, just when the spines have not yet fully developed. Nevertheless, research studies carried out in northern Mexico demonstrated that dry matter (DM) potential yield was low in harvesting safflower at the beginning stages of capitulum formation (E50) (5143 kg ha⁻¹) and capitulum clearly separated from the younger leaves (E55) (7750 kg ha⁻¹) [8 - 10].

A way to avoid the rejection of the safflower forage due to its spines in the nutrition of ruminants is to use spineless safflower varieties. These cultivars have been developed mainly to allow easier manual harvesting of the seeds and flowers [11]. This characteristic of the spineless safflower constitutes a great advantage when used as forage for animals. Spineless safflower forage has been preserved with good nutritional quality as silage [12], and excellent results have been obtained when included as hay and silage in rations for dry and dairy-producing cows [7]. Another advantage of the spineless safflower cultivars is that forage may be harvested at a more advanced phenological stage due to the absence of spines, which allows an increase in forage DM yield. Although the information has been generated concerning the use of spineless safflower as forage, there is little evidence with regard to its forage DM yield and its nutritional composition in comparison to spiny safflower cultivars harvested in different phenological phases. The objective of this study was to compare two cultivars of spineless safflower forage with two spiny cultivars of safflower forage harvested in four phenological stages regarding the nutritional composition and forage DM and nutrients yield. The hypothesis of the study was that delaying the harvest from the beginning of capitulum formation (E50) until the beginning of flowering (E61) in spineless safflower cultivars would increase forage DM and nutrients yield when compared to spiny safflower cultivars harvested between stages E50 and E55 while maintaining an acceptable nutritional composition.

2. MATERIALS AND METHODS

2.1. Location of the Experiment

The experiments were carried out during the production cycles of autumn-winter 2017/2018 and 2018/2019 at the La Laguna Experimental Station in the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), located in Matamoros, Coahuila, Mexico (25° 32' N, 103° 14' O and 1150 m above sea level). The soil at the experimental site was a loamy-clayed texture, with a depth greater than 1.8 m. The availability of water was 150 mm m⁻¹ [13], the organic carbon content was 0.75%, and the pH was 8.14 [14].

2.2. Treatments Assessed

The research consisted of the comparison between the behavior of four safflower cultivars harvested in four phenological stages under a randomized complete blocks design with four repetitions in a 4 × 4 factorial arrangement (64 experimental plots). Factor A was the cultivars, and Factor B was the phenological stage. The cultivars were: CD868, Selkino, Gila, and Guayalejo, with the first two being spineless and the other two spiny; all of them were developed by

INIFAP. The phenological stages during harvest were: (E): beginning of capitulum formation (E50), capitulum clearly separated from the younger leaves (E55), distinguishable medium and intermediate external bracts (E59), and beginning of flowering (E61) [15]. The harvest was made 76, 84, 92, and 100 days after sowing (das) in E50, E55, E59, and E61, respectively. All cultivars were harvested simultaneously at each harvest date.

2.3. Soil Preparation

Seedbed preparation was performed through disk plough at a depth of 0.30 m, followed by double disking and zero-slope levelling. Nitrogen and phosphorus fertilizer dose was calculated considering the safflower extraction capacity: 250 kg N ha⁻¹ and 80 kg P₂O₅ ha⁻¹. The nitrogen source was urea (46% N), and monoammonium phosphate (52% P₂O₅) was used as the phosphorus resource. For the latter, the full dosage was applied during sowing, and N was distributed by 20%, 40%, and 40% during sowing and during the first and second irrigations, respectively. Soil preparation and fertilization were the same for both growth cycles. No potassium fertilizer application was made because soils in this region have high potassium content, with average values of 3030 kg ha⁻¹ at a depth of 0.30 m [14].

2.4. Sowing and Agronomic Handling of the Crop

The sowing was made by hand on dry soil on December 13, 2017 (Experiment 1) and on December 13, 2018 (Experiment 2) on a total surface of 2240 m². The seeding rate was 50 kg ha⁻¹ with germination of 85%. Each experimental plot was set in 12 rows, each 10 m long with a separation between lines of 18 cm (21.6 m²). The useful plot was 5 m long on the 10 central furrows (9 m²). Plants were subsequently thinned to leave a 160 m⁻² plant population density. On the same sowing date, a 150 mm irrigation depth was applied. During the cycle, three irrigations were made (37, 61, and 83 das), with an irrigation depth of 130 mm. The phenological stages when irrigation was applied after sowing were: rosette, elongation of the stem, and the initial formation of the capitulum. Weeds were controlled by hand with a hoe.

2.5. Response Variables

The yields for fresh forage and DM were determined at harvest. The content of DM was determined from a 0.4 m² sample randomly taken from the useful plot. For this purpose, a 0.74 m sample was taken from three of the central furrows of each plot. The sampled plants were weighted fresh, then pre-drying was performed for five days under the protection of a greenhouse, after which the samples were dried at 65 °C in a forced-air oven for 48 to 72 hours until a constant weight was attained [16, 17]. The DM yield was estimated by multiplying the fresh forage yield by the percent of DM forage in each useful plot. Leaf area index (LAI) was measured using AccuPAR model Lp-80 PAR/LAI Ceptometer (Decagon Devices, Inc., Pullman, WA, USA). The LAI measurements were collected before each one of the four harvest treatments. Three readings per plot were taken between 1200 and 1400 h solar time. Three measurements were made above and the other

three below the canopy, parallel to the ground surface. The probe was positioned at a 45° angle with respect to rows. Plant height measured from ground level to plant apex of 10 random plants within each plot was determined before harvest. The plants sampled to estimate the DM content were used to analyze the nutritional value of the forage. The dry forage samples were ground in a Wiley® mill (Thomas Scientific, Swedesboro, NJ, USA) with a 1 mm mesh. The nitrogen content of each sample was determined using the Dumas Combustion Method number 990,03 of AOAC using Thermo Scientific Flash 2000 equipment, and the result was multiplied by 6.5 to obtain the percent of crude protein (CP) [18]. The Neutral Detergent Fiber (NDF) and the Acid Detergent Fiber (ADF) were analyzed in accordance with Goering and Van Soest [19]. The “*in vitro*” DM digestibility (IVDMD) was

obtained from a ground dry forage sample placed in a Daisy incubator [20]. The content of net energy for lactation (NE_L) was obtained following the methodology proposed by Weiss *et al.* [21]. Yields of CP (CPY) and NE_L (NE_LY) per hectare were obtained by multiplying the contents of CP and NE_L by the DM yield per hectare estimated for each experimental plot. Digestible dry matter yield (DDMY) per hectare was obtained by multiplying IVDMD of forage by the DM yield per hectare.

2.6. Climate during Growing Seasons

Prevailing weather conditions for both years and the average values of 30 years during the same growing period are shown in Fig. (1). Meteorological data were obtained from a weather station located at the experimental site.

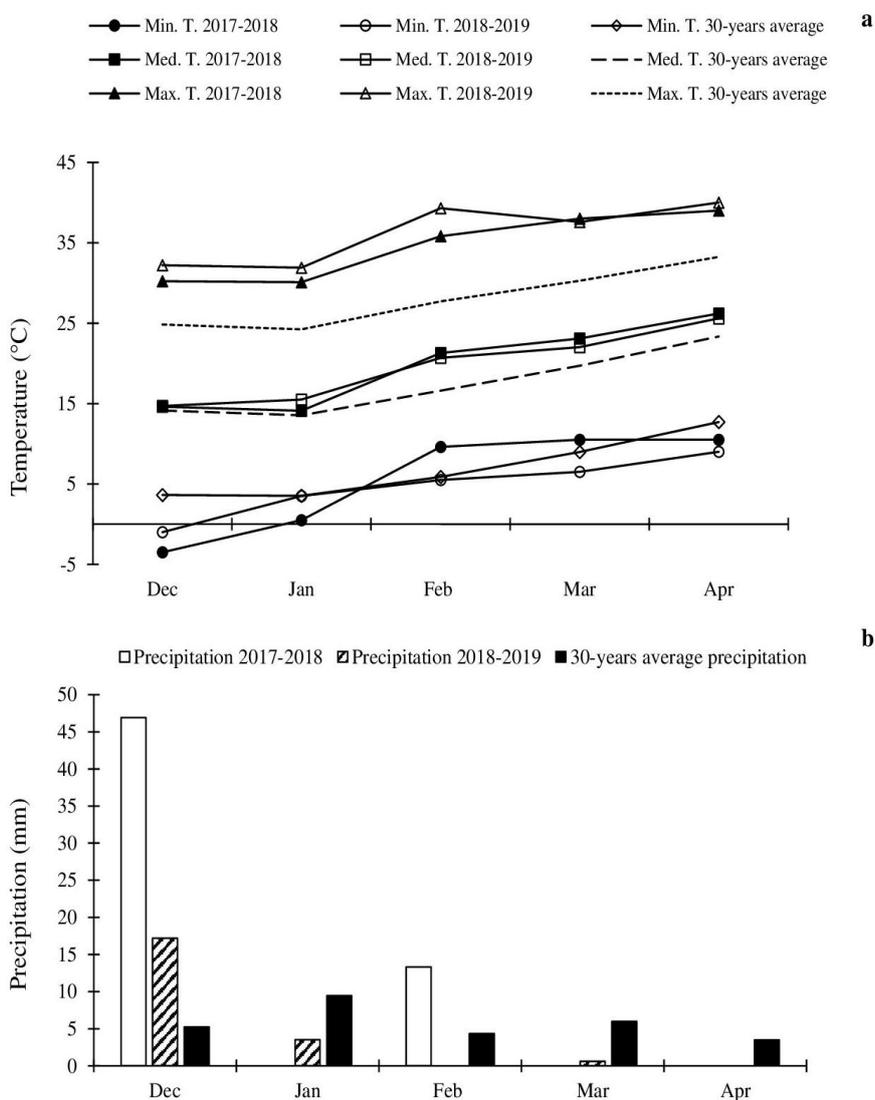


Fig. (1). Monthly temperature (a) and precipitation (b) during the safflower growing season in the two years of study and the average values of 30 years (1990-2019) at the La Laguna Experimental Station, Mexico.

Table 1. Combined analysis of variance and significance for nutritional composition and nutrient yields variables of four safflower cultivars harvested in four growth stages during the 2017-2018 and 2018-2019 growing seasons.

Effect	DMY (kg ha ⁻¹)	CP (g kg ⁻¹)	CPY (kg ha ⁻¹)	NDF (g kg ⁻¹)	ADF (g kg ⁻¹)	IVDMD (g kg ⁻¹)	NE _L (MJ kg ⁻¹ DM)	NE _L Y (MJ ha ⁻¹)	DDMY (kg ha ⁻¹)
Cultivar (C)	0.0001	0.0001	0.0001	0.5527	0.9270	0.0036	0.0031	0.0003	0.0823
Phenological Stage (E)	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
E × C	0.3094	0.8984	0.3522	0.1837	0.0574	0.1243	0.1582	0.2828	0.5114

Yield (Y); Dry Matter (DM); Crude Protein (CP); Neutral Detergent Fiber (NDF); Acid Detergent Fiber (ADF); In-vitro DM digestibility (IVDMD); Net Energy for Lactation (NE_L); Digestible DM (DDM).

Table 2. Nutrient yields and nutritional composition of four safflower cultivars harvested in four phenological stages in the growing seasons 2017-2018 and 2018-2019.

Variables	CD868	Gila	Guayalejo	Selkino	SE	P value
Nutrient yields (kg ha⁻¹)						
DMY [†]	8124 a	7970 a	7969 a	6341 b	1075	0.0001
CPY	1735 a	1715 a	1785 a	1487 b	119	0.0001
NE _L Y (MJ ha ⁻¹)	41783 a	42589 a	41800 a	35293 b	4513	0.0003
DDMY	4877 a	4788 a	5041 a	4474 a	574	0.0823
Nutritional composition (g kg⁻¹DM)						
CP	222 b	226 b	233 ab	247 a	19.1	0.0001
NDF	477 a	467 a	474 a	475 a	15.6	0.5527
ADF	389 a	386 a	388 a	390 a	27.8	0.9270
IVDMD	627 b	650 ab	640 b	674 a	18.0	0.0036
NE _L (MJ kg ⁻¹ DM)	5.23 b	5.48 ab	5.36 b	5.73 a	0.201	0.0031

Y (yield); Dry Matter (DM); Crude Protein (CP); Neutral Detergent Fiber (NDF); Acid Detergent Fiber (ADF); In-vitro DM Digestibility (IVDMD); Net Energy for Lactation (NE_L). Mean values in the same column followed by the same letter are not significantly different (Tukey P ≤ 0.05). SE: Standard Error.

2.7. Data Analysis

The data obtained from this study were analyzed by using PROC MIXED from SAS version 9.3 [22]. A combined analysis of the data was performed using a randomized complete blocks design with four repetitions in a 4 × 4 factorial arrangement. Levene homogeneity of variances test was used to test the homogeneity of the variance, and it was found that the variances were homogeneous (P > 0.05). The analysis considered the block and the year as random variables, while the treatments were considered as fixed variables. Yields of DM, CP, NE_L, DDM, and concentrations of CP, NDF, ADF, and IVDMD were considered as fixed variables. The Tukey Kramer test was used at a level of P ≤ 0.05 to verify measurements. Also, a linear regression analysis was performed (P < 0.05) in order to determine the relationship between NDF and IVDMD with CP, NE_L, and IVDMD in the forage.

3. RESULTS AND DISCUSSION

3.1. General Evaluation of Statistical Analysis

The combined analysis of the data using the year as a random effect shows no interaction between phenological stages and cultivars in all evaluated variables (P > 0.05) (Table 1). Then, the interaction was removed from the final model. The nutritional composition and the nutritional yield of safflower were affected by both the phenological stages and the cultivars significantly (P < 0.05) (Table 1).

3.2. Nutritional Composition and Energy Contribution of Forage

The spineless cultivars Selkino and CD868 showed better or equal nutritional composition than the one observed for the spiny cultivars Gila and Guayalejo. All cultivars showed similar content of ADF and NDF. Regarding CP concentration, the better values occurred in Selkino and Guayalejo, while Selkino and Gila were outstanding in NE_L and IVDMD concentrations (Table 2). These differences between safflower cultivars were not observed in a study by Reta *et al.* [23], where a higher ADF concentration was reported in CD868 cultivar (372 g kg⁻¹) than that observed in the Selkino cultivar (341 g kg⁻¹).

Crude protein concentrations in all safflower cultivars (222 to 247 g kg⁻¹) were higher than those observed (119 g kg⁻¹ to 156 g kg⁻¹) in other studies, where spiny and spineless cultivars were evaluated [7, 12, 23 - 25]. Other chemical composition parameters of forage such as NDF (467 g kg⁻¹ to 477 g kg⁻¹), ADF (339 g kg⁻¹ to 390 g kg⁻¹), and NE_L (5.23 MJ kg⁻¹ to 5.73 MJ kg⁻¹ DM) were similar to those found in other studies, with values of NDF from 421 g kg⁻¹ to 462 g kg⁻¹, 331 g kg⁻¹ to 374 g kg⁻¹ of ADF [7, 12, 23 - 26] and from 5.15 MJ kg⁻¹ to 5.52 MJ kg⁻¹ DM of NE_L by Reta *et al.* [23]. Also, the levels of IVDMD observed in the current research (640 g kg⁻¹ to 674 g kg⁻¹) were similar to those observed by Landau *et al.* [7] and Ochoa-Espinoza *et al.* [24] in a spineless cultivar. Considering the nutrient composition of safflower, Landau *et al.* [7] (2004) indicated that forage of this crop could be used as silage for producing dairy cows.

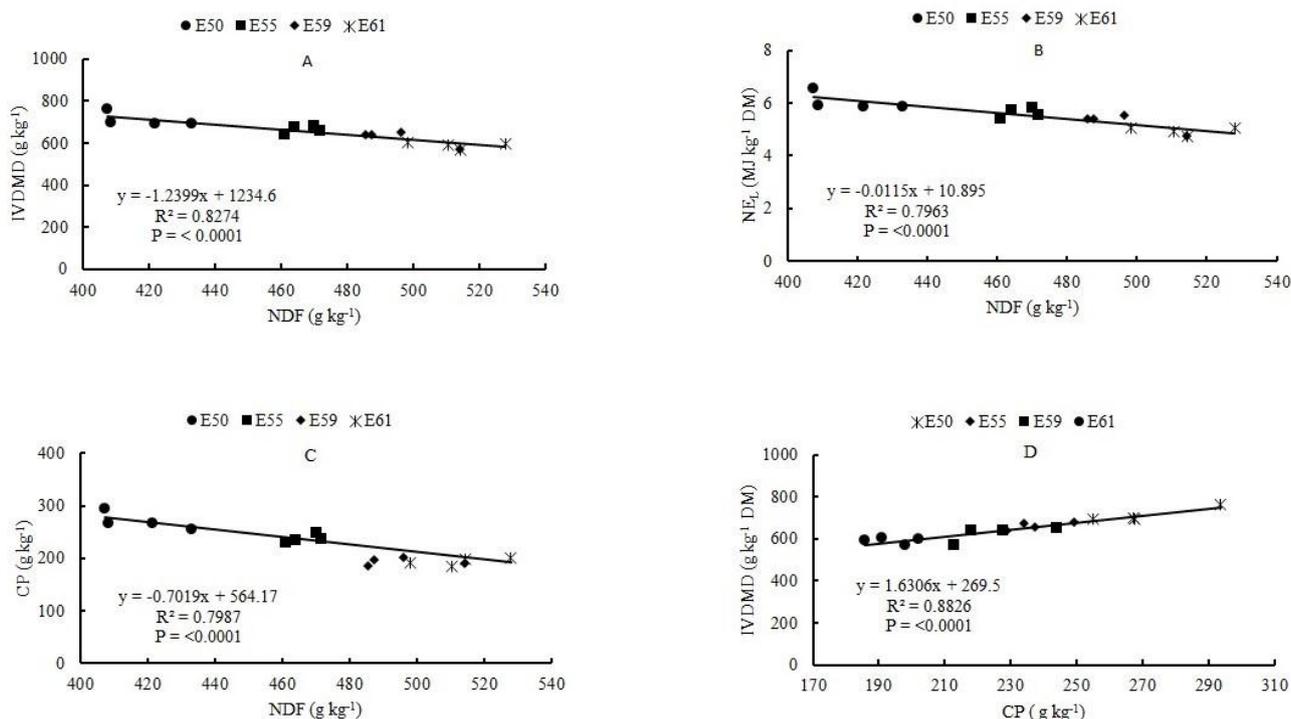


Fig. (2). Relationship between Neutral Detergent Fiber (NDF) and In-Vitro Dry Matter Digestibility (IVDMD) with Protein Content (CP), Net Energy for Lactation (NE_L), and IVDMD in the forage of four safflower cultivars harvested in four phenological stages (E) in the growing seasons 2017-2018 and 2018-2019.

Table 3. Forage nutritional composition and nutrient yields of four safflower cultivars harvested in four phenological stages (E) in the growing seasons 2017-2018 and 2018-2019.

Variables	E50 [†]	E55	E59	E61	SE*	P value
Nutrients yield (kg ha ⁻¹)						
DM [†]	4461 d	6835 c	8292 b	10816 a	1075	0.0001
CP	1190 d	1607 c	1853 b	2071 a	119	0.0001
NE _L (MJ ha ⁻¹)	26702 d	38256 c	43526 b	52978 a	4513	0.0001
DDMY	3144 d	4519 c	5168 b	6350 a	574	0.0001
Nutritional composition (g kg ⁻¹ MS)						
CP	271 a	237 b	226 b	194 c	19.1	0.0001
NDF	418 c	467 b	496 a	513 a	15.6	0.0001
ADF	351 c	376 b	410 a	416 a	27.8	0.0001
IVDMD	712 a	663 b	626 c	591 d	18.0	0.0001
NE _L (MJ kg ⁻¹ DM)	6.02 a	5.61 b	5.27 c	4.94 c	0.201	0.0001

Dry Matter (DM); Crude Protein (CP); Neutral Detergent Fiber (NDF); Acid Detergent Fiber (ADF); In-vitro DM Digestibility (IVDMD); Net Energy for Lactation (NE_L). Mean values in the same column followed by the same letter are not significantly different (Tukey P ≤ 0.05). Stage: Beginning of capitulum formation (E50), capitulum clearly separated from the younger leaves (E55), distinguishable medium and intermediate external bracts (E59), and beginning of flowering (E61). SE: Standard Error.

The spineless cultivar CD868 produced DM yields and nutrients similar to those obtained from the spiny cultivars Gila and Guayalejo, while the spineless cultivar Selkino had smaller yields of DM, CP, and NE_L than the aforementioned three cultivars. However, Selkino produced a similar DDMY to the other three cultivars (Table 2). Differences in nutrient yields between Selkino and the other cultivars were associated with variations in agronomic traits. It is important to note that Selkino had a lower plant height at harvest (93 cm) compared

with that achieved in the other cultivars (108 to 113 cm). Under this condition, Selkino was able to accumulate more DM in leaf (53%) and less in stem (47%) than the other three cultivars, where the accumulation of DM in the stem was 58 to 60% and in leaf from 40 to 42%. This behavior potentially decreased its DM yield but increased its IVDMD. Therefore, Selkino equaled the DDMY obtained by the other three cultivars. Landau *et al.* [7] reported a higher *in vitro* DM digestibility in leaves (729 g kg⁻¹) than in stems (546 g kg⁻¹) of safflower forage.

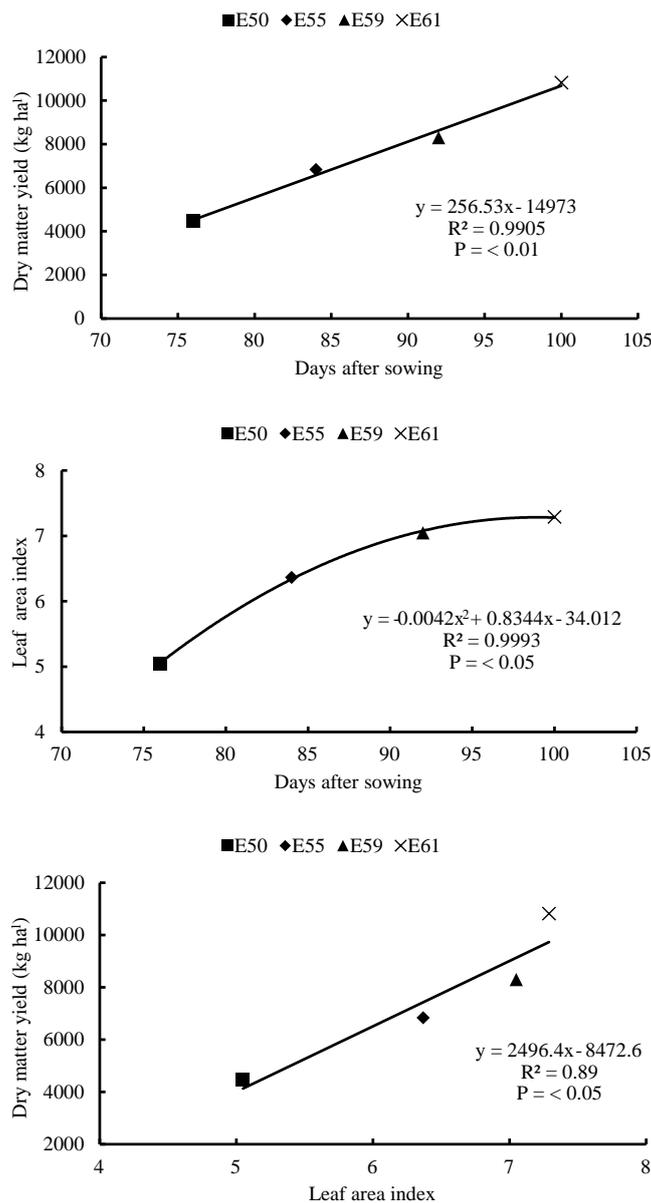


Fig. (3). Relationship between phenological stage (E), Leaf Area Index (LAI), and dry matter yield of four safflower cultivars harvested in four phenological stages in the growing seasons 2017-2018 and 2018-2019.

As the safflower harvest was carried out at a later phenological stage, the nutritional composition of the forage was less due to reductions in CP content and increases in fiber content. The parameter with a greater variation in the nutritional composition was CP (271 g kg⁻¹ to 194 g kg⁻¹), but its values were higher (152 g kg⁻¹ to 83 g kg⁻¹ DM) [23] or similar (272 g kg⁻¹ vs. 125 g kg⁻¹ DM) than those reported by others studies [26, 27]. Regarding fiber concentration, the values for NDF increased (P < 0.05) from 418 g kg⁻¹ to 513 g kg⁻¹, while the values of ADF increased from 351 g kg⁻¹ to 416 g kg⁻¹. This higher fiber content in the phenological stages with greater maturation was related to decreases in the content of CP, IVDMD (712 g kg⁻¹ to 591 g kg⁻¹), and NE_L (6.02 MJ kg⁻¹ to 4.94 MJ kg⁻¹ DM) in the forage (Fig. 2). In the last phenological stage evaluated in the study (E61), safflower

forage was characterized by contents of CP of 194 g kg⁻¹, NDF of 513 g kg⁻¹, ADF of 416 g kg⁻¹, IVDMD of 591 g kg⁻¹, and 4.94 MJ kg⁻¹ of NE_L. The nutrient composition of safflower forage is considered acceptable when compared to that obtained in other traditional forages such as oat harvested at the beginning of heading in north-central México. At the heading stage, oat forage is characterized by containing 112 g kg⁻¹ CP, 349 g kg⁻¹ ADF, 547 g kg⁻¹ NDF and 5.4 MJ kg⁻¹ DM of NE_L [28].

Regarding this response of the safflower, Corleto *et al.* [27] indicated that the decrease in CP content and the increase in fiber concentration was associated with a lesser leaf proportion and a larger stem in the biomass of the crop in the harvests that were carried out in the phenological stages with greater maturation. Another study states that the increase in

fiber in safflower forage in the phenological stages with greater maturation was due to the translocation of soluble cellular content from the leaves and stems to the seeds [26]. In other forages, the relation between the digestibility of the fiber and the nutritional composition of the forage showed a positive relationship between fiber digestibility and CP content [29, 30]. However, fiber digestibility has been negatively related to the concentrations of ADF and NDF in the forage [30, 31].

3.3. Dry Matter Yield, Nutrients, and Energy

Yields of DM, CP, NE_L , and DDM presented a linear response with significant increases at every phenological stage when the harvest was delayed from E50 to E61. The greater yields for DM (10816 kg ha⁻¹), CP (2071 kg ha⁻¹), NE_L (52978 MJ ha⁻¹) and DDM (6350 kg ha⁻¹) occurred ($P < 0.05$) at the phenological stage E61 (Table 3). Delaying harvesting at stage E59 increased DM yield up to 8292 kg ha⁻¹, while the harvest at stage E61 allowed for DM yield up to 10816 kg ha⁻¹. The linear increase of DM yield when safflower grows from E50 (70 das) to E50 (76 das) is clearly associated with the higher DM accumulation ratio (265 kg ha⁻¹ day⁻¹) at the physiological stage of E61 by the crop. Flemmer *et al.* [15] indicated that the growth rate of safflower is related to stem elongation besides the formation and growth of branches and inflorescences after the E50 stage.

Furthermore, as the plant grows, the expansion of the leaf area also increases, which improves the interception of solar radiation. The leaf area increment in the present study is in line with the quadratic response of LAI observed between the stages E50 and E61 (Fig. 3), which also influenced the increase of DM yield. Reta *et al.* [23] observed a similar DM yield in safflower (9336 kg ha⁻¹) harvested at the E61 phenological stage. In contrast, Cazzato *et al.* [25] reported a higher DM yield (11600 kg ha⁻¹) than the DM production found at E61 when safflower was harvested at the 25% flowering stage. The increase of nutrient yields when harvesting from E50 to E61 follows the same pattern as the increase of DM yield when harvesting at the same physiological stages. Therefore, the yields of CP, NE_L , and digestible DM are clearly explained by the effect of DM yield since the percentage of these nutrients in forage decreased as the harvest progressed from E50 to E61. This response allowed safflower to produce the highest nutrient yields at the E61 stage.

The null difference in the interaction cultivars × harvest age indicates that the greater yields of DM obtained in the study may be attained with spiny and spineless cultivars. However, when spiny cultivars are used, the highest forage yield free of spines is obtained during the phenological stage E50, since in later stages, the spines on the leaves and inflorescences are already present. The forage in phenological stage E55 may be considered acceptable since the spines present on the plant are not yet fully developed. This is important because due to the spiny nature of traditional safflower cultivars, farmers can decide whether or not to use this forage in the diet of dairy cattle since cows are more susceptible than goats and sheep to ulcerations of the mouth caused by spines present in traditional safflower forage [32].

The use of spineless safflowers allowed the production of forage free of spines until the last phenological stage (E61) evaluated in the study. Since the nutritional composition of the safflower forage maintained an acceptable level during the two last phenological stages (E59 and E61), the increase in DM yield at phenological stage E61 (58%) allowed the spineless safflowers to increase their yields by 29% for CP, by 39% for NE_L , and by 41% for digestible DM as compared to the yields of spiny safflower harvested during phenological stage E55, which is the latest stage when forage with undeveloped spines may be obtained.

CONCLUSION

Spineless safflower cultivars harvested at phenological stage E61 (beginning of flowering) showed a greater forage potential than spiny cultivars harvested at phenological stage E55 (capitulum clearly separated from the younger leaves), producing forage free of spines while maintaining an acceptable nutritional composition. Although the forage nutritional composition decreased when the harvest was delayed, DM yield increments observed counteracted losses in nutrient concentrations, allowing safflower to achieve the highest nutrient yields at the E61 stage. Therefore, spineless safflower cultivars could be a good forage alternative for the nutrition systems of ruminants due to their higher yields of DM (58%), CP (29%), NE_L (39%), and digestible DM (41%). Further research is needed to determine the best method for forage conservation of these cultivars and their potential effect on the productivity of ruminants.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No humans or animals were used in this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The data that support the findings of this study are available from the corresponding author, [D.G. R-. S.], on special request.

FUNDING

This research was financed by the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (México) and by the Universidad Autónoma Agraria Antonio Narro-Unidad Laguna (México).

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

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