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AGRO PRODUCTIVIDAD

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AGRO PRODUCTIVIDAD

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Analysis of water productivity and social impact on forage sorghum (*Sorghum bicolor* (L.) and maize (*Zea mays* L.) in the Comarca Lagunera, Mexico

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ABSTRACT

Objective: The purpose of the study was to compare the profitability, efficiency, and productivity indexes of water in forage sorghum (*Sorghum bicolor* (L.) Moench) and maize (*Zea mays* L.) production in the Comarca Lagunera in 2019.

Design/methodology/approach: Mathematical models were used to estimate the efficiency and productivity indexes of water used in sorghum and maize production. Data used in the mathematical models was obtained from official statistical sources.

Results: The water efficiency and physical productivity results were 226 L kg^{-1} and 4.42 kg m^{-3} and 221 L kg^{-1} and 4.52 kg m^{-3} for sorghum and maize, respectively. The efficiency and economic productivity indexes of water were 82.87 and 49.96 m^3 per USD profit and $12,067 and $20,018 USD profit per hm^3. Nevertheless, social efficiency was higher in maize (7.7 employments hm^{-3}) than sorghum (7.1 employments hm^{-3}), which required 8% more water to produce an employment unit.

Study Limitations/Implications: Models should be used to compare different productivity indexes of sorghum and maize in the Comarca Lagunera which has a shortage of water resources.

Findings/Conclusions: In the case of the Comarca Lagunera, maize was more productive and efficient than sorghum in physical, economic and social terms (employment creation); however, further research must take into consideration dynamic models.

Keywords: Water footprint, Forage production, Water, Efficiency, Productivity.

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INTRODUCTION

The scarcity of water resources impacts agricultural activities, reducing the crop yield potential (Reyes-González *et al.*, 2020). Maize is the most important crop in Mexico: it is the main food source for humans, but it is also an excellent livestock forage. In 2019,

Image by gawchar555 at Pixabay

the domestic production of forage maize reached 15,569,846.80 tons (t), generating an economic revenue of approximately $10.2 billion Mexican pesos ($10,198,617,458.29 pesos). Meanwhile, the production sorghum amounted to 3.3 million tons, reaching a domestic production value of 1.2 billion pesos (SADER, 2019). According to SIAP (2019), the Comarca Lagunera produced 12.53 and 25.34% of the total forage sorghum and maize production in the country (37,860.24 ha, 76.36% of which was used for forage maize and 23.63%, for forage sorghum). As a whole, the production amounted to 1.5 million tons of forage (9.73% of the domestic production), generating an overall economic revenue of more than $1.18 million pesos —79.3 and 20.87% was obtained from maize and sorghum, respectively (SIAP, 2019).

Agriculture —as the activity that consumes most water resources— demands strategic actions promoting increased efficiency in the use of water during the input-output transformation (Mancosu *et al.*, 2015). Conventional methodologies have failed to identify the problem: how to assess the water footprint with specific data. However, the use of mathematical models based on physical, economic, and social water footprints provides certainty about the origin of the data to be processed. An output that can be replicated in different settings is obtained (Ríos-Flores and Navarrete-Molina, 2019; Ríos-Flores *et al.*, 2018). Optimization models provide solutions to problems involving the maximization or minimization of an objective function with a system of strictly delimited equations (Ramírez-Barraza *et al.*, 2019).

Agricultural production should focus on improving yield per unit area (Perales-García *et al.*, 2019). Physical and monetary productivity indexes that can be used as eco-efficiency, yield, and environmental pressure indexes are useful tools for producers or decision-makers (Ríos-Flores *et al.*, 2014).

The Comarca Lagunera shows a high rate of productivity. However, since the water resource supply does not satisfy the demand, the region also suffers water scarcity, representing an allocation conflict between different users (Ramírez-Barraza *et al.*, 2019). It is estimated that more than 683.15 hm^3 are extracted from the subsoil in this area, while the average annual recharge only amounts to 534.10 hm^3 (Tovar-Triana, 2021). The purpose of this study was to compare the profitability, efficiency, and productivity indexes of the water used in the production of forage sorghum (*Sorghum bicolor* (L.) Moench) and maize (*Zea mays* L.) crops with groundwater irrigation, using pumping and gravity irrigation, in the Comarca Lagunera during 2019. This comparison would be used to selected the most efficient crop.

MATERIALS AND METHODS

Geographical location of the study area

The Comarca Lagunera study area is located between 26° 00’ and 26° 10’ N and 104° 10’ and 103° 20’ W, at an altitude of 1,119 m (Acevedo- Peralta *et al.*, 2017). According to García (2004), the Comarca Lagunera has a desertic climate with low atmospheric humidity and an annual rainfall of 260 mm.

Official statistical sources used

RF_i=Physical yield of the *i*-th crop (t ha^{-1}). Where $RF=VBP/P$; the sources of the *VBP* (Gross Value of Production), P=Annual physical production during the 2019 agricultural closure according to SIAP-SADER (2021).

LR_i=Irrigation sheet of the *i*-th crop (m^3) (INIFAP-CENID-RASPA, 2006).

EC_i=Hydraulic conductivity efficiency of the *i*-th crop ($0<EC<1$).

Where *EC* is the Hydraulic conductivity efficiency (INIFAP-CENID-RASPA, 2006).

The weighted price for the aggregation level of both forages (overall forage production for both crops) is provided by the following equation, in which "Q_i" is the production and "P_i" is the price per ton:

$$Weighted\ price = \frac{\sum_{i=1}^{n} Q_i * P_i}{1\sum_{i=1}^{n} Q_i}$$

P_i=Price of the *i*-th crop product (in MX\$ t^{-1}), for which $p=VBP/P$, where the *VBP* sources (gross value of production) and P=annual physical production (t) (SIAP-SADER, 2021).

PC=Exchange rate in Mexican pesos (MX$) per each USD ($20.50 MXN) (Banco Mundial-XE, 2021).

Mathematical models used

The following equation was used to determine the weighted cost per hectare:

$$Weighted\ cost = \frac{\sum_{i=1}^{n} S_i * C_i}{1\sum_{i=1}^{n} S_i}$$

C_i=Production cost per hectare of the *i*-th crop (MX\$ ha^{-1}) (FIRA, 2019).

S_i=Harvested area of the *i*-th crop (ha) (SIAP-SADER, 2021).

$g_i=U_i$=Profit per hectare of the *i*-th crop (USD\$ ha^{-1})$=RF_i\,(p_i/PC)-(C_i/PC)$.

The following equation was used to estimate the weighted profit per hectare of maize and sorghum crops:

$$Weighted\ profit = \frac{\sum_{i=1}^{n} S_i * g_i}{1\sum_{i=1}^{n} S_i} = \frac{\frac{1}{PC}\sum_{i=1}^{n}\left[S_i * RF\left(P_i-\left(C_i / RF_i\right)\right)\right]}{\sum_{i=1}^{n} S_i}$$

J_i=Number of daily wages invested per hectare in the *i*-th crop (FIRA, 2019).

Table 1. Mathematical models used to determine productivity.

Variable	Model for an individual crop
PFA (L kg^{-1})	$Y = 10^4 * LRi * (RFi * ECi)^{-1}$
EFA (kg m^3)	$Y = 10^{-1} * RFi * ECi * LRi^{-1}$
EEA (m^3 USD^{-1})	$y = \dfrac{10^4 \left({LR_i}/{EC_i} \right)}{RFi\left({P_i}/{PC} \right) - \left({C_i}/{PC} \right)}$
PEA (Thousands of USD earned per hm^{-3} of water)	$y = 10^2 g_i EC_i (LR_i)^{-1}$
PSA (Jobs hm^{-3})	$y = \dfrac{25}{72} \dfrac{J_i}{(LR_i / EC_i)}$
ESA (m^3 $Jobs^{-1}$)	$y = \dfrac{2.88 * 10^6 LR_i}{J_i EC_i}$

PFA=Physical Productivity; PEA=Economic Productivity; PSA=Social Productivity; EFA=Physical Efficiency; EEA=Economic Efficiency; and ESA=Social Efficiency. All these values are related to the water used in the individual crop production or for crop aggregates designed by Ríos-Flores & Navarrete-Molina (2019) and Ríos-Flores *et al.* (2020).

i=i-th crop under a specific irrigation system (groundwater and gravity irrigation) (FIRA, 2019).

Number of working days per year per worker=6 days per week times 48 weeks per year=288=1 equivalent employment (FIRA, 2019).

RESULTS AND DISCUSSION

Productivity efficiency of forage sorghum and maize

Table 2 shows the total harvest of both crops: forage sorghum and maize (37,860.24). In 2019, over 100 thousand hectares were planted in the Comarca Lagunera; two out of every five hectares (almost 40%) were planted with these forages.

The cost-benefit ratios (RB/C) for sorghum and maize were 1.09 and 1.13, respectively. For every dollar invested in forage, sorghum obtained a 9% profit, while maize obtained a higher profit (13%). Although profits are reported in this study, Ríos-Flores *et al.* (2016) reported a loss of −23.4 million pesos in their study of *Triticum vulgare* in the Mexicali Valley, despite the large volume of water invested in irrigation (low yield m 3 kg^{-1}).

Social efficiency of forage sorghum and maize

An investment of 18.13 daily wages (145.04 working hours) was required to produce one sorghum commercial hectare, while 19.62 daily wages (156.96 working hours) were required to produce one maize commercial hectare. The number of daily wages (J) invested per sorghum and per maize hectare generated the equivalent of 493.58 employments to harvest forage sorghum in 8,948.74 and 1,473.57 employments to harvest forage maize in 28,911.5 ha. Overall, a total of 1,967.15 employments were created.

Table 2. Macroeconomic statistical indicators of forage maize and sorghum crops irrigated with groundwater by pumping and gravity irrigation (Comarca Lagunera, 2019).

Macroeconomic Variable	Forage sorghum	Forage Maize
Harvested area (ha)	8,948.74	28911.5
Annual production (t)	351,334.99	1161998.78
Gross value production (millions of USD)	$ 12.05	$ 47.70
Yield (Green forage; t ha^{-1})	39.26	40.19
Price (USD t^{-1})	$ 34.30	$ 39.3
Incomes (USD ha^{-1})	$ 1,347	$ 1,581
Costs (USD ha^{-1})	$ 1,239.39	$ 1,403
Exchange rate (MX per USD)	$ 20.5 MXN per 1 USD	
Profits (USD ha^{-1})	$ 107	$ 178
Cost-Benefit ratios	1.09	1.13
M^3 of water used per ha	8,888.90	8,888.90
Daily wages per ha (J)	18.13	19.62
Irrigation cost ha^{-1} (USD)	$ 149	$163

Developed using SIAP data (2019).

The social water efficiency (ESA) reported for sorghum was 141,203 m^3 $employment^{-1}$. Meanwhile, forage maize required a smaller volume of water resource to generate one employment (130,479 m^3 $employment^{-1}$). Sorghum cultivation generated 7.1 employments hm^{-3}, as a result of the use of a greater water volume; on its part, according to the PSA, maize cultivation generated 7.7 employments hm^{-3} surpassing sorghum. Maize was more efficient, since sorghum required 8% more water to produce an employment unit. These results surpass the values recorded in the Comarca Lagunera by Ríos-Flores *et al.* (2015) for alfalfa (0.037), forage oats (0.68), rye grass (0.76 employments hm^{-3}). García-García *et al.* (2013) and others reported the generation of 24 to 62 employments hm^{-3} in the vegetable and fruit production, while in greenhouses more employments were created (190 employments hm^{-3}).

Water efficiency of forage sorghum and maize

According to SADER (2020), forage sorghum and maize used 8,888.9 m^3 ha^{-1} of water. The area sown with both crops amounted to 37,860.24 ha (8,948.74 hectares of sorghum and 28,911.55 hectares of maize). If we multiply 37,860.24 ha by the total number of m^3 used (8,888.9 m^3), we find that both crops used 336.53 hm^3. On the one hand, sorghum producers invested $0.017 USD in water per m^3, while maize producers invested $0.018 USD (SADER, 2020). On the other hand, in Spain the cost ranged from 0.59 to 0.17 € m^{-3} j (García-García *et al.*, 2013), while, in California, the productivity of water amounted to 0.20 € m^{-3}, €0.70 m^{-3}, and €5.00 m^{-3} for maize, almonds, and strawberries, respectively (Fereres, Goldhamer, & Parsons, 2003).

Forage sorghum used 226 L kg^{-1} and forage maize 221 L kg^{-1}, suggesting that forage maize was more efficient than sorghum, since it can produce the same biomass kg using only 97.78 % of the water volume used by forage sorghum (Table 3). We obtained less efficient results than those reported by Pedroza-Sandoval *et al.* (2014) (175 L kg^{-1} for maize). Values for dryland (875 to 710 L kg^{-1}) and for irrigation (586 to 671 L kg^{-1}) were reported by Alvarez *et al.* (2016). This improved use of water in the Comarca Lagunera can be attributed to the varieties used.

The production of forage sorghum and maize amounted to 4.42 kg m^{-3} and 4.52 kg m^{-3}, respectively (PFA) (0.98; Table 4). Similar values (3.60 kg m^{-3} to 2.18 kg m^{-3}) were reported by Garcia-Garcia *et al.* (2013) in Spain for artichoke using three different irrigation options. In the study about *Triticum vulgare* by Ríos-Flores *et al.* (2016), lower values (0.321 kg m^{-3}, 0.668 kg m^{-3} and 0.667 kg m^{-3}) were obtained in Ensenada, Mexicali Valley, and Baja California. Lower values were likewise reported in the province of Punjab, Pakistan, regarding wheat (0.43 kg m^{-3}) and cotton (1.12 kg m^{-3}) (Shabbir *et al.*, 2012), as well as regarding wheat in China (0.9 kg m^{-3}) and the United States of America (1.3 kg m^{-3}) (Brauman, Siebert, & Foley, 2013).

Producing one dollar of profit requires 82.87 m^3 of water in the case of sorghum, while forage maize requires almost half the water (49.96 m^3) and the index was equal to 1.66 (Table 3). Water-wise, forage sorghum was less efficient, since it required almost double the amount of water (177 %) than maize to produce the same amount of profit. Sorghum economic productivity reached $12,067 USD of profit, while forage maize had a higher revenue ($20,018 USD), using the same volume of water. Compared with the indexes reported by Ríos-Flores *et al.* (2015) —70,000 USD hm^{-3} (sorghum) and 40,000 USD hm^{-3} (maize) PEA indexes— This study has values far below the ones reported in 2015. The above statement is also reported by Pedroza-Sandoval *et al.* (2014) with a $32,683 USD index for forage maize. The variation in the efficiency of the crops can be attributed to the high temperature that influences the photosynthesis rate of crops; forage sorghum is more sensitive to different types of stress (Chadalavada, Kumari, & Kumar, 2021).

Table 3. Efficiency and productivity indexes of water, capital, and labor of forage sorghum and maize crops using groundwater (Comarca Lagunera, 2019).

Index	Units	A: Forage Sorghum	B: Forage Maize	c=a/b
EFA	m^3 kg^{-1}	0.226	0.221	1.02
EFA	L kg^{-1}	226	221	1.02
PFA	kg m^{-3}	4.42	4.52	0.98
EEA	m^3 of water used for each USD of profit (m^3 USD^{-1})	82.87	49.96	0.94
PEA	USD profit per hm^3 of water in production	$ 12,067	$ 20,018	0.60
ESA	m^3 of water used per each job generated	141,203	130,479	1.08
PSA	Jobs generated per hm^3 (Jobs hm^3)	7.1	7.7	0.92
Price of water	USD per hm of water used	$ 16,765	$18,336	0.91
PEA/Price of water	Dimensionless	0.72	1.09	

PFA=Physical Productivity; PEA=Economic Productivity; PSA=Social Productivity; EFA=Physical Efficiency; EEA=Economic Efficiency; and ESA=Social Efficiency. All these values are related to the water used in the individual crop production or for crop aggregates designed by Ríos-Flores & Navarrete-Molina (2019) and Ríos-Flores *et al.* (2020).

CONCLUSIONS

Mathematical models can be indicative of the sustainability assessment of natural resources; consequently, a more comprehensive analysis of the said resources can be carried out. Production- and water-wise, forage maize was more efficient than forage sorghum, which would benefit the Comarca Lagunera by saving water resources. Further research must take into consideration dynamic models that cover environmental variables, in order to obtain a better explanation of the crop productive behavior.

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AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Mathematical models to estimate forage production in southeastern Coahuila, Mexico

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ABSTRACT

Objective: To calibrate two non-linear models, in three intermediate triple hybrids, by theoretically comparing the accumulation of dry matter in relation to the days after sowing (das).

Methodology: The cuts were made every 14 days, from 30 to 170 days after sowing, and were adjusted to the Logistic and Richards models. The experimental design was a randomized block, with three replications.

Results: The models explained most (83%) of the total variability of dry matter (DM) yield in maize observed in the field. The best fit model was the Logistic model (cultivar AN447) and the Richards model (cultivar A7573), both with R^2=0.98. The maximum yield simulated with the Richards model was observed in AN447 (22,616 kg DM ha^{-1}) and the lowest in AN388 (10,970 kg DM ha^{-1}).

Limitations/Implications: The results can only be applied to the study case, as a consequence of the limitations imposed by the variety, climate, and soil conditions. Therefore, no general explanation can be developed and the conclusions should be treated with caution.

Conclusion: The Logistic model enables a more precise simulation of the dry matter yield in maize, using the days after sowing as an independent variable.

Keywords: *Zea mays* L., mathematical modeling, and goodness of fit.

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INTRODUCTION

Maize (*Zea mays* L.) is considered the most cultivated cereal worldwide (FAO, 2019). However, climatic variability and management have generated uncertainty and instability in the productivity of this crop; therefore, calibrated simulation models have become a viable tool to study its behavior (Flores *et al.*, 2013). The Logistic (Nelder, 1961) and Richards (1959) models are the most commonly used to describe the growth of plants, animals, and/or other organisms (Villegas *et al.*, 2019). However, their calibration takes

Image by Matan Ray Vizel at Pixabay

into consideration the physiological aspect of the plant and its productivity; therefore, as more information is generated, the model fits with greater accuracy (Sinclair and Seligman, 1996). Another important aspect is the validation of the model, carried out comparing the data obtained in the field versus the data obtained by the model and its subsequent application in regions with similar characteristics to the region where it was validated (Boons *et al.*, 1993). In forage species, the models have a good fit (experimental data *vs.* simulated data); such is the case of *Panicum maximun* Cv. Mombaza and *Pennisetum purpureum* Cv. Cuba CT-115 (Rodríguez *et al.*, 2007; Thornley and France, 2007). Many mathematical models simulate maize's development and growth (Lizaso *et al.*, 2011). However, some of these models are too sophisticated and their calibration, validation, and implementation require a large number of parameters and specific skills. Consequently, they are still exclusively for scientific use (Sau *et al.*, 2012) and farmers have a difficult time adopting them (Heng *et al.*, 2009). Therefore, the objective was to calibrate two non-linear models, in three intermediate triple hybrids, by theoretically comparing the accumulation of dry matter in relation to the days after sowing.

MATERIALS AND METHODS

Study area: The work was established in the "El Bajío" experimental area of the Universidad Autónoma Agraria Antonio Narro (UAAAN), in Saltillo, Coahuila, Mexico (25° 23' 12.7" N, and 101° 00' 9.8" W, at 1783 m.a.s.l.). The climate is temperate semi-dry, with temperatures that surpass 18 °C and fall below 0 °C. The average annual accumulated precipitation is 340 mm (García, 2004). Figure 1 shows the weather conditions during the study (Red Universitaria de Observatorios Atmosféricos, UAAAN).

Three intermediate triple maize hybrids (AN447, AN388, and A7573) from the Mexican Corn Institute were used; they were established on April 8th, 2017. The materials were distributed in a randomized block design, with three replications. Each replication consisted of five 9-m long furrows, generating 18 experimental units. The land was plowed, twice harrowed, and furrowed at 0.8 m. Plant density was 86,000 plants ha^{-1}. Irrigation

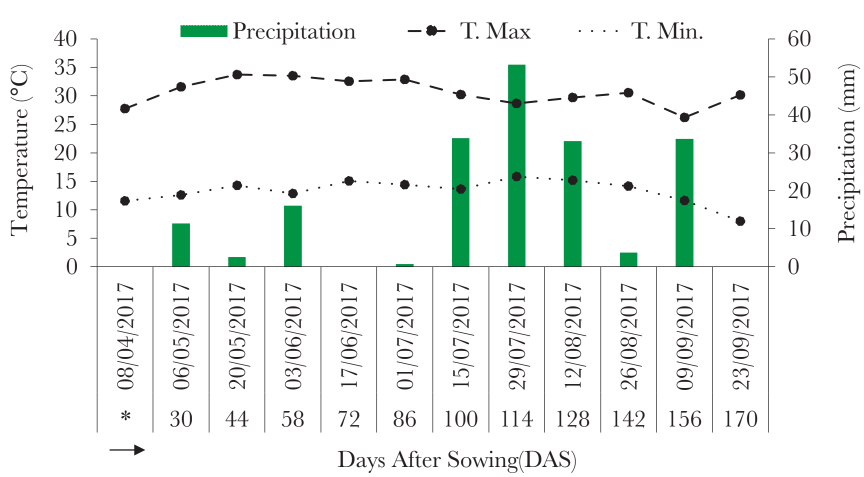

Figure 1. Average maximum and minimum temperatures, and fortnightly accumulated precipitation during the study period (April 8th to October 21st, 2017). *Sowing.

was applied once a week at field capacity, by drip irrigation, using drip tape (wall thickness 6 mil), with a 15-cm distance between drippers. The predominant soil has a sandy-clay-loam texture, with 62, 10, and 20% sand, silt, and clay, respectively; it has 3.02% organic matter and 1.25 g cm^3 apparent density, determined at the beginning of the experiment. Cuts were made every fourteen days from May 6th (30 das) to September 23rd, 2017 (170 days). Samples were taken from three plants and brought to constant dry weight, in a POM-246F forced air stove, at 55 °C during 72 h, in order to determine the accumulation of dry matter. Relationships were established between cutting age after sowing and biomass yield. To estimate the dynamics of growth and dry matter production, two non-linear models were considered: Logistics and Richards (Table 1). The regressions were adjusted with the Statistical Package for Social Sciences software (SPSS, 2011), in which the significance of the correlation coefficients was calculated ($p<0.05$).

RESULTS AND DISCUSSION

Goodness of fit of the Logistic and Richards models

The best model of the AN447 and AN388 hybrids was obtained with the Logistic model; however, the A7573 presented a better fit with the Richards model. The R^2 was 0.98 (AN447), 0.96 (AN388), and 0.98 (A7573) (Table 1). This behavior tends to form a sigmoid curve, similar to the growth dynamics of forage plants (Thornley and France 2007; Martínez *et al.*, 2010). In the case of maize, the field values obtained with other models (*e.g.*, MCWLA-Maize) differed by 2% from those estimated by the model with $R^2=0.70$, showing good prediction capacity (Tagarakis and Ketterings, 2017).

Estimation of variety AN447 dry matter

The precision with which the models explain the DM production in the AN447 hybrid (depending on the age of the plant) was higher in the Logistic model ($R^2=0.98$), than in Richards model ($R^2=0.84$), indicating a lower variability of the Logistic model *vs*. Richards (Figure 2). Although the Logistic and Richards models had predicted 22,536 kg DM ha^{-1} and 22,616 kg DM ha^{-1} DM accumulation, respectively, the actual highest accumulation

Table 1. Non-linear models used to estimate the dry matter yield (W_t) in three maize hybrids with irrigation, as the age of the plant increases in southeastern Coahuila, Mexico.

Model	Funtional form	Differential form
Logistic	$W_t=\dfrac{W_0W_f}{W_0+\left(W_f-W_0\right)e^{-\mu t}}$	$\dfrac{dW_t}{dt}=\mu W\left(1-\dfrac{W}{W_f}\right)$
Richards	$W_t=\dfrac{W_0W_f}{\left[W_0^n+\left(W_f^n-W_0^n\right)e^{-kt}\right]^{1/n}}$	$\dfrac{dW_t}{dt}=\dfrac{k}{n}W\left(1-\left(\dfrac{W}{W_f}\right)^n\right)$

Where: W_0=Initial dry weight, W_f=Maximum or final dry weight, μ=Relative or specific growth rate, e=Base of natural logarithms, t=Time. For the case of the Richards model: n=Form parameter, and k=Constant parameter. The values of μ and k were obtained by simple regression. The values of n were considered according to Thornley, J. H. M. and France, J. (2007).

Table 2. Goodness of fit models for the dry matter yield of three maize hybrids, developed from 30 to 170 days after sowing, at 14-day intervals in southeastern Coahuila, Mexico.

	Cultivars					
	AN447	**AN388**	**A7573**	**AN447**	**AN388**	**A7573**
	Logistics model			**Richards model**		
R^2	0.98	0.96	0.94	0.84	0.83	0.98

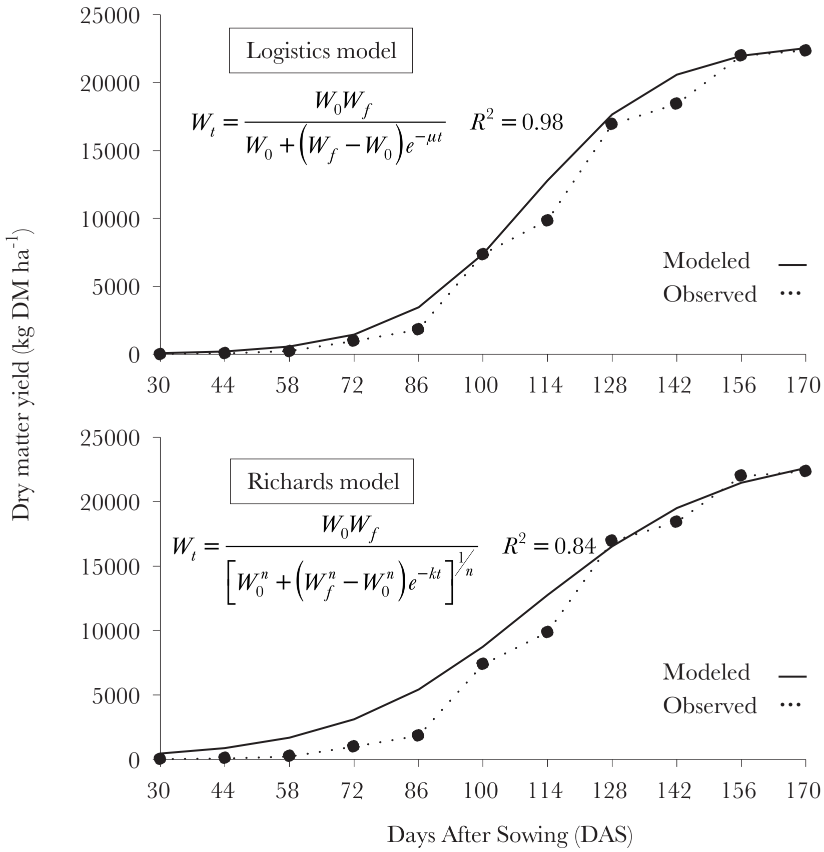

Figure 2. Dry matter yield (kg DM ha^{-1}) of AN447 maize hybrid obtained in the field and simulated with the Logistic and Richards models. Production cycle: April 8th to September 23rd, 2017. Location: southeastern Coahuila, Mexico.

of DM observed in the field at 170 das was 22,361 kg DM ha^{-1}. Both models overestimated the performance by 7% (Logistic) and 12% (Richards); however, the behavior registers a turning point from 156 das. This suggests that the optimal harvest time for this hybrid is after the said date, as reported by Machado *et al.* (1983). These data match the findings of González *et al.* (2014), Diaz *et al.* (2018), and Tornés (2016), who estimated average yields of 20,680 kg DM ha^{-1} at 125 das, obtained with the FAO AquaCrop simulation model. In this regard, determining the optimal harvest time based on simulated values allows a maximum handling of the study materials (Castro *et al.*, 2017), taking into account their growth, which can vary according to management, species, cultivar, and edaphoclimatic conditions (Torres *et al.*, 2012).

Estimation of variety AN388 dry matter

The values estimated with the Logistic model are closer to the observed values than those obtained with the Richards model, since the adjustment generated a correlation of 0.96 and 0.83, respectively. Throughout the study, the variation of DM accumulation in the observed values ranged from 12 to 13,261 kg ha^{-1}. In the Logistic model, the simulated yields varied between 190 and 12,070 kg DM ha^{-1}, while the Richards model recorded variations between 287 and 10,970 kg DM ha^{-1}. Both models overestimated the yield by 2%, which suggests good agreement and little variability. Días and Villalobos (2018) validated the FAO AquaCrop model and reported yields of 28,600 kg DM ha^{-1}. The average difference with the simulations was 670 kg DM ha^{-1} (6% underestimation). This result indicates good biomass predictions (R^2=0.96) and a strong significant relationship between simulated and observed values. Conde *et al.* (2004) calibrated the DSSAT_CERES model for forage maize and overestimated the actual yield by 5-12% for a temperate region of Mexico, which suggests a good fit. In contrast, Arce *et al.* (2017), under similar conditions, estimated a 49.3% decrease in forage maize yield (1-1.7 t ha^{-1}) using AquaCrop, which shows a high degree of variability.

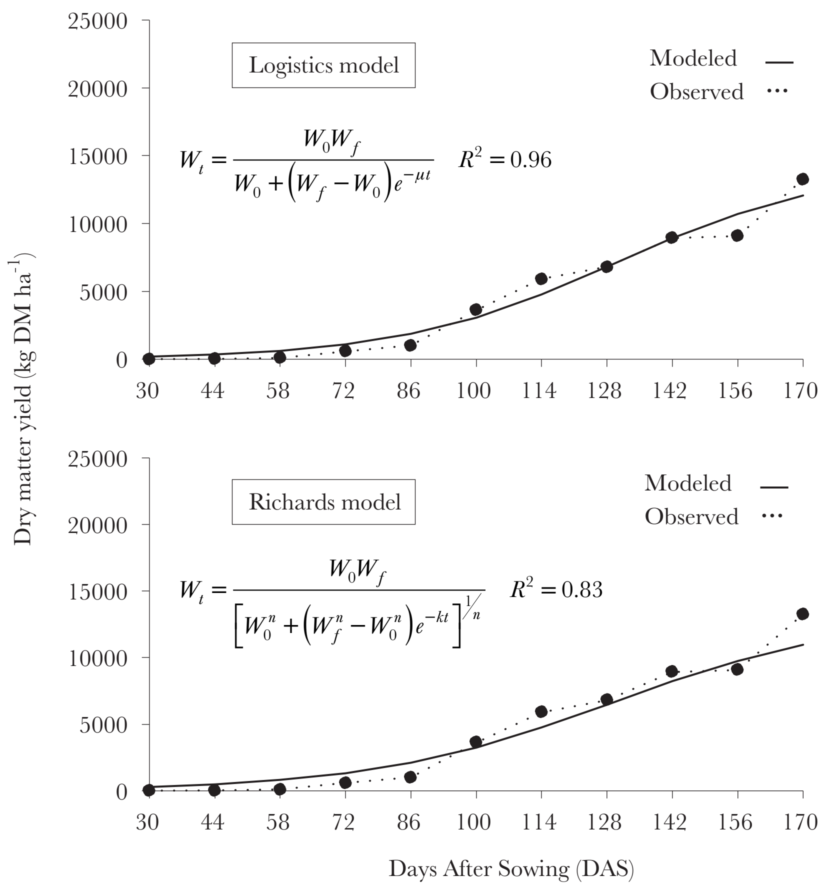

Figure 3. Dry matter yield (kg MS ha^{-1}) of AN388 maize hybrid obtained in the field and simulated with the Logistic and Richards models. Production cycle: April 8th to September 23th, 2017. Location: southeastern Coahuila, Mexico.

Estimation of variety A7573 dry matter

The inflection point of cultivar A7573 took place at 156 das and the maximum value (18,603 kg DM ha^{-1}) was recorded at 170 das (Figure 4). On average, the Logistic model and the Richards model overestimations reached 1 and 2%, respectively. Therefore, both models adequately explain the growth of cultivar A7573, as a result of the good fit of both models —which have correlation coefficients of 0.94 (Logistics) and 0.98 (Richards). The range of dry matter yield in the observed values, from the beginning to the end of the experiment, ranged from 20 to 18,603 kg ha^{-1}. There was a 1 and 2% difference between the values obtained with the Logistic and Richards models regarding the observed values from 30 to 114 das. Subsequently, both models overestimated the yield by approximately 18%. Nouna *et al.* (2000) recorded average differences of 7.27 and 11.71% between the observed and simulated values; differences up to 10% were considered as an acceptable data.

Overall, the highest yields observed were registered by the cultivar AN447, with 9,098 kg DM ha^{-1} average values; meanwhile, the simulated Logistic model recorded 9,880 kg DM ha^{-1} and the Richards model registered 10,291 kg DM ha^{-1} ($p>0.05$); the

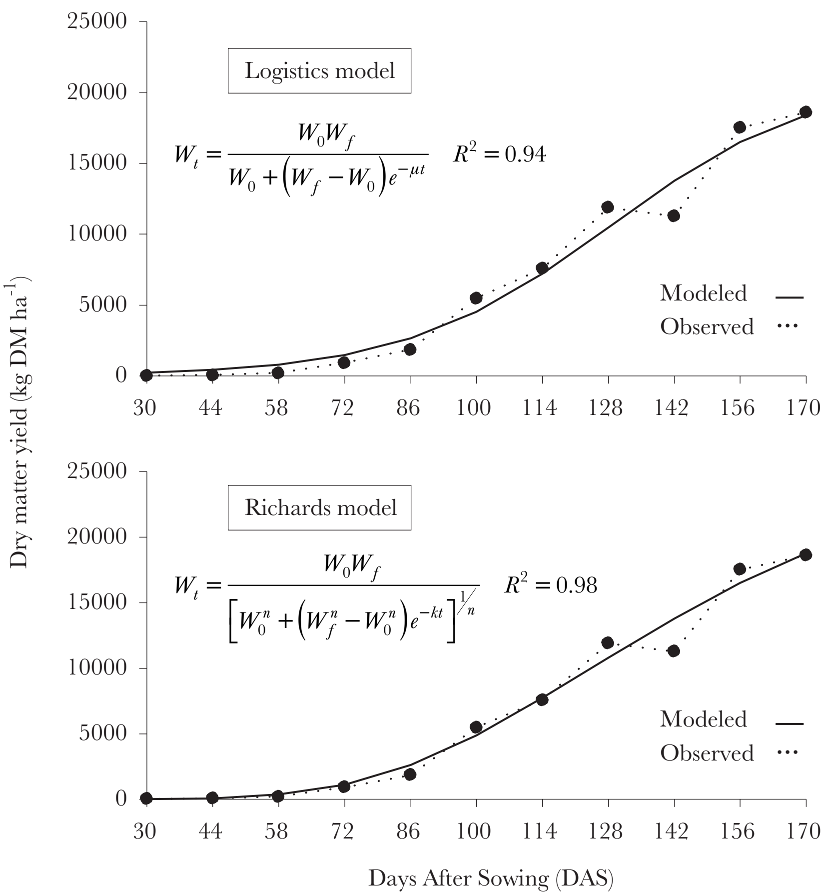

Figure 4. Dry matter yield (kg DM ha^{-1}) for A7573 maize hybrid obtained in the field and simulated with the Logistic and Richards models. Production cycle: April 8th to September 23rd, 2017. Location: southeastern Coahuila, Mexico.

overestimation was 8.5% (Logistic) and 13% (Richards). The lowest average yields were registered in the cultivar AN388, with 4488, 4585, and 4406 kg DM ha^{-1}, in the observed, Logistic, and Richards models, respectively. The Logistic model had an 2% overestimation, while the Richards model had an 1.8% underestimation. Paredes *et al.* (2014) mention that the prediction error is reduced when fewer differences are found between the observed values and those generated by the models, which indicates a correct parameterization of the forage yield curve.

CONCLUSIONS

The adjustment of the models accounted for 83% of the total variability of the dry matter yield. The Logistic model had the best fit for the AN447 and AN388 hybrids, while the Richards model had the best fit for the A7573. However, both models underestimated the observed performance. The A7573 and AN388 hybrids were the best and least fit, respectively.

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AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Chemical pregerminative promoters in *Zea mays* L. seed

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ABSTRACT

Objective: Plant life begins at germination. Stimulating germination with chemical methods can be advantageous. The pregerminative treatment of four promoters was determined in two maize genotypes (G1, G2).

Design/methodology/approach: Sixty seeds per Petri dish were used as experimental unit with three repetitions, organized in a completely randomized 2×4 factorial design. Two factors were taken into consideration: A) Genotypes (G1, G2); and B) four pregerminative promoters. The genotypes were Antelope G1 and yellow Antelope G2. Meanwhile, the pregerminative promoters were salicylic acid ($C_7H_6O_3$) (SA), citrulline ($C_6H_{13}N_3O_3$) (CI), humic substances derived from leonardite (HS), and tap water (TW), in 1000-ppm concentrations. The following variables were evaluated: germination percentage (GP)/days^{-1}, radicle diameter (RD), radicle length (RL), and number of lateral seminal roots (NSR). An analysis of variance and Tukey tests ($\alpha \leq 0.05$) were performed.

Results: The germination promoters were highly significant in both genotypes, as well as during the promoter-genotype interaction. G1 and G2 means showed a higher growth and development for humic substances (HS) during germination in the NSR.

Study Limitations/Implications: Germination can be inhibited, if the promoters are overweighted.

Findings/Conclusions: The best genotype and germination promoter (G2) had a 94% effectiveness and HS at 1000 ppm. CI and SA registered the lowest GP.

Keywords: Seed, biostimulant, seminal roots.

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INTRODUCTION

Maize (*Zea mays* L.) is the third most consumed food by humans and animals worldwide, after wheat and rice; therefore, it is important to study the physiological mechanisms of germination, growth, and development (Assem, 2015). Germination includes a series of processes —from seed imbibition to radicle emergence (Doria, 2010). Some of the visual indicators used to assess these processes include: radicle emergence, coleoptile emergence, mesocotyl elongation, and emergence of lateral seminal root (Sáenz and Cassab, 2021). Visual indicators have

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different sequences and are influenced by temperature, germination, and emergence. They were recorded earlier in warm soils conditions than in cold ones (Nielsen, 2016); however, the seed germination and vigor of maize is related to its metabolic activity during the first hours (El-Maarouf-Bouteau H., 2022).

Several pregerminative treatments —such as biostimulants and phytohormones— are used to promote germination. Biostimulants include humic substances, microorganisms, plant and animal products, microbial inoculations, etc. which improve germination and root growth (Calvo *et al.*, 2014; Hasanuzzaman and Responses, 2019). Root growth and water absorption are enhanced by humic substances (HS) (Olk *et al.*, 2018, Canellas *et al.*, 2015; du Jardin, 2015). Furthermore, Sarropoulou *et al.* (2016) mention that metabolism and transport of citrulline has been observed in *Arabidopsis thaliana* and *Cucurbitaceae* crops. In studies performed with cherry slides, there were direct effects on *in vitro* rooting. Phytohormones do not only provide control and protect germination under stress processes, they also enhance the tolerance of germination under low-temperature environments (Steven *et al.*, 2019). Dawood *et al.* (2019) and Raskin (1992) mention that salicylic acid (SA) is a phenolic compound that acts as an endogenous regulator of physiological and biochemical processes in the germination and seedling stages. SA increases root size in maize seedlings and, at low concentrations, increases germination in beans (Haas *et al.*, 2015, Rodríguez-Larramedi *et al.*, 2017, Dawood *et al.*, 2019).

Several maize species found in the La Laguna region are affected by genotype and environment. In the last 30 years, climate change has caused a persistent statistical deviation with extreme temperature variations and limited water resources (Inzunza-López *et al.*, 2011; CONAGUA, 2022). The interaction of two maize genotypes and promoters will favor germination (Ortiz-Timoteo *et al.*, 2018). This study was developed to assess four germination promoters in two maize genotypes.

MATERIALS AND METHODS

Study area

The experiment was conducted in October 2019, at Universidad Autónoma Agraria Antonio Narro-Unidad Laguna, located in the City of Torreón, Coahuila (25° 31' 11" N and 103° 25' 57" W, 1123 m.a.s.l.). According to the Köppen climate classification (as modified by García (2004)), the area has a BWh, warm desert climate, with a maximum temperature of 40 °C, minimum of 6 °C, and average rainfall of 250 mm.

Establishment of the experiment

Sixty maize (*Zea mays* L.) seeds of genotype (G1) white Antelope and genotype (G2) yellow Antelope Y were used per Petri dish. Damaged seeds were discarded. Subsequently, they were disinfected using 10 mL/L of sodium hypochlorite at 1% and left to settle for 15 minutes. Then they were washed with drinking water (FAO, 2001) and the promoters were sprayed three consecutive days, along with SA, CI, HS, and leonardite by-products in 1000 Mg kg^{-1} concentrations and running water as control solution (Table 1), until the germination percentage was observed.

Table 1. Physical and chemical characteristics of the tap water used in the experiment under greenhouse conditions.

Chemical elements	Mg kg^{-1}
Iron Fe^3	0
Zinc Zn^+	0.02
Copper Cu^+	0.03
Manganese Mn^+	0.01
Boron B^+	0.99
Sodium Na^+	120
Potassium K^+	13
Calcio Ca^+	288
Magnesium Mg^+	29
Nitrates NO_3	23.03
Phosphate phosphorus	0.08
Phosphorus Diacino H_2PO_4	0.25
Sulfate of SO_4	643.6
Carbonates CO_3	0
Bicarbonates HCO_3	170.83
Chlorides Cl	198.52
Physical parameters	
pH	7.80
Electrical conductivity mS/cm	2.21
Sodium absorption ratio	1.8
Interchangeable sodium (%)	0.38

Variables assessed

The following variables were measured: germination percentage (GP) (Moreno *et al.*, 2018; Caroca *et al.*, 2016), radicle diameter (RD), radicle length (RL), and number of lateral seminal roots (NSR). Regarding germination percentage (GP), seed with a ≥2 mm radicle length were considered to be germinated seeds. PG was calculated using the following formula (Caroca *et al.*, 2016):

$$GP = \left[\left(No.\ of\ germinated\ seeds\right)/\left(No.\ of\ sown\ seeds\right)\right]\times 100$$

A vernier Truper CALDI-6MP with a 30 cm rule was used to estimate the radicle diameter (RD) and the radicle length (RL), and the number of secondary seminal roots was counted.

Statistical analysis

A completely randomized 2×4 factorial design with 3 replications was used. Each Petri dish represented an experimental unit. The study factors were the G1 and G2 genotypes, factor A and its promoters, Factor B (SA, CI, HS, and TW). An analysis of variance and a comparison of means (Tukey, P<0.05) were performed, both using the SAS statistical package (SAS, 2009).

RESULTS AND DISCUSSION

Germination behavior

The analysis of variance (Table 2) shows the effects of genotype (G1, G2) and of pregerminative treatments (promoters) on the germination percentage, where it was highly significant for the three days of % GP1, % GP2, and % GP3 ($p<0.0001$) with ($p\leq 0.05$). On their part, the pregerminatives (promoters) were significant only for % GP1 and % GP3 days, while they were significant for all three days of the G1 and G2 × promoters interaction. A coefficient of variation of 41, 8, and 6% was recorded for % GP1, % GP2 and % GP3 days, respectively. In the case of GP, RD, RL, and NSR, they were highly significant only for G1 and G2 × promoters and for RD and RL with one ($p<0.0001$) and only for promoters in RL ($p<0.0001$), and between G1 and G2 with NSR ($p<0.0001$). Moreover, they were significant for G1 and G2 in GP (0.0001), RL ($p<0.0028$), and promoters in NSR ($p<0.0019$), as well as for G1 and G2 × promoters interaction for GP (0.0005) ($p<0.0007$) with coefficients of variation of 6% for GP, RD, and RL and 17% for NSR.

Behavior of the germination variable means of genotypes 1 and 2

The behavior of means with respect to genotypes G1 and G2 germination in the double-entry Table 3 on day GP1 was statistically different for G2. Meanwhile, G1 was statistically different on days GP2 and GP3, with respect to G1 and G2. However, with CI, HS, and AL, these promoters had outstanding results on day GP1, while with SA and HA they behaved similarly on day GP2 and day GP3, respectively. On their part, GP matched G1 and HS promoters. Regarding the RD variable, the mean is statistically the same for genotypes G1, G2, as well as for the promoters. In the case of RL, the mean between genotypes G1 and G2 belonged to G2 and, in the case of the promoters, it matched CI and

Table 2. Analysis of variance (mean squares and statistical significance) for the germination variables of days 1, 2, and 3 (% GP1, % GP2, % GP3), radicle diameter (RD), radicle length (RL), and lateral seminal roots (SR).

VS	GL	Germinal variables						
		% GP1	% GP2	% GP3	GP	RD	RL	NSR
Genotypes	1	782**	630**	551**	551**	0.010	21*	5**
Promoter	3	42	36	99	99	0.012	57**	0.685*
Genotypes * Promoter	3	40*	105*	228*	228*	0.124**	53**	0.852*
Error	16	7	9	22	22	0.007	1.69	0.087
Total	23	1142	1203	1894	1894	0.536	379	11
CV (%)		41	8	6	6	6	6	17

VS=variation sources; Germination percentage of day 1, 2, and 3=% GP1, % GP2, % GP3; germination percentage=GP; radicle diameter=RD; radicle length=RL; and number of lateral seminal roots=SR. ** Highly significant * Significant, Coefficient of variation (CV) (Tukey, 0.05).

TW. In relation to NSR, the G1 and G2 genotypes mean belonged to G2 and, in the case of promoters, it was HS.

Figure 1 shows the behavior of the germination percentages of genotypes G1 and G2 on days 1, 2, and 3, when no significant difference was recorded for G1 with regard to promoters on day 1; meanwhile, in the case of G2, CI, HS, and TW promoters were statistically the same. Moreover, on day 2, promoters were the same for both genotypes. However, on day 3, genotypes G1 and G2 were the same only for the HS promoter, while CI had the lowest result.

Germination percentage

The findings of this experiment suggest that the highest germination percentage for varieties belongs to G1 and that, when they were exposed to germination promoters, HS achieved a higher germination for both G1 and G2 (Table 2 and 3). These results match the findings of authors such as Rodrigues *et al.* (2017) and Šerá and Novák (2011), who assessed the effect of treating maize seeds with a humic acid-based commercial product and found that humic acid promotes a greater growth of seedlings, in addition to greater dry

Table 3. Behavior of G1 and G2 means during germination.

Variable		SA	CI	HS	TW	Average
GP1	G1	0.66 a	1.33 a	0.66 a	0.66 a	0.83 b
	G2	4.66 b	13.66 a	16.76 a	14.00 a	12.25 a
	Average	2.66 b	7.50 a	8.66 a	7.33 a	
GP2	G1	38.66 b	42.33 ab	47.33 a	40.66 b	42.25 a
	G2	39.66 a	25.66 c	30.66 bc	32.00 b	32.00 b
	Average	39.16 a	34.00 b	39.00 ab	36.33 ab	
GP3	G1	78.33 c	87.66 ab	94.33 a	82.00 bc	85.58 a
	G2	83.00 a	65.00 b	78.00 a	77.66 a	76.00 b
	Average	80.83 ab	76.33 b	86.16 a	79.83 ab	
GP	G1	78.33 c	87.66 ab	94.33 a	82.00 bc	85.58 a
	G 2	83.00 a	65.00 b	78.00 a	77.66 a	76.00 b
	Average	80.83 ab	76.33 b	86.16 a	79.83 ab	
RD	G1	1.36 b	1.43 b	1.66 a	1.46 b	1.48 a
	G2	1.46 a	1.60 a	1.20 b	1.50 a	1.44 a
	Media	1.41 a	1.51 a	1.43 a	1.48 a	
RL	G1	14.73 b	25.03 a	24.13 a	25.96 a	22.46 b
	G2	24.8 b	25.46 ab	19.93 c	27.16 a	24.34 a
	Average	19.77 c	25.25 a	22.03 b	26.56 a	
NSR	G1	0.50 c	1.36 b	2.20 a	1.23 b	1.32 b
	G2	2.26 a	2.03 a	2.20 a	2.43 a	2.23 a
	Average	1.38 b	1.70 b	2.20 a	1.83 ab	

Germination percentage of days 1, 2, and 3=% GP1, % GP2, and % GP3; Genotype 1 and 2=G1 and G2; Salicylic Acid=SA; Citrulline=CI; Humic Substances=HS; Tap water=TW; Germination percentage=GP; Radicle diameter=RD; Radicle length=RL; and Number of lateral seminal roots=SR.; Means with the same letter in each column (a, b) or per row (a, b, and c) are not different (Tukey, 0.05).

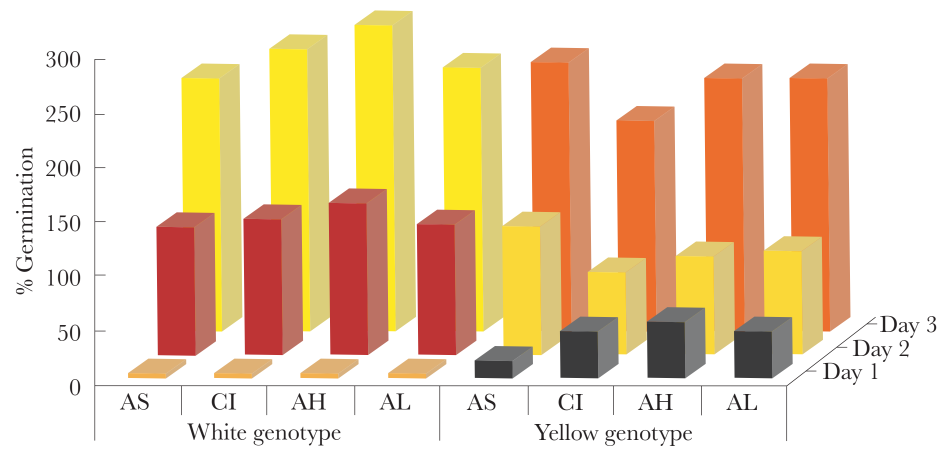

Figure 1. Germination behavior of G1 and G2 with regard to promoters, salicylic acid (SA), citrulline (CI), humic acid (HA), and tap water (TW), throughout days 1, 2, and 3.

mass of maize sprouts. These results have a positive influence on the emergence rate index, up to doses of 158 mL 100 kg^{-1} seeds. For their part, Šerá and Novák (2011) used seeds of Lamb's Quarters (*Chenopodium album* agg.) and determined that the main differences in germination and length of sprouts occurred during the first days of the experiment.

Radicle diameter

No statistical differences in radicle diameter were found between either genotypes G1 and G2 (Table 3). El-Mergawi (2019) and Rodríguez-Larramedi *et al.* (2017) mention that high doses (20 mM) with salicylic acid can inhibit germination, affecting growth and root development. For their part, Pertuit *et al.* (2001) mentioned that high doses of leonardite-based humic substances also inhibited root growth and sprouts.

Radicle length

Table 3 shows a greater development of the radicle length in G2 and, in the case of germination promoters, citrulline (CI) and tap water (TW) were the same. Sarropoulou *et al.* (2016) carried out studies with cherry slides which had direct effects on *in vitro* rooting, as well as in the accumulation of proline in the leaves and roots.

Number of seminal roots

The largest number of seminal roots shows that the promoter with humic substances (HS) is statistically different from the other promoters; in the case of genotypes, it belonged to G2 (Table 3). Authors like Olk *et al.* (2018) mention that humic acids favor root development. Qin and Leskovar (2020) mention that, in studies made with tomato, pepper, lettuce and watermelon cultivars, the biomass of roots, leaves, and sprouts was higher after transplantation, improving the growth rate at a faster rate.

CONCLUSIONS

When pregerminative treatments were applied and a promoter × genotype interaction was carried out, germination was highly significant in promoters and between two genotypes. For the GP and NSR variables, the leonardite-based HS obtained a greater response for germination and root development (in the case of G1 and G2), while it decreased for GP, RL, and NSR when salicylic acid and citrulline were used. Therefore, we conclude that the HS pregerminative treatment with G2 favors germination by 94%, when exposed to a 1000 mg kg^{-1} stimulus. This increases the number of seminal roots and the germination percentage in three days. This result will be of interest for further studies where pregerminative treatments can be manipulated to ensure higher GP on certified seeds.

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AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Forage yield of African star grass (*Cynodon nlemfuensis* Vanderyst) at different cut heights

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ABSTRACT

Objective: To determine the effect of cutting height, season, and year on the forage yield of African Star grass (*Cynodon nlemfuensis* Vanderyst) in Loma Bonita, Oaxaca, México.

Design/Methodology/Approach: A randomized block design was used in plots divided using a factorial arrangement and three replications. The treatments consisted of the combination of cutting heights, seasons, and years of evaluation with four repetitions. The following variables were taken into consideration: fresh and dry forage yield (kg ha^{-1}), leaf weight (kg FM ha^{-1}), stem weight (kg FM ha^{-1}), dead material (kg ha^{-1}), leaf-steam ratio, and total yield per hectare (kg ha^{-1}). An analysis of variance was carried out and the means were compared using Tukey's test.

Results: The cutting heights did not have an effect on the DM yield. The uniformity cut can be made between 7 and 10 cm. DM performance for the 6 characters evaluated was better in 2018 than in 2016.

Study limitations/Implications: Applying dolomite calcium to increase soil pH, N, P, and K levels under irrigated conditions could improve growth rates and DM production.

Findings/Conclusions: Cutting heights did not have an impact on forage yield. The uniformity cut could be made at a 7-10 cm height. The rainy season had a higher dry matter yield (3,310 kg ha^{-1}) than the dry season (1,902 kg DM ha^{-1}) and the cold front season (1,914 kg DM ha^{-1}).

Key words: *Cynodon nlemfuensis*, African Star grass, dry matter, forage yield.

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INTRODUCTION

The African Star grass (*Cynodon nlemfuensis* Vanderyst) is native to Rhodesia, Africa (Barrón-Arredondo *et al.*, 2020). This grass has a C_4 photosynthetic mechanism (Huang *et al.*, 2020). It is a major crop in the tropical and subtropical regions of the world due to its productive potential for animal feed, ease of establishment, persistence under grazing, and crude protein content (7.6-13.1%) (Dormond *et al.*, 1998; Hernández *et al.*, 2004; Brighenti *et al.*, 2020; Torres *et al.*, 2020). Ruminants feed on this forage species, in extensive and intensive grazing systems (Namihira *et al.*, 2019), and it can be used with cut-and-carry, hay making or ensilage systems.

As a result of their evaluation of the forage yield of African Star grass in Tabasco, Mexico, in three seasons of the year, Barrón-Arredondo *et al.* (2020) recorded the highest yield of fresh forage in the rainy season, with 10,041.8 kg FM ha^{-1}. This value was significantly different from that obtained in the dry and cold front seasons, when 5,851.3 and 5,851.5 kg FM ha^{-1} were recorded, respectively. However, the dry matter yield did not vary significantly, with averages of 2,649.8, 2,213.0, and 1,990.5 kg DM ha^{-1} for the rainy, cold front, and dry seasons, respectively. Therefore, in order to increase the biomass yield of African Star grass per unit of area, management strategies should be implemented (Barrón-Arredondo *et al.*, 2020) to protect the meristematic tissues associated with grass regrowth (Taiz and Zeiger, 2006). This process would identify the use level of the grassland and the grazing intensity, avoiding overgrazing and, consequently, achieving a better use of the areas destined for animal production. In Loma Bonita, Oaxaca, Mexico, local ranchers grow the African Star grass without fully understanding either its productive behavior throughout the year or its fresh forage yield and dry matter per hectare. These data would allow them to enhance animal productivity in the lower basin of the Papaloapan river. Therefore, the objective of this study was to determine the effects of cutting height, season, and year on the forage yield of African Star grass in Loma Bonita, Oaxaca, Mexico.

MATERIALS AND METHODS

The research was carried out in the experimental plots of the Zootechnical Post of the Universidad del Papaloapan, Loma Bonita Campus, Oaxaca, Mexico. It is located at 18° 06' N, 95° 52' W, and 25 m.a.s.l. Rainfall reaches 1,845 to 1,910 mm and the average temperature is 24.7 °C (Soto *et al.*, 2019).

For the establishment, we used 40-50 cm long stolons with developed roots and a 90-day regrowth age. The soil conditions were: 4.8 pH; 2.6% organic matter; 29.7, 35.3, 32.0, 148, and 107 mg kg^{-1} of N, P, K, Ca, and Fe; and sandy loam texture (53, 29, and 18% sand, silt, and clay, respectively).

From 2015 to 2018, the plots were fertilized with sheep manure (2,500 kg ha^{-1}) during the pasture rest, and the grass was fertilized using the 100-00-00 of the N, P_2O_5, K_2O formula, plus urea (46-00-00). In December 2015, a uniformity cut was made with 40 sheep, which initially remained only two days in each grassland to avoid overgrazing; the cut was standardized to a 10-cm average height with garden shears. This procedure guaranteed that 48 sheep (28 ewes and 20 lambs of approximately 4 months of age) could graze for five days in each plot, once the sampling sites had been isolated.

We used a randomized block design, in split plots, with a factorial arrangement and three replications. The experimental plots were traced in four 43-m long × 15-m wide African Star grass pastureland. Each experimental plot measured 5.0 m × 10.75 m (experimental unit = 53.75 m^2). Over the course of a two-year evaluation (2016 and 2018), the treatments combined four cutting heights (7, 8, 9, and 10 cm) with three production seasons: dry (March-May), rainy (June-August), and cold front (November-December). Figure 1 shows the temperature and precipitation data for the evaluation years.

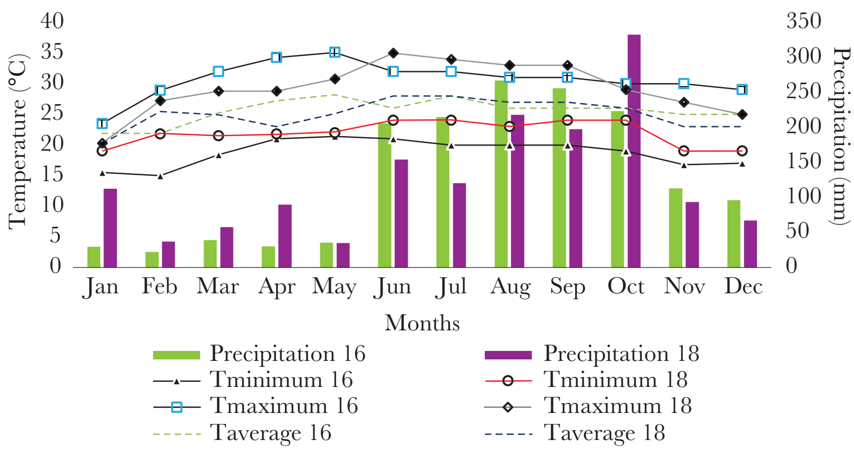

Figure 1. Precipitation (mm) and maximum, minimum, and average temperature (°C) in Loma Bonita, Oaxaca, Mexico (2016 and 2018).

The following variables were studied: fresh forage yield, leaf weight, stem weight, dead material, leaf-stem ratio, and dry matter yield. Fresh forage yield (FFY, kg ha^{-1}) was estimated from a 250-g sample harvested in a 0.25 m^2 area. The leaf blade and the stems of the plant were separated from this sample to obtain the weight of leaves (Wl, kg ha^{-1}), the fresh stems (Ws, kg ha^{-1}), and the dead material (Dm, kg ha^{-1}).

The leaf-stem ratio (LSR) was obtained by dividing the leaf component by the stem yield. To determine the dry matter yield (kg DM ha^{-1}), 250-g samples of harvested fresh forage (kg FM ha^{-1}) were collected 30 days after the uniformity cut and were put in paper bags, labeled, and dried in a forced air stove at 65 °C for 72 h. Subsequently, the dry weight was determined using a Scout Pro® digital scale.

The statistical analysis of the information consisted of an analysis of variance (Padilla *et al.*, 2019) performed under a randomized block experimental design, in subdivided plots with a factorial arrangement and three repetitions. The SAS 9.4 statistical package (SAS, 2013) was used to perform a mean comparison test (Tukey, $p\leq0.05$) of the significant variables.

RESULTS AND DISCUSSION

Influence of the year and seasons in the African Star grass production

There were significant statistical differences ($p\leq0.01$) between the evaluation years; the year 2018 stood out regarding 5 of the 6 variables measured for the African Star grass (Table 1). This situation is the result of the correct agronomic management of the grasslands and a better distribution of rain during the said year (Figure 1). In 2018, the average dry matter yield, in the rainy, dry, and cold front seasons was 2,811.7 kg DM ha^{-1}, 30% higher than in the average yield of 2016 (Table 1). In a study of African Star grass carried out in Tabasco, Mexico, an average of 2,017.7 kg DM ha^{-1} was recorded in the same seasons of the year (Barrón-Arredondo *et al.*, 2020). Meanwhile, in an evaluation carried out on *Cynodon plectostachyus* under tropical climate conditions in Ecuador, after 30 days of grass regrowth and an average of four cuts, a 2,413.7 kg

DM ha^{-1} yield was obtained, which amounted to 2,850.9 kg DM ha^{-1}, according to the nutritional contribution received by the grass (Vera *et al.*, 2019). A similar yield was obtained in the present research for the year 2018.

In 2016, only the leaf-stem ratio (0.65) was favored, a situation attributed to the recent establishment of the grasslands. This situation could change over time, as a consequence of grazing by animals and the soil compaction caused by their trampling. Rodriguez *et al.* (2015) indicate that the yield decreases over time, even when the stocking rate is adequate, mainly as a consequence of the said compaction. In this regard, Ramón-Castro *et al.* (2015) reported a leaf:stem ratio of 0.6 in African Star grass, six weeks after the uniformity cut.

The 1,533.1 mm rainfall registered in 2018 accounts for the effect of the year on the African Star grass yield. Although it was similar to the 2016 rainfall (Figure 1), it was better distributed among the seasons, allowing a better yield among the components. In addition, the higher yield recorded in 2018 is the result of a greater accumulation of leaf and stem biomass. However, as a consequence of a higher productivity, senescent or dead material also increased (Table 1).

It is evident that the time factor (years) caused changes in the grass variables measured, mainly due to the climatic conditions that caused different responses in the forage accumulated in the grasslands used. Villalobos-Villalobos and WingChing-Jones (2019) mentioned that temperature, solar radiation, relative humidity, cloudiness, and water content in the soil —which change from one year to the next— directly affect the grassland's growth and speed of recovery for its subsequent use.

The sampling seasons caused significant differences ($p \leq 0.01$) in the 6 characters evaluated in African Star grass. Statistical significance indicates that the time of year influenced the behavior of the different variables. The FFY, Wl, Ws, Dm, LSR, and DM characteristics made the rainy season stand out (Table 1).

The dry matter yield was higher in the rainy season (3,310.4 kg DM ha^{-1}), surpassing the cold front season (1,914.6 kg DM ha^{-1}) and the dry season (1,902.3 kg DM ha^{-1}) by 42.2% and 42.5%, respectively (Table 1). In this regard, Barrón-Arredondo *et al.* (2020) recorded 2,649.8 kg DM ha^{-1} in the rainy season, exceeding the dry matter

Table 1. African Star grass (*Cynodon nlemfuensis*) forage yield, depending on years and seasons (dry, rainy, and cold front; average of two years). Loma Bonita, Oaxaca, Mexico.

Variable	X	2016	2018	MSD	Dry season	Rainy season	Cold front	MSD
FFY (kg ha^{-1})	8419.2	6837.5 b	10000.9 a	1279.1	6580.2 b	12234.4 a	6443.0 b	2126.1
Wl (kg ha^{-1})	2748.4	2216.6 b	3280.3 a	486.7	1790.7 b	4418.8 a	2035.9 b	701.9
Ws (kg ha^{-1})	4312.9	3490.6 b	5135.3 a	668.7	3408.9 b	6225.5 a	3304.6 b	1082.7
Dm (kg ha^{-1})	1357.4	1130.4 b	1584.5 a	130.0	1308.7 ab	1589.0 a	1102.6 b	359.4
LSR	0.63	0.65 a	0.62 b	0.006	0.52 c	0.71 a	0.62 b	0.01
DM (kg ha^{-1})	2375.8	1939.9 b	2811.7 a	320.8	1902.3 b	3310.4 a	1914.6 b	590.4

FFY=Fresh forage yield, Wl=Fresh leaf weight, Ws=Fresh stem weight, Dm=Dead material, LSR=Leaf-stem ratio, DM=Dry matter, X=Average value, MSD=Minimum significant difference (Tukey $p \leq 0.05$), abc=Different letters within rows indicate a significant difference ($p \leq 0.05$).

yield per hectare of the dry season (1,990.5 kg) and the cold front season (2,213.0 kg) by 25% and 16.5%, respectively. These results are lower than those obtained in a very humid tropical climate in Costa Rica (where it rains up to 4,000 mm per year) and a production of 4,796 kg DM ha^{-1} $cycle^{-1}$ was recorded (Villalobos-Villalobos and WingChing-Jones, 2019).

African Star grass yield per grassland

The study showed a statistically different DM yield ($p \leq 0.05$) between grasslands in which rotational grazing had been enabled. Grasslands I, II, and III surpassed grassland IV in the FFY, Wl, Wt, Dm, and DM variables, but not in LSR. Consequently, the DM productivity recorded in grasslands I, II, and III was 27.6, 25.6, and 12.2% higher, respectively, than the productivity obtained in grassland IV (2,041.8 kg ha^{-1}) (Table 2).

The results indicate that soil fertility has an impact on the DM yield of African Star grass, since grassland IV is located in an area with a slight slope; although the evaluations were carried out three years after the grasslands had been established (under similar management between them), soil fertility could affect the results obtained. This information should be taken with caution, because the soil of each grassland was not analyzed.

In Tejupilco, State of Mexico, with a semi-warm climate with summer rains, *Cynodon plectostachyus* was subject to an agronomic and chemical composition evaluation and differences were found in grassland height, net accumulation of forage, living material, dead material, leaves, and stems. Specifically, DM yield changed from 728 to 1,193 kg DM ha^{-1} (López-González *et al.*, 2010).

African Star grass yield, depending on cutting height

The modifications to the cutting height did not affect FFY and DM (Table 3), which means that a 7-10 cm cutting height did not affect the African Star grass' productive performance.

In their study about *Cynodon plectostachyus*, Namihira *et al.* (2019) reported that a 5-cm height was less favorable for morphological components than a 15-cm height, because regrowth height mainly depends on the amount of nonstructural carbohydrates present in the plant. Additionally, the largest amount of carbohydrates stored in African Star grass is located in the root crown; therefore, Namihira *et al.* (2019) suggest refraining from

Table 2. Components associated with forage yield per grassland for African Star grass (*Cynodon nlemfuensis*).

Variable	Grasslands I	Grasslands II	Grasslands III	Grasslands IV	MSD	SE
FFY	9205.6 a	9080.1 a	8132.8 ab	7258.3 b	1131.2	308.1
Wl	2979.3 a	2961.6 a	2652.6 ab	2400.2 b	383.9	104.5
Ws	4716.5 a	4637.2 a	4171.8 ab	3726.4 b	580.6	158.2
Dm	1509.8 a	1479.0 ab	1306.7 bc	1134.2 c	186.2	50.8
LSR	0.62 a	0.61 a	0.62 a	0.62 a	0.2	0.01
DM	2604.7 a	2565.3 a	2291.4 ab	2041.8 b	318.7	86.8

FFY=Fresh forage yield (kg ha^{-1}), Wl=Fresh leaf weight (kg ha^{-1}), Ws=Fresh stem weight (kg ha^{-1}), Dm=Dead material (kg ha^{-1}), LSR=Leaf-stem ratio, DM=Dry matter (kg ha^{-1}). MSD=Minimum significant difference (Tukey $p \leq 0.05$), SE=Standard Error, ab=Different letters within rows indicate a significant difference ($p \leq 0.05$).

using 5-cm cutting heights when the temperature falls below 20 °C, during the cold front season in Mexico, because it could affect the grassland forage production for the following growing season.

African Star grass behavior, depending on the interaction of years per seasons

In both 2016 and 2018, the rainy season favored the leaf-stem ratio (Table 4). The FFY, Wl, and Ws yield components also increased in 2018; the resulting increase in grass productivity was represented by a greater amount of leaf and stem dry matter (Table 4).

The interaction detected between years × seasons indicates that African Star is a perennial grass with a creeping habit, whose stolons can reach 3.0 m in length. It grows roots at the nodes. It forms a dense cover, lignifying during the drought, although it grows rapidly in the rainy season and low temperatures stop its rapid growth during the cold front season. Therefore, proper management of this grass must be ensured to guarantee its persistence for years (Enríquez *et al.*, 2013).

Table 3. Components associated with the forage yield of African Star grass (*Cynodon nlemfuensis*), depending on cutting heights (CH), average of two years and three study seasons.

Variable	CH7	CH8	CH9	CH10	DMS	EE
FFY	8300.9 a	8623.3 a	8246.4 a	8506.3 a	1594.2	411.9
Wl	2696.9 a	2817.3 a	2677.2 a	2802.3 a	533.0	137.7
Ws	4252.9 a	4399.6 a	4233.5 a	4365.9 a	812.6	209.9
Dm	1351.1 a	1406.5 a	1333.4 a	1338.8 a	260.1	67.2
LSR	0.62 a	0.62 a	0.61 a	0.62 a	0.2	0.01
DM	2347.2 a	2425.8 a	2336.3 a	2393.9 a	446.2	115.3

FFY=Fresh forage yield (kg ha^{-1}), Wl=Fresh leaf weight (kg ha^{-1}), Ws=Fresh stem weight (kg ha^{-1}), Dm=Dead material (kg ha^{-1}), LSR=Leaf-stem ratio, DM=Dry matter (kg ha^{-1}). MSD=Minimum significant difference (Tukey $p \leq 0.05$), SE=Standard Error, ab=Different letters within rows indicate significant difference ($p \leq 0.05$).

Table 4. African Star grass (*Cynodon nlemfuensis*) yield during a two-year evaluation with three study seasons. Loma Bonita, Oaxaca, Mexico.

Variable	2016			2018			MSD	SE
	Dry season	Rainy season	Cold front	Dry season	Rainy season	Cold front		
FFY	6660.4 c	9333.3 b	4518.8 d	6500.0 c	15135.4 a	8367.3 b	1594.2	379.0
Wl	1815.9 d	3362.5 b	1471.5 d	1765.4 d	5475.0 a	2600.3 c	533.0	128.6
Ws	3401.0 c	4755.7 b	2315.3 d	3416.8 c	7695.3 a	4293.8 b	812.6	194.5
Dm	1443.6b c	1215.5 c	732.0 d	1317.8 bc	1962.5 a	1473.2 b	260.1	62.4
LSR	0.53 d	0.71 a	0.64 b	0.52 e	0.71 a	0.61 c	0.2	0.01
DM	1913.6 c	2560.9 b	1345.2 d	1891.1 c	4060.0 a	2484.0 b	446.2	106.7

FFY=Fresh forage yield (kg ha^{-1}), Wl=Fresh leaf weight (kg ha^{-1}), Ws=Fresh stem weight (kg ha^{-1}), Dm=Dead material (kg ha^{-1}), LSR=Leaf-stem ratio, DM=Dry matter (kg ha^{-1}). MSD=Minimum significant difference (Tukey $p \leq 0.05$), SE=Standard Error, ab=Different letters within rows indicate significant difference ($p \leq 0.05$).

CONCLUSIONS

No effect of the cutting heights was recorded for the forage yield of African Star grass. The uniformity cut could be made between 7 and 10 cm high. A higher dry matter yield in the 6 dry matter yield traits was registered in 2018 than in 2016. The rainy season surpassed the dry matter yield (3,310 kg ha^{-1}) of both the dry season (1,902 kg DM ha^{-1}) and the cold front season (1,914 kg DM ha^{-1}) in Loma Bonita, Oaxaca.

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AP AGRO PRODUCTIVIDAD

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Forage yield of Guinea grass (*Megathyrsus maximus* Jacq.) using mineral fertilization during two seasons of the year

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ABSTRACT

Objective: To determine the dry matter yield of Guinea grass (*M. maximus* Jacq.) in response to mineral fertilization with N-P-K in a tropical humid climate.

Design/Methodology/Approach: We conducted an experiment with Guinea grass in Loma Bonita, Oaxaca, Mexico, during the cold front season (November 2018 to February 2019) and the dry season (March to May 2019). The following N-P-K fertilization formulations were used: 00-00-00, 100-00-00, 140-20-00, 180-40-20, 200-00-00, 240-40-20, 260-60-40, and 300-00-00. The response variables were: plant height (cm), chlorophyll content, and dry matter yield (kg ha^{-1}).

Results: The fertilization with the highest nitrogen, phosphorus, and potassium levels ($p \leq 0.05$) increased the dry matter yield, the chlorophyll content, and the height of the Guinea grass above the control.

Study Limitations/Implications: Mineral fertilization improved the productive performance of Guinea grass. However, studying more seasons is necessary to validate the results obtained.

Findings/Conclusions: The fertilizer formulas with 260-60-40 and 240-40-20 N-P-K units improved the productive performance of Guinea grass. Consequently, the season of the year had a considerable influence on grass yield in a humid tropical climate.

Key words: Gramineae, humid tropic, chlorophyll, dry matter.

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INTRODUCTION

Given their geographical position on the planet, the humid tropical regions of Mexico concentrate a high diversity and richness of forage resources (Ellis & Martínez, 2010). Nevertheless, livestock currently requires the production and preservation of forage resources that are scarce throughout the year, particularly during the dry season. Ruminant production is important in Oaxaca and it requires forage for animal feed. In 2018, 381,985 ha of forage grasses were reported in this state; they provided an average fresh yield of 34.1 t ha^{-1}. In the district of Tuxtepec, 163,507 ha with 38.0 t ha^{-1} of forage yield were recorded, while, in Loma Bonita, 10,470 ha with 43.6 t ha^{-1} of fresh forage yield were recorded (SIAP, 2019).

Guinea grass is one of the most important forage species used to feed domestic ruminants in regions with tropical and subtropical climates. This is the result of its great potential to produce dry matter per unit of area, wide adaptability, forage quality, easy establishment, and its capacity to support a high stocking rate (Munari *et al.*, 2017). An emerging problem is that the plants are not fertilized, which impacts their development and reduces their quality and biomass yield (Shintate *et al.*, 2017). In this sense, fertilization is an alternative that increases grass productivity by influencing the generation of photoassimilates during photosynthesis (Silveira *et al.*, 2017).

There are few studies about the fertilization of cut tropical grasses in the humid tropical region of Mexico. Throughout the region, Guinea grasslands are fertilized with the 100-00-00 formula (Joaquín *et al.*, 2009). In addition, Joaquín *et al.* (2020) recommended using the 100-50-50 fertilizer formula (N, P_2O_5, and K_2O, respectively) to obtain a better seed yield with the said Poaceae in Loma Bonita, Oaxaca, Mexico. Evidently, in order to increase the biomass yield, the nutritional management of this grass must be identified at different seasons of the year (*e.g.*, cold front and dry seasons).

The objective of the present study was to quantify the biomass yield of Guinea grass fertilized with nitrogen, phosphorus, and potassium under the environmental conditions of Loma Bonita, Oaxaca, Mexico, during the cold front and dry seasons. The assumption was that fertilizing with high levels of these elements improves the components associated with the growth and dry matter yield of this grass.

MATERIALS AND METHODS

Geographical location of the study area

The research work was carried out in Loma Bonita, Oaxaca, Mexico (18° 06’ N, 95° 53’ W), at 25 m.a.s.l. The weather is warm humid (Am) in 81.7% of the territory, with abundant rainfall in summer, an average temperature of 25 °C, and an annual rainfall of 1,845.2 mm. However, the Papaloapan weather station reported 2,135.3 mm of rain per year. The dominant soil type are acrisols (INEGI, 2005).

Vegetal material and establishment

In June 2018, 24 experimental units were sown with *Megathyrsus maximus* Jacq cv. Guinea vegetative material, after digging 40-cm deep times 20-cm wide holes. In October 30, a uniform cut was made with gardening shears. On November 16, the cold front season samplings of the growth and dry matter yield of the grass began. This season started on November 2018 and finished on February 2019 and is characterized by low intensity rains and low temperature (Figure 1). The dry season was evaluated from March to May 2019. The dry season is characterized by high temperatures and low rainfall. During both seasons, samples were collected every seven days from the same plants.

Treatments and experimental design

Eight fertilizer formulas (FM) with nitrogen, phosphorus, and potassium were applied to Guinea grass: 00-00-00 (FM1), designated as control treatment, 100-00-00 (FM2), 140-20-00 (FM3), 180-40-20 (FM4), 200-00-00 (FM5), 240-40-20 (FM6), 260-

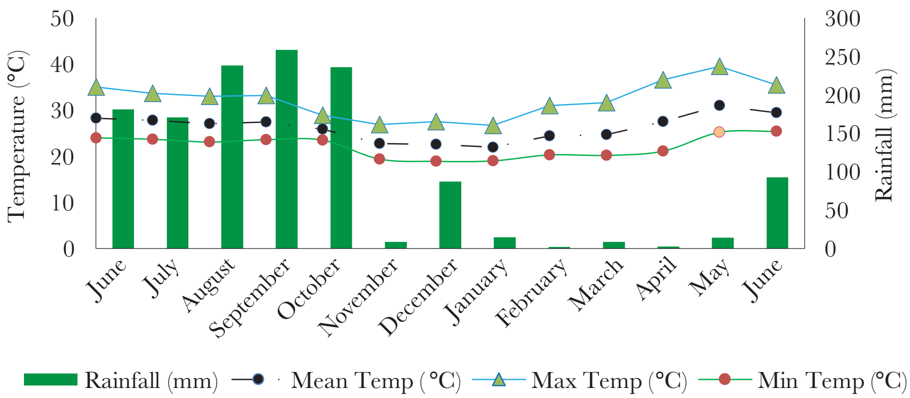

Figure 1. Precipitation (mm), and maximum, minimum, and average temperature (°C), in Loma Bonita, Oaxaca, Mexico (June 2018-June 2019).

60-40 (FM7), and 300-00-00 (FM8). Urea (46-00-00), diammonium phosphate (18-46-00), and potassium chloride (00-00-60) were used as nutrient sources. The tested fertilizer formulas were defined based on a soil analysis that was done prior to the establishment of the grass and contemplated ≤300 units of N, ≤60 units of P, and ≤40 units of K per ha, in order to determine a dose that does not have an economic impact on the producer.

The grass was homogeneously fertilized in November (cold front season) and in March (dry season), before a 15-cm uniformity cut was made in each case and when the regrowth began. In each season, the soil was fertilized after it had rained or when there was enough moisture in the soil.

The experimental design was completely randomized with eight treatments (fertilizer formulas) applied to 3.0-m long × 2.0 m wide (6 m^2) experimental units and three replications. The evaluations were made in the central part of each experimental plot. The size of the useful plot was 4.2 m^2.

Response variables

The response variables were: plant height (cm), chlorophyll content in the leaves, and dry matter yield. Plant height was measured with a flexible metal tape, from ground level to the top of the plant, with its leaves fully extended. The chlorophyll content of leaves was measured in five plants per plot, using a Minolta® portable SPAD-502 chlorophyll meter, that establishes the greenness index, which is directly related to the chlorophyll content of the leaves (Rincón & Ligarreto, 2010). The production of fresh biomass (kg ha^{-1}) was determined by weighing the plant material from the aerial part of the plant on a 5.0-kg digital scale, with a 1.0 g margin of error; this result was used to calculate the dry matter yield (kg ha^{-1}). The methodology consisted of taking a 200-g sample of fresh forage from each of the useful plots and placing it in a forced air stove at 65 °C for 72 hours or until the sample reached a constant weight which could be used to calculate the dry matter percentage.

Statistical analysis

The statistical analysis of the data was carried out separately for each season (cold front and dry season), based on a completely randomized design, with eight fertilization treatments and three repetitions, using the linear model:

$$Y_{ij}=\mu+T_i+E_{ij}$$

where Y_{ij} is the response variable, μ is the general mean of the experiment, T_i is the effect of the fertilization treatments, and E_{ij} is the source of variation associated with the experimental error. The analysis of variance was carried out using Proc GLM for Windows version 9.4 (SAS, 2013) and the means of the variables whose treatments showed them to be statistically different were compared using Tukey's test ($p \leq 0.05$).

RESULTS AND DISCUSSION

Plant height

Table 1 revealed that, when Guinea grass received different doses of fertilization with nitrogen or its combination with phosphorus and potassium, plant height (PH) in the cold front season always was statistically superior than control. The maximum PH was 148.4 cm and was obtained with the 260-60-40 treatment (Table 1), although it was not different from the PH obtained with the 180-40-20 formula on 135.2 cm tall plants ($p \leq 0.05$); in the dry season, the plant height response was lower than in the cold front season (Table 1).

Other researchers suggest that for grasses to grow, vegetative structures, elongation, and development of leaves according to their genetic nature must be present. These characteristics are influenced by rainfall, temperature, light, and soil fertility (Costa *et al.*, 2017). Muñoz *et al.* (2007) indicated that Guinea grass adapts to average annual rainfalls of 800 to 1,200 mm; although it tolerates droughts, Guinea grass recovers quickly at the beginning of the rainy season and it keeps a green foliage. The plant height was different between the cold front and dry seasons, because the rain and temperature data promoted a differential growth between seasons (Figure 1).

Chlorophyll content of leaves

In the cold front season, the chlorophyll content of Guinea grass increased in the treatments that received fertilization. They were statistically different ($p \leq 0.05$) than control, from day 14 to day 84 of sampling (Table 2). Such behavior confirmed that high nitrogen fertilization had a positive correlation with the color (green) of the leaves and it affected their chlorophyll content.

In Brazil, the application of nitrogen doses of 0, 50, 100, 150, and 200 kg ha^{-1} to *Brachiaria decumbens* resulted in an increase of the color (green) of the leaves, with intervals of 32 to 54 and 30 to 48 SPAD units, in the first and second cut, respectively (da Silva *et al.*, 2013). In another study, Salman *et al.* (2016) reported foliar chlorophyll values of 48.4 for marandu grass and 40.7 for Guinea grass; these values are consistent with the results of the present work.

Table 1. Plant height (cm) of Guinea grass (*Megathyrsus maximus*) depending on fertilization in the cold front and dry seasons. Loma Bonita, Oaxaca, Mexico.

F	E	FM1	FM2	FM3	FM4	FM5	FM6	FM7	FM8	Dhs	CV
14	1	54.7e	60.5d	57.4e	67.3b	66.9bc	65.2bc	64.1c	71.0a	2.8	4.5
	2	38.3a	33.0a	40.3a	37.3a	41.00a	37.0a	32.3a	30.0a	11.2	10.9
21	1	66.5e	73.0d	75.7cd	91.0a	77.3c	83.7b	83.6b	84.4b	3.2	4.1
	2	40.7bc	36.0cd	40.7bc	39.3bc	42.3ab	45.7a	38.0bcd	34.3d	4.7	4.2
28	1	69.7d	85.3c	83.0c	98.7a	84.4c	92.1b	96.2ab	92.7b	5.6	10.3
	2	42.3bc	38.3cd	41.3bcd	42.3bc	48.3b	48.0a	41.7bcd	37.7d	4.4	3.7
35	1	72.7e	92.2d	90.3d	106.2a	93.6cd	99.4b	108.7a	98.7bc	6.0	11.3
	2	43.7abc	41.0bc	42.0bc	45.0ab	45.0ab	47.7a	44.0ab	39.0c	4.7	3.8
42	1	80.7e	103.3bcd	96.1d	110.6ab	98.7cd	105.5bc	114.9a	102.2bcd	8.5	10.1
	2	46.0bc	43.0bc	43.3bc	46.7ab	46.7ab	50.3a	47.0ab	41.7c	4.9	3.8
49	1	84.2d	107.1bc	100.3c	115.8ab	107.0bc	112.7ab	121.0a	103.6c	8.4	10.3
	2	46.3cde	43.7e	45.3de	50.0ab	48.3bcd	52.0a	49.3abc	45.7de	3.1	2.3
56	1	89.7e	111.7bcd	103.1d	121.9ab	108.4cd	115.8bc	128.4a	106.7cd	11.3	3.6
	2	48.3c	44.3d	47.3cd	53.0b	47.7cd	57.7a	51.0bc	47.7cd	3.8	2.8
63	1	92.0d	113.3bc	108.9c	126.1ab	110.0c	118.2bc	133.6a	108.4c	14.1	4.3
	2	49.0ef	57.7ab	48.0ef	55.7bc	51.7cde	61.0a	55.0bcd	51.3de	4.3	2.9
70	1	93.6d	116.8c	112.4c	128.1ab	111.4c	119.2bc	136.2a	110.0c	11.4	3.5
	2	50.0b	68.0a	54.7b	57.0ab	53.3b	61.7ab	57.7ab	57.0ab	11.8	7.3
77	1	97.1d	121.7bc	115.1c	129.4ab	115.4c	120.7bc	140.6a	112.3c	11.3	3.4
	2	52.0c	81.0a	61.3bc	62.7bc	60.3bc	66.7b	64.7b	64.0b	11.9	6.4
84	1	99.8c	125.9b	123.1b	135.2ab	126.8b	128.4b	148.4a	126.1b	14.1	6.4
	2	54.7c	87.3a	66.0bc	67.7b	64.7bc	71.0b	71.0b	70.3b	11.6	5.9

F=Sampling date (d), E=Season (1: cold front of november 2018-february 2019), (2: march-may 2019 drought), FM1=00-00-00, FM2=100-00-00, FM3=140-20-00, FM4=180-40-20, FM5=200-00-00, FM6=240-40-20, FM7=260-60-40, FM8=300-00-00 de N-P-K-, Dhs= Significant honest difference (Tukey, p≤0.05), CV=Coefficient of variation (%), a,b,c… different letters in columns mean significant differences.

However, during the dry season, the behavior of chlorophyll in leaves was lower than in the cold front season (Table 2), as a consequence of the scarcity of edaphic moisture from March to May 2019 (Figure 1). C_4 plants (such as *Megathyrsus maximus*) can present biochemical and anatomical modifications whose aim is to increase their photosynthetic efficiency through adjustments in leaf area. These adjustments modify their root/aerial part ratio, potentially increasing the concentration of chlorophyll in the leaves, all intervened by edaphic moisture content and nutrient availability (Barragán & Cajas, 2019).

Guinea grass dry matter yield

During the cold front season, the dry matter production of *M. maximus* increased as a result of fertilization (Table 3). From day 21 to 84, the 240-40-20 and 260-60-40 NPK formulas stood out, with the exception of days 63 and 77, when the presence of strong winds bent plants, modifying the previous tendencies. Overall, these ternary formulas surpassed the control in DM production, including the rest of the fertilization treatments.

Table 2. Chlorophyll (SPAD units) of Guinea grass (*Megathyrsus maximus*), depending on fertilization in the cold front and dry seasons. Loma Bonita, Oaxaca. Mexico.

F	E	FM1	FM2	FM3	FM4	FM5	FM6	FM7	FM8	Dhs	CV
14	1	44.4b	50.7a	52.0a	50.0a	45.9b	44.8b	51.6a	50.6a	3.0	4.9
	2	20.6b	38.5a	32.5ab	21.6b	25.8b	24.4b	32.5ab	22.3b	11.9	15.4
21	1	40.3c	47.6a	46.1ab	46.0ab	47.2a	43.9b	48.2a	47.5a	3.2	4.3
	2	22.3a	32.6a	35.9a	37.3a	38.5a	38.5a	35.9a	32.6a	20.5	21.1
28	1	35.0c	40.0bc	40.3bc	50.7a	45.2ab	40.6bc	43.2b	43.2b	5.7	4.8
	2	25.8bc	38.8a	22.0c	32.5ab	32.6ab	32.6ab	21.6c	35.9ab	10.2	11.9
35	1	29.4d	37.3c	38.8abc	40.0abc	37.5bc	43.0ab	44.1a	41.3abc	5.8	5.1
	2	21.6c	25.8bc	38.5a	35.9ab	38.8a	38.8a	43.9a	38.5a	10.7	10.7
42	1	31.9c	41.2b	43.0ab	40.0bc	45.5ab	43.3ab	40.7b	50.0a	8.0	7.2
	2	33.9ab	52.7a	32.5ab	22.3b	20.6b	32.5ab	22.3b	32.5ab	22.2	25.2
49	1	31.0c	43.9ab	42.0ab	40.8ab	44.6ab	44.6ab	39.3bc	47.9a	8.5	7.3
	2	25.8bc	33.7ab	38.8a	25.8bc	21.6c	35.9a	38.5a	38.8a	9.2	10.0
56	1	31.5b	39.6ab	40.7a	43.0a	42.5a	40.9a	43.7a	45.2a	8.4	7.8
	2	35.9a	35.9a	21.6a	38.5a	37.3a	21.5a	32.5a	25.8a	20.5	23.2
63	1	30.7c	43.4b	42.8b	42.6b	39.6b	41.9b	44.6b	50.6a	5.6	4.7
	2	21.6a	38.5a	37.3a	32.5a	22.3a	37.3a	38.8a	21.6a	26.7	30.3
70	1	29.9c	39.6ab	41.9a	43.3a	34.7bc	40.9a	40.8a	43.7a	5.3	5.1
	2	25.9ab	43.9a	25.8ab	38.8ab	32.5ab	22.3b	25.8ab	37.3ab	21.4	24.0
77	1	33.0c	42.7a	41.2ab	40.7abc	32.7c	42.7a	44.1a	41.4ab	8.5	7.5
	2	22.7c	38.3a	22.3c	20.0c	35.9ab	25.8bc	21.3c	23.9c	11.7	15.6
84	1	32.9b	41.0ab	39.1ab	39.9ab	38.5ab	42.9a	41.6a	41.5a	8.4	7.6
	2	23.3c	38.8a	25.6c	26.7c	35.9ab	28.2bc	26.3c	27.3c	8.3	10.2

F=Sampling date (d), E=Season (1: cold front of november 2018-february 2019), (2: march-may 2019 drought), FM1=00-00-00, FM2=100-00-00, FM3=140-20-00, FM4=180-40-20, FM5=200-00-00, FM6=240-40-20, FM7=260-60-40, FM8=300-00-00, Dhs=Significant honest difference (Tukey, $p \leq 0.05$), CV=Coefficient of variation (%), a,b,c... different letters in columns mean significant differences.

At 42 days of sampling, during the cold front season, the Guinea grass yield was 4,674 and 4,449 kg DM ha^{-1} with the 240-40-20 and 260-60-40 formulas, respectively (Table 3). A trial carried out by Homen *et al.* (2010) with *Megathyrsus maximus* in Venezuela (2,450 mm of rain and an average temperature of 26.5 °C) recorded a yield of 4,376 and 5,097 kg DM ha^{-1} on days 35 and 42; they recommended cutting the grass 42 days after regrowth for animal feed. At a greater cutting interval, a PH and forage accumulation increase was reported for Guinea grass, as a possible consequence of the high presence of stems and dead material, which modify the leaf-stem ratio in the grass —a behavior that can affect the efficiency with which animals use the grassland (Ramírez *et al.*, 2009).

The dry matter yield was lower in the dry season than in the cold front season (Table 3), as a consequence of the lack of moisture from March to May 2019 (Figure 1), which hindered the grass' potential to induce the development of meristematic tissues and promote the growth of the grass.

Table 3. Dry matter yield (kg ha^{-1}) of Guinea grass (*Megathyrsus maximus*), depending on fertilization in the cold front and dry seasons. Loma Bonita, Oaxaca, Mexico.

F	E	FM1	FM2	FM3	FM4	FM5	FM6	FM7	FM8	Dhs	CV
14	1	269.1e	535.0b	428.2c	333.4d	307.2de	717.3a	544.7b	551.5b	56.9	15.7
	2	243.5b	99.5b	637.2a	223.3b	226.1b	81.3a	98.1b	44.3b	222.8	28.1
21	1	561.3f	1578.5b	1073.2e	1578.7b	1228.7cd	1768.7a	1779.7a	1192.4d	112.4	13.9
	2	196.0b	466.3ab	683.2a	388.8ab	347.2ab	216.8b	233.7ab	130.3b	454.0	10.2
28	1	705.7e	2296.2b	1415.8d	2000.3c	1945.0c	2952.5a	2929.5a	1827.6c	290.1	14.9
	2	329.0bc	656.8a	504.8ab	537.0ab	453.7abc	328.8bc	452.1abc	193.3c	284.9	23.3
35	1	1398.0d	2617.8bc	2282.0c	2540.0bc	3041.2b	4332.7a	4438.4a	2283.7c	566.4	17.8
	2	789.7ab	513.6bcd	974.1a	713.6abc	493.0bcd	422.9cd	701.2abc	282.4d	306.9	17.7
42	1	1465.0d	3043.0bc	2715.3c	2642.0c	3005.7bc	4674.0a	4449.3a	2460.4c	848.1	10.2
	2	834.1abc	888.8ab	1229.9a	930.7ab	563.0bc	536.5bc	831.2abc	401.3c	448.0	20.4
49	1	1646.0d	3805.8ab	3777.8ab	3055.5bc	3940.7a	4048.9a	4557.3a	2677.8c	853.1	18.8
	2	930.9ab	1254.7a	857.1ab	1263.2a	659.7b	662.7b	1231.5a	580.8b	503.5	19.2
56	1	1443.9e	3712.1cd	3759.7cd	5220.8b	3978.4c	5346.2ab	6110.3a	3069.4d	860.8	17.3
	2	1168.3b	1529.0a	447.2d	1504.8a	772.3c	830.9c	1758.9a	750.1c	261.6	8.5
63	1	2154.9d	6824.8b	6039.1b	6468.0b	6338.1b	4221.7c	9125.5a	4639.7c	848.1	12.3
	2	884.1c	1728.0ab	1225.3bc	1867.5a	1337.3bc	1044.5c	2111.5a	985.3c	518.7	13.1
70	1	3452.0f	4830.0e	6411.0d	7617.0bc	6996.0dc	9156.0a	8340.0ab	5224.0e	1130.7	16.2
	2	1643.5abc	2301.3abc	2513.1ab	2597.1ab	1362.7bc	1445.3bc	2716.0a	1222.9c	1268.1	22.7
77	1	3565.0f	5201.0e	8699.0c	97302.0b	6061.0d	12545.3a	9898.0b	5490.0de	832.2	21.8
	2	2752.0bc	4743.5a	2653.1bc	2727.2bc	1311.6c	1921.9c	3761.1ab	1796.8c	1833.0	23.9
84	1	2023.0e	5832.0c	6067.0bc	5613.0c	4154.0d	7053.0ab	7665.0a	6711.0abc	1130.7	16.9
	2	2885.3bc	5043.5a	2953.1bc	2860.5bc	1711.6c	2155.2c	3961.1ab	2463.5bc	1628.9	19.2

F=Sampling date (d), E=Season (1: cold front of november 2018-february 2019), (2: march-may 2019 drought), FM1=00-00-00, FM2=100-00-00, FM3=140-20-00, FM4=180-40-20, FM5=200-00-00, FM6=240-40-20, FM7=260-60-40, FM8=300-00-00 de NPK, Dhs=Significant honest difference (Tukey, $p \leq 0.05$), CV=Coefficient of variation (%), a,b,c… different letters in columns mean significant differences.

CONCLUSIONS

The productive performance of Guinea grass varied depending on the environmental conditions. Precipitation was a determining factor, because the highest productions of dry matter (DM) occurred in the cold front season, when the 240-40-20 and 260-60-40 formulas generated the highest yield of dry matter and chlorophyll in leaves; the latter formula also promoted greater plant height. In the dry season, the opposite phenomenon occurred, since the dry matter productivity decreased in comparison with the cold front season.

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Evaluation of three Buffel grass varieties, in northern Tamaulipas

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ABSTRACT

Objective: To evaluate the vegetative, reproductive, production, and forage quality characteristics of three Buffel grass varieties from northern Tamaulipas.

Design/Methodology/Approach: A pot trial was established under open field conditions, at CERIB-CIRNE-INIFAP in Río Bravo, Tamaulipas, on 04/15/2016 and 03/10/2017; the experiment was carried out with a completely randomized design, with 30 replications by material. The morphological, reproductive, production, and chemical composition characteristics of Milenio, Regio, and Titán Buffel grass varieties forage were evaluated.

Results: The year accounted for 62.3% of the variation in the stem and leaf characteristics. The genotype contributed 35.4% and G*Y interaction, 2.3%. Regarding the reproductive variables, the genotype accounted for 62% of the results, the G*Y interaction, 25%, and the year, 13%. The genotype and the year accounted for 44% and 47% of the variance in forage production and quality, respectively. The G*Y interaction had no statistical effect.

Limitations/Implications: The evaluation was carried out under pot conditions, which implies that the roots of the plants have limited growth and that the expression of some of the characteristics of the material may be restricted.

Findings/Conclusions: The major differences between the three varieties were found in their morphological characteristics: duration of the flowering stage, reproductive stems proportion, panicle length, exertion of the panicle from the main stem, number of florets per panicle, and seed weight.

Keywords: Forage, apomictic, elite, adaptation, alternative.

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INTRODUCTION

The semi-arid zone covers central and northeastern Tamaulipas, northern Nuevo León, and northeastern Coahuila. The most representative plant communities are the sclerophyll

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shrubland or chaparral. In southern Texas and northeastern Mexico, the herbaceous stratum has low diversity and is mainly composed of grasses such as: Relaxgrass [*Aegopogon cenchroides* (Humb. & Bonpl. ex Willd.)], Blue Grama [*Bouteloua gracilis* (Willd. ex Kunth) Lag. ex Steud.)], Hairy Grama (*Bouteloua hirsuta* Lag.), Deergrass (*Muhlenbergia rigida* Kunth), Woodland Muhly [*Muhlenbergia rigens* (Benth) A.S. Hitch.], and *Setaria* sp. (González, 2003). Buffel grass (*Cenchrus ciliaris* or *Pennisetum ciliare* (L.) Link) is a perennial, bunchgrass, rhizomatous species native to Africa (Ibarra *et al.*, 2013). In the state of Tamaulipas, Buffel grass is used to modify two types of natural vegetation: the thorn scrub in the northern part and the thorn lowland rain forests in the center and south of the state (Garza *et al.*, 2010). In addition, it is a drought-tolerant grass; drought is a recurring phenomenon especially in northern Tamaulipas that is aggravated by the presence of the midsummer heat (Sánchez *et al.*, 2018). Currently, erratic precipitation accompanied by temperatures above 38 °C has spread to the central and southern areas (SMN-CONAGUA, 2021), where most of the state's livestock is raised. This fast-growing species is resistant to intensive grazing and has a higher seed production than native species; these characteristics make it valuable as forage in the state of Tamaulipas (Garay *et al.*, 2017). Therefore, as a result of this evaluation, we expect to find the contrasting differences between the three varieties; these differences are caused by their genetic origin and the adaptation area where they have been tested. The objective of this experiment was to evaluate seed production and yield and forage quality of three Buffel grass varieties in northern Tamaulipas.

MATERIALS AND METHODS

Vegetable material

Milenio Buffel grass variety is apomictic and was obtained by selection of accessions from the Germplasm Bank of Buffel grass from Texas A&M University, USA. These lines come from an initial collection carried out in South Africa in 1976 by North American researchers (García, 2003). In 2007, this variety was registered in the National Catalog of Plant Varieties (NCPV) of the National Seed Inspection and Certification Service of México Department of AgricultureNational Seed Inspection and Certification Service of México Department of Agriculture, with the number CEN-003-060608. Titán and Regio varieties were also obtained in 2007 from a regional collection of germplasm in the highlands of San Luis Potosí, México (Beltrán *et al.*, 2017) and have CVV registration numbers CEN00160608 and CEN00260608, respectively. The morphological variables were measured using the SAGARPA-SNICS technical guide (2014), during the early cycle of seeds sown in April (2016) and March (2017) at the Campo Experimental Río Bravo, Tamaulipas (25° 57' N and 98° 01' W; 25 m.a.s.l.). This experiment was established under open field conditions in 40×40 cm pots with approximately eight kilos of substrate. Thirty pots were planted of each variety (Titán, Regio, and Milenio). The pots were irrigated every 10 days. The reported data correspond to the average of two years. During the experiment, ambient temperature data (maximum, minimum, and average) and precipitation (mm) were taken at the weather station located in the experimental field.

The fresh biomass was divided into stem, leaves, and panicle and the weight of each component was obtained. Subsequently, they were dried in an oven at 60 °C for 72 h.

Once dehydrated, they were ground in a Revolving Knife Mill for laboratory use (Thomas Model 4 Wiley® Mill) with a 1-mm sieve. The crude protein (CP) content of the forage was determined by the Kjeldahl method. Neutral detergent fiber (NDF), acid detergent fiber (ADF), and lignin in leaves and stems were analyzed using the method described by Van Soest *et al.* (1991). The proximal analysis was performed based on the official data of the AACC Methods. Total protein content (official method 46-13.01) and crude fiber (official method 32-10.01) were determined by the Kjeldahl method. The combined analysis of variance was performed with a completely randomized model; the variation sources were varieties or genotypes and years, with 30 repetitions by variety. Simple Pearson correlations were also made between vegetative and reproductive variables using the statistical analysis system version 9.31 (SAS, 2006). Mean comparisons were made with Tukey test ($p<0.05$). This work does not include tables of the correlations between the variables; only some results are quoted.

RESULTS AND DISCUSSION

Morphological differences between varieties

The analysis of variance (Table 1) showed that the effect of the year was the factor with the highest statistical value: it accounted for 62.3% of the variation of the stem's and the leaf's 12 vegetative characteristics, while the genotype contributed 35.4% and the G*Y interaction, less than 2%. The variation sources did not have a significant effect on the number of axillary bayonets (NAB); consequently, the stability of this varieties is considered a good descriptive characteristic. In contrast, the main stem height was highly variable. In this experiment, the 2016 and 2017 ambient temperature records were: 33 and 34 °C (maximum); 28 and 28 °C (average); and 22 and 22 °C (minimum), respectively. Total rainfall ranged from 155 mm in 2016 to 131 mm in 2017. The climatic variable that showed a difference was the amount of rainfull: 15% more in 2016 than in 2017.

Table 1. Mean squares for vegetative variables.

SV	df	PH (cm)	MSH (cm)	NB	NN	DT (mm)	NAB
G	2	388.1 ns	1129.7*	6 ns	16.9 ns	14.7*	0.3 ns
Y	1	74.9 ns	2155.5*	1229**	326.9*	31.0*	0.2 ns
G*Y	2	1305.3**	1951.9*	11 ns	9.0 ns	1.7*	2.1 ns
CV		14.6	15.9	15.3	19.6	20.8	13.5
SV	**gl**	**L3L**	**W3L**	**LFL**	**WFL**	**LA3L**	**LAFL**
		(cm)				**(cm²)**	
G	2	193.8*	25.8*	426.3*	62.3*	72368**	125662*
Y	1	49.0 ns	0.02 ns	2492.4**	202.3*	5683 ns	342605**
G*Y	2	125.3*	17.3*	90.0 ns	3.5 ns	295 ns	7063 ns
CV		17.9	11.4	9.8	19.2	13.6	15.3

SV: source of variation; df: degrees of freedom; G: genotype; Y: year; G*Y: genotype per year interaction. PH: plant height; MSH: main stem height; NB: number of bunches; NN: number of nodes in the main stem; DT: Diameter of stem; NAB: number of axillary bayonets; 3L: third leaf; FL: flag leaf (L: length, W: width, and LA: leaf area). CV: Coefficient of variation. Statistical significance: ns=not significant; *=.001; **0.001.

In contrast to the vegetative characteristics, the genotype had a greater effect on the reproductive variables (62%), followed by the G*Y interaction (25%). Finally, the year had a lower statistical weight in the expression (13%). The three sources of variation had a highly significant effect on seed weight, unlike the rest of these characteristics (Table 2). This result can be expected, because grasses produce seed in a staggered manner and part of that production is vain or of very low weight, as a result of changes in the length of the seed filling period. This period can be affected by intra-plant competition between bunches and translocation of photoassimilates to the grain (Ryan *et al.*, 2018). The effect of the genotype is associated with this response. Genetic variability influences the components of seed yield, as Lopes *et al.* (2017) have reported for *Paspalum* genus interspecific hybrids.

The characteristic that best differentiates the Milenio variety was the height of the main stem (98.0 cm); in contrast, the stems of the Regio and Titán varieties were 8.1 cm and 10.8 cm longer, respectively. The rest of the stem and the leaf morphological characteristics showed no statistical differences or very little contrast (Table 3). Greater differences were expected in this evaluation, as a consequence of the genetic origin of the Milenio variety. This material was derived by introduction of accession PI409443,

Table 2. Mean squares for reproductive variables.

SV	df	DA	DFS	RSP	LP (cm)	EX (cm)	NFP	W1000S (g)
G	2	6311**	33*	19 ns	41**	30.1 ns	1737.2*	5.8*
Y	1	609*	132**	595**	89**	298.5**	0.13 ns	5.2*
G*Y	2	8 ns	2 ns	48*	11 ns	51.3 ns	3162.8**	4.4*
CV		2.0	12.0	23.5	11.1	17.5	21.2	16.6

SV: source of variation; df: degrees of freedom; G: genotype; Y: year; G*Y: genotype per year interaction. DA: days to anthesis; DFS: duration of the flowering stage; RSP: reproductive stems proportion; LP: length of the panicle; EX: exertion of the panicle from the main stem; NFP: number of florets per panicle; and W1000S: weight of 1000 seeds. CV: Coefficient of variation. Statistical significance: ns=not significant; *=.001; **0.001.

Table 3. Comparison of means between Buffel grass varieties. Phenotypic characteristics of the stem and leaf. Río Bravo, Tamaulipas, México, 2016 and 2017.

Variety	MSH (cm)	ATS (cm)	NB	NN	NAB	DT (cm)
Milenio	80.0±13.3 a	98.0±19.3 a	7.2±6.2 c	10.4±1.9 a	10.3±2.7 a	4.0±1.8 a
Regio	72.5±12.2 b	87.9±14.6 b	11.1±7.4 a	10.3±2.1 a	9.9±2.5 a	3.5±1.6 b
Titán	73.0±12 ab	87.7±14.3 b	9.8±7.8 b	9.8±1.9 a	10.5±2.6 a	3.3±1.2 b
	L3L (cm)	**W3L (cm)**	**LFL (cm)**	**WFL (cm)**	**LA3L (cm^2)**	**LAFL (cm^2)**
Milenio	2.8±0.7 a	34.0±6.1 a	8.5±2.3 a	24.3±7.9 a	294±21 a	192±58 a
Regio	1.9±0.6 b	33.7±6.7 a	8.4±2.5 a	25.3±8.3 a	277±34 a	173±61 a
Titán	1.9±0.7 b	30.0±6.8 b	7.5±2.6 b	21.4±8.1 b	222±48 c	107±77 b

MSH: main stem height; NB: number of bunches; NN: number of nodes; NAB: number of axillary bayonets; DT: Diameter of stem; 3L: third leaf; FL: flag leaf (L: length, W: width, and LA: leaf area). Values with different literals between columns are statistically different (Tukey, p=0.05).

collected in Aberdeen, Cape Province, South Africa (32° 29’↱0”↱ S, 24° 4’↱0” E, at an altitude of 915 m.a.s.l.), where the average temperature is 16.7 °C (USDA-ARS-GRIN, 2021). The Regio and Titán varieties were derived from regional collections in the highlands of San Luis Potosí, México at altitudes ranging from 1,800 to 1,950 m.a.s.l. (Beltrán *et al.*, 2017). Meanwhile, the Milenio variety formed 31% and 20% less bunches by plant than the Regio and Titán varieties (García *et al.*, 2003). The Milenio variety has adapted to the extreme climatic conditions of northeastern Mexico (Coahuila, northern Nuevo León, and Tamaulipas), in localities with an altitude between 50 and 200 m.a.s.l., an average temperature of 23.5 °C, and rainfall ranged 400 and 750 mm. In this experiment, the mean temperature during the growing season was 28 °C, both in 2016 and 2017. According to these data, the mean temperature was 4.5 °C higher than the temperature recorded during the past decade (García *et al.*, 2003).

In this evaluation, the earliest variety was Milenio with 68±3 days at anthesis, while the Regio and Titán varieties were later with 78±4 days and 89±5 days, respectively (Table 4). These results match the findings of Beltrán *et al.* (2017), who indicate that the Titan variety is less precocious. A characteristic of the panicle structure of the Milenio variety is the length of the peduncle. In this case it was measured on the main stem, but all the reproductive stems show that it is longer (9.2±5.2 cm). The panicle measured 11.5±2.3 cm. The Regio variety had similar results (Table 4). Kizima *et al.* (2014), using a direct seeding method, report that seed production has the following components: proportion of fertile stems (0.50); stem diameter (2.35 cm), stem height (88 cm), length (12 cm), and inflorescence width (13.2 cm). Meanwhile, the average data of the three varieties in this experiment was as follows: proportion of fertile stems (0.29); stem diameter (2.2 cm), stem height (76.3 cm), panicle length (11.1 cm), and number of florets per panicle (87) (Table 4). No significant correlation was observed between the number of florets per panicle and the following variables: stem diameter, plant height, and number of total stems. The correlation values were less than 5%. Conde *et al.* (2011) report differences between genotypes regarding the production of florets per panicle. The materials with the highest number of florets were Nueces (82) and T-1754 (80), while Común (41) and Formidable (52) had a lower production and the average number of florets (64) was recorded in

Table 4. Comparison of the means of reproductive characteristics between Buffel grass varieties. Río Bravo, Tamaulipas, México 2016 and 2017.

Variety	DA	DFS	RSP	LP (cm)	EX (cm)	W1000S (g)	NFP
Milenio	68±3 c	9±3 a	0.33 a	11.5±2.3 a	9.2±5.2 a	3.6±0.6 a	92±20 a
Regio	78±4 b	9±3 a	0.29 b	11.9±2.5 a	7.3±3.8 b	3.2±0.8 b	90±21 a
Titán	89±5 a	8±2 b	0.25 b	10.0±2.5 b	7.4±3.8 b	3.1±0.7 b	80±22 b
mean	78	8	0.29	11.1	7.9	3.3	87

DA: days to anthesis; DFS: duration of the flowering stage; RSP: reproductive stems proportion; LP: length of the panicle; EX: exertion of the panicle from the main stem; NFP: number of florets per panicle; and W1000S: weight of 1000 seeds. Values with different literals between columns are statistically different (Tukey, p=0.05).

Victoria, Tamaulipas, México. Meanwhile, during this experiment, an average of 87 was obtained over two years. In this evaluation, no incidence of rice blast disease (*Pyricularia grisea*) was observed; this is the main disease of susceptible genotypes such as the Buffel Común and its incidence and severity increase under drought stress (Díaz *et al.*, 2007). The presence of panicle ergot (*Clavices* sp.) may be registered when the temperature drops by 15 °C during the flowering stage. This disease affects seed production.

Agronomic and chemical characteristics of the forage

In this experiment, a significant effect of the genotype was detected on yield and forage quality variables (Table 5). This effect helped to explain 44% of the variance, while the year factor accounted for 47% and the G*Y interaction had no statistical weight in any of these variables. The sources of variation had no effect on the amount of lignin in the biomass. Unlike the vegetative and reproductive variables, the genotype significantly contributed to explain the results.

The growing season or seasonal variations have an effect on the amount of NDF and ADF, factors that determine the forage quality (Flores, 2009). The growing season influences the production of dry biomass: the highest forage production was obtained during the spring (4.7 t MS ha^{-1}), subsequently decreasing in winter (0.7 t MS ha^{-1}) (Garza *et al.*, 2005; Flores, 2009; Garza *et al.*, 2010). In this experiment the year factor led to significant variations on yield and forage quality. Dumont *et al.* (2015) point out that climate change has a twofold impact on livestock: (i) on the chemical characteristics of forages and (ii) on animals themselves (intake and digestive processes). The production of fresh and dry weight per plant in 2016 was 10% and 19% higher than in 2017 (Table 6). The chemical composition of the fiber portion of the forage (NDF, ADF, LIG, HE, and CE) was 3-6% higher in 2016 than in 2017. On the one hand, there was no difference in crude protein between years (average: 7.3%). On the other hand, differences between genotypes were observed. The Regio variety produced the highest amount of protein (8.1%).

Table 5. Mean squares of forage production and chemical composition.

SV	df	BFW (g)	BDW (g)	CP	NDF	ADF	LIG	HE	CE	AS
					(%)					
G	2	3463.4*	895.1*	4.7*	16.0**	5.8**	0.108 ns	10.4**	6.4**	0.36*
Y	1	94508.3**	29670.1**	0.04 ns	21.8**	7.6**	0.088 ns	3.6**	6.1**	2.88**
G*Y	2	2361.0 ns	811.0*	0.03 ns	2.3 ns	1.4 ns	0.008 ns	1.6 ns	2.4 ns	0.22 ns
CV		14.9	16.4	9.3	0.41	1.6	6.1	1.9	1.6	2.2

SV: source of variation; df: degrees of freedom; G: genotype; Y: year; G*Y: genotype per year interaction. BFW: total biomass fresh weight; BDW: total biomass dry weight (biomass data per plant); CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; LIG: lignin; HE: hemicellulose; CE: cellulose; and AS: ash. CV: Coefficient of variation. Statistical significance: ns= not significant; *=.001; **0.001.

Table 6. Means comparison between Buffel grass varieties. Characteristics of forage production and chemical composition. Rio Bravo, Tamaulipas, México, 2016 and 2017.

Variety	BFW (g)	BDW (g)	CP	NDF	ADF	LIG	HE	CE	AS
				(%)					
Milenio	57.3 b	24.2 c	7.5 b	65.4 c	40.1 b	4.9 a	25.3 b	35.2 c	11.9 b
Regio	62.6 a	30.5 a	8.1 a	68.6 a	41.0 b	4.7 a	27.6 a	36.4 b	12.9 a
Titán	56.5 b	27.9 b	6.3 c	67.4 b	42.1 a	4.8 a	25.3 b	37.2 a	12.8 a
Year									
2016	61.9 a	30.4 a	7.3 a	68.2 a	41.7 a	4.9 a	26.5 a	36.8 a	13.1 a
2017	55.8 b	24.5 b	7.3 a	66.0 b	40.4 b	4.7 a	25.6 b	35.7 b	12.3 b
mean	58.8	27.5	7.3	67.1	41.1	4.8	26.1	36.2	12.5

BFW: total biomass fresh weight; BDW: total biomass dry weight (biomass data per plant); CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; LIG: lignin; HE: hemicellulose; CE: cellulose, and AS: ash. Values with different literals between columns are statistically different (Tukey, p=0.05).

CONCLUSIONS

The year factor accounted for 62.3% of the variation of the stem's and the leaf's 12 vegetative characteristics, while the genotype contributed 35.4% and the G*Y interaction, 2.3%. Regarding the reproductive variables, the genotype accounted for 62% of the results, the G*Y interaction, 25%, and the year, 13% (the lowest statistical value). The characteristic that best differentiates the Milenio variety from the Regio and Titán varieties was the height of the main stem (98.0 cm). The rest of the stem and the leaf morphological characteristics showed no statistical differences or very little contrast The earliest variety was Milenio (68±3 DA). The genotype accounted for 44% of the variance in forage production and quality; meanwhile, the year factor accounted for 47% and the G*Y interaction was not significant. In 2016, the production of fresh and dry weight per plant was 10% and 19% higher than in 2017. The chemical composition of the fiber portion of the forage (NDF, ADF, LIG, HE, and CE) was 3-6% higher in 2016 than in 2017. The variations in this experiment were attributed to the genotype. The Regio variety produced the highest amount of protein (8.1%).

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Yield and nutritional value of *Moringa oleifera* Lam, forage at different population densities

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ABSTRACT

Objective: To assess the aerial biomass yield and nutritional value of *Moringa oleifera* at densities of 50,000 (D1), 100,000 (D2), and 200,000 (D3) plants ha^{-1}.

Design/methodology/approach: The experiment was established under a randomized complete block design, with a split-plot arrangement and three replications. From 155 days after sowing, 5 cuts were made every 28 days. The following variables were assessed: total dry matter (TDM) and leaf dry matter (LMD) yield (kg ha^{-1}) and crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber (ADF) content (g kg^{-1}).

Results: An interaction between densities and cuts was observed. Regarding TDM yield, D1 surpassed D2 and D3, by 71 and 83%, respectively, in cuts 1 and 2; however, D3 showed the highest TDM yield ($P<0.05$) in cuts 3 and 4, surpassing D2 by 47% and D1 and D2 by 46 and 76%, respectively. The highest SDM yield occurred in D1, in cuts 1 and 2 (561 and 852 kg ha^{-1}, respectively); while D3 obtained the highest values in cuts 3 and 4 (901 and 1054 kg ha^{-1}, respectively). An 11% CP content reduction ($P<0.05$) was observed by the density increased from D1 to D2 (222 *vs.* 198 g kg^{-1}). In regard to NDF and ADF values, no differences ($P>0.05$) were found between the densities assessed.

Limitations/Implications: Planting density in *Moringa oleifera* determines the forage yield potential and nutritional value.

Findings/Conclusions: *Moringa oleifera* grown in semi-arid conditions at a density of 50,000 ha^{-1} plants and with 28-day cutting intervals showed the best productive behavior (yield and protein concentration).

Key words: Forage tree, morphological composition, semi-arid.

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INTRODUCTION

The state of Tamaulipas allocates approximately 4.98 million hectares (62% of its territory) to livestock, 75% of which is used to develop this activity through extensive grazing systems, mainly in native grasslands (SAGARPA, 2010) and crop residues with low nutritional value (Katoch and Tripathi, 2020). Additionally, the instability of climatic conditions resulting from climate change affects the livestock sector (Hatfield *et al.*, 2020), especially during the dry season, when forage production is reduced by more than 90% (Garay *et al.*, 2019).

The use of forage trees to feed ruminants can be an option to maintain a more sustainable animal production (García *et al.*, 2006). Such is the case of *Moringa oleifera* Lam., a tree species native to the tropical forests of northeastern India (Ramachandran *et al.*, 1980). In Mexico, *Moringa oleifera* has been adapted to tropical regions with the following characteristics: an altitude of less than 600 masl, an absolute minimum temperature of 15 °C, a bimodal/monomodal rainfall regime, an annual rainfall of up to 1000 mm, and drained soils that do not prevent the oxygen passage to the roots (Olson and Alvarado-Cárdenas, 2016).

This plant achieved approximately 12.85 t ha^{-1} $year^{-1}$ dry matter yields, with up to 240 and 800 g kg^{-1} of crude protein and *in vitro* digestibility, respectively (Zheng *et al.*, 2016). However, Zheng *et al.* (2016) and Rojas-García *et al.* (2021) have reported that, in the case of forage trees, topological arrangements and planting density can modify the yield and nutritional values of forage. Therefore, the purpose of this study was to assess the forage yield and nutritional value of different population densities of *Moringa oleifera*, in the climate and soil conditions of Tamaulipas, Mexico.

MATERIALS AND METHODS

The study was performed under rain-fed agriculture conditions, from July to November 2017, in the Zoological Research Station "Ing. Herminio García González" of the Facultad de Ingeniería y Ciencias, Universidad Autónoma de Tamaulipas, located in the municipality of Güémez, Tamaulipas, Mexico (23° 56' 26" N and 99° 05' 59" W, at 193 masl). The clayey soil is moderately alkaline (pH 8.4), has an 0.84 dS m^{-1} electrical conductivity, and 4.43 and 0.264% of organic matter and nitrogen, respectively. It has a BS_1(h') hw climate (Vargas *et al.*, 2007), characterized by summer rains and scarce rain the rest of the year. Table 1 shows the weather conditions that occurred during the evaluation period.

Three population densities were assessed: 50,000 (D1), 100,000 (D2), and 200,000 (D3) plants ha^{-1}. A 2×2 m (4 m^2) useful plot was established in the center of a 4×4 m (16 m^2) experimental plots. Soil preparation consisted of two crossed patterns made with a drag harrow (discs) and one with a power harrow (rotavator); the latter was used to incorporate plant debris and to crumble the soil. The seeds were sown on February 5, 2017. Two

Table 1. Accumulated rainfall and minimum and maximum temperature of the experimental site, recorded per cut during the evaluation period (2017).

Variable	Cut 1 05-aug	Cut 2 01-sep	Cut 3 29-sep	Cut 4 27-oct	Cut 5 24-nov
Precipitation (mm)	10	61	98	128	7
Maximum Temperature (°C)	42	42	39	35	35
Minimum Temperature (°C)	21	21	17	10	7

botanical seeds (without pregerminative treatment) were placed per point at a 2-cm depth, 0.10, 0.20, and 0.40 m apart from each other; there was a distance of 0.50 m between rows. Thirty days after sowing, thinning was conducted to obtain densities D3, D2, and D1. In addition, supporting irrigation was used at field capacity and weeding was performed every 14 days to ensure the establishment of the crop.

On July 7, 2017 (155 days after sowing), a uniform cut was made 25 cm above ground level and the soil was fertilized with 100, 50, and 50 kg ha^{-1} of N, P_2O_5, and K_2O, respectively. Urea (46% N), calcium triple superphosphate (46% P_2O_5), and potassium chloride (60% KCl) were used as sources. Then, five plants were selected from each useful plot using the five diagonal point sampling method (four corners and the center) and they were labeled. Subsequently, five samples were taken (every 28 days), harvesting all the regrowths (branches) above the uniformity cut. Height of regrowth (HR, cm) was evaluated, along with total dry matter (TDM), leaf dry matter (LDM), and stem dry matter (SDM) yields (t ha^{-1}). HR was measured before each cut from the uniform level cut up to the canopy's upper edge. In the case of the yield, regrowths were harvested (25 cm from the ground) and separated into leaves (compound leaves) and stems, then weighed on a digital scale (ADAM® Core balance™ CQT2601 model) to obtain the green matter weight. Afterwards, fresh leaf (250 g) and stem (100 g) subsamples were taken and placed separately in kraft paper bags and then put in a stove of forced air circulation (Thermo Scientific™ Heratherm™ OGS100 model) at 60 °C for 72 h. Subsequently, they were weighed on a digital scale (ADAM® Core balance™ CQT2601 model) to determine their dry weight and calculate the LDM, SDM, and TDM.

A hammer mill (Thomas Model 4 Wiley® Mill) was used to determine the nutritional value of samples that had been previously ground with a 1-mm sieve. Crude protein (CP) and ash (AS) content was determined using AOAC methodologies (1990) and neutral detergent fiber (NDF) and acid detergent fiber (ADF) content, using methodology Van Soest *et al.* (1991). Hemicellulose (Hem) was obtained using the following formula:

$$Hem = NDF - ADF$$

Data was analyzed with the GLM procedure (SAS, 2002), based on a completely randomized split-plot arrangement design with three replications. The large plot was the population density and the small plot, the cuts. Means were compared using the Tukey's test ($\alpha = 0.05$).

RESULTS AND DISCUSSION

Significant differences ($P<0.05$) between densities, cuts, and density × cut interaction became evident as a result of the analysis of the yield of total, leaf, and stem dry matter (DM) and height of regrowths (HR) in the evaluation period (Table 2).

D1 surpassed D2 by 71% (686 *vs.* 399 kg ha^{-1}) and D3 by 83% (1004 *vs.* 550 kg ha^{-1}) in TDM yield in cuts 1 and 2, respectively (Figure 1). However, D3 obtained the highest TDM yield ($P<0.05$) in cuts 3 and 4, surpassing D2 by 47% (1028 *vs.* 700 kg ha^{-1}) and D1 and D2 by 46 and 76% (1193 *vs.* 816 and 678 kg ha^{-1}), respectively (Figure 1).

Table 2. Significance of yield of total, leaf, and stem dry matter, and height of regrowth.

Variable	Source of variation		
	Density	**Cut**	**Density × Cut**
Total dry matter (t ha^{-1})	**	**	**
Leaf (t ha^{-1})	**	**	**
Steam (t ha^{-1})	**	**	*
Regrowth height (cm)	**	**	**

*: P≤0.05, **: P≤0.01.

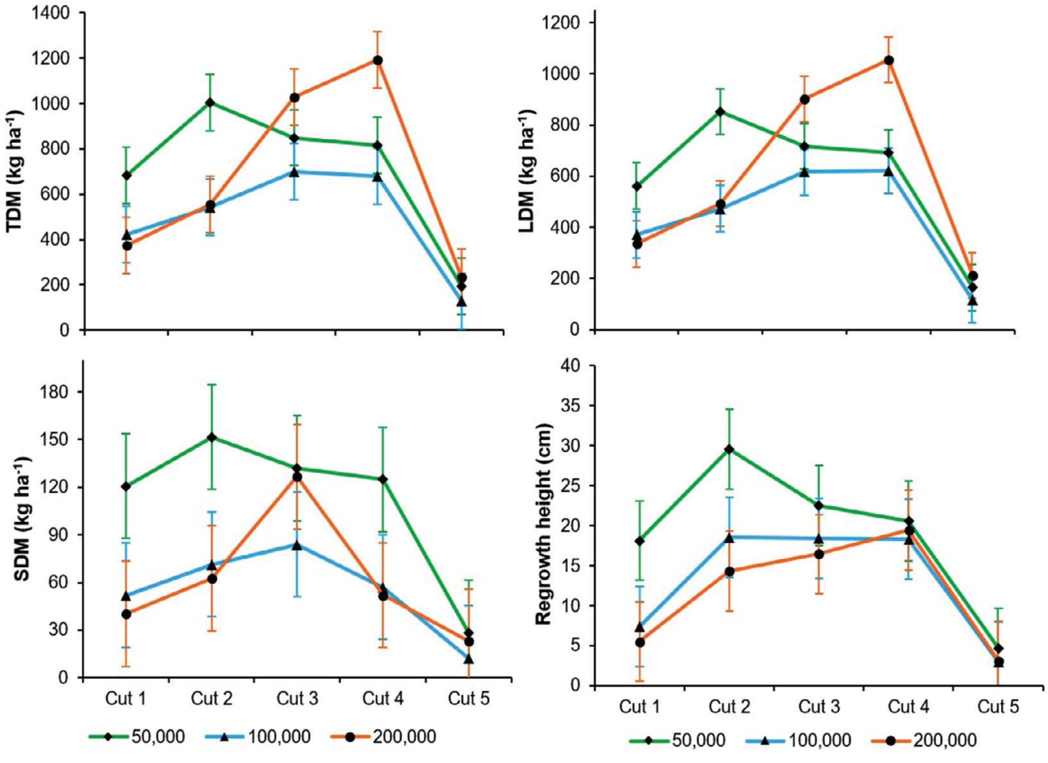

Figure 1. Yield of total dry matter (TDM), leaf dry matter (LDM), and stem dry matter (SDM) and height of regrowth in *Moringa oleifera* Lam. with different population densities (50,000, 100,000 and 200,000 plants ha^{-1}). Bars indicate least significant difference (Tukey; α=0.05).

D1 had the highest LDM yields in cuts 1 and 2 (561 and 852 kg ha^{-1}); meanwhile, in cuts 3 and 4, D3 obtained the highest LDM (901 and 1054 kg ha^{-1}, respectively) (Figure 1). D1 showed the highest TDM yields only in cuts 1, 2, and 4 (121, 152, and 125 kg ha^{-1}), while TDM values were similar (P>0.05) between D2 and D3 and ranged from 12 to 127 kg ha^{-1} (Figure 1). D1 showed the HR values in the first two cuts: 18 *vs*. 6 cm and 30 *vs*. 16 cm, respectively (P>0.05). A positive trend was observed in TDM, LDM, and HR values, as well as in cuts 1-4; however, values decreased significantly towards the end of the evaluation (cut 5) (Figure 1).

The CP content decreased ($P<0.005$) by 11% (222 *vs.* 198 g kg^{-1}) when D1 increased to D2; no differences were found ($P>0.05$) when D1 increased to D3 (Table 3). Ash content was 4% higher ($P<0.05$) in D1 than in D2 and D3 (103 *vs.* 99 g kg^{-1}). No differences were found ($P>0.05$) between the assessed densities of the NDF, ADF, and hemicellulose values (Table 3).

Bopape-Mabapa *et al.* (2020) reported that, in species with arboreal or shrubby growth habit, there is a positive correlation between planting density and aerial biomass yield. In this regard, Zheng *et al.* (2016) reported a 165% increase in TDM yield in moringa, when density increased from 15,625 to 250,000 plants ha^{-1}. However, no differences were found in TDM yield, within the first year that the same species was established. These results were obtained with both high —250,000 to 750,000 plants ha^{-1} (Reyes *et al.*, 2006)— and low —62,500 to 125,000 plants ha^{-1} (Manh *et al.*, 2005)— densities.

The variable agroecological conditions from one region to another can account for the difference in the results obtained in this study. Additionally, when a positive relationship between population density and biomass yield was found, rainfall replaced the gravimetric moisture demand (Zheng *et al.*, 2016).

The most suitable environmental conditions for the growth of this tropical species were observed in cut 3 (17-39 °C, 98 mm) and cut 4 (10-35 °C, 128 mm) (Table 1). The highest density (D3) had a positive response: a biomass yield increase of 85 and 16% regarding cut 2 and 3 (Figure 1). With a lower plant population, water availability per plant is greater, resulting in a higher biomass yield (Mabapa *et al.*, 2017). Consequently, under humidity restriction conditions (Table 1) —such as in the case of cut 1 (10 mm) and cut 2 (61 mm)—, the lowest density (D1, 50,000 plants ha^{-1}) had the highest TDM yields (Figure 1).

The height of regrowth has a positive correlation with plant density (Sosa-Rodríguez *et al.*, 2017). However, the results of this study were different: the highest values were obtained with the lowest density (D1), because this density had greater availability of nutrients, water, and space than D2 and D3 (Figure 1). The height of regrowth differed between densities and, therefore, might explain the variability observed in the nutritional value. According to Guzmán-Maldonado *et al.* (2015), the height of the plant at harvest influences the nutrient content in moringa leaves; the same phenomenon took place with CP content in this study, since D1 ranked first regarding height of regrowth and concentration of this nutrient. Likewise, these results differ from the findings of Reyes *et al.* (2006) for the case of

Table 3. Nutritional value of *Moringa oleifera* Lam. with different population densities.

Variable (g kg^{-1})	**Population density (plants ha^{-1})**		
	D1 (50,000)	**D2 (100,000)**	**D3 (200,000)**
Crude protein	222 a	198 b	215 ab
Neutral detergent fiber	258 a	260 a	251 A
Acidic detergent fiber	126 a	123 a	116 A
Hemicellulose	132 a	137 a	135 A
Ash	103 a	99 b	99 B

Means with different letters within the same row are statistically different (Tukey, $\alpha=0.05$).

Nicaragua; they did not report any effect of density on CP concentration and plant height, with the exception of the results obtained for the average density (500,000 plants ha^{-1}) in the first year of evaluation, which did not have influence on CP.

Meanwhile, NDF, ADF, hemicellulose, and ash content showed a similar behavior to that reported by Zheng *et al.* (2016), who assessed moringa at densities between 15,625 and 250,000 plants ha^{-1}, and found that they did not have a significant influence on the structural carbohydrates content. However, those densities did impact ash content, which decreased with increasing density. This situation could be the result of the reduced range (28 days) between cuts. Therefore, lowest density plants did not have enough time to fully develop. Additionally, plants need to maintain a balance in their chemical composition through compounds synthesis. These compounds include structural carbohydrates, which provide support and rigidity to the plant (Mendieta-Araica *et al.*, 2012).

Several factors could have influenced ash, such as the physicochemical characteristics of the soil (Lukhele and Van Ryssen, 2003; Rubio-Sanz and Jaizme-Vega, 2022) and the maturity status of the plant (Méndez *et al.*, 2018). Finally, the low availability of minerals for each plant in the soil at densities with higher number of plants per area unit also had an impact.

CONCLUSIONS

Moringa oleifera established at a 50,000 plants ha^{-1} density, with a planting pattern of 0.50 and 0.40 m between row and plant, respectively, and 28-day cutting intervals achieved the best productive behavior (yield and protein concentration). Additionally, its establishment requires less seed and labor.

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AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Effect of genotype on the production and quality of sweet sorghum juice [*Sorghum bicolor* (L.) Moench]

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ABSTRACT

Objectives: To evaluate the production of sweet sorghum juice (*Sorghum bicolor* (L.) Moench) and sugar, the physicochemical parameters of juice during extraction, and to quantify the differences between genotypes.

Design/Methodology/Approach: We established an experiment under irrigation with 10 sweet sorghum genotypes in southern Sonora, México, during the autumn-winter agricultural cycle. We used a randomized complete block design with four replications. The sowing dates were 03/15/2015 and 02/20/2016. The variables were: days to flowering (DF); weight of fresh biomass (t ha^{-1}): whole plant (WTo), stem (WSt), leaf (WLf), and panicle (WP). After extraction, we determined juice weight (WJ), bagasse weight (WBz), juice volume (JV), and extraction efficiency (EFx:WJ/WSt). The juice was sieved to remove impurities. Temperature (°C), pH, and soluble solids (°Brix) were determined at extraction time.

Results: The sources of variation had a significant effect on the production of biomass, juice, and sugar. The year explained 53% of variation, the genotype 36%, and the interaction (G×A) only 5%. On average, the production of fresh stem biomass was 38 t ha^{-1}, with 28% efficiency in juice extraction. The SWS686 and SWS694 genotypes exceeded both the average and the control (M81E) in juice production. Juice production in 2016 was higher (31%) than in 2015. In average, juice values were of 32 °C, 12.9 °Brix, and pH 3.8.

Study limitations/implications: The decrease in the content of soluble solids and spontaneous fermentation during juice conservation at room temperature can limit the use of sweet sorghum in areas where temperatures of >30 °C prevail during the post-harvest stage.

Findings/conclusions: The environment and the genotype affected the production and quality of sweet sorghum juice. It is necessary to make a complete life cycle analysis that indicates the challenges and opportunities to improve the efficiency of the processes to obtain sweet sorghum juice.

Keywords: Sweet sorghum, Genotype, Biomass, Sugars.

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INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) Moench] is an exotic resource in Mexico. Native grain genotypes come from Africa (Venkateswaran *et al.*, 2019), while sweet sorghum germplasm comes from the USA and India. Both are the basis for genetic improvement (Williams-Alanis *et al.*, 2020). Sucrose is the main soluble sugar obtained from sugar cane (*Saccharum officinarum* L.), with approximately 75% of the world supply (Zhao *et al.*, 2014). It is also the most abundant ingredient in sweet sorghum juice (66%), which can also have glucose (25%), fructose (13%), and minerals such as nitrogen, phosphorus, Cl^{-1}, Na^{+}, K^{+}, Mg^{2+}, and Ca^{2+} (Olszewska-Widdrat *et al.*, 2019). Bioproducts such as ethanol are obtained by direct fermentation of the juice (Fagundes *et al.*, 2021). Sweet sorghum in semi-arid climates is used for biomass production (Mishra *et al.*, 2017). It is also a low-cost alternative non-food energy crop that can simultaneously provide juice and bagasse (Zegada and Monti, 2012). Genetic improvement of sweet sorghum has focused mainly on developing genotypes with high sugar yields. Morphological characters such as plant height and stem diameter are related to juice production (Shinde *et al.*, 2012). In Mexico, sweet sorghum has been experimentally grown to obtain green forage (Nava-Berumen *et al.*, 2017), sugars (Montes-Garcia *et al.*, 2019), and syrup as an alternative to replace corn fructose (Arvizu-Castro *et al.*, 2016). In northwestern Mexico, the adaptation of sweet sorghum crops is being evaluated, since some genotypes are photosensitive (Oliveira *et al.*, 2019) and susceptible to disease (Xavier *et al.*, 2017). The objective of the experiment was to evaluate the differences between genotypes, and to measure the effect of genotype and environment on the production of juice and sugar, as well as the physicochemical parameters of juice during extraction.

MATERIALS AND METHODS

Study area

The experiments were established in the same area in Block 2328 Valle del Yaqui, in Agua Blanca, Municipality of Villa Juárez, Sonora, Mexico (27° 7’ 45.58” N, 109° 50’ 14.57” W), at an altitude of 20 m. The climate is very dry and hot BW (h) hw. The mean annual temperature is 23.3 °C. The average annual rainfall is 340 mm, and July, August, and September are the rainiest months. Occasional frosts can occur from November to March.

Soil and climatic factors in the experiments

Before establishing the experiment in 2015, we conducted a soil sampling of the experimental site at a depth of 30 cm in order to determine its physical and chemical properties. We took 15 subsamples (0.5 kg) to form a composite sample of 5 kilograms. We assessed the following chemical properties: pH, measured with a potentiometer in a soil:water ratio of 1:2; electrical conductivity (CE1:5); soil organic matter (SOM), obtained with the Walkley-Black wet digestion method; Kjeldahl nitrogen, using a semi-micro method; carbon:nitrogen (C:N) ratio, calculated with SOM and Kjeldahl nitrogen results; extractable phosphorus, obtained with the Olsen method, for the analysis of available P, K, Fe, Cu, Zn, and Mn, and KCl for exchangeable acidity, Ca, and Mg. These methodologies are a standard for assessing soil properties as described by Castillo-Valdez *et al.* (2021). We recorded the climatic data during the February-August crop cycle: maximum and minimum temperature, evaporation, and rainfall, all of them taken from the Agua Blanca weather station DDR-148-EMA-26071-03.

Biological material

The genotypes used were: 1) experimental varieties of sweet sorghum (SWS) —603, 657, 658, 662, 691, 686, 694, and 817— with six selection cycles derived from the segregation of sweet sorghum Keller × Dale, both of them US public varieties (Montes *et al.*, 2013); RB-Cañero, the first Mexican variety of sweet sorghum (Montes *et al.*, 2019); and genotype M81E (control), a public domain commercial variety of sweet sorghum, released in Meridian, Mississippi, USA (Broadhead *et al.*, 1981), which was previously assessed in some towns of Sonora, Mexico (Ochoa *et al.*, 2011).

Conduction of experiment in the field

We planted ten sweet sorghum genotypes in five rows of 5 × 0.80 m for each material in a randomized complete block design with four replications. The useful plot comprised two central furrows. The irrigation frequency was based on the phenological stages of the crop. We established a surface irrigation system in the autumn-winter agricultural cycle of 2015 and 2016. The first irrigation was carried out prior to sowing, and three auxiliary irrigations were subsequently applied during the cycle. The first auxiliary irrigation was applied 40 days after sowing, the second 30 days after the first, and the third 25 days later. The sowing dates of the experiments were March 15, 2015 and February 20, 2016. After emergence, plant density was adjusted to 12 plants per meter (150,000 plants ha^{-1}).

The agronomic management of the crop followed the INIFAP technological package for this region (Ochoa *et al.*, 2011). The applied fertilization dose was 180-80-00. Half the nitrogen and all the phosphorus were applied with the pre-sowing irrigation, while the remaining nitrogen was included in the first auxiliary irrigation. As regards days to flowering (DF), a flowering of between 25 and 50% was observed from the sowing date until the appearance of 50% of plant panicles in the plot. The assessment was conducted in June and July. The plots were harvested in order according to the same phenological stage. At the dough stage of grain, about 20 days after flowering, the entire plants were manually cut at ground level. The largest accumulation of sugars in the stem occurs during this stage (Montes *et al.*, 2013).

All plants in the useful plot were cut and the following variables were observed: weight of fresh biomass per plot, and its separate components —whole plant (WTo), stem (WSt), leaf (WLf), and panicle weight (WP). The final data were reported in t ha^{-1}. The ratio to total fresh weight (WSt:WTo) was measured. Extraction was carried out with a 9HP electric artisanal roller mill. The bagasse (WBz) and juice (WJ) were weighed at the end of the extraction. Finally, extraction efficiency (EFx: WJ/WSt) was calculated and the extracted juice volume (JV) was measured in a graduated cylinder. The final data are expressed in L ha^{-1}.

Juice parameters

The juice was extracted with a mill using stems, free of leaves and panicles, of all genotypes. Then it was sieved to remove impurities. Subsequently, the total volume was divided into three subsamples and placed in 250 ml plastic bottles. At the time of extraction, the pH and temperature (°C) of each juice sample were determined using a portable potentiometric pH meter (Hanna® Instruments PHE-HI98127). Brix degrees or soluble sugars were determined with a manual refractometer (REF113/Brix/ATC 0-32).

These data were used to conduct a combined analysis of variance with a randomized complete block design with two factors: genotypes (G=10) and production years (A=2). We used Tukey's means test ($p<0.05$), with the statistical software SAS version 9.3.1.

RESULTS AND DISCUSSION

Environmental conditions

The soil analysis results showed a clay loam texture with pH 7.4, electrical conductivity of 0.25 dSm^{-1}, sodium (580 ppm), and 1.2% organic matter. The macronutrients found in soil were nitrogen (25.8 ppm), phosphorus (19.7 ppm), and potassium (400 ppm); the micronutrients found were Fe (1.92 ppm), Mg (10 ppm), Zn (4.8 ppm), Cu (0.68 ppm), and Bo (0.76 ppm). The soil is slightly alkaline, has a medium-fine texture, no salinity problems and a low organic matter content, with medium levels of nitrogen and phosphorus, a low potassium content, and is deficient in microelements Fe, Cu, and Bo, but with a sufficient level of Zn. The region's soils are characterized by a clay loam texture, are poor in organic matter (<2%), and have a pH close to neutrality (Moreno-Ramos *et al.*, 2014). Sweet sorghum is a crop that adapts to loam to light sandy soils, but grows better in loam and sandy loam soils. It can tolerate a wide range of pH (5.0-8.5) and soil drainage conditions. It can even be cultivated on marginal soils (Zegada and Monti, 2012). The most important element for the growth of sweet sorghum is nitrogen, since the latter is related to the accumulation of biomass in stem and leaf (Olson *et al.*, 2013). Montes-García *et al.* (2013) reported 60 to 62 t ha^{-1} of stem biomass with inorganic nitrogen levels between 60 and 120 kg ha^{-1}. In regions with a minimum temperature of 13.9 °C and a maximum of 36.9 °C, with rainfall levels of 600 to 700 mm, the cultivation of sweet sorghum can yield a production of 70 to 80 t ha^{-1} of fresh biomass (Rao *et al.*, 2013), even without complementary irrigation. The site where the experiments were established, which belongs to the southern region of Sonora, has a virtually constant climate (Moreno-Ramos *et al.*, 2014). During the experiments (2015 and 2016), no rain and a high evaporation were recorded (Table 1). In the arid and semi-arid regions of Arizona, USA, the cultivation of sweet sorghum requires between 900 and 1,300 mm of water per crop cycle. The M81E variety produces 39,000 liters ha^{-1} of juice, can consume up to 1190 mm, and requires 33.4 mm of water per hectare per liter of juice (Martínez-Cruz *et al.*, 2015). These experiments were conducted under irrigation conditions. Therefore, the amount of precipitation was a secondary climatic variable, since precipitation occurred at the end of the cycle. Water availability for irrigation is an important factor for sweet sorghum production in the southern region of Sonora.

Table 1. Climatic conditions of the study area.

	Month							Accumulated
Year 2015	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	
Evaporation (mm)	94.5	121.9	148.3	185.7	195.6	208.5	174.3	1128.8
Rain (mm)	11	21	0	0	3.2	3.9	79.4	118.5
Year 2016								
Evaporation (mm)	104.2	131.8	167.4	201.2	207.3	218.4	179.1	1209.4
Rain (mm)	0.6	16	0	0	4.5	30.6	57.4	109.1
Year 2015								**mean**
Tmax (°C)	27.4	30.7	30.2	33.3	35.7	37.2	36.8	33±1.8
Tmin (°C)	11.3	11.9	12.8	13.4	22.0	25.7	25.5	17.5±2.2
Year 2016								
Tmax (°C)	26.7	27.2	30.8	32.8	36.0	37.1	35.9	32.4±2.1
Tmin (°C)	8.6	9.8	11.1	13.8	21.3	25.5	24.9	16.4±2.3

Juice production according to genotypes

The analysis of variance showed a significant effect of the variation sources on the production of biomass, juice, and sugar. The year or environment explained 53% of the variation, the genotype explained 36%, and the interaction (G×A) only 5%. We observed a significant difference between genotypes regarding fresh biomass and juice production (Table 2). The genetic diversity of sweet sorghum has been widely documented from a morphological and molecular point of view, and it constitutes a genetic resource that differs from grain sorghum in its ability to accumulate biomass and sugar (Mullet *et al.*, 2014). The SWS686, SWS691, and RB-Cañero genotypes exceeded the control in total biomass production by 10 to 18 t ha^{-1}, which represents between 18 and 32%. The same genotypes exceeded the control in juice production by 36%, due to their higher extraction efficiency (Table 2). The control produced almost 50% of bagasse or fibrous fraction. Materials with low fiber percentages are more susceptible to lodging, and to attacks by pests and microorganisms (Souza *et al.*, 2016). In this experiment, we obtained an average of 53±12 t ha^{-1} of total biomass. It has been reported, for example, that the RB-Pirulí variety of sweet sorghum produces large amounts of total fresh biomass, which generate up to 121 t ha^{-1} and 26 thousand L ha^{-1} of juice (Montes-García *et al.*, 2019). The variability between sweet sorghum genotypes in the accumulation of biomass and sugars can be attributed to precocity (Viator *et al.*, 2015; Souza *et al.*, 2016). In these experiments, the genotypes with the longest growing season produced more biomass. Sweet sorghum genotypes were differentiated according to their precocity. The SWS603 genotype was the earliest with 85 days to flowering and 106 days to dough stage. There were no significant differences between the SWS materials 657, 658, 662, 694, and 817, at 98 and 120 days, respectively.

The late genotypes group (SWS686, M81E, RB-Cañero, and SWS691) took between 102 and 118 days to flower, and between 123 and 139 days to dough stage. In this experiment, the plant's leaves and panicle constituted 33% of the total biomass (Table 2)

Table 2. Biomass and juice production. Average data for years 2015 and 2016.

Genotype	Weight fresh (t ha^{-1})						VJ (L ha^{-1})	EFx	RSt:To
	WTo	WSt	WHf	WP	WJ	WBz			
M81E	52 cd	36 b	10 bc	6 bc	9 bc	27 c	7,578 b	0.25 b	0.69
SWS603	44 ef	31 cd	10 bc	7 b	8 cd	23 cd	6,381 c	0.25 b	0.70
SWS657	46 de	31 cd	10 bc	5 bc	9 bc	22 cd	8,055 bc	0.29 ab	0.67
SWS658	54 cd	35 bc	14 ab	9 a	9 bc	27 c	7,428 bc	0.24 b	0.65
SWS662	37 f	23 d	9 c	4 de	7 d	17 d	5,536 c	0.28 ab	0.62
SWS686	60 bc	42 ab	15 ab	3 e	13 a	29 bc	12,788 a	0.33 a	0.70
SWS691	75 a	52 a	18 a	5 cd	14 a	38 a	12,790 a	0.27 ab	0.69
SWS694	65 b	43 ab	15 ab	7 b	13 a	31 abc	10,983 ab	0.30 ab	0.66
RB-Cañero	71 ab	51 a	14 ab	4 de	14 a	38 a	12,827 a	0.26 b	0.72
SWS817	50 cd	35 bc	10 bc	3 e	11 ab	23 cd	9,972 ab	0.33 a	0.70
Means	**55**	**38**	**13**	**5**	**11**	**27**	**9,434**	**0.28**	**0.68**
SD	12	9.2	3.0	1.9	2.8	6.7	2,801	0.03	
CV	4.6	4.1	4.1	2.8	3.8	4.0	3.0	8.6	
Year									
2015	42 B	31 B	8 B	4 B	8 B	23 B	7,560 B	0.26 B	
2016	66 A	44 A	16 A	6 A	13 A	31 A	11,040 A	0.30 A	

Wto: weight of whole plant. Fresh weight: WSt: stem; WLf: leaf; WP: panicle; WJ: juice; and WBz: bagasse. EFx: extraction efficiency. Control (+): M81E. WSt:WTo: stem ratio:total fresh weight. SD: standard deviation. CV: coefficient of variation. Means with different letter differ significantly (Tukey, 0.05).

and were not processed during extraction because they are considered waste, since their inclusion together with the stem produces sugar loss in the juice (Viator *et al.*, 2015). Therefore, the model genotype is the one with the highest proportion of stems. The RB-Cañero and SWS817 genotypes had a stem ratio of 0.70 in relation to the total biomass, with an extraction efficiency of 0.33. On average, we obtained 28% juice extraction. This amount varied between genotypes and years (Table 2). Rao *et al.* (2013) reported differences between agricultural cycles, but not between genotypes, with 44% juice extraction from the stem, free of panicle and leaves. Genotypic differences in the fibrous fraction of the bagasse may be a factor related to grinding efficiency. Li *et al.* (2018) found that the stem's fresh biomass is a complex structure, with anatomical heterogeneity, and even in chemical composition. The average juice production was 9,400±2,800 L ha^{-1} (Table 2). Due to its capacity to adapt to changes in soil moisture in semi-arid regions, the potential of sweet sorghum as an annual crop is important for juice production (da Silva *et al.*, 2019). These data suggest that handling juice volumes on a larger scale implies a technological challenge in planning and operation, during and after juice extraction, for the efficient production of sugars and their derivatives (Aguilar-Uscanga and Montes-García, 2017).

Juice characteristics

The analysis of variance showed a significant effect of the genotype on juice parameters during extraction. These variables are important, since they affect the juice fermentation process. Brix degrees or soluble solids correlate with the amount of sucrose, the main substrate for the sweet sorghum juice fermentation (Dutra *et al.*, 2013). It has been observed that sucrose decreases with time in sugar cane juice with pH=3. A temperature increase reduces the amount of sucrose in the juice. In addition, the pH level affects the concentration of sucrose, because the latter is degraded by the action of contaminating bacteria (Arvizu *et al.*, 2016). The average values of the stem juice variables, taken at the time of extraction, were 32.6±2.3 (T °C), 3.8±0.7 (pH), 14.9±2.4 (°Brix), as shown in Table 3. The concentration of soluble solids and the pH of the juice can vary significantly between genotypes of sweet sorghum, as observed in this experiment. Dávila-Gómez *et al.* (2011) reported values between 10 and 13.2 °Brix and a pH between 4.43 and 4.85 in sweet sorghum juice.

In these experiments, the pH of the juice averaged 3.8. The SWS 686 genotype reached a pH value of 2.8, 26% lower than the average (Table 3). Sweet sorghum juice has low pH levels (4 to 5); in addition, these values can vary between genotypes (Holou and Stevens, 2012; Freita *et al.*, 2014). Temperature and pH affect not only yeast growth, but also enzymatic activity, which is directly related to the efficiency of ethanol production (Lu *et al.*, 2017). Significant differences have been reported in ethanol concentration, productivity, and yield at 37 and 40 °C, with pH values between 4 and 6, using sweet sorghum juice (Pilap *et al.*, 2018). pH is important because it controls metabolism and affects the composition of microbial communities present in the juice. Even a change in percentage can affect their growth. Few native bacteria in sweet sorghum juice can survive

Table 3. Parameters of sweet sorghum juice. Average data for 2015 and 2016.

Genotype	T (°C)	°Brix	pH
M81E	29 e	14.7 c	3.4 e
SWS603	34 bc	17.7 a	3.5 de
SWS657	32 d	14.6 c	3.7 d
SWS658	33 d	11.5 d	5.2 a
SWS662	36 a	17.8 a	3.5 de
SWS686	33 d	17.0 ab	2.8 f
SWS691	33 d	14.6 c	3.5 de
SWS694	30 e	10.5 d	4.9 b
RB-Cañero	30 e	16.1 bc	4.2 c
SWS817	35 ab	14.7 c	3.4 e
Means	**32.6**	**14.9**	**3.8**
SD	2.3	2.4	0.7
CV	14.4	6.1	5.2
Year			
2015	31.7 B	14.3 B	3.5 B
2016	33.4 A	15.4 A	4.1 A

Control (M81E). SD: standard deviation and CV: coefficient of variation. Means with the same letter differ significantly (Tukey, 0.05).

even at pH 4.7 (Jin and Kirk, 2018). The optimal conditions to produce bioethanol from sweet sorghum are pH 5.5, a temperature of 28 °C, with a maximum theoretical yield efficiency of 0.75 (Ebrahimiaqda and Ogden, 2018).

CONCLUSIONS

Environmental conditions allowed us to identify efficient sweet sorghum genotypes for the production of biomass and juice. There are genotypic differences and seasonal changes in the juice quantity and quality. Bagasse percentage is a genotypic characteristic that affects the amount of juice. The SWS686 and SWS694 genotypes exceeded the average and the control M81E in juice production. Juice production was higher in 2016 (31%) than in 2015.

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AP AGRO PRODUCTIVIDAD

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Leucaena leucocephala (Lam.) de Wit as protein supply for heifers

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ABSTRACT

Objective: To estimate the percentage of Leucaena in the diet of heifers fed with Taiwan grass, in order to maximize the efficiency of the ruminal supply of microbial Nitrogen (ERSMN).

Design/Methodology/Approach: Five heifers were randomly selected for each of the five treatments (0, 20, 40, 60, and 80% supplementation with Leucaena), according to a 5×5 Latin square experimental design. We determined the ruminal supply of microbial nitrogen (RSMN), ERSMN, and the urea-N by measuring N and purine derivatives in urine. Subsequently, we predicted the duodenal RSMN, the rumen nitrogen balance (RNB), and the urea cost with the Large Ruminant Nutrition System (LRNS v. 1.0.33) model.

Results: The inclusion of Leucaena improved ($P \leq 0.05$) the RSMN. The ERSMN estimated by purine derivatives had a quadratic response ($P \leq 0.05$) at the inclusion level of Leucaena in the diet. The RNB, the cost of urea, and the urea-N increased ($P \leq 0.05$) with a higher inclusion percentage of Leucaena. The maximum ERSMN and N balance were obtained with 20% Leucaena in the ration.

Study Limitations/Implications: The expression of the nitrogen utilization potential of Leucaena for microbial protein synthesis in this study was likely restricted by the limited availability of non-fiber carbohydrates (NFC). Further studies must be conducted to determine the most affordable source of NFC to match Leucaena nitrogen utilization in the rumen.

Findings/Conclusions: Leucaena could be used as an efficient protein source for heifers at a 20% inclusion in their diet.

Key words: *Leucaena leucocephala*, Microbial protein, Purine derivatives, LRNS.

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INTRODUCTION

Leucaena [*Leucaena leucocephala* (Lam.) de Wit] is known in the tropic as a leguminous quality forage, as a result of its high protein and low fiber contents, as well as its moderate tannin levels (Quero-Carrillo *et al.*, 2014). Several studies have shown that Leucaena improves animal response when used as a protein supplement (Madera *et al.*, 2013; Ku

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Vera *et al.*, 2014; Solorio *et al.*, 2016). However, increasing levels of Leucaena in the diet have been reported to cause a nitrogen (N) loss in urine (Hung *et al.*, 2013; Piñeiro-Vázquez *et al.*, 2017). N loss in animal production systems has important nutritional and environmental effects, including an increase in greenhouse gas emissions. Agriculture is estimated to contribute approximately 8 to 10% to global emissions (O'Mara, 2011). In this regard, ruminants contribute to methane and nitrous oxide emissions (IPCC, 2007; Rotz, 2018). The main source of nitrous oxide in ruminants is urea-N in urine (Hoogendoorn *et al.*, 2010). Reducing urea-N in urine provides the best opportunity to diminish N excretions and this can be achieved through an efficient feeding strategy (Nasiru *et al.*, 2014). Optimizing the use of N in the ruminant diet would be an alternative to reduce N loss in urine, since it improves the synthesis of microbial protein, expressed as microbial N (MN). In forage-based diets, MN is produced in the rumen and requires NH_3 as N and structural carbohydrates (SC) as energy (AFRC, 1993); therefore, the amount of MN that reaches the small intestine depends on the availability of SC and NH_3 in the rumen (Sniffen & Robinson, 1987). There are several methods to estimate the MN supply, including the use of urine purine derivatives (PD) (Chen & Gomes, 1992).

The use of simulation models can be an alternative to predict the amount of N entering the duodenum. Both the Cornell Net Carbohydrate and Protein System (CNCPS) —a system that estimates protein and energy requirements, developed by the University of Cornell— and its 5.0 version —known as Large Ruminant Nutrition System (LRNS v. 1.0.33, Fox *et al.*, 2004)— use a mechanistic model to quantify the supply of bacterial N to the duodenum. In addition, these models predict the rumen N balance, which indicates whether or not the rumen bacterial N requirements are met. When the rumen N balance is positive, excess N is excreted as urea and contributes to the energetic cost of urea synthesis. Non-fiber carbohydrate (NFC) supplements for the rumen are an option to improve the efficiency of microbial synthesis in this organ (Poppi & McLennan, 1995). Products such as citrus pulp, molasses, polished rice, and sorghum can be used as optional NFC sources in ruminant diet in the tropic (Harper *et al.*, 2019). Some studies report that using these supplements in diets with Leucaena in dual-purpose cows improves the efficiency of N use in the diet (Flores-Cocas *et al.*, 2019, Arjona-Alcocer *et al.*, 2020). However, the inclusion levels of Leucaena and the NFC source that would allow capturing the N of this forage is still uncertain. The objective of this work was to estimate the adequate percentage of Leucaena in the diet of heifers fed with *Cenchrus purpureus* cv. Taiwan grass that would both maximize the efficiency of the ruminal supply of microbial N and reduce the excretion of urea-N.

MATERIALS AND METHODS

The two-stage work evaluated the effect on the ruminal supply of microbial N (RSMN) of different Leucaena inclusion levels in the diet of heifers. Stage 1 —conducted on 18-month-old heifers with a live body weight of 295±19 kg— evaluated the effect of the Leucaena inclusion level (determined through purine derivatives) on RSMN. Stage 2 validated the LRNS v. 1.0.33 model (Fox *et al.*, 2004) as a tool to predict the effect of the Leucaena inclusion level on RSMN.

Stage 1. Ruminal supply of microbial N through purine derivatives

The research was conducted at the Facultad de Medicina Veterinaria y Zootecnia of the Universidad Autónoma de Yucatán, in Mérida, México. The region has a tropical climate, an average temperature of 26.8 °C (García, 2004), and an average annual rainfall of 984.4 mm. Five crossbred heifers (*Bos taurus* × *Bos indicus*) with an average live weight of 295±19 kg were used to determine the RSMN through purine derivatives; the heifers were kept in metabolic cages inside a roofed, wall-less building with concrete floor. The basal ration consisted of fresh, chopped Taiwan grass and Leucaena forage, harvested at 60 days of regrowth. Heifers were fed *ad libitum*, allowing a 15% rejection of the amount of food offered the previous day. The estimated DM intake was 7 kg head^{-1} day^{-1}. The grass levels of this intake were increasingly substituted with Leucaena (0, 20, 40, 60, and 80%) for the experimental treatments. Heifers were fed once a day, with the complete feed amount offered at 9:00 h. The N content in Leucaena was 25.6 g kg^{-1} of DM, which equals 160 g of protein per kg^{-1} of DM. During this stage, the total urine volume was collected for 24 h in a 20-L plastic container; 1000 mL of a 10% sulfuric acid solution were added in order to maintain a pH$<$3 and avoid the volatilization of N. Urine aliquots (100 mL) were obtained from the total daily volume and then frozen at −4 °C, awaiting the chemical analyses that determined purine derivatives and urea-N (Chen & Gomes, 1992).

Purine derivatives (PD) and microbial N synthesis. Allantoin and uric acid were determined with colorimetry, using a DU-650 spectrophotometer (Beckman Instruments, USA) according to the methodology described by Chen *et al.* (1993). The amount of PD absorption was calculated from the PD excretion (allantoin and uric acid), based on the relation derived from the equation of Chen & Gomes (1992):

$$Y = 0.385 \text{ kg } PV0.75 + 0.85 * X$$

The supply of microbial protein, expressed as microbial nitrogen (SMN), was estimated by PD excretion in urine, based on Chen & Gomes (1992):

$$SMN(g\ N\ d^{-1}) = X(mmol\ d^{-1}) * 70/0.116 * 0.83 * 1000 = 0.727 * X$$

where X and Y are PD absorption and excretion in $mmol\ d^{-1}$, respectively.

Efficiency of microbial N in the ruminal supply. The ERSMN was calculated using the following formula:

$$ESRNM = microbial\ N\ (g\ d^{-1})\ kg^{-1}\ DOMR$$

where *DOMR* is the digestible organic matter in rumen (assuming that ruminal digestion is 650 g kg^{-1} of organic matter digested in the total tract);

$$DOMR = IDOM \times 0.65$$

where *IDOM* is the ingested digestible organic matter, according to ARC (1980).

Urinary urea-N excretion. The concentration of urea was determined using the urease modified Berthelot reaction, a colorimetric method. The urea-N excreted in urine was determined based on the existent relation between the molecular weight of urea and the molecular weight of its N content (46.65%). The amount of excreted urea (g) was previously obtained. These values were used in the following relation:

$$urea\text{-}N\ (g) = urea\ (g) \times 0.4665.$$

Stage 2. Validation of RSMN with the LRNS model

Database. To validate the LRNS model (Fox *et al.*, 2004), we input the observed values for each cow per sampling period (n=25), using dry matter (DM) intake averages per each 5-day period.

Model entries. Table 1 presents a summary of the supplies, animal characteristics, and environment input into the model. The information about the nutritional composition of feed was entered into the model's feed library (Table 2).

Intake and bacterial N predictions with the LRNS model. The LRNS model (Fox *et al.*, 2004) was used to predict the rumen N balance (RNB, requirement %), the urea cost (Mcal d^{-1}), and the RSMN (g d^{-1}).

Experimental design and statistical analyses

In Stages 1 and 2, an analysis of variance with a 5×5 Latin square experimental design (Cochran & Cox, 1990) was conducted for the PD, RSMN, ERSMN, urea-N, RNB, and

Table 1. Inputs used to evaluate DM and bacterial N intake predictions with the LRNS model.

Description	Heifers	Description	Units
Animal:		**Environment:**	
Animal Type	Growth/Finishing	Additive	None
Age (mo)	18	Supplemented fat	Not
Sex	Female	Wind Speed (kph)	16
Current Body Weight (kg)	295±19	Previous Temperature (°C)	26
Mature Body Weight (kg)	550	Previous Humidity (%)	80
Body Weight	Live Weight	Current Temperature (°C)	26
Breed Type	Cross Bred (Dual Purpose)	Current Humidity (%)	80
		Sunlight Exposure (h)	4
Grade	Low Marbling (22% body fat)	Storm Exposure	None
		Hair Depth (cm)	0.64
Production:		Floor Mud Depth (cm)	0
Condition score (scale 1 to 9)	5	Hide	Average
Breeding System	*Bos taurus*×*Bos indicus*	Hair Coat	No Mud
Bull's Breed	Holstein	Cattle Panting	None
Dam's Breed	Brahman	Minimum Night Temperature (°C)	18
		Activity	Confinement

Table 2. Inputs of the dietary composition into the model to predict microbial N using the LRNS model.

Composition	Taiwan	Leucaena
DM (% as feed basis)	23.7	32.6
NDF (% DM)	67.0	58.0
Lignin (% NDF)	7.0	16.0
CP (% DM)	6.0	16.0
Ether Extract (% DM)	2.3*	0.7*
Ash (% DM)	5.1*	5.3*
Soluble Protein (% CP)	46.0*	25.0*
Non-protein N (% SP)	2.2*	5.0*
NDIP (% CP)	2.2*	33.5*
ADIP (% CP)	0.9*	12.5*

Cenchrus purpureus cv. Taiwan, Leucaena (*Leucaena leucocephala*); DM: dry matter; NDF: neutral detergent fiber; CP: crude protein; NDIP: neutral detergent insoluble protein; ADIP: acid detergent insoluble protein; *LRNS version 1.0.33 Tropical Feed Library.

urea cost variables, using the SAS generalized linear model procedure (PROC GLM) (2002). The following model was used:

$$Y_{ijk}=\mu+P_i+A_j+T_k+e_{ijk}$$

where Y_{ijk} is the dependent variable, μ the overall mean, P_i the effect of the ith period, A_j the effect of the j^{th} animal, T_k the effect of the k^{th} Leucaena level, and e_{ijk} the experimental error.

The least square means of Leucaena levels were estimated using the LSMEANS option, while the means ($P<0.05$) were compared with Tukey's test. The differences between means ($P<0.05$) were accepted as statistically significant. In addition, we conducted a response surface analysis with orthogonal contrasts (Kaps & Lamberson, 2017) to evaluate the linear, quadratic, and cubic effects of the Leucaena level on the study variables. In Stage 1, the SAS REG procedure (PROC REG) (2002) was used to conduct a regression analysis in order to obtain the equation ($y=a+bx+cx^2$) that can determine the optimal Leucaena level where the ERSMN is maximized. In this equation, the values of "x" (Leucaena level; DM%) was calculated equating to zero the first derivative of the equation —where "y" (ERSMN, g N/kg DOMR) is maximal. To validate the LRNS, we analyzed the mean difference of the variables (DM intake, N intake, and RSMN) —experimentally observed and predicted by LRNS—, by conducting a variance analysis with the SAS GLM procedure (PROC GLM) (2002). We used the following model:

$$Y_{ij}=\mu+T_i+e_{ij}$$

where Y_{ij} is the dependent variable, μ the overall media, T_i the effect of the i^{th} treatment (observed v. predicted), and e_{ij} the experimental error.

RESULTS AND DISCUSSION

Stage 1. Effect of diet on ERSMN

In this study, the Leucaena inclusion level in the diet had no effects (P>0.05) on the total PD excretion and the RSMN. However, the ERSMN values were affected with a quadratic response (P<0.05) by the Leucaena level in the diet, while the effect of the diet on the urea-N excretion showed a positive linear response (P<0.05) (Table 3). The maximum ERSMN was observed with 20% Leucaena (33.4 g N kg^{-1} DOMR), while the ERSMN values were similar with 40 and 60% Leucaena (31 g N kg^{-1} DOMR) (Table 3). These results agree with those found by Hung *et al.* (2013) in buffaloes fed with increasing levels of Leucaena (0 to 45%). The maximum ERSMN (33.4 g N kg^{-1} DOMR) in Hung's study was observed with 20% Leucaena.

Stage 2. Effect of diet on RSMN as predicted by LRNS

The Leucaena level in the diet had a significant impact (P<0.05; Table 3) on rumen nitrogen balance (RNB), as a N requirement percentage. The optimal RNB value was reached at 20% Leucaena. These results show that both the ERSMN observed by PD and the ERSMN predicted by LRNS reached their optimal level when heifers were fed with 20% Leucaena. Above this level, the excretion of (observed) urea-N and the (predicted) urea cost increased linearly. These results match the increase of RNB after this level (Table 3).

The effect of the Leucaena level in the diet showed a positive linear response on the observed excretion of urea-N (P<0.05) and the predicted urea cost (P<0.05) (Table 3). These results could be explained by a lack of energy in rumen for the capture of N, which

Table 3. Efficiency in the ruminal supply of microbial nitrogen and predictions made with the LRNS model for rumen nitrogen balance in heifers supplemented with Leucaena.

Leucaena (% DM)	Observed (PD)				Predicted (LRNS)		
	TPD (mmol d^{-1})	RSNM (g N d^{-1})	ERSNM (g N kg^{-1} DOMR)	Ureic N (g N d^{-1})	RNB (% Req)	Urea cost (Mcal d^{-1})	RSNM (g N d^{-1})
0	74.1 ^{a}	47.8 ^{a}	14.8 ^{bc}	4.93 ^{b}	90.2 ^{e}	0.00 ^{d}	59.0 ^{bc}
20	112.6 ^{a}	72.7 ^{a}	33.4 ^{a}	13.5 ^{b}	100.6 ^{d}	0.03 ^{d}	67.2 ^{ab}
40	98.7 ^{a}	61.3 ^{a}	31.3 ^{ab}	16.6 ^{b}	107.6 ^{c}	0.06 ^{c}	71.8 ^{ab}
60	102.8 ^{a}	66.0 ^{a}	31.7 ^{ab}	46.6 ^{a}	112.6 ^{b}	0.12 ^{b}	77.2 ^{a}
80	109.5 ^{a}	58.1 ^{a}	24.4 ^{ab}	56.1 ^{a}	117.0 ^{a}	0.15 ^{a}	79.6 ^{a}
RMSE	18.5	13.1	7.77	12.1	0.37	0.01	5.9
P-value	0.0911	0.1168	0.0339	0.0005	<.0001	<.0001	0.001
L	*	NS	NS	***	***	***	***
Q	NS	NS	**	NS	***	NS	NS
C	NS	NS	NS	NS	**	*	**

PD: Purine Derivatives; LRNS: Large Ruminants Nutrition System; TPD: Total of purine derivatives; RSMN: Ruminal supply of microbial N; ERSMN: Efficiency in the RSMN; DOMR: Digestible organic matter in rumen; RNB: Rumen N balance (required %); RMSE: Root mean square error. L: Linear contrast; Q: Quadratic contrast; C: Cubic contrast. Means with the same superscript in the same column do not differ significantly (P>0.05). *P<0.05; ** P<0.01; *** P<0.001; NS=Not significant.

is the factor that most frequently limits microbial growth (Clark *et al.*, 1992). Karsli & Russell (2001) have pointed out that energy supply is commonly the major limiting factor for microbial growth in rumen. Meanwhile, Orskov (1992) has indicated that microbial protein synthesis can be maximized by synchronizing the availability of fermentable energy and degradable N for rumen microorganisms. Consequently, when the heifers' diet is supplemented with >20% Leucaena as a protein source, a source of energy that allows the capture of the N supplied by Leucaena should be provided to avoid the loss of urea-N in urine. Figure 1 shows that the N intake increases linearly ($P<0.05$), while the ERSMN increases quadratically ($P<0.05$) up to a certain level, after which it starts to drop.

According to the equation ($y=17.49436808+(0.60784137\ x)+(-0.00687749\ x2)$), the inflection point for the optimal level of the RSMN yield (31 g N kg^{-1} MODR) was reached with 44% Leucaena. This result contrasts with the RNB, whose optimal N level in rumen is reached with 20% Leucaena. Therefore, the mathematical equation does not match the dynamics of microorganisms in rumen.

Comparison of data observed with PD *v*. data predicted with the LRNS model

There were no differences ($P>0.05$) between the general averages of DM intake, N intake, and RSMN observed by PD and predicted with the LRNS model (Table 4). This result confirms the usefulness of the LRNS model for the nutritional assessment of tropical forages in ruminant diets.

Figure 2 shows the relation between the intake of N and RSMN in heifers. The N intake increases linearly ($P<0.05$), while the RSMN reaches its maximum value with 20% Leucaena (72.7 g N d^{-1}) ($P>0.05$). A similar response regarding the relation of the intake of N and RSMN was observed with the data predicted with LRNS. The intake of N and the RSMN had a positive linear effect ($P<0.05$) when Leucaena levels in the diet

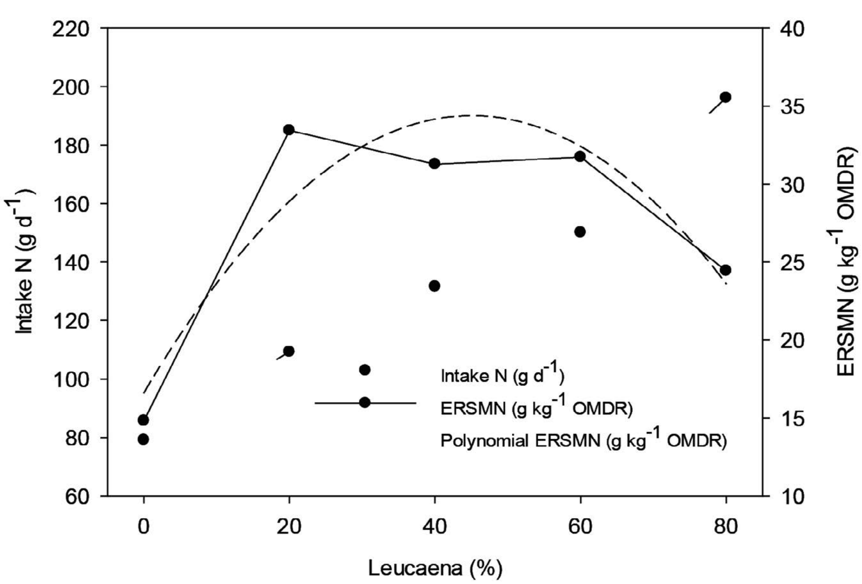

Figure 1. Relation between N intake and the efficiency in the ruminal supply of microbial N (ERSMN, g N kg^{-1} DOMR) in heifers fed with different percentages of Leucaena in their diet.

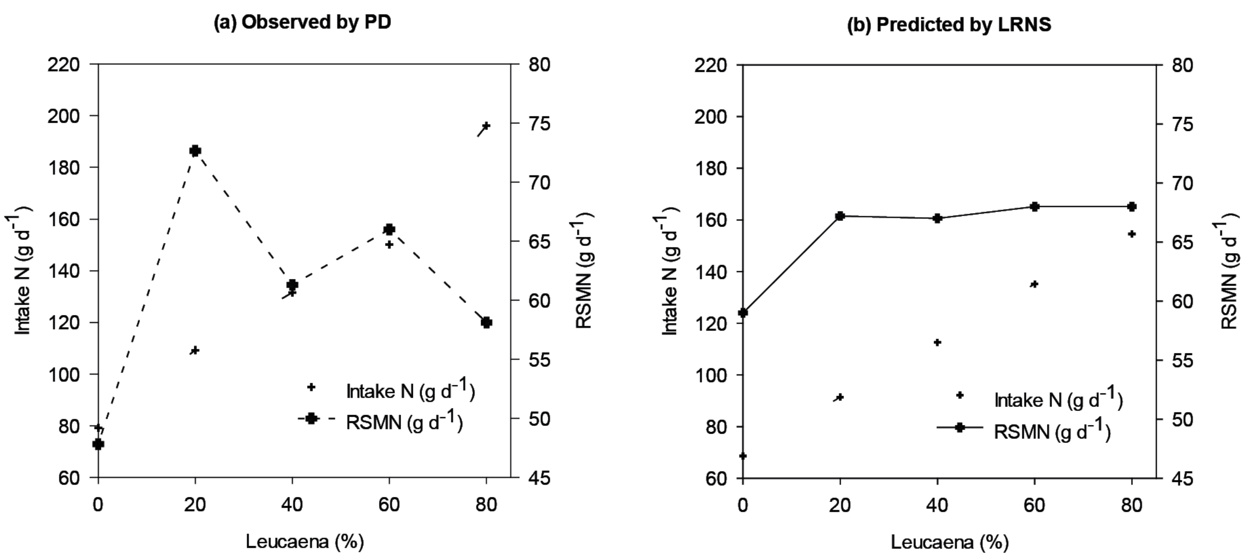

Figure 2. Comparison of the relation between variables: intake of N (g d^{-1}) and ruminal supply of microbial N (RSMN, g d^{-1}) observed by PD (a); and intake of N (g d^{-1}) and RSMN predicted with LRNS (b).

Table 4. Comparison between dry matter intake, N intake, and RSMN observed by PD *v*. LRNS predictions in heifers fed with Leucaena levels.

Variable	Dry Matter Intake (kg d^{-1})	N Intake (g d^{-1})	RSMN (g d^{-1})
Observed by PD	7.02[a]	133.2[a]	61.1[a]
Predicted by LRNS	7.08[a]	112.5[a]	65.7[a]
RMSE	0.88	45.2	14.2
P-value	0.7974	0.1114	0.2612

Means with the same superscript in the same column do not differ significantly ($P>0.05$). PD: Purine Derivatives; LRNS: Large Ruminants Nutrition System; RMSE: Root mean square error. RSMN: Ruminal supply of microbial N.

increased. However, the maximum RSMN value (67 g d^{-1}) remained slightly constant with ≥20% Leucaena. The results for the RSMN match the behavior of urea cost, which increases linearly starting from 20% Leucaena (Table 4).

CONCLUSIONS

Leucaena supplementation significantly increased the RSMN in heifers. In addition, urea-N excretion increased as the Leucaena supplementation level increased. The ERSMN and the RNB reached their optimal level when the heifers' diet was supplemented with 20% Leucaena. For higher levels, we suggest including NFC sources in the diet to capture the N supplied by Leucaena and avoid the loss of N in urine.

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We certify that no conflict of interests exists with any financial organization regarding the material discussed in this manuscript. We thank Ángel Trinidad Piñeiro Vázquez (ScD) for kindly providing us with the heifer urine samples.

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AP AGRO PRODUCTIVIDAD

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In vitro fermentative characteristics and chemical quality of Guinea grass with organic and chemical fertilization

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ABSTRACT

Objective: To evaluate the chemical quality and *in vitro* fermentative characteristics of *Panicum maximum* cv. Guinea grass, in order to determine its optimum cutting point under four fertilization schemes.

Methodology: Guinea grass was fertilized with chemicals (F1), vermicompost (F2), compost (F3), and compost + leachate (F4). The grass was cut at 20, 35, 50, 50, 65, 80, and 105 days. The neutral detergent fiber (NDF), acid detergent fiber (ADF), crude protein (CP) contents were determined, along with the dry matter (DMD), NDF (NDFD), and ADF (ADFD) degradations, as well as the *in vitro* gas production. The experimental design was a 4×6 factorial arrangement within a completely randomized design; fertilization and cutting days were used as factors.

Results: F3-65 d, F1-65 d, F4-80 d, and F1-8 d had higher NDF content; F1-65 d, higher ADF; F1-20 d, higher CP ($p \leq 0.05$); F4-50 d and F2-50 d, higher gas production; F4-20, F4-35, F4-50, F2-20, F2-35, F1-20 d, higher DMD, F2-20 and F4-20 d, higher NDFD; F4-20, F4-35, F4-65, and F2-20 d, higher ADFD ($p \leq 0.05$).

Limitations/Implications: The lack of previous research studies about the organic fertilization of Guinea grass.

Conclusions: Fertilization with vermicompost or compost + leachate improves chemical content, *in vitro* gas production, and degradation of Guinea grass.

Key words: *Panicum maximum*, *in vitro* degradation, *in vitro* gas, bromatological.

INTRODUCTION

Panicum maximum species are tolerant to trampling and drought. They produce a high volume of quality forage, with great palatability and digestibility. These grasses grow in conditions of 0 to 1,500 meters above sea level and rainfall between 800 and 3,500 mm.

Image by Ralph at Pixabay

They need medium to high fertility, well-drained soils with a pH of 5 to 8 and have a tiller growth system. In addition, its biomass productivity and nutritional quality make *P. maximun* a forage alternative for ruminants (Andrade-Solórzano *et al.*, 2020).

The *in vitro* gas production method uses volume measurement to assess the rate and degree of fermentation of forages for ruminants. *In vitro* ruminal fermentation systems are adapted to forage feeding conditions; consequently, the gas produced is directly related to microbial fermentation (Amanzougarene and Fondevila, 2020). The objective of this research was to evaluate the chemical quality and biogas production of *Panicum maximum* cv. Guinea grass under four fertilization arrangements.

MATERIALS AND METHODS

Experimental Site

The work was carried out at the Facultad de Medicina Veterinaria y Zootecnia No. 2 of the Universidad Autónoma de Guerrero, located in the municipality of Cuajinicuilapa, Guerrero.

Forage and Fertilization

The ground was harrowed twice. *Panicum maximum* cv. Guinea grass was established in August 2017. Three stripes were established 1 m apart from each other. Each row included four rows separated by 20 cm. The sowing density was 6 kg ha^{-1}. The following fertilization arrangements were used: F1=inorganic, 120-60-00 of NPK; F2=application of 10 t ha^{-1} vermicompost; F3=10 t ha^{-1} compost; and F4=10 t compost + three applications of a 20% leachate dose, at 7-day intervals, using a backpack sprayer with a 40-pound pressure adjustable nozzle. The samplings were carried out 20, 35, 50, 65, 80, and 105 days after the homogenization cut.

In vitro gas production

For gas measurement, biodigesters (experimental unit) were prepared using a 120-mL serological pipette, following the method described by Torres-Salado *et al.* (2019) and using Guinea grass with different fertilization at each cutting age. Accumulated biogas production was measured at 2, 4, 6, 8, 10, 12, 24, 48, and 72 h as described by Hernández-Morales *et al.* (2018). At 72 h of incubation, the content of the biodigesters was filtered into ANKOM® bags and the dry matter (DMD), neutral detergent fiber (NDFD), acid detergent fiber (ADFD) degradations were determined, according to the methodology proposed by Hernández- Morales *et al.* (2018).

Chemical analysis

On the one hand, the crude protein (CP) and ashes (As) contents of Guinea grass —with different fertilizations at each cutting age— were determined following the methods described by the AOAC (2005). On the other hand, the neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined with the method described by Van Soest *et al.* (1991).

Statistical analysis

The variables of the chemical analysis and *in vitro* gas production were examined with the SAS GLM procedure (SAS Institute Inc, 2011), in a completely randomized design with a 6×4 factorial arrangement, considering the cutting days (20, 35, 50, 65, 80, and 95 days) and the type of fertilization (F1, F2, F3, and F4) as factors. The comparison of means was carried out with the Tukey test ($p \leq 0.05$).

RESULTS AND DISCUSSION

Fertilizing grasses with nitrogen affect the chemical quality of forages. Additionally, it causes productive and environmental damage. Consequently, organic fertilization is an agroecological alternative for the improvement of the chemical quality of forages (Álvarez *et al.*, 2016). The highest NDF contents were observed in the F3-65, F1-65, F4-80, and F1-80 d interactions, while in ADF there were achieved with F1-65 d (Table 1). These high NDF levels are observed at these ages, because, as the grass grows, it develops a support structure mainly composed of lignin and cellulose (Gándara *et al.*, 2017), components of NDF and ADF. The highest CP content was recorded in F1-20 d and the highest values occurred in the first 20 d (Table 1): as a forage ages, its CP content decreases, because the nitrogen concentration declines, as the tissues mature and less protein compounds are synthesized (Gándara *et al.*, 2017).

Regarding the cutting age, no differences between fertilizations are identified at 20 and 35 d, but a trend towards a difference starts as the grass grows older, since the use of biological fertilizers (F2, F3, and F4) decreases the detergent fiber content. In contrast, the protein content does not show a clear difference between F1 (which shows the highest values) and the rest of the biological fertilizations (F2, F3, and F4) at different cutting ages (Table 1). Sánchez-Santillan *et al.* (2021) reported lower values for aruana grass than in this study, at similar cutting ages and using the same fertilizers. They reported 17.4% CP at 20 d of cutting with chemical fertilization, 79.9% NDF at 65 d with vermicompost fertilization, and 46.8% ADF at 95 d with chemical fertilization. It should not be assumed that these differences in chemical content are only related to the type of fertilization, but that agronomic management also interferes with the chemical content of Guinea (Table 1) and aruana (Sánchez-Santillán *et al.*, 2021) grasses.

The *in vitro* gas production method is used to evaluate the fermentation rate and degree of ruminant feeds, because it is correlated with *in vivo* parameters (Amanzougarene and Fondevila, 2020). Therefore, it has been used in the present study to infer the microbial digestion of fertilizations on different cutting days and their gas production (mainly carbon dioxide and methane) as final catabolites of fermentation (Amanzougarene and Fondevila, 2020). Therefore, F4 and F2, both at 50 d of cutting, showed the highest gas production ($p \leq 0.05$) in the evaluated hours (Table 2). However, at each measurement hour, there are no differences ($p > 0.05$) between fertilizations on the different cutting days (Table 2). Overall, Table 2 shows a great variability of gas production in each measurement hour; this is assumed to be caused by the chemical nature of carbohydrates, since gas production is the result of the fermentation of acetate, propionate, and butyrate (Amanzougarene and Fondevila, 2020). Values lower than the interactions recorded in the present study

Table 1. Chemical analysis of Guinea grass at different regrowth age under different chemical and organic fertilization scheme.

Fertilization	Age (days)	NDF (%)	ADF (%)	Hemi (%)	CP (%)	Ash (%)	OM (%)
F1	20	64.6 h	34.6 l	30.0 defgh	20.3 a	12.8 ab	87.2 lm
F2	20	65.3 h	36.6 k	28.7 ghij	10.9 d	13.6 a	86.4 m
F3	20	71.6 g	39.6 j	32.0 ab	9.9 e	12.0 bcd	88.0 jkl
F4	20	64.3 h	35.0 kl	29.3 fghij	13.6 b	11.8 cde	88.2 ijk
F1	35	74.4 def	42.2 ghi	32.2 ab	12.5 c	11.0 ef	89.1 hi
F2	35	72.9 efg	41.9 hi	31.1 bcde	8.0 fg	11.6 cde	88.4 ijk
F3	35	75.1 cde	43.9 efg	31.1 bcde	6.8 hi	10.9 ef	89.1 hi
F4	35	73.4 efg	40.4 ij	32.9 a	7.0 gh	7.3 l	92.7 b
F1	50	78.6 b	46.8 cd	31.8 abc	8.6 f	8.7 ijk	91.3 cde
F2	50	71.3 g	40.5 ij	30.8 bcdef	5.7 jkl	12.2 bc	87.8 kl
F3	50	77.6 bc	46.8 cd	30.8 bcdef	5.2 jklm	9.7 gh	90.3 fg
F4	50	75.0 cde	42.8 fgh	32.2 ab	7.1 gh	6.2 m	93.8 a
F1	65	81.5 a	52.9 a	28.6 ghij	6.2 hij	8.1 jkl	91.9 bcd
F2	65	75.2 cde	44.1 efg	31.2 bcde	6.0 ijk	9.0 hij	91.0 def
F3	65	79.0 ab	47.6 c	31.5 abcd	5.8 ijk	9.0 hij	91.0 def
F4	65	70.8 g	42.7 fgh	28.0 ij	5.9 ijk	10.0 fg	90.0 gh
F1	80	79.2 ab	50.4 b	28.8 ghij	5.3 jklm	7.8 kl	92.2 bc
F2	80	74.6 de	43.4 efgh	31.2 abcde	5.9 ijk	9.0 hij	91.0 def
F3	60	77.9 b	48.2 c	29.7 efghi	4.8 lmn	9.4 ghi	90.6 efg
F4	80	71.8 fg	44.1 efg	27.6 j	6.0 ijk	11.1 e	88.9 i
F1	95	76.5 bcd	48.2 c	28.3 hij	4.3 n	9.4 ghi	90.6 efg
F2	95	74.8 de	45.0 de	29.8 defgh	5.2 klmn	9.1 ghi	90.9 efg
F3	95	79.0 ab	50.6 b	28.4 hij	4.4 mn	8.9 hij	91.1 def
F4	95	74.6 de	44.4 ef	30.3 cdefg	5.8 ijk	11.1 de	88.9 ij
SEM		0.54	0.55	0.18	0.43	0.21	0.21

Different literals within each column indicate significant difference ($p \leq 0.05$).
NDF=neutral detergent fiber; ADF=acid detergent fiber; Hemi=hemicellulose; CP=crude protein; OM=organic matter; SEM=standard error of the mean.

(Table 2) were reported by Sánchez-Santillan *et al.* (2021), who fertilized aruana grass with vermicompost, compost, compost + leachate, and chemical fertilizers, at 20, 35, 50, 65, and 90 d of cut.

The determination of *in vivo* forage digestibility is expensive and laborious, it harms animal welfare, and is unsuitable for routine analysis; therefore, the *in vitro* method correlates well with *in vivo* digestibility (Gosselink *et al.*, 2004). The highest DMD ($p \leq 0.05$) was recorded with F4 at 20, 35, and 50 d; F2 at 20 and 35 d; and F1 at 20 d, without differences between them ($p > 0.05$; Table 3). NDFD is used to predict the energy content of the evaluated substrate and animal performance (Hoffman *et al.*, 2006), as well as to improve the prediction of animal weight gain measurement (Hoffman *et al.*, 2007). Therefore, Guinea grass could be considered to be in a vegetative state, with F2 and F4

Table 2. Cumulative biogas production (mL g^{-1} DM) of Guinea grass at different regrowth age under different chemical and organic fertilization schemes.

Fertilization	Age (day)	2 h	4 h	6 h	8 h	10 h	12 h	24 h	48 h	72 h
F1	20	21 hij	40 ef	40 i	46 h	51 jk	56 gh	87 h	107 gh	153 efgh
F2	20	46 a	57 a	63 abcde	73 abc	85 ab	99 a	140 ab	177 ab	219 a
F3	20	36 bcdef	49 cd	52 h	58 fg	65 ghi	74 def	97 fgh	119 efgh	147 gh
F4	20	33 cdefg	50 bcd	58 defgh	67 bcde	75 cde	84 cde	132 abcd	170 abc	206 abc
F1	35	12 k	29 g	29 j	32 i	40 l	41 i	88 h	114 fgh	150 fgh
F2	35	40 abc	52 abcd	61 bcdef	69 abcd	81 abc	92 abc	140 ab	185 a	216 a
F3	35	37 abcdef	49 cd	52 h	58 fg	67 efghi	77 de	113 defg	129 defg	152 efgh
F4	35	31 defg	52 abcd	66 abc	75 ab	88 a	99 a	141 ab	179 ab	223 a
F1	50	18 jk	37 f	37 i	40 h	47 k	51 hi	91 gh	111 fgh	148 gh
F2	50	45 a	57 ab	67 ab	76 a	87 a	101 a	146 a	184 a	208 abc
F3	50	38 abcde	51 abcd	52 h	58 fg	64 hi	77 de	128 abcde	150 bcde	166 defg
F4	50	42 ab	57 a	68 a	76 a	88 a	97 ab	138 abc	159 abcd	212 ab
F1	65	19 ijk	35 fg	35 ij	40 h	46 k	49 hi	53 i	67 i	107 i
F2	65	39 abcd	52 abcd	58 efgh	63 def	68 defghi	74 def	106 efgh	127 defg	155 efgh
F3	65	37 abcde	50 abcd	54 fgh	61 efg	68 defghi	77 de	116 cdef	137 defg	157 efgh
F4	65	41 abc	50 bcd	60 cdefg	65 cdef	72 cdefgh	80 cde	122 bcde	155 abcd	195 abcd
F1	80	28 fghi	50 bcd	51 h	54 g	66 fghi	72 ef	84 h	94 hi	104 i
F2	80	35 bcdefg	55 abc	65 abcd	72 abc	74 cdefg	86 bcd	122 bcde	142 cdef	171 defg
F3	80	35 bcdefg	51 abcd	60 cdefg	67 bcde	77 bcd	92 abc	121 bcde	150 bcde	182 bcde
F4	80	26 ghij	46 de	56 efgh	67 bcde	75 cdef	78 de	114 def	147 bcde	181 bcdef
F1	95	32 cdefg	51 abcd	52 h	58 fg	60 ij	64 fg	94 fgh	92 hi	128 hi
F2	95	26 ghij	49 cd	57 efgh	66 cdef	74 cdefg	83 cde	124 abcde	139 cdefg	171 defg
F3	95	30 efgh	48 cd	53 gh	60 efg	72 cdefgh	82 cde	111 defg	154 abcd	180 cdef
F4	95	18 jk	40 ef	52 h	60 efg	68 defghi	72 ef	113 defg	134 defg	172 defg
SEM		1.0	0.7	1.0	1.2	1.3	1.7	2.4	3.3	3.5

Different literals within each column indicate significant difference ($p \leq 0.05$).
SEM=standard error of the mean.

at 20 d of cutting (Hoffman *et al.*, 2007), since they presented degradations greater than 70% (Table 3). Maturity, growth conditions, and forage management at cutting and after cutting are assumed to be responsible for the variability of the NDFD values (Reuss, 2001). Sánchez-Santillan *et al.* (2021) reported higher DMD and NDFD values in aruana grass, fertilized with compost + leachate at 20, 35, and 50 days of cutting, than in the present study (Table 3).

Identifying factors that limit cell wall degradation is a complex process (Ramírez *et al.*, 2002). Consequently, the ADFD refers to the portion of the cell wall composed of cellulose and lignin, which are associated with the ability to digest forage. Therefore, the highest values were F4 at 20, 35, and 65 d and F2 at 20 d ($p \leq 0.05$; Table 3). Lower ADFD values were reported in Bermudagrass (*Cynodon dactylon*), Mulato II grass (*Brachiaria* *hybrid*), Palisade grass (*Brachiaria brizantha*), Star grass (*Cynodon nlemfuensis*), Quackgrass (*Elytrichia repens*), Gamba grass (*Andropogon gayanus*), Guinea grass (*Panicum maximun*), Para grass (*Brachiaria mutica*), and Pangola grass (*Digitaria decumbens*), with 56 days of regrowth and without any fertilization (Almaraz-Buendía *et al.*, 2019); for its part, Guinea grass was fertilized with vermicompost and compost + leachate at 35 and 50 d (Table 3).

From another perspective, the Guinea grass with 20 d had the greatest degradations as a result of its chemical content, which stands out for its lower amount of cellulose and lignin and greater amount of cellular content. This is assumed to be associated with the maturity degree of the cut; as the plant matures, it undergoes physiological changes and develops the xylem tissue for water transport, accumulates cellulose, and begins the lignification process, resulting in a cell wall that is difficult for ruminal bacteria to adhere to and to digest (Hoffman *et al.*, 2007).

Table 3. Degradations of dry matter and detergent fibers of Guinea grass at different regrowth age under different chemical and organic fertilization schemes.

Fertilization	Age (days)	DMD (%)	NDFD (%)	ADFD (%)
F1	20	63.39 abc	61.62 ab	59.75 abcd
F2	20	73.25 a	70.18 a	69.45 a
F3	20	38.23 fghi	28.72 cde	25.11 jk
F4	20	71.73 ab	70.61 a	68.81 a
F1	35	50.51 cdefg	47.32 abcde	44.05 efghi
F2	35	62.46 abc	35.6 bcde	58.56 abcde
F3	35	34.79 ghi	26.88 de	25.19 jk
F4	35	64.55 abc	62.87 ab	64.41 ab
F1	50	43.22 defghi	38.52 abcde	37.7 ghij
F2	50	56.95 bcde	50.64 abcde	47.44 defgh
F3	50	41.26 efghi	32.66 bcde	28.57 jk
F4	50	58.71 abcd	55.98 abcd	56.51 abcde
F1	65	32.6 hi	26.7 de	27.34 jk
F2	65	42.14 efghi	33.98 bcde	29.51 ijk
F3	65	42.65 efghi	36.25 bcde	33.14 hijk
F4	65	56.01 bcde	61.27 abc	62.77 abc
F1	80	27.87 i	18.29 e	17.89 l
F2	80	43.09 defghi	34.76 bcde	28.66 jk
F3	80	53.76 cdef	49.67 abcde	48.78 cdefg
F4	80	53.61 cdef	48.11 abcde	53.35 bcdef
F1	95	31.47 hi	20.97 e	21.23 k
F2	95	46.29 defgh	39.79 abcde	38.79 fghij
F3	95	51.73 cdef	46.98 abcde	44.78 efgh
F4	95	52.75 cdef	47.72 abcde	49.68 bcdefg
SEM		1.53	2.01	1.91

Different literals within each column indicate significant difference ($p \leq 0.05$). DMD=dry matter degradation; NDFD=neutral detergent fiber degradation; ADFD=acid detergent fiber degradation; SEM = standard error of the mean.

CONCLUSIONS

Fertilization with biological products, such as vermicompost or compost + leachate, improve the chemical content, gas production, and *in vitro* degradation of Guinea grass with regard to chemical fertilization or compost. It has been confirmed that the maturity of grass decreases its protein content, increases the components of the cell wall, and decreases its degradation in *in vitro* tests.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Forage yield of *Urochloa* grass cv Camello I and II at different cutting frequencies and intensities

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ABSTRACT

Objective: To evaluate the productive performance of forage from hybrid grasses of the genus *Urochloa* at different cutting intervals and intensities.

Design/Methodology/Approach: The cultivars Camello I (GPB025) and Camello II (GPB07) were evaluated at different intervals (5 and 7 weeks) and cutting intensities (10 and 20 cm). The research was carried out under seasonal conditions, from 2020 to 2021. The experimental design was a randomized complete block. The following variables were evaluated: yield of total dry matter (TDM), leaf (DMl), stem (DMs), inflorescence (DMin), and senescent matter (DMsm); as well as plant height, basal cover, specific leaf area (SLA), and leaf area index (LAI). DMl, DMs, DMin, and DMsm are the morphological components of the TDM.

Results: On average, harvesting the forage at a 7-week interval and with a 20-cm intensity results in higher TDM, DMl, and DMs (66, 46, and 85%, respectively) than those obtained when harvesting is carried out at a 5-week interval and with a 20-cm intensity.

Study Limitations/Implications: Agronomic management of grasses is a factor that affects forage yield and sward persistence. Cutting grasses at different intervals and intensities generates adequate management strategies aimed to increase yields and sward persistence.

Findings/Conclusions: The highest yield of total dry matter in both cultivars —according to their morphological component, plant height, and leaf area index— was obtained when the residual forage was harvested at a 7-week interval and a 20-cm height.

Keywords: Forage yield, hybrid grasses, cultivars (GPB025) and (GPB07), cutting interval and intensity.

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INTRODUCTION

Forage grasses are the basis of ruminant feeding in different livestock production systems. However, they have been classified as a poorly available food source, mainly in tropical and subtropical regions, given the edaphoclimatic conditions of those regions (Maldonado-Peralta *et al.*, 2019; Núñez-Torres and Rodríguez-Barros, 2019). Inadequate forage species have been selected and established in areas for which they are not suitable. However, the forage yield of grasses largely depends on factors such as the genotype and agronomic management (Gándara *et al.*, 2017). The agronomic management includes the intensity and frequency of defoliation, which are factors that modify the forage yield (Hernández *et al.*, 2012; Rojas-García *et al.*, 2018). When Cruz *et al.* (2017) evaluated Chetumal grass (*Brachiaria humidicola*) at different intervals (21 and 28 days) and grazing intensities (severe: 9-11 and light: 13-15 cm), they obtained greater forage accumulation when light grazing took place every 28 days (9,771 kg DM ha^{-1}) than with severe grazing (8,337 kg DM ha^{-1}). In cultivar Mulato II (*Urochloa* hybrid) grass evaluated at different intervals (21 and 28 days) and residual grazing intensities or heights (severe: 17-20 and light: 22-25), Cruz-Sánchez *et al.* (2018) determined that the forage accumulation obtained when grazing took place every 28 days was higher with light grazing (11,504 kg DM ha^{-1}) than with severe grazing (9,775 kg DM ha^{-1}). In this sense, in order to establish new grass cultivars, previous studies must be carried out to evaluate their productive performance, just as in the case of the grasses evaluated in this research, of which there is scarce information. Therefore, the objective of this research was to evaluate the forage yield of hybrid grasses of the genus *Urochloa* cultivars Camello I (GPB025) and Camello II (GPB07), at different cutting intervals and intensities.

MATERIALS AND METHODS

Study site location

The research was carried out from 2020 to 2021, at the "Posta Zootécnica Ingeniero Herminio García González" of the Facultad de Ingeniería y Ciencias, Universidad Autónoma de Tamaulipas, located at 23° 56' 26.5" N and 99° 05' 59.9" W, at 193 m.a.s.l. (INEGI, 2015).

Edaphoclimatic characteristics

The experimental site has a semi-arid warm climate, classified as BS_1(h') hw (Vargas *et al.*, 2007). The average annual temperature and precipitation are 24 °C and 940 mm, respectively (SMN, 2010); the maximum and minimum temperatures were recorded during the evaluation period, along with the monthly accumulated precipitation (Table 1).

The soil is classified as clayey, with an alkaline pH of 8.3 (Garay-Martínez *et al.*, 2018), for this, a soil analysis was carried out to determine the content of organic matter (OM), nitrogen (N), phosphorus (P), potassium (K), iron (FE) and zinc (Zn) (Table 2). Fertilization was not applied.

Plant material, treatments, and agronomic management

Urochloa hybrids [cultivars Camello I (GPB025) and Camello II (GPB07)] were used. The treatments were the combination of both cultivars at 5- and 7-week intervals and 10- and 20-cm cutting intensities. Swards sown in 2017 with a manual seeder at a distance of 15 cm between plants were evaluated. Prior to the evaluation, a uniformity cutting was made depending on the cutting intensity. Subsequently, the forage contained within 1 m^2 was harvested every 5 and 7 weeks with 10- and 20-cm intensities.

Table 1. Monthly accumulated temperature and precipitation during the evaluation period, in Güémez, Tamaulipas, Mexico.

	Maximum Temperature	Minimum Temperature	Rainfall
2020	**(°C)**	**(°C)**	**(mm)**
May	34	22	115
June	36	22	90
July	38	23	66
August	37	22	80
September	34	20	164
Octuber	34	16	77
November	30	14	18
2021	**(°C)**	**(°C)**	**(mm)**
March	38	14	22
April	32	19	49
May	35	21	105
June	35	22	87
July	36	21	57
August	39	22	79

Table 2. Chemical and physical characteristics of the soil of the experimental site.

pH	TN	OM	TCa	P	K	Fe	Zn	Sand	Slime	Clay	SAR
	%			mg kg^{-1}				%			
8.2	0.27	4.5	45.3	0.93	0.71	2.82	0.26	4.8	32.7	62.5	0.72

TN: Total Nitrogen; OM: Organic matter; TCa: Total carbonates; P: phosphorus; K: Potassium; Fe: iron; Zn: zinc; SAR: sodium adsorption ratio.

Evaluated variables

Plant height and basal cover

Three readings were made with a graduated wooden rule to obtain the average height at each cutting intensity within each experimental unit (1 m^2), measuring the height (cm) from the soil surface to the most homogeneous point of the leaves' apex. The coverage was estimated with a 1 m^2 steel frame, divided in 100 cm^2 grids; the frame was placed in the experimental unit (1 m^2) and the empty spaces were counted to subsequently estimate the coverage.

Yield of total dry matter and of each morphological component

The forage of each 1 m^2 experimental unit was harvested and immediately weighed to determine the yield of green forage (GF). Later, 300 g of sample were selected and separated into their morphological components: leaf (leaf blade + pod), stem, and dead matter (leaf blades with more than 50% chlorotic tissue). To estimate the leaf area, 10 leaf blades were taken from 5 stems per sample and measured with the Cl 202 Leaf Area Meter (CID Bio-Science® Inc., USA). All samples were dried in an OMS60 forced air stove (Thermo

Scientific®, USA) at 60 °C until constant weight was obtained. Each morphological component was weighed before (GF) and after (DM) drying on a CQT 2601 analytical balance (ADAM®, USA) to determine the percentage content of dry matter.

Specific Leaf Area (SLA) and Leaf Area Index (LAI)

The specific leaf area (cm^2 g^{-1}) was estimated dividing the leaf area by its dry weight. To estimate the leaf area index, the total leaf area was divided by the ground-level surface (1 m^2, in the case of this research).

Statistical analysis

The variables were analyzed with the GLM procedure (SAS, 2003), with a randomized complete block design, and the Tukey test was applied for the comparison of means ($P=0.05$).

RESULTS AND DISCUSSION

Dry matter accumulation and morphological composition

The interval and intensity of defoliation had a significant effect ($P>0.05$) on the accumulation of total dry matter (TDM) and the dry matter of its individual morphological components. Regardless of the cultivar and cutting intensity, the highest TDM accumulation ($P>0.05$) was obtained when the forage was harvested at 7-week intervals. Several researchers (Cruz-Sánchez *et al.*, 2018; Cruz-Hernández *et al.*, 2020) have determined this performance prolonging the cutting interval of the longer regrowth period it provides to the sward. In this regard, decreasing the cutting intensity (from 11-15 to 13-15 cm) and increasing the cutting interval (from 21 to 28 days), Cruz *et al.* (2017) obtained a higher dry matter accumulation of the cultivar Chetumal (*Brachiaria humidicola*), with a light harvesting (13-15 cm) at a 28-day interval. The accumulation was 16 % higher than that obtained with a severe harvesting (9-11 cm). In this sense, Cruz-Sánchez *et al.* (2018) obtained a greater accumulation of annual dry matter of Mulato II at different grazing intensities (severe: 17-20 cm and light: 22-25 cm) and intervals (21 and 28 days), with a light grazing every 28 days. The greater forage accumulation obtained with a lower intensity harvest can be attributed to the residual leaf area in the sward after the forage is harvested: a greater leaf area results in an increase of the photosynthetic rate, which favors the increase in regrowth speed (Difante *et al.*, 2011). In this sense, no significant statistical difference ($P>0.05$) was recorded between cultivars and cutting intensities in the seventh week for TDM, DMl, DMs, and DMin; however, cultivar Camello II obtained 14 and 13% more TDM yield than cultivar Camello I with 10 and 20 cm intensities, respectively.

Regarding leaf yield, the cultivar Camello II obtained 21 and 15% more leaf yield when it was harvested at a 7-week interval, with 10- and 20-cm intensities, respectively, (Table 3). The stem yield showed a similar performance, obtaining the highest accumulation at a 7-week interval ($P<0.05$). In this case, the cultivar Camello II accumulated 10 and 17% more stem at 10 and 20-cm intensities, respectively. Regarding the DMsm yield, no significant statistical differences ($P>0.05$) were recorded between cultivars, intervals, and cutting intensities.

Table 3. Productive performance of *Urochloa* hybrids at different cutting intervals and intensities, in Güémez, Tamaulipas, Mexico.

Cultivar	Interval (weeks)	Intensity (cm)	DMI	DMs	DMin	DMsm	TDM
			kg DM ha^{-1}				
Camello I	5	10	388 c	21 b	38 c	12 a	461 c
Camello II		10	473 c	36 b	48 c	19 a	576 c
Camello I		20	529 c	33 b	68 c	14 a	644 bc
Camello II		20	655 bc	48 b	52 c	28 a	785 bc
Camello I	7	10	727 bc	218 a	188 a	15 a	1149 ab
Camello II		10	917 ab	240 a	151 ab	22 a	1331 a
Camello I		20	985 ab	218 a	225 a	18 a	1447 a
Camello II		20	1150 a	262 a	207 a	37 a	1657 a

DMI: leaf dry matter; DMs: stem dry matter; DMin: inflorescence dry matter; DMsm: dry matter of the senescent matter; TDM: total dry matter; different lowercase letters (a, b, c) in each column indicate a statistical difference (Tukey, P=0.05).

The greatest leaf accumulation is obtained with a light defoliation. In this sense, Rojas-García *et al.* (2018) recorded greater leaf accumulation in the cultivar Cobra (*Brachiaria* hybrid) harvested at 35 days with a 15-cm intensity (1,200 kg DM ha^{-1}) than with a 10-cm intensity (980 kg DM ha^{-1}). Similarly, when Torres *et al.* (2020) evaluated the cultivar Cobra at different grazing intensities (light: 15 cm and severe: 10 cm) at a 35-day grazing interval, they recorded greater leaf accumulation with a light grazing (5,323 kg DM ha^{-1}) than with a severe grazing (4,213 kg DM ha^{-1}); furthermore, stem accumulation was greater with a light grazing intensity (2,286 kg DM ha^{-1}) than with a severe grazing intensity (1,898 kg DM ha^{-1}). In this regard, Rojas-García *et al.* (2018) mention that the leaf content in the Cobra grass forage is more digestible and has a higher protein content than the stem.

Differences were detected ($P<0.05$) in the morphological and structural characteristics of *Urochloa* hybrids at different cutting intervals and intensities. The plant height variable ($P<0.05$) was affected by both the frequency and the intensity of cutting (Table 4). Regardless of the cultivar and cutting interval, the highest heights were obtained when the forage was harvested at a 20-cm height. In this sense, when they evaluated the effect of the cutting interval (14, 28, and 42 days) in two hybrids of the genus *Cynodon*, Silva *et al.* (2016) recorded higher plant heights when the forage was harvested at a longer interval (42 days). Regarding the specific leaf area, no significant statistical differences were registered ($P>0.05$); however, statistical differences ($P<0.05$) were observed between cutting intervals and intensities regarding the leaf area index variable —which indicates that the leaf area increases as the cutting interval is extended and the cutting intensity decreases. When they evaluated different hybrid cultivars of the genus *Urochloa* at 4, 6, and 8 weeks of regrowth, Garay *et al.* (2020) recorded a higher leaf area index for harvests with longer intervals (8 weeks); in this research, a similar performance was registered at 7 weeks. More leaves accumulated, when higher heights or longer cutting intervals are maintained in Mulato II grass; the improved production of those leaves increases the photosynthetic rate as well as the leaf area index (Yasuoka *et al.*, 2018).

Table 4. Morphological and structural characteristics of *Urochloa* hybrids at different cutting intervals and intensities, in Güémez, Tamaulipas, Mexico.

Cultivar	Interval (weeks)	Intensity (cm)	Plant height (cm)	Basal coberture (cm^2)	Specific leaf area ($cm^2\ g^{-1}$)	Leaf area index
Camello I	5	10	23 c	69 c	165 a	1.4 bcd
Camello II		10	24 c	68 c	152 a	1.3 bcd
Camello I		20	37 b	75 abc	146 a	1.2 cd
Camello II		20	37 b	78 ab	154 a	1.1 d
Camello I	7	10	37 b	71 bc	170 a	2.1 a
Camello II		10	39 ab	70 bc	160 a	1.8 ab
Camello I		20	50 a	72 bc	146 a	2.0 a
Camello II		20	51 a	83 a	163 a	1.7 abc

Different lowercase letters (a, b, c, d) in each column indicate a statistical difference (Tukey, P=0.05).

Regarding basal cover, statistical differences were registered ($P<0.05$). A lower basal cover was recorded for harvests with shorter intervals (5 weeks) and greater intensity (10 cm). This phenomenon represents a greater degradation of the meadow over time, another response to the current environmental conditions (Euclides *et al.*, 2019).

CONCLUSIONS

The highest yield of total dry matter in both cultivars determined based on morphological component, plant height and leaf area index was obtained for harvests with 7-week intervals and a 20-cm high residual forage.

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AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Growth rate, leaf:stem ratio, and height of crotalaria (*Crotalaria juncea* L.) at different planting densities

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ABSTRACT

Objective: To evaluate growth rate, leaf:stem ratio, and height of crotalaria (*Crotalaria juncea* L.) plants sown at different densities, in the dry tropics of the state of Guerrero, Mexico.

Design/Methodology/Approach: The treatments consisted of four planting densities: 400,000, 200,000, 100,000 plants ha^{-1}, and overseed. A growth analysis was also carried out at 30, 38, 45, 52, 60, 68, and 75 days of growth, when the pod was fully developed. The variables evaluated were: growth rate, leaf:stem ratio, plant height, and their correlation.

Results: The best growth rate (577 kg DM ha^{-1} d^{-1}) was obtained at a 400,000 plants ha^{-1} planting density, at 75 days of development. Likewise, the best plant height rate was obtained with this planting density (281 cm). Meanwhile, the leaf:stem ratio had a different behavior, obtaining its highest rate at 30 days. Regardless of the age of the plant, the following descending order was recorded: 100,000 > overseed > 200,000 > 400,000 plants ha^{-1}, with 0.65, 0.60, 0.59, and 0.55 ($p<0.05$), respectively.

Study Limitations/Implications: This is a very important study for future researches, because the crotalaria variables studied in this research have never been evaluated before and they are fundamental for forage production.

Findings/Conclusions: The recommended planting density is 400,000 plants ha^{-1}. Acceptable leaf:stem ratio and growth rate were reported at 45 days of development; the plant height reached 186 cm.

Keywords: Growth rate, height, leaf:stem, densities.

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INTRODUCTION

Cattle raising faces challenging environmental conditions in the dry tropics of Mexico (Castro-Rincón *et al.*, 2018). Livestock production is a major activity, as a result of its

Image by Karynne Grabovski at Pixabay

contribution to food production (SIAP, 2019). Currently, society requires food security and must produce more with less resources (FAO, 2020; SIAP, 2021); this includes the search for a solution to water shortage. This situation demands the acceleration of productive systems, through the search for alternatives that improve livestock feed efficiency (Burbano-Erazo *et al.*, 2019), using affordable technology that enables the sustainable development of rural communities (García *et al.*, 2018). Although pulses are beneficial, in most of the livestock systems their use as green manure or feed is rare (Castro *et al.*, 2017; Russelle *et al.*, 2007; Peters and Lascano, 2003). Crotalaria (*Crotalaria juncea* L.) is mainly used as vegetable coverage, green manure, environmental nitrogen-fixing plant, and as biological control for soil nematodes (Wang *et al.*, 2002; Timossi *et al.*, 2016). This genus is a pulse with a strong and bush-type development (Pereira, 2006). It is an annual crop and, on its early stages, it can regrow after its cut. The stems are fibrous and semi-woody; it has erect habits and narrow leaves, with seeds of different color, contained in pods (Silva *et al.*, 2016). In the different areas of the state of Guerrero, there are periods of food shortage. The alternative is the use of different species which —provided at different concentrations (mainly as flour)— allows producers to supplement better-quality food during the different seasons of the year. Additionally, growing crotalaria significantly improves soil quality (Lemaire et al., 2014). Therefore, the objective of this study was to evaluate growth rate, leaf:stem ratio, and plant height of crotalaria, at different planting densities, in the dry tropics of the state of Guerrero.

MATERIALS AND METHODS

Experimental plot location

The study was carried out from July to October, 2020, in an experimental plot in Tecuescontitlán, Tepecuacuilco de Trujano, Guerrero, Mexico (18° 08’ N and −99° 33’ W, a 782 m.a.s.l). The climate is subhumid warm with summer rains (790 mm annual average rainfall) and a 26 °C average temperature (García, 2004).

The soil has a 7.3 pH, 0.3 dS m^{-1}, and 2.1 % organic matter. Figure 1 shows the maximum, medium, and minimum temperatures, as well as the weekly rainfall accumulation

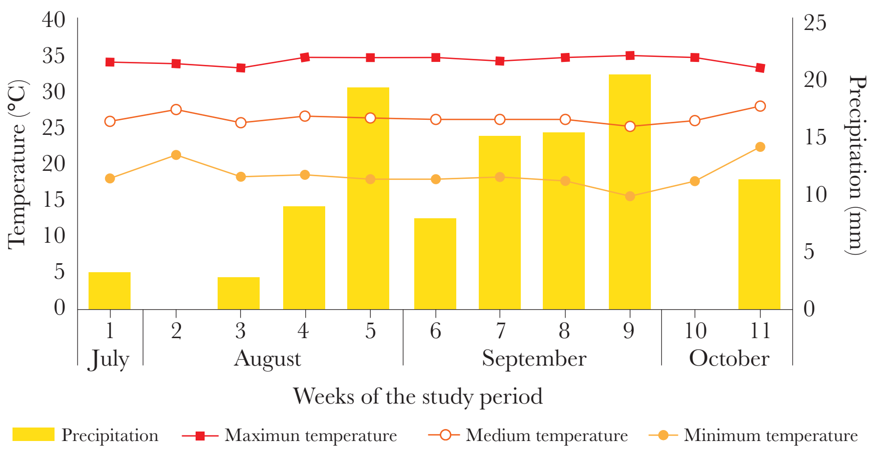

Figure 1. Maximum, medium, and minimum temperatures and weekly rainfall accumulation recorded during the experiment.

during the study period. The data were obtained from weather station 12,092, located in Tonalapa del Sur, 51 km away from the experimental plot.

Plot management

The experimental plot was established on July 30, 2020, during the rainy season. The land was prepared with traditional techniques (fallow, two harrows, and furrow). Sixteen experimental units were used; they were made up of 5 × 5 m plots —randomly distributed and with 3 repetitions. Four planting densities (treatments) were evaluated. The seeds were sown by hand, placing them in the furrows, at a depth that doubled the size of the seed. The distance between furrows was 50 cm and the distance between plants was 5, 10, and 20 cm, resulting in 400,000, 200,000, and 100,000 plants ha^{-1} per each distance. For its part, control was overseed (approximately 380,000 plants ha^{-1}). No irrigation or fertilizers were used and weed control was carried out by hand. From the 30 days after the emergence, samplings were carried out at 8-day intervals, until the plants reached their reproductive stage and had fully developed seeds.

Evaluated variables

Growth rate

Growth rate was determined dividing dry matter yield by the cutting time that passed between cuts.

Leaf:steam ratio

The leaf:stem ratio was determined dividing dry weight (kg ha^{-1} of the morphology fractions of the leaf) by the stem.

Plant height

Plant height was measured one day before each cut, using a wooden rule (cm), from the ground to the highest component of the plant. The height of 20 randomly selected plants was recorded per repetition.

Statistical analysis

The data was analyzed using a completely randomized block design, with a divided plots and three replications arrangement. The PROC GLM of SAS 9.2 (2009) was used and a comparison of the means of the treatments was carried out using the Tukey's Test (α=0.05). Potential simple regressions were carried out, comparing growth rate with the meadow height. The significance of the correlation coefficients ($p<0.05$) was calculated; an analysis of variance and a comparison of means were also carried out (Tukey: $p<0.05$).

RESULTS AND DISCUSION

Growth rate

Table 1 shows the growth rate (GR) of crotalaria, at different planting densities and cutting ages. Regardless of the cutting age, the highest and the lowest rates for this variable were obtained with 400,000 and 100,000 plants ha^{-1} planting densities, recording 317 and 66 kg ha^{-1} d^{-1}, respectively ($p<0.05$). The planting density with the highest GR was 400,000 plants ha^{-1}, at 68 and 75 days, obtaining 556 and 577 kg ha^{-1} d^{-1}, respectively. Overseed also obtained the highest values, recording 546 kg ha^{-1} d^{-1}, at the 75th cutting day. The lowest GR was obtained with the 200,000 and 100,000 plants ha^{-1} planting densities, which recorded 16 and 13 kg ha^{-1} d^{-1}, respectively ($p<0.05$).

Growth rate reflects the dry matter yield, as Tripathi *et al.* (2013) mentioned in their study about crotalaria and different spaces between plants: a higher plant yield can be obtained increasing planting density. These results match the findings of this research. Meanwhile, Richena *et al.* (2020) recorded a higher dry matter yield production and, therefore, a higher growth rate 135 days after the emergence, while pod production started 90 days after the emergence.

Leaf:stem ratio

Table 2 shows the leaf:stem ratio of crotalaria crops, at different planting densities and cutting ages. Regardless of the cutting age, this variable behaved in the following order (from highest to lowest): 100,000 > overseed > 200,000 > 400,000 plants ha^{-1}, with 0.65, 0.60, 0.59, and 0.55, respectively ($p<0.05$). Thirty days after the cutting, the highest leaf:stem ratio was obtained (1.69 average). This average diminished 86% (0.23) when the forage was cut at 75 days ($p<0.05$). The highest and the lowest leaf:stem ratio were obtained with a 100,000 plants ha^{-1} planting density, in the 30th and 75th days after the cutting, recording 2.00 and 0.13 ratios, respectively ($p<0.05$). This leaf:stem ratio trend has been recorded for several tropical pulses (Sosa *et al.*, 2008). A 1.2 average was recorded for these pulses, depending on the cutting and the season of the year. Meanwhile, Rojas-García *et al.* (2017) reported that several alfalfa varieties had a 0.88-1.55 leaf:stem ratio. Something similar happened with this tropical species, at early stages.

Plant height

Table 3 shows crotalaria height, at different planting densities and cutting ages. Regardless of planting density, the height of the plants had a significant increase during the vegetative development stage ($p<0.05$). At day 75, the 200,000 and 100,000 plants ha^{-1} planting densities recorded the shortest plants (276 and 265 cm, respectively), while

Table 1. Forage yield (kg ha^{-1} d^{-1}) of crotalaria, at different planting densities and cutting ages.

Density (plants ha^{-1})	Age at cut (days after the emergency)							Average
	30	38	45	52	60	68	75	
400,000	22 ^{Af}	90 ^{Ae}	172 ^{Bd}	315 ^{Ac}	491 ^{Ab}	556 ^{Aa}	577 ^{Aa}	317 ^{A}
200,000	16 ^{BCf}	53 ^{Be}	78 ^{Ce}	143 ^{Bd}	193 ^{Cc}	236 ^{Cb}	270 ^{Ba}	141 ^{C}
100,000	13 ^{Ce}	27 ^{Cde}	48 ^{Dcd}	64 ^{Cbc}	83 ^{Db}	113 ^{Da}	118 ^{Ca}	66 ^{D}
Overseed	18 ^{Bg}	97 ^{Af}	199 ^{Ae}	314 ^{Ad}	374 ^{Bc}	476 ^{Bb}	546 ^{Aa}	289 ^{B}
Average	17 ^{g}	67 ^{f}	124 ^{e}	209 ^{d}	285 ^{c}	345 ^{b}	378 ^{a}	

Means with the same lower-case letter in the same column (^{abcd}) and capital letters (^{ABCD}) in the same column are not statistically different (Tukey: $\alpha=0.05$).

Table 2. Leaf:stem ratio of crotalaria, at different planting densities and cutting ages.

Density (plants ha^{-1})	Age at cut (days after the emergency)							Average
	30	38	45	52	60	68	75	
400,000	1.27 Ba	0.85 Ab	0.49 ABc	0.45 Ac	0.28 Bd	0.27 Bd	0.27 Ad	0.55 C
200,000	1.66 ABa	0.80 Ab	0.44 ABc	0.36 Bc	0.32 Ac	0.33 Ac	0.25 Ac	0.59 B
100,000	2.00 Aa	0.72 Cb	0.69 Ab	0.47 Ac	0.30 ABcd	0.26 Bcd	0.13 Bd	0.65 A
Overseed	1.83 Aa	0.70 Bb	0.40 Bbc	0.36 Bbc	0.32 Ac	0.32 Ac	0.28 Ac	0.60 B
Average	1.69 a	0.76 b	0.50 c	0.41 c	0.30 c	0.29 c	0.23 d	

Means with the same lower-case letter in the same column (abcd) and capital letters (ABCD) in the same column are not statistically different (Tukey: α=0.05)

Table 3. Plant height (cm) of crotalaria, at different planting densities and cutting ages.

Density (plants ha^{-1})	Age at cut (days after the emergency)							Average
	30	38	45	52	60	68	75	
400,000	59 Cf	112 Be	186 Ad	239 Ac	267 Ab	278 Aa	281 Aa	203 A
200,000	71 Af	111 Be	174 Bd	227 Bc	235 Cc	266 Bb	276 Ba	194 C
100,000	68 ABg	114 Bf	168 Be	212 Cd	239 Cc	253 Cb	262 Ca	188 D
Overseed	66 Bf	117 Ae	185 Ad	227 Bc	251 Bb	271 Ba	278 Aba	199 B
Average	66 e	113 d	178 c	226 bc	248 b	267 ab	274 a	

Means with the same lower-case letter in the same column (abcd) and capital letters (ABCD) in the same column are not statistically different (Tukey: α=0.05).

the 400,000 plants ha^{-1} and overseed planting densities obtained the tallest plants (281 and 278, respectively, which are statistically equal).

Dubeux *et al.* (2019) evaluated planting densities and inoculants for crotalaria and reported heights of 200 and 300 cm, 75 days after the sowing. These values are very similar to those found in this study. Meanwhile, Sosa *et al.* (2008) reported a relationship between plant height, dry matter yield, and the coverage percentage of tropical pulses. These results are also similar to the findings of this study. Finally, Abdul-Baki *et al.* (2001) reported 220-320 cm plant heights, 100 days after sowing. These results are also similar to those recorded in this study.

Growth rate (kg ha^{-1} d^{-1}) regression equations with meadow height (cm)

Figure 2 shows the regression coefficient (R^2), between growth rate (kg ha^{-1} d^{-1}) and crotalaria leaf (%), at different planting densities. This research determined that meadow height is similar to the four associations. However, growth rate has a high variation, which depends on the planting density; in this study, the highest growth rate was recorded with a 400,000 plants ha^{-1} planting density. Overall, all regressions recorded a potential trend and a high ratio. The average R^2 ($p<0.001$) was 0.98. As a reference, a higher growth rate resulted in higher height (and vice versa) for the four planting densities of crotalaria.

These results are similar to those reported by Rojas-García *et al.* (2021), who evaluated the correlation between forage yield and meadow height in white clover with orchard grass

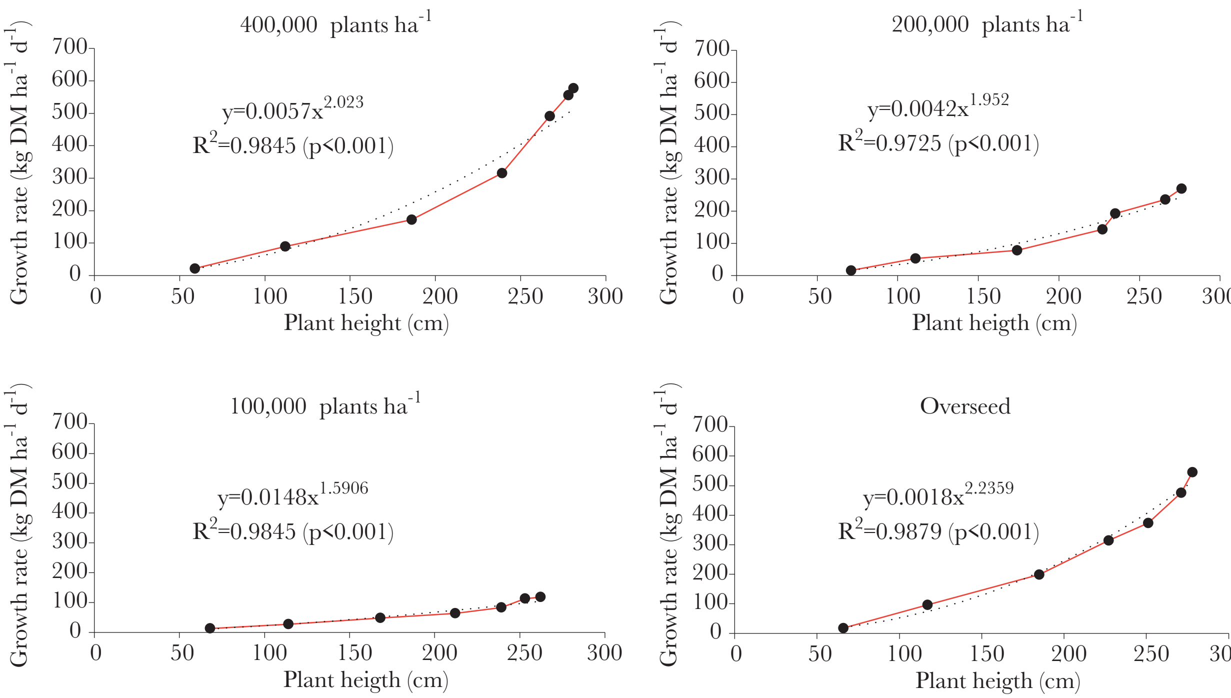

Figure 2. Regression coefficient between growth rate (kg ha^{-1} d^{-1}) and meadow height (cm) of crotalaria, at different planting densities and cutting ages.

and ryegrass. They recorded a high ratio (R^2: 0.805) ($p<0.001$), resulting in a reliable reference to determine the cutting age or grazing guidelines. Several researchers evaluated pulses and grasses and reinforced these references (Rojas *et al.*, 2016; Teixeira *et al.*, 2007).

CONCLUSION

The 400,000 plants ha^{-1} planting density reported an acceptable leaf:stem ratio and growth rate, at 45 days of development, recording a 186 cm height. Heigh measurement is a reliable method to determine growth rate, as a result of its high correlation.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Thermal sum in the determination of the phenological stages of Chihuahua and Cuauhtémoc oats in Güémez Tamaulipas

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ABSTRACT

Objective: To determine the thermal sum requirements of *Avena sativa* (Cuauhtémoc and Chihuahua varieties) as Growing Degree Days (GDD) per phenological stage and their effect on the accumulation of total forage and the accumulation of each morphological component, in Güémez, Tamaulipas.

Design/Methodology/Approach: The mechanical sowing (120 kg ha^{-1} seed dose) was carried out in 6×6 m plots with four replications of Cuauhtémoc and Chihuahua varieties. Subsequently, the plot was irrigated and fertilization works were carried out. The treatments consisted of two varieties and six phenological stages (Zadok's scale: Z2, Z3, Z4, Z7, Z8, Z9) in a randomized complete block design.

Results: It was found that 1,923.5 and 1,831.5 GDD were obtained from November to March, respectively; these results are a crop requirement for the fulfillment of the biological cycle of the Chihuahua and Cuauhtémoc varieties. Yields by morphological component depended on the observed phenological stage. The highest leaf yield ($P<0.05$) was obtained in stage Z3 (stem elongation): 2.7 t ha^{-1} in Cuauhtémoc (accumulation: 1,032 GDD) and 3.6 t ha^{-1} in Chihuahua (accumulation: 980 GDD).

Study Limitations/Implications: These results can only be applied to the evaluation area, as a result of the intervention of the environment on these physiological responses.

Findings/Conclusions: In Güémez, Tamaulipas, the Chihuahua oat variety requires 1,923.5 GDD to complete its biological cycle, while Cuauhtémoc requires 1,831.5 GDD. The difference between the thermal accumulation of the varieties and the phenological stages has an impact on the total forage yield and the yield of each morphological component.

Keywords: Forage, *Avena sativa*, Growing Degree Days, temperature, Güémez Tamaulipas.

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INTRODUCTION

In Mexico, livestock activities are carried out in 56% of the country's surface and 35.6 million heads of cattle are distributed in different milk- or meat-oriented farms

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or livestock production units (SIAP, 2020). The country ranks 8^{th} in world meat production; additionally, it is one of the 10 countries that concentrate 90 % of beef exports (COMECARNE, 2021).

The different farms or livestock production units partly or exclusively depend on the availability of forage in swards or rangelands to satisfy most of their nutritional requirements. This area offers the greatest possibilities of reducing costs with more productive and higher quality species (Zamora *et al.*, 2002). However, greater availability of forage is associated with the quantity and quality of the moisture available in the soil, as well as the temperature and light hours available to the plants. Therefore, a decrease in temperature and light hours during the winter season results in a decrease of forage yield in swards and rangelands (Sosa *et al.*, 2008). This is the result of the effects on the physiological activity of plants, such as the decrease in photosynthetic activity derived from a lower CO_2 absorption caused by the inactivation of the Rubisco enzyme. This enzyme is the protein responsible for the absorption of this gas, a requirement to increase the photosynthetic rate and to obtain the carbohydrates needed to increase plant development (Azcón-Bieto *et al.*, 2008; Yepes and Silveira, 2011).

In addition, daytime temperatures are related to the duration of the crop cycle, since the thermal sum during this period (unit of measure: Growing Degree Days or GDD) determines the period from sowing to ripening. This period can differ from one year to another, while the thermal sum remains relatively stable within the same region (Liu *et al.*, 2020).

The lower availability of forage during winter season can be counteracted by the use of winter crops, such as oats (*Avena sativa* L.), through an efficient use of humidity and temperature. Oats have a short cycle and are resistant to frost, if this phenomenon takes place before flowering and grain filling (CIREN, 1989; Sánchez *et al.*, 2014; Bilal *et al.*, 2017). This crop stands out among the livestock industry: 80% of the national production is used as animal feed, in the form of hay, green forage, silage or grain (Espitia *et al.*, 2012; Villazón *et al.*, 2017). Therefore, the objective of this research was to determine the thermal sum requirements of *Avena sativa* (Cuauhtémoc and Chihuahua varieties) as Growing Degree Days (GDD) per phenological stage and their effect on the accumulation of total forage and the accumulation of each morphological component, in Güémez, Tamaulipas.

MATERIALS AND METHODS

Location of the experimental site and climatic characteristics

The research was carried out from November 2021 to March 2022 at the "Posta Zootécnica Ingeniero Herminio García González", located in the municipality of Güémez, Tamaulipas, Mexico (23° 56' 28" N, 99° 06' 24" W), at 190 m.a.s.l. The local climate is semi-arid warm [BS1 (h') hw]; it mostly rains on summer, but up to 10% of the rains occur in winter (Vargas *et al.*, 2007)]. Temperature data were recorded from the meteorological station of the Centro Nacional de Innovación y Transferencia de Tecnología en Agricultura de Precisión, Facultad de Ingeniería y Ciencias, located in the Posta Zootécnica.

Treatments and experimental unit

The varieties used were Chihuahua and Cuauhtémoc. The sowing was carried out in 6×6 m plots with four replications per variety; the sowing density was 120 kg ha^{-1} of seed with 85% germination. The soil was prepared with a cross harrowing; subsequently, broadcast sowing was carried out and a heavy branch was used to cover the seed. Three sprinkler irrigations were applied at field capacity, at the time of sowing, tillering (beginning), and flowering. The 120-40-00 (NPK) fertilization dose was divided into two applications: half at the time of sowing and half at the tillering stage. A 125 g a.i. ha^{-1} dose of the Propiconazole® fungicide was used to control incidences of rust (*Puccinia* spp.).

The treatments consisted of two varieties of oats and six phenological stages, according to the Zadok's Scale: Z2 (tillering), Z3 (stem elongation), Z4 (booting), Z7 (milk development), Z8 (dought development), and Z9 (ripening). The samplings were carried out when more than 50% of the plants presented the stage described above. To determine the experimental units, the plots were divided into six 6 m^2 areas, one per each stage. A 1 m^2 area was delimited within each of those areas.

Variables

Plant height and light interception: Before each sampling, 10 random measurements were made in each experimental unit with a 100 cm graduated ruler (precision: 1 mm), from the base of the plant to the top, without stretching the leaves or panicles. The rule was then laid on the ground and the length shaded by the leaves was recorded as a percentage (100 cm=100%). Such measurement required that the sun rays hit plants in a straight vertical direction.

Forage accumulation and morphological composition: In each sampling, each experimental unit was harvested 5.0 cm above the ground and the samples were immediately weighed on a CQT 2601 analytical balance (ADAM®, USA). Later, at the laboratory, the harvested forage was placed in an OMS60 forced air stove at 60 °C (Thermo Scientific®, USA) until a constant weight was achieved and the dry matter yield was obtained.

The morphological composition was evaluated with a subsample (20% of the harvested forage), which was separated into leaf (leaf blade+pod), stem, panicle, and senescent material. The dry weight data was used to determine the contribution (t ha^{-1}) of the leaf (DMl), stem (DMs), panicle (DMp), and senescent matter (DMsc) components to the yield of total dry matter (TDM).

Growing Degree Days: The days after sowing were recorded and, subsequently, the GDDs for each phenological stages were calculated. However, each crop has minimum and maximum threshold temperatures within which the crop continues to develop; in the case of oat, the minimum and maximum temperatures are 5 and 30 °C, respectively (Servin *et al.*, 2018). The GDDs were calculated as the difference between the average daily temperature and the minimum threshold temperature, the base temperature required by the crop (Liu *et al.*, 2020).

$$GDD = \sum_{i=1}^{n} \left(\frac{Tmax + Tmin}{2} - TUMin \right)$$

Where *Tmax* and *Tmin* are the maximum and minimum daily temperatures and *TUMin* is the crop's minimum threshold temperature.

Statistical analysis. An analysis of variance was performed with the GLM procedure (SAS, 2003), in a randomized complete block design. The Tukey test was applied for the comparison of means (P=0.05).

RESULTS AND DISCUSSION

Figure 1 shows that the average daily temperatures during the evaluation period ranged from 8 to 31 °C, with variations of the coldest and hottest days in each month. Additionally, it shows the minimum and maximum threshold temperatures of the oat crop (*i.e.*, the temperatures at which the crop develops progressively). For this evaluation, the average daily temperatures were within the range of the threshold temperatures for the oat crop.

The varieties had only numerical differences in terms of the number of days between phenological stages with respect to the sowing date. Likewise, the different DDS for each stage propitiate different GDD between varieties (Table 1). Therefore, the thermal sum between varieties during the crop cycle was different from one variety to another. The Chihuahua variety had 1,803 °C GDD: 92 °C hotter than the Cuauhtémoc variety (which appeared 5 days earlier than the Chihuahua variety, in terms of the length of the crop cycle). Regarding the development cycle of the oat crop (considered a winter crop) in Güémez, Tamaulipas, oats are considered a forage option from November to March, when the temperature mostly ranges from <30 °C to >5 °C. The remainder of the year, the yield and quality of the crop are compromised by the increase in rates with physiological and biochemical variables (Liu *et al.*, 2020). In addition to compromising the CO_2 absorption due to the inefficiency of the Rubisco enzyme and the photosynthetic rate, it is negatively affected as a response variable (Azcón-Bieto *et al.*, 2008).

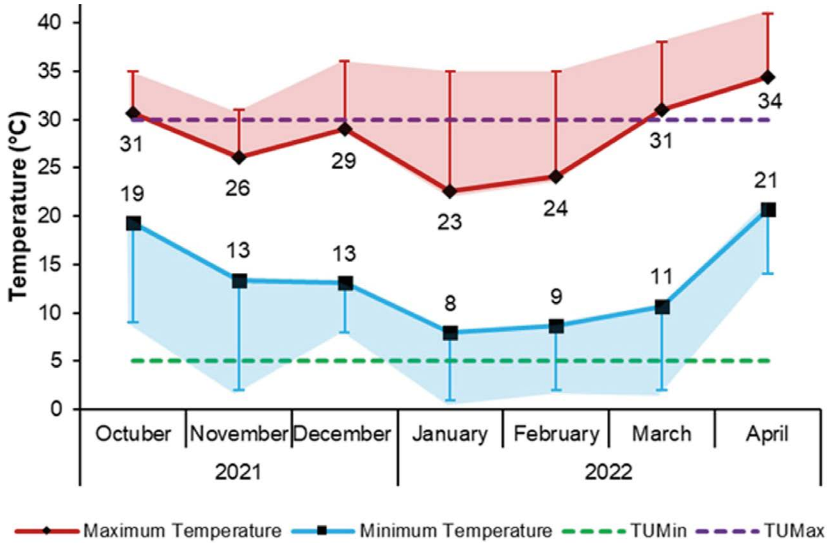

Figure 1. Monthly maximum and minimum temperatures during the evaluation period and maximum (TUMax) and minimum (TUMin) threshold temperatures of *Avena sativa* in Güémez, Tamaulipas, Mexico.

Regarding the plant height variable, differences were found between varieties in stages Z4, Z7, Z8, and Z9; the Chihuahua variety recorded the highest height (P<0.05) with 80, 105, 118, and 106 cm, respectively. Meanwhile, there was only a statistical difference (P<0.05) in the light interception percentage, between variables in stage Z7 (milk development). However, the highest percentages of interception per variety occurred in stage Z3 (Table 1).

The dry matter yield for the Chihuahua and Cuauhtémoc varieties showed differences in total dry matter and the dry matter of each component (P<0.05). Regarding the total dry matter, the Chihuahua variety presented a higher yield with 4.6, 5.4, 5.8, and 4.1 t ha^{-1}, except in stages Z2 and Z7 (Figure 2); this is a response to the longer biological cycle time, which has been reported to obtain a higher dry matter yield in late cycle species (Espitia *et al.*, 2012).

Table 1. Growing Degree Days (GDD), plant height, and light interception in the phenological stages of Cuauhtémoc and Chihuahua oats, in Güémez, Tamaulipas, Mexico.

PS	DAS		DDD (°C)		Height (cm)		Interception (%)	
	Cu	Ch	Cu	Ch	Cu	Ch	Cu	Ch
Z2	34	39	542	617	31 a D	35 a C	67 a DE	67 a ABC
Z3	65	72	439	415	65 a BC	69 a B	93 a A	96 a A
Z4	77	86	94	125	70 b BC	80 a B	92 a AB	96 a A
Z7	105	110	319	285	88 b C	105 a A	78 b BC	90 a AB
Z8	119	124	185	205	99 b A	118 a A	75 a DC	78 a BC
Z9	135	140	254	277	93 b AB	106 a A	60 a E	64 a C

PS=Phenological stages; DAS=Days after sowing; DDD=Degree days of development.
Different letters in the same column (ABC) and same row per component (abc) indicate statistical difference (Tukey, α=0.05). PS (EF): Phenological stage, DAS (DDS): Days after sowing, Z2: tillering, Z3: stem elongation, Z4: booting, Z7: milk development, Z8: dough development, Z9: ripening.

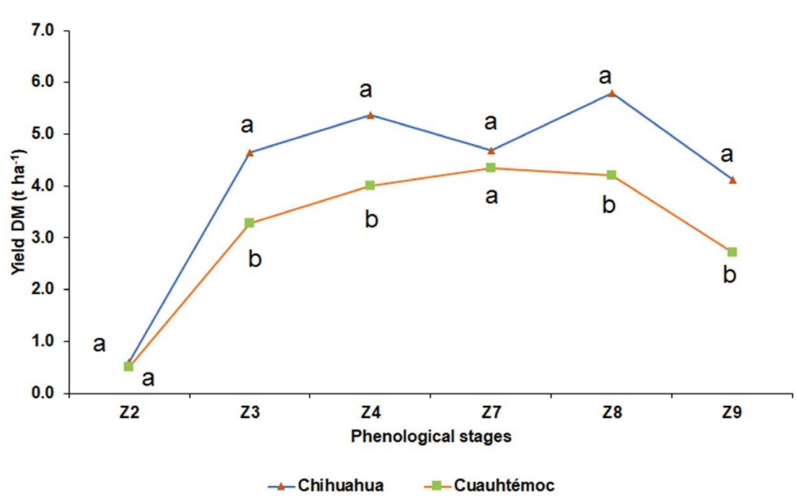

Figure 2. Total dry matter yield of Chihuahua and Cuauhtémoc oats at different phenological stages. Different letters present a statistical difference between cultivars for each phenological stage (Tukey, α=0.05). Z2: tillering, Z3: stem elongation, Z4: booting, Z7: milk development, Z8: dough development, Z9: ripening.

Table 2. Morphological composition (t ha^{-1} of dry matter) of Cuauhtémoc and Chihuahua oats at different phenological stages.

PS	Leaf		Stem		Panicle		SM	
	Cu	Ch	Cu	Ch	Cu	Ch	Cu	Ch
Z2	0.5 a C	0.6 a E	0.0 a B	0.0 a B	0.0 a C	0.0 a D	0.0 a D	0.0 a E
Z3	2.7 a A	3.6 a A	0.5 a AB	0.8 a AB	0.0 a C	0.0 a D	0.0 b D	0.3 a DE
Z4	1.7 b B	2.5 a B	1.0 a A	1.3 a A	0.7 a BC	0.9 a C	0.6 a BC	0.7 a C
Z7	1.5 a B	1.7 a C	1.0 a AB	1.2 a A	1.1 a BC	1.2 a BC	0.7 a CD	0.6 a CD
Z8	0.6 b C	1.1 a D	1.0 b A	1.5 a A	1.8 a A	1.9 a A	0.8 b BC	1.3 a B
Z9	0.0 a D	0.0 a F	0.0 a B	0.0 a B	1.1 b AB	1.5 a AB	1.6 b A	2.6 a A

PS=Phenological stages; SM=Senescent Material.
Different letters in the same column (ABC) and same row per component (abc) indicate statistical difference (Tukey, α=0.05). PS (EF): Phenological stage, Msc: senescent matter, Z2: tillering, Z3: stem elongation, Z4: booting, Z7: milk development, Z8: dough development, Z9: ripening.

The highest leaf yields were obtained in stage Z3 for both varieties ($P<0.05$); however, there were no statistical differences between them ($P>0.05$) with 2.7 and 3.6 t ha^{-1} yields for Cuauhtémoc and Chihuahua, respectively. Likewise, higher leaf yields have been related to forage quality; therefore, when higher leaf yields are recorded, higher protein contents are also present, as established by Mendoza-Pedroza *et al.* (2021) for the Chihuahua variety, where the highest leaf yield (5,288 kg) provides 1,100 kg ha^{-1} of protein at 75 DDS. In this research, the highest yield was recorded at 65 and 72 DDS in the Cuauhtémoc and Chihuahua varieties. The higher leaf content in the forage yield favors its digestibility, as a result of the increased efficiency of the ruminal microbiota (Carmona, 2007).

The contribution of each component to the dry matter yield is determined by its phenological stage. Both varieties have a 100% leaf content in the tillering stage, which descends in later stage. Simultaneously to that descent, the dry matter of stem, panicle, and senescent material increases its contribution to the yield, until a higher percentage of senescent matter and panicle is obtained (Figure 3). This behavior is expected from most cereals during their development: the total yield has shown a growth curve, where the highest dry matter yield occurs in the last stages of grain filling; afterwards, the amount of dry matter decreases as a consequence of the increased contribution of senescent matter (Wilson *et al.*, 2020). Likewise, these changes in the morphological proportion determine the quality of the forage, because a higher percentage of leaf results in maximum protein values; otherwise, a higher percentage of senescent matter is recorded during ripening (Ramírez-Ordóñes *et al.*, 2013), increasing the percentage of lignin, a component that hinders its decomposition by means of the ruminal microbiota (Carmona, 2007).

Finally, Sánchez *et al.* (2014) indicate that, between the milky and doughy grain stages, the proportion of the leaf in the yield is approximately 18%. This value is similar to that obtained in this research for both varieties in the Z8 stage. Therefore, the component-ratio behavior is relatively stable between development stages.

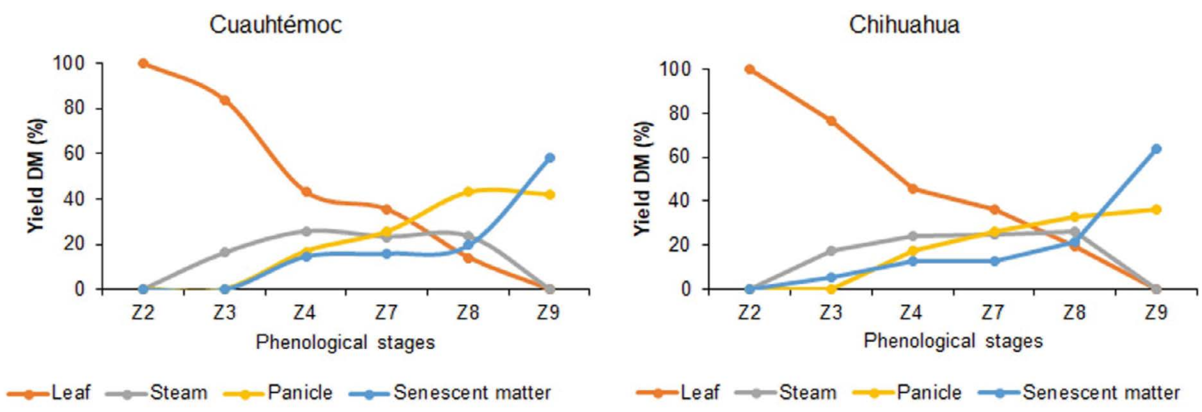

Figure 3. Contribution of each morphological component to the dry matter yield of *Avena sativa* Cuauhtémoc and Chihuahua varieties in different phenological stages. Z2: tillering, Z3: stem elongation, Z4: booting, Z7: milk development, Z8: dough development, Z9: ripening.

CONCLUSIONS

In Güémez, Tamaulipas, the Chihuahua oat variety requires 1,923.5 GDD to complete its biological cycle, while Cuauhtémoc requires 1,831.5 GDD. The difference between the thermal accumulation of the varieties and the phenological stages has an impact on the total forage yield and the yield of each morphological component.

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AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Economic evaluation and productive performance of lambs finished with concentrate and corn stover

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ABSTRACT

Objective: To evaluate the effect of two concentrates and corn stover in the productive performance and economic impact of finishing hair lambs in pens.

Design/Methodology/Approach: Twenty hair lambs (Dorper×Katahdin) with an initial live weight of 33.3±2.9 kg were grouped into ten blocks (two lambs per block) and then were randomly assigned to two treatments: T1) commercial concentrate + corn stover and T2) experimental concentrate + corn stover (with an 80:20 ratio). The total weight gain (TWG), average daily gain (ADG), dry matter intake (DMI), feed conversion (FC), feeding costs, gross value of TWG, gross profit margin and economics of feed efficiency (EE) were evaluated. An analysis of variance was performed under a completely randomized block design. The means were compared with the Tukey test (α=0.05).

Results: There were no differences in TWG, ADG, FC, and DMI ($P>0.05$). T1 has higher costs (US$41.91) per ton of feed. Production costs of diets and feeding were lower for T2, which also showed the best economic feed efficiency (EE=26.6%).

Study Limitations/Implications: Their availability throughout the year is the advantage of using agro-industrial and agricultural by-products (*e.g.*, corn stover) in total mixed diets to finish ovines.

Findings/Conclusions: Lambs finishing is profitable when the producer formulates and prepares his own diet, reducing feeding production costs without affecting productive variables.

Keywords: Hair sheep, feedlot finishing, economic impact.

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INTRODUCTION

In Mexico, the largest sheep meat production is obtained from extensive and semi-extensive systems, where average daily gain is low (120-150 g d^{-1}) and the lamb mortality rate is high (30-40%) (González-Garduño *et al.*, 2013). Consequently, lambs finished under this system take longer to get to market, although the production cost is relatively low. In recent years, taking into account the competitive price and the high demand for sheep meat, producers —mainly from central and northern Mexico— have chosen to finish lambs in intensive systems: the grain-based total mixed diets provided to lambs allows them to fulfill their genetic potential for growth (Mendoza-Martínez *et al.*, 2007). These systems have then made it possible to reduce fattening periods by improving dry matter intake, average daily gain, and feed efficiency. However, production costs undergo a considerable increase, since they require facilities and total mixed diets (Macedo and Castellanos, 2004).

The feed used in intensive systems to fatten sheep includes total mixed diets or commercial concentrates combined with 20-30% forage. Alfalfa is generally used as forage, due to its year-long availability, particularly in central-northern Mexico (Muñoz-Osorio *et al.*, 2016; Rodríguez-Hernández *et al.*, 2019). The cost of commercial concentrates has become increasingly high and expensive for producers; however, approximately 50% of sheep farmers still depends on them (Muñoz-Osorio *et al.*, 2015). One strategy to reduce feeding costs is having producers formulate and prepare their own diets, based on regionally available supplies, combined with corn stover as a source of fiber. Although Guerra-Medina *et al.* (2015), Sun *et al.* (2018) and others have already researched the nutritional feasibility of using concentrates with corn stover in the diet of fattening sheep, there is scarce information available about the economic impact of implementing this nutritional strategy for sheep fattening. Therefore, it is necessary to carry out an economic analysis of sheep fattening with total mixed diets that include corn stover. Thus, the objective of this study was to evaluate the use of two concentrates with corn stover on the productive performance and economic impact of finishing hair lambs in pens.

MATERIALS AND METHODS

Location and description of the study area. The research was conducted from July to August 2019, at the “Mezquitalito” ranch, located in the municipality of Autlán de Navarro, Jalisco (212° SW latitude, 19° 45’ 55” N and 104° 19’ 55” W, at 890 m.a.s.l.). The climate of the region is predominantly semi-dry, with most of the rains concentrated in summer and an average annual temperature of 23.5 °C (García, 2004).

Animals and handling. Twenty F1 hair lambs (Dorper×Katahdin, 33.3±2.9 kg LW) were used. In average they were 3.5-months old. Prior to the start of the experiment, the lambs received an antiparasitic prophylactic treatment (200 mcg Ivermectin/kg LW, subcutaneous route; Ivermectin, Sanfer Laboratory, Mexico City, Mexico) and vitamins (1 ml of A-D-E, intramuscular route; Vigantol, Bayer, Mexico City). The animals were housed in individual pens that included a feeder and a drinker (*ad libitum* water). The lambs adapted to the experimental diets 10 days before the performance test. The evaluation was made in the following 30 days.

Treatments. At the beginning of the test, the lambs were grouped into ten blocks, each with two lambs with similar initial LW (blocking factor). Subsequently, two treatments were randomly assigned to each block: T1) commercial concentrate + corn stover (n=10) and T2) experimental concentrate + corn stover (n=10) at an 80:20 ratio. The experimental diet was formulated (2.8 Mcal of ME kg^{-1} DM and 15% CP) in order to obtain a 300 g d^{-1} gain (NRC, 2007). Table 1 shows the ingredients and chemical composition of the experimental diets.

Evaluated variables. Productive performance: Initial (day 1) and final (day 31) weight (kg) was recorded before the morning feeding. Additionally, the weight of food offered and rejected the previous day was recorded every day, in order to calculate the dry matter intake (DMI). The amount of feed offered on the first day of the test was 1.5 kg/lamb; subsequently the amount was adjusted daily, taking into consideration an approximate rejection rate of 10%. The availability of clean water and the health status of the animals were checked

Table 1. Ingredients and chemical composition of the experimental diets.

Ingredients, kg t^{-1}	T1	T2
Maxiengorda[1]	800	-
Ground corn	-	570
Soybean meal	-	100
Wheat bran	-	80
Vegetable oil	-	20
Minerals	-	20
Urea	-	10
Corn stover	200	200
Chemical composition (%)		
Dry matter	90.0	90.7
Crude protein	13.0	15.0
Fat	4.9	5.3
Fiber	14.4	9.9
Ashes	4.0	3.1
Acid detergent fiber	13.0	12.6
Neutral detergent fiber	25.4	24.3
Total digestible nutrient, TDN	76.5	77.1
Energy from de diet (Mcal kg^{-1})		
Digestible energy, DE	3.4	3.4
Metabolizable energy, ME	2.8	2.8
Net energy for maintenance, NEm	1.8	1.8
Net energy for growth, NEg	1.2	1.2

[1]Sorghum, yellow corn, soybean meal, canola meal, corn gluten, molasses, flavoring, vitamins (A, D, E), minerals (calcium, cobalt, phosphorus, iron, manganese, potassium, and zinc), and antioxidant (B.H.T.); $TDN=91.0246-0.571588*NDF$ (Cappelle *et al.*, 2001); $DE=TDN\times0.044$ (NRC, 1985); $ME=0.82\times DE$ (NRC, 1985); $NEm=1.37\times ME-0.14ME^2+0.01ME^3-1.12$ (NRC, 1985); $NEg=1.42\times ME-0.17ME^2+0.012ME^3-1.65$ (NRC, 1985).

daily. The total weight gain (TWG, kg) was calculated based on the difference between the final weight and the initial weight; meanwhile, average daily gain (ADG, g d^{-1}) was obtained by dividing TWG between the number of days of the test. The consumption of DM (DMI, kg d^{-1}) was calculated based on the difference between the food offered and rejected each day and finally it was multiplied by the DM% of the food. Feed conversion (FC) was calculated as the DMI:ADG ratio.

Economic impact: All the supply costs that were used to formulate the diet and the live lamb price per kilogram in the market were gathered. The data were reported in US dollars (US$), considering an exchange rate of 1 US$=$20.2321 Mexican pesos. The experimental diets' production costs (US$ t^{-1} MS) were calculated multiplying the price (US$/ t^{-1} MS) of the ingredients by the amount (t^{-1}) used in each diet, adding the total at the end. Subsequently, some of the economic parameters described below were estimated using the methodology proposed by Mahrous *et al.* (2021). The feed cost was estimated multiplying the diet cost times the total DMI in the period, while the gross value per TWG was determined multiplying the total gain in the period times the kg price of live lamb (US$2.1). The gross profit margin was calculated as the difference of the TWG gross value and the feed cost. Finally, the economics of feed efficiency (EE) was obtained dividing the percentage of the gross profit margin by the feed cost.

Statistical analysis. An analysis of variance was performed with a randomized complete block design using the PROC GLM of the SAS statistical package (SAS, 2011). The means were compared using a Tukey test ($P \leq 0.05$).

RESULTS AND DISCUSSION

Table 2 shows the productive performance results: there were no differences between treatments ($P>0.05$). Sheep ADG was 250 g d^{-1}, similar to the results reported in hair sheep (Muñoz-Osorio *et al.*, 2016), but lower (300 g d^{-1}) than the expectations (NRC 2007). On the one hand, Guerra-Medina *et al.* (2015) reported a 301 g d^{-1} ADG in Katahdin×Dorper lambs that consumed concentrates with 15% of corn stover; however, they consumed a similar amount of energy and protein (1.2 Mcal kg^{-1} of ME and 13.3% of CP) than the diets in this study. On the other hand, Vicente-Pérez *et al.* (2020) reported a 343 g d^{-1} ADG in Katahdin lambs that consumed the same experimental concentrate

Table 2. Productive performance of lambs finished with concentrates and corn stover.

	T1	T2	S.E.M.	P
Number of lambs (n)	10	10	-	-
Initial weight, kg	34.39	34.21	0.31	0.57
Final weight, kg	42.17	41.37	0.83	0.36
Total weight gain, kg	7.78	7.16	0.76	0.43
Average daily gain, g d^{-1}	259	239	25.50	0.43
Dry matter intake, kg d^{-1}	1.47	1.31	0.09	0.11
Feed conversion, kg kg^{-1}	5.93	5.93	0.43	0.99

T1=Commercial concentrate - corn stover (80% - 20%); T2=Experimental concentrate - corn stover (80%-20%); S.E.M.=Standard error of the mean.

than T1, but with 20% pine sawdust as fiber source. Additionally, the feed conversions of both treatments were higher than in sheep exploited in southeastern Mexico. This is an important variable for the profitability of fattening. Munoz-Osorio *et al.* (2015) reported feed conversions of 5.0 (in raised pens) and 3.2 (in ground-level pens) in intensive feedlot systems. In both systems the lambs were fed commercial-brand or farm-made feeds combined with tropical grasses.

Table 3 shows the production costs of the experimental diets. The costs of T1 increased by US$41.91 per ton of feed. Therefore, T2 is a viable option to finish sheep in the same time, at a lower diet cost. The high cost of grains and cereals has put the subsistence of production systems at risk —a risk that intensifies when the producer depends on commercial brand foods. A study in Yucatan reported that 47% of the producers fatten their lambs with commercial feed, while the rest prepare their own diets and a few others buy their feed from informal suppliers (Muñoz-Osorio *et al.*, 2015). In this sense, this study reached economically important results: producers are encouraged to seek advice and prepare their own diets with locally available supplies.

Table 4 shows feed costs, gross value per weight gain, gross profit margin and economic feed efficiency per fattening lamb finished with concentrates and corn stover. The gross

Table 3. Production costs (US$ t^{-1} DM) of experimental diets for lambs finished with concentrates and corn stover.

Ingredients	Price, (US$ t^{-1} MS)	T1		T2	
		Quantity (t^{-1})	Cost (US$)	Quantity (t^{-1})	Cost (US$)
Corn stover	176.45	0.2	35.29	0.2	35.29
Maxiengorda	386.51	0.8	309.21	-	-
Concentrate	334.12	-	-	0.8	267.30
TOTAL	-	1.0	344.50	1.0	302.29

T1=Commercial concentrate-corn stover (80%-20%); T2=Experimental concentrate-corn stover (80%-20%).

Table 4. Gross profit margin and economic feed efficiency obtained in hair lambs finished with concentrates and corn stover.

Items	T1	T2	S.E.M.	P
Cost of feed, US$ kg^{-1} DM	0.34	0.30	-	-
Total DM intake, kg $animal^{-1}$	44.23	39.33	2.79	0.11
Total DM intake cost (US$ $animal^{-1}$)	15.24	11.90	0.90	<0.01
Market price, US$ kg^{-1} live body weight	2.1	2.1	-	-
Profit of total weight gain[1] (US$ $animal^{-1}$)	16.53	15.22	1.62	0.43
Gross profit margin[2] (US$ $animal^{-1}$)	1.30	3.32	1.09	0.09
Economics of feed efficiency[3] (%)	7.56	26.56	7.96	0.04

T1=Commercial concentrate-corn stover (80%- 20%); T2=Experimental concentrate-corn stover (80%-20%); [1]US$ of total weight gain/animal; [2]Difference between profit of total weight gain and total DM intake cost (Mahrous *et al.*, 2021); [3]Gross profit margin/Total DM intake cost × 100 (Mahrous *et al.*, 2021); S.E.M.=Standard error of mean.

value for live weight gain (P=0.43) and gross profit margin (P=0.09) were not affected by the concentrate source of the diet. However, feed cost (P<0.01) and economic feed efficiency (P≤0.04) improved with T2. Finishing lambs with a commercial concentrate and corn stover (T1) is US$3.34 more expensive than with diets based on concentrates available in the region and corn stover (T2). T1 obtained a higher income per kilogram of live weight gained than T2 (US$16.53 *vs.* US$15.18); however, as a consequence of its higher feed costs, the gross profit margin per finished lamb was US$2.02 lower. Finally, based on its economics of feed efficiency, T2 is more profitable (EE=26.6%) and consequently much better than T1 (EE=7.6%). Therefore, the evidence suggests that finishing hair lambs with the T2 diet proposed in this study is feasible.

Rebollar *et al.* (2015) mention that the purchase of animals and feed are headings that require the greatest investments among all production costs in a feedlot. Duarte and Olmedo (2013) mention that, when concentrates are used in diets, the fattening time shortens, total DM intake increases, and feed conversion improves. In addition, the Net Profit increases as the variable costs (*e.g.*, feed costs) are reduced without affecting production parameters. Meanwhile, Muñoz-Osorio *et al.* (2015) determined that most producers sell their lambs at live lamb prices (91.18%), because it represents the main source of economic income in their system; their results match the findings of this study, which took into consideration the price for the sale of live animals.

Herrera-Toscano and Carmenate-Figueredo (2018) suggest that selecting local resources to feed lambs reduces production costs, particularly of forages (*e.g.*, leguminous trees and agricultural by-products). Likewise, Gutierrez *et al.* (2014) indicate that using agricultural by-products (*e.g.*, sugarcane tips) in total mixed diets for lambs improves the production parameters and economic profitability of these production systems.

CONCLUSIONS

The use of concentrate made from locally available supplies and corn stover (80:20 ratio) for lamb finishing improves the profitability of the fattening, as a result of the lower production cost of the diet, without facing the negative effects on the weight gain of commercial concentrates.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Effect of sunflower oil (*Helianthus annuus*) on *in vitro* ruminal fermentation and emission of gases

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ABSTRACT

Objective: To evaluate the production of total gas, methane (CH_4), and *in vitro* fermentative variables in diets for sheep with 1 and 2% sunflower oil.

Design/Methodology/Approach: Serological pipettes with 0.5 g of treatment and 50 ml of culture medium were incubated at 39 °C for 24, 48, and 72 h. The production of total gas, CH_4, and degradation of dry matter (DEGDM), neutral detergent fiber (DEGNDF), acid detergent fiber (DEGADF), as well as the production of volatile fatty acids (VFA) and ammoniacal nitrogen ($N\text{-}NH_3$) were estimated. The experimental design was completely randomized.

Results: Total gas production increased ($p<0.05$) at 48 and 72 h and decreased ($p<0.05$) at 24 h as the oil increased. CH_4 production at 24 and 48 h did not present differences ($p>0.05$); a linear decrease ($p<0.05$) was quantified at 72 h. DEGDM increased ($p<0.05$) at 24 and 48 h and decreased ($p<0.05$) at 72 h. DEGNDF and DEGADF increased ($p<0.05$) at 48 h. Butyric acid content and $N\text{-}NH_3$ decreased ($p<0.05$) at 48 h.

Study Limitation/Implications: A >2% inclusion of sunflower oil in the diet can reduce the degradability of the food and the microbial protein.

Findings/Conclusions: Including up to 2% of sunflower oil in diets for lambs does not affect the fiber degradation and is an alternative to reduce the amount of methane emissions released into the environment.

Keywords: degradation, bacteria, methane, *in vitro*, lambs.

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INTRODUCTION

An increase in greenhouse gasses (GG) is a significant contributor to climate change (Patra, 2014; Hill *et al.*, 2016). Livestock farming contributes 18% of global greenhouse gas emissions, of which the enteric methane (CH_4) of ruminants makes up 37% of the total anthropogenic production (Steinfeld *et al.*, 2006; Key & Tallard, 2012; Kumar *et al.*, 2014). CH_4 is produced during rumen fermentation and represents a loss of 2 to 15% of

Image by Susanne Jutzeler, Schweiz, at Pixabay consumed energy (Kumar *et al.*, 2009; Eckard *et al.*, 2010). Climate change and energy loss during food fermentation in ruminants awakens an interest in the study of different biotechnological alternatives that minimize the production of enteric CH_4 (Eckard *et al.*, 2010; Patra, 2014), without affecting rumen metabolism.

A strategy to reduce CH_4 production in ruminants is the use of fats and oils in diet (Grainger and Beauchemin, 2011; Bodas *et al.*, 2012; Hristov *et al.*, 2013). Peanut, rapeseed, corn, and soy oils affect fatty metabolism in rumen and act as CH_4 suppressors (Wang *et al.*, 2016). Other researchers have used coconut oil to reduce CH_4 production in sheep (Machmuller *et al.*, 2000) and heifers (Jordan *et al.*, 2006). Therefore, this study aims to evaluate the *in vitro* production of total gas, methane, and fermentative variables in the diets of fattening lambs with two levels of sunflower oil.

MATERIALS AND METHODS

Location of study area

The study was carried out in the laboratory of the Nutrición Animal y Microbiología Ruminal y Genética Microbiana del Posgrado en Recursos Genéticos y Productividad – Ganadería, Campus Montecillo, Colegio de Postgraduados, Montecillo, Texcoco, State of México.

Treatments

Treatments consisted of three iso protein diets (Table 1), developed based on NRC (2007), in order to meet the nutritional requirements of finishing lambs. The ingredients of these diets (Table 1) were ground on a mill (Thomas Willey, USA) with a 1-mm mesh.

Table 1. Experimental diets for finishing lambs.

Ingredients	T1	T2	T3
Composition (g kg^{-1} MS)			
Alfalfa	120	120	120
Corn grain	550	550	550
Corn stubble	150	140	130
Soybean meal	100	100	100
Sunflower oil	0	10	20
Wheat bran	60	60	60
Mineral premix	20	20	20
Chemical composition			
Dry matter, %	90.85	91.41	92.66
Crude protein, %	15.47	15.23	15.15
Ether extract, %	3.74	5.28	7.74
Neutral detergent fiber, %	33.14	30.75	31.29
Acid detergent fiber, %	20.07	18.13	18.56
Ashes, %	6.38	6.71	6.81

T1: Complete diet without sunflower oil; T2: Complete diet with 1% sunflower oil; T3: Complete diet with 2% sunflower oil.

Chemical analysis

In treatments, the following elements were determined: dry matter (DM), crude protein (CP), ashes (A), ether extract (EE), according to AOAC (2005). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were quantified using an ANKOM 200/220 analyzer (USA), based on the method of Van Soest *et al.* (1991).

Culture medium, traps for the capture of biogas and biodigesters

They were prepared following the methods described by Sánchez-Santillán *et al.* (2015), Hernadez-Morales *et al.* (2018), and Torres-Salado *et al.* (2018).

Total gas and methane production

Total gas production was quantified by displacing saturated saline solution at 24, 48, and 72 h of incubation, according to the description of Torres-Salado *et al.* (2018). CH_4 production was measured at 24, 48, and 72 h in accordance with the technique described by Prada-Matiz and Cortés-Castillo (2011).

Variables of rumen fermentation

In vitro degradation of dry matter (DEGDM) was calculated according to Hernández-Morales *et al.* (2018). ANKOM® bags were heat sealed to determine NDF, using the ANKOM® Technology Method in accordance with Van Soest *et al.* (1991).

Volatile fatty acids (VFA) were analyzed in accordance with the description of Sánchez-Santillán and Cobos-Peralta (2016).

For the concentration of total bacteria, a direct counting technique was employed in a Petroff-Hausser® chamber (Hausser #39000, Electron Microscopy Sciences, USA) and an Olympus® EX51 microscope (Olympus, USA), at 1000X magnification, in accordance with the description of Sánchez-Santillán and Cobos-Peralta (2016).

Statistical analysis

The experimental design was completely randomized (eight independent repetitions per treatment). Data was analyzed using the GLM procedure of SAS® (SAS, 2011). Comparison of means was done with Tukey's test (α=0.05). Orthogonal polynomials were used in order to test the linear and quadratic effect resulting from the gradual increase of sunflower oil.

RESULTS AND DISCUSSION

The production of partial gas and total gas increased ($p<0.05$) lineally at 48 and 72 h of incubation, as sunflower oil content increased in treatments (Table 2). This increase is assumed to be the consequence of a greater digestibility of diets, since Menke *et al.* (1979) mention a high correlation between *in vitro* gas production and apparent degradability of dry matter. At 24 h of incubation, partial gas production decreased ($p<0.05$) lineally, perhaps as a result of cellulolitic bacteria, protozoa, and methanogenic archaea toxicity (Dohme *et al.*, 1999).

Partial production at 24 and 48 h and accumulated production displayed no difference among treatments ($p>0.05$). However, at 72 h of incubation, CH_4 production decreased ($p<0.05$) lineally. Long chain fatty acids in oils are toxic to methanogenic archaea in rumen, which brings about a decrease in CH_4 production (Patra & Yu, 2013).

In vitro degradation of dry matter (DEGDM) increased ($p<0.05$) lineally at 24 and 48 h of incubation as sunflower oil content in the diet increased (Table 3). However, DEGDM decreased ($p<0.05$) lineally at 72 h of incubation. The increase in DEGDM up to 48 h of fermentation is attributed to the fermentation of non-structural carbohydrates, while at 72 h, the linear decrease could be caused by the slow fermentation of carbohydrates in diet fiber (Gatachew *et al.*, 1998; Toral *et al.*, 2011).

In vitro degradation of neutral detergent fiber (DEGNDF) and acid detergent fiber (DEGADF) show no difference between treatments ($p>0.05$) at 24 and 72 h of incubation. However, DEGNDF and DEGADF increased ($p<0.05$) lineally at 48 h of incubation. This effect could be attributed to a low percentage of sunflower oil in diet, as it has been reported that >6% DM contents in diet have decreased fiber digestibility in rumen — through the direct action of fatty acids on the cellular membrane of bacteria or through indirect effects in the availability of Ca^{2+} and Mg^{2+} (Palmquist, 1991).

Table 3 shows that, on the one hand, pH increased ($p<0.05$) lineally as the level of sunflower oil rose at 24 and 48 h. This could be the result of grain content in diets, given that they have fest fermenting carbohydrates. On the other hand, pH decreased ($p<0.05$) at 72 h of incubation, which may be related to the accumulation of organic acids in the *in vitro* system, where there is no flow of organic acids towards other systems (Dehority *et al.*, 2003). Total bacteria count decreased ($p<0.05$) lineally at 24 and 72 h of incubation between treatments. Ley de Coss *et al.* (2013) reported a total bacteria concentration of 109 ml^{-1}, while Dehority (2003) published values of 1010 to 1012 bacteria mL^{-1} in rumen. These values are higher than those obtained in this study, since high fat content

Table 2. Production of partial gas and total gas and methane totals in diets prepared with sunflower oil.

Incubation (h)	Treatment			SEM	Value of p	
	T1	T2	T3		Linear	Quadratic
Total gas production (ml g^{-1} MS)						
24	102.85^{a}	83.94^{b}	91.70^{ab}	2.5640	0.0429	0.0063
48	37.93^{b}	47.66^{a}	49.14^{a}	1.6980	0.0044	0.1841
72	9.43^{b}	12.36^{ab}	15.11^{a}	0.6640	0.0001	0.9337
Total	143.19^{b}	151.60^{ab}	162.16^{a}	3.0350	0.0086	0.8560
Methane gas production (ml g^{-1} MS)						
24	38.09	36.54	40.06	1.5470	0.6230	0.4570
48	13.25	13.48	13.18	0.3590	0.9442	0.7359
72	6.96^{a}	4.72^{b}	4.55^{b}	0.3120	0.0002	0.0332
Total	61.79	56.80	58.88	1.554	0.4512	0.3011

^{a,b} Mean values with different letters in a row indicate differences ($p \leq 0.05$).
T1: Complete diet without sunflower oil; T2: Complete diet with 1% sunflower oil; T3: Complete diet with 2% sunflower oil; SEM: Standard error of the mean.

Table 3. Fermentative characteristics of diets prepared with sunflower oil.

Incubation (h)	Treatment			SEM	Value of p	
	T1	T2	T3		Linear	Quadratic
In vitro degradability of dry matter (%)						
24	41.80[b]	41.49[b]	47.85[a]	1.0030	0.0050	0.0410
48	48.08[b]	61.38[a]	68.48[a]	2.7750	0.0002	0.3414
72	72.75[a]	67.48[b]	68.98[ab]	0.8650	0.0430	0.0370
In vitro degradability of neutral detergent fiber (%)						
24	43.51	47.90	45.06	1.087	0.5564	0.1456
48	45.85[b]	52.29[a]	54.85[a]	1.252	0.0009	0.2719
72	57.87	55.66	56.98	0.499	0.4553	0.1057
In vitro degradability of acid detergent fiber (%)						
24	20.30	14.44	22.06	1.367	0.5314	0.0224
48	25.14[b]	37.19[a]	44.81[a]	2.578	0.0001	0.3976
72	49.79[a]	46.28[b]	50.39[a]	0.674	0.6396	0.004
pH						
24	5.48[b]	5.52[ab]	5.58[a]	0.015	0.0041	0.6903
48	5.54	5.52	5.58	0.013	0.2149	0.1564
72	5.64	5.60	5.60	0.009	0.0687	0.2707
Total bacteria (10^{-9} cells mL^{-1})						
24	6.16[a]	5.68[a]	4.56[b]	0.233	0.0016	0.3676
48	5.68	6.20	6.88	0.492	0.3435	0.9451
72	6.00	5.30	5.12	0.170	0.0243	0.4274

[a,b] Mean values with different letters in a row indicate differences ($p \leq 0.05$).
T1: Complete diet without sunflower oil; T2: Complete diet with 1% sunflower oil; T3: Complete diet with 2% sunflower oil; SEM: Standard error of the mean.

in diet reduces the number of protozoa as well as bacterial concentration in rumen (Yang *et al.*, 2009), especially fibrolytic bacteria, which are more sensitive to fat content in diet (Patra and Yu, 2013). Table 4 shows that the acetic and propionic acid ratio did not display any difference between treatments at 24 and 72 h of incubation ($p>0.05$). Butyric acid content decreased ($p<0.05$) lineally at 48 h of incubation. The inclusion of oil in diets did not result in the decrease of the total concentration of fatty acids during the measured incubation times ($p>0.05$). On the contrary, Toral *et al.* (2011) reported that the total fatty acid content diminished with diets including 20 g kg^{-1} of sunflower oil and 10 g kg^{-1} of fish oil in adult sheep. The H_2 generated, along with the presence of methanogenic archaea, increase methane production by using H_2 and CO_2 as a source of energy (Kim *et al.*, 2012). Meanwhile, Jordan *et al.* (2006) have observed that using coconut oil decreases the total VFA concentration, because it reduces the digestibility of dry matter and NDF and ADF components.

Table 4. Volatile fatty acid (mM L^{-1}) and ammoniacal nitrogen (mg dL^{-1}) concentration in the *in vitro* fermentation of sunflower oil-based diets for sheep.

Incubation (h)	Treatment			SEM	Value of p	
	T1	T2	T3		Linear	Quadratic
Acetic (mM L^{-1})						
24	36.76	35.38	37.23	0.451	0.663	0.102
48	41.78	44.44	40.81	0.687	0.515	0.024
72	42.68	42.12	41.64	0.404	0.331	0.965
Propionic (mM L^{-1})						
24	21.70	20.84	21.61	0.280	0.891	0.194
48	24.02	24.90	23.31	0.420	0.494	0.186
72	28.64	29.22	28.45	0.196	0.685	0.118
Butyric (mM L^{-1})						
24	15.38	15.33	14.63	0.182	0.098	0.379
48	16.54^{a}	17.08^{a}	15.51^{b}	0.211	0.008	0.002
72	15.92	16.12	16.07	0.122	0.631	0.648
A:P Ratio						
24	1.69	1.70	1.73	0.025	0.608	0.930
48	1.79	1.79	1.75	0.020	0.543	0.659
72	1.49	1.44	1.46	0.015	0.495	0.295
Ammoniacal nitrogen (mg dL^{-1})						
24	23.62	24.73	24.49	0.485	0.496	0.541
48	31.44^{a}	27.41^{b}	27.22^{b}	0.666	0.002	0.066
72	29.49^{b}	35.27^{a}	32.40^{ab}	1.030	0.196	0.031

^{a,b} Mean values with different letters in a row indicate differences ($p \leq 0.05$).
T1: Complete diet without sunflower oil; T2: Complete diet with 1% sunflower oil; T3: Complete diet with 2% sunflower oil; A:P acetic-propionic; SEM: Standard error of the mean.

CONCLUSIONS

The inclusion of sunflower oil in sheep diet has had a positive influence on the *in vitro* degradability of dry matter, without affecting fiber degradability. Therefore, it could be used as an alternative to reduce methane emissions in ruminants.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Lignocellulosic biomass production in six cultivars of *Cenchrus purpureus* (Schumach.) Morrone in the tropics

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ABSTRACT

Objective: To determine the cellulose, hemicellulose, and lignin production in six *Cenchrus purpureus* cultivars, with and without fertilization, harvested every 21 days during 168 days.

Design/methodology/approach: The evaluated cultivars were CT-115, elephant grass, king grass, Maralfalfa, Roxo, and Taiwan, with and without fertilization. The experimental design was a randomized complete block with a split-plot arrangement and four replications. The fertilization dose was 141-43-20 of NPK. Forage was harvested every 21 days during six months in the rainy season. We tested the samples for dry matter (DM), ash, NDF, ADF, hemicellulose, cellulose, and lignin. We performed a nonlinear regression analysis. We determined biomass production with the estimated values and the percentage of each component. The goodness-of-fit indicators were: R^2, the coefficient of determination (R), and the model selection criterion (MSC). The parameters and goodness-of-fit coefficients were analyzed in ANOVA with the SAS software using the GLM procedure. Means were compared with Tukey's test.

Results: The Maralfalfa cultivar presented a higher cellulose production (3,786 kg ha^{-1}), similar ($p>0.05$) to the elephant grass and Taiwan cultivars, which presented values of 3,451 kg ha^{-1} and 3,329 kg ha^{-1}, respectively, and different ($p<0.05$) from CT-115 and king grass, which produced more than 1 t ha^{-1} of cellulose.

Limitations/implications: Cellulose production increased by the effect of fertilization.

Findings/conclusions: The fertilized Maralfalfa, elephant grass, and Taiwan cultivars produced more than 4,000 kg ha^{-1} of cellulose, 3,000 kg ha^{-1} of hemicellulose, and 1,000 kg ha^{-1} of lignin.

Keywords: Biomass, Cellulose, Paper, Hemicellulose, Lignin.

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INTRODUCTION

In the last years, there has been an increased interest in lignocellulosic materials (an array of materials of forestry, agricultural or urban origin). This interest has drawn attention to the use of residual materials from forests, crops, and the timber industry (FAO, 2001). Cellulose, lignin, and hemicellulose can be obtained from said materials in an approximate ratio of 4:3:3, with significant variations depending on the species (Mohammad, 2008). Traditionally, wood has been the preferred material in paper pulp manufacture and, therefore, the primary source of lignocellulosic material. The increased demand for this type of raw material, together with economic and environmental problems, requires the research of alternative sources of lignocellulosic materials other than wood. The *Cenchrus purpureus* (Schumach.) Morrone species, called Napier, Uganda, or elephant grass, is native to Africa. It has been introduced in tropical and subtropical areas for livestock feeding purposes. The paper industry is looking for lignocellulosic resources other than trees (because of their scarcity) or sugar cane (because of a shift toward biofuels). It has opted for the tropical species *C. purpureus*, which has the world's highest potential for lignocellulosic biomass production, due to its erect growth habit, vigorous strains, and large height (Reyes-Castro *et al.*, 2018). Elephant grass and king grass have cellulose and hemicellulose contents of 22.6% and 20.9%, and 23.6% and 21.9%, respectively (Cardona *et al.*, 2013). For the CT-115 cultivar, the reported percentages are 32.9% and 28.8% (Valenciaga *et al.*, 2009). For Maralfafa, Mateus *et al.* report 33.8% and 22.5% (2012). The contents of cellulose and lignin vary greatly, depending on harvest age, soil fertility, variety of grass, and its management, among other factors. Percentages vary between 34% and 39% for cellulose and between 3% and 8% for lignin, considering grasses harvested in weeks 4 and 24 of regrowth for different cultivars of *C. purpureus* (Faria *et al.*, 2017). On average, the cellulose concentration increments for these cultivars ranged from 20% to 40% at 28 to 140 days of regrowth. These data help estimate the theoretical production of paper for each cultivar. Madakadze *et al.* (2010) compared the pulp characteristics of elephant grass and switchgrass (*Panicum virgatum* L.) for paper manufacture. They found that elephant grass had↱—cellulose contents of 45.6% and lignin contents of 17.7%; the corresponding contents for switchgrass were 41.2% and 23.9%. The yields for paper pulp were 48% for switchgrass and 50% for elephant grass. These and other characteristics, such as Kappa numbers, and fiber length and freshness, prove the feasibility of using these grasses to produce paper pulp. Agroecological conditions for the cultivation of *C. purpureus* with the required characteristics to produce cellulose and obtain paper are adequate in the tropical areas of Mexico. However, due to a rapid growth rate and variation between cultivars of this species, an assessment is necessary to choose the age of regrowth with the greatest biomass and lignocellulose production rate for each cultivar. Therefore, the objective of our research was to determine the biomass production of cellulose, hemicellulose, and lignin in six cultivars of *C. purpureus*, with and without fertilization with nitrogen, phosphorus, and potassium, harvested every 21 days for 168 days during the rainy season in the tropics of Veracruz.

MATERIALS AND METHODS

The experiment was implemented from July 2010 to January 2011 at the La Posta experimental station in Paso del Toro, Veracruz (19° 00' 49" N and 96° 08' 19" W), at 12 masl (INEGI 2009). The prevailing climate in the area is sub-humid tropical Aw_2 (Vidal 2005); with a rainfall of 1,461 mm, a relative humidity of 77.4%, and an average temperature of 25 °C, with a maximum of 35 °C and a minimum of 15 °C. The soil type is vertisol and has an acidic pH (4.9 to 5.4), a clay sandy loam texture, medium content of organic matter (1.6%-2.6%), and inorganic nitrogen (7-21 ppm), phosphorus (17.6-78.5 ppm), potassium (174 ppm), calcium (1,590-1,936 ppm), and magnesium (288-418 ppm). We used cultivars of CT-115, elephant grass, king grass, Maralfalfa, Roxo, and Taiwan. Each plot was subdivided into two areas: one with and the other one without fertilization. The experimental design was a randomized complete block with a split-plot arrangement and four replications per plot, representing each grass cultivar. The land was prepared with subsoil, fallow, double harrow, and 80 cm furrows. We used three-node cuttings planted every 80 cm on the furrow slope, making sure two of the three nodes remained buried at a 45° angle from the surface. The sowing took place in June 2009, while the assessments were conducted from July 2010 to January 2011, after cutting to level. The sampling area on each cut date was 5.76 m^2 (or three furrows), 2.4 m wide per 2.4 m long. We made a total of eight cuts. At the beginning of the experiment, we cut to level at 25 cm. The fertilization dose was 141-43-20 with the following formula: 200 kg of urea, 50 kg of diammonium phosphate, and 200 kg of the 20-10-10 mixture, rationed in two applications. The first was made eight days after cutting to level, and the second 60 days afterwards. Aerial biomass (green matter) was harvested at 25 cm above the ground every 21 days, for six months, during the rainy season. When sampling, we recorded the production of dry matter (DM) of the whole plant in each parcel, took a subsample of 300 g, minced it, dried it at 55 °C in a forced air furnace until reaching constant weight, and finally ground it with a Willey mill using a 1 mm mesh. The chemical composition of DM and ash of these dehydrated and ground subsamples (A.O.A.C., 2000) was determined using an Ankom Fiber Analyzer. In duplicate, neutral detergent fiber (NDF), acid detergent fiber (ADF), and lignin were determined using the 72% sulfuric acid method; hemicellulose and cellulose were calculated by difference (Van Soest *et al.*, 1991). With the results of biomass yield at each sampling moment in kg DM ha^{-1}, we proceeded to perform a nonlinear regression analysis using the Gompertz model and applying the Powell algorithm as a minimization method. We used the Micromath Scientist® software with the Gompertz function:

$$Y = A\exp^{-\exp(-\mu(x-B))}$$

Where Y=biomass yield (kg DM ha^{-1}); x=time (days); A=maximum biomass production (kg DM ha^{-1}); B=inflection point, indicating the end of growth and the beginning of decrease (days); and μ=growth rate (kg DM ha^{-1} day^{-1}). To determine the production of lignocellulosic biomass (lignin, hemicellulose, and cellulose) for each period, we multiplied the estimated values of biomass in kg DM ha^{-1} (obtained using the Gompertz model) by the percentage of cellulose, hemicellulose, and lignin. We did so every 21 days during the

168 days of the experiment. Using the nonlinear Gompertz model, we adjusted the data and obtained the curve parameters of cellulose, hemicellulose, and lignin production for each cultivar.

Experimental design and statistical analysis

The obtained parameters and the goodness-of-fit coefficients were analyzed with ANOVA in a randomized complete block design in a split-plot arrangement —where large plots were cultivars and small plots were split into areas with and without fertilization— and four replications. The data were analyzed with the SAS software (2007) using the GLM procedure. Means were compared using Tukey's test ($p<0.05$).

RESULTS AND DISCUSSION

Maximum production values of cellulose, hemicellulose, and lignin biomass from the whole plant in the different cultivars of *C. purpureus*, obtained with the Gompertz model, can be observed in Table 1. The variance analysis results show that cultivars presented differences ($p<0.05$) in lignocellulosic biomass. The Maralfalfa cultivar presented the highest cellulose production (3,786 kg ha^{-1}), statistically similar ($p>0.05$) to the elephant grass and Taiwan cultivars, whose values were 3,451 kg ha^{-1} and 3,329 kg ha^{-1} respectively, and statistically different ($p<0.05$) from the CT-115 and king grass cultivars, with variations of more than 1 t ha^{-1} of cellulose. Maralfalfa, elephant grass, and Taiwan cultivars produce more biomass DM ha^{-1}, which accounts for the differences and turns them into a renewable option for the Mexican paper industry. Hemicellulose and lignin are part of the elimination process that the paper industry contemplates in its protocols for paper production. It is therefore important to have materials with low concentrations of these components in dry matter and aerial biomass production per hectare (Segura *et al.*, 2008). In this study, the CT-115, king grass, and Roxo cultivars produced 2,133 kg ha^{-1}, 1,519 kg ha^{-1}, and 2,105 kg ha^{-1} of hemicellulose, respectively, and 550 kg ha^{-1}, 530 kg ha^{-1}, and 688 kg ha^{-1} of lignin. The king grass cultivar excelled in both components (hemicellulose and lignin), being statistically different ($p<0.05$) from elephant grass, Maralfalfa, and Taiwan cultivars. It is worth noting that this maximum in production,

Table 1. Maximum production (A) of cellulose, hemicellulose, and lignin kg ha^{-1} from different *C. purpureus* cultivars at a regrowth age of 168 days, obtained using the Gompertz model.

Cultivars	Cellulose		Hemicellulose		Lignin	
	Mean	SEM	Mean	SEM	Mean	SEM
CT-115 (6)	2701^{c}	258	2133^{ab}	199	550^{ab}	150
Elephant (5)	3431^{ab}	328	2623^{a}	239	702^{ab}	190
King grass (5)	2079^{c}	328	1519^{b}	239	530^{b}	190
Maralfalfa (6)	3786^{a}	258	2983^{a}	199	932^{ab}	150
Roxo (6)	2633^{bc}	258	2105^{ab}	199	688^{ab}	150
Taiwan (5)	3329^{ab}	328	2645^{a}	239	1017^{a}	190

^{ab} Different literals within the column, of the different response variables are, statistically significant ($p<0.05$). Tukey means test=0.05. ( )= repetitions.

obtained using the parameter (A) of the Gompertz model, was estimated with a regrowth age of 168 days or more. This coincides with what Martínez *et al*. (2010), and Rodríguez *et al*. (2013) concluded: the Gompertz model is the one that best adjusts the data.

The results of fertilization with or without NPK in *C. purpureus* regarding cellulose, hemicellulose, and lignin production in kg ha^{-1} are shown in Table 2. Cellulose production increased ($p<0.05$) as an effect of fertilization, and biomass production reached 4,007 kg ha^{-1}—two times the amount compared to other cultivars without fertilization. We observed a similar trend in the production of hemicellulose and lignin per hectare, with 3,031 kg ha^{-1} and 963 kg ha^{-1} for fertilized treatments. In non-fertilized plants, hemicellulose reached 1,513 kg ha^{-1} and lignin 462 kg ha^{-1}. The fertilization effect in tropical soils has always been beneficial for increasing biomass production. In this regard, Oliveira *et al*. (2015) found a linear effect when applying nitrogen to the Cameroon Piracicaba variety, which yielded 58 t DM ha^{-1} $year^{-1}$, using a dosage of 1,600 kg ha^{-1} of N. In another study, Ramos *et al*. (2013) show an increment in biomass production due to fertilization in OM-22 and king grass cultivars. The yields, in this case, reached about 150 t DM ha^{-1} $year^{-1}$. However, the response of cultivars is not always similar, since CT-115 had a moderate response to nitrogen application.

Table 3 shows the percentage chemical composition of DM, ash, cellulose, hemicellulose, and lignin of the whole plant, and the yield of the six cultivars of *C. purpureus* with and without fertilization harvested at a regrowth age of 168 days. We observe a slightly higher effect of fertilization with NPK on the increment in cellulose content: 36.3% without fertilization and 38.1% when fertilized. There is also a decrease in hemicellulose, from 30.0% to 28.1%. Regarding lignin content in the whole plant, there were no observed effects of fertilization ($p<0.05$), so the average value remained at 8.3%. Cane pulp is a sub-product used in the paper industry for being a good source of cellulose, but it has a much higher lignin production (16%) (Bhatti and Khan, 1996) as compared to the percentages found in the

Table 2. Maximum production (A) of cellulose, hemicellulose, and lignin kg ha^{-1}, with or without fertilization (NPK), in *C. purpureus* at a regrowth age of 168 days or more.

Treatment	Cellulose		Hemicellulose		Lignin	
	Mean	EEM	Mean	EEM	Mean	EEM
Fertilized (18)	4007^a	88	3031^a	66	963^a	86
Unfertilized (15)	1786^b	106	1513^b	80	462^b	110

[ab] Different literals within the column, of the different response variables are, statistically significant ($p<0.05$). Tukey means test=0.05. ( )=repetitions.

Table 3. Fertilization with NPK and its effect on percentage chemical composition and yield of *C. purpureus* cultivars harvested at a regrowth age of 168 days.

Treatment	DM	Ashes	Cellulose	Hemicellulose	Lignin	Yield (kg DM ha^1)
Fertilized	30.8^a	7.4^b	38.1^a	28.1^a	8.2^a	9991^a
Unfertilized	27.9^a	9.5^a	36.3^a	30.0^a	8.4^a	4438^b

[ab] Different literals within the column of the different response variables are, statistically significant ($p<0.05$). Tukey means test=0.05.

grasses we evaluated. Due to the effect of fertilization, DM production doubled its yield, that went up from 4,438 kg DM ha^{-1} to 9,991 kg DM ha^{-1} ($p<0.05$).

Figure 1a shows the behavior of each cultivar due to the effect of fertilization. Maralfalfa and elephant grass cultivars responded rapidly to fertilization, presenting a cellulose yield of more than 4,000 kg DM ha^{-1} in a short period (115 days), during which the curve became asymptotic. The Taiwan cultivar did not respond so fast, its asymptote beginning at 168 days. Still, its cellulose values are higher than 4,000 kg DM ha^{-1}. Rengsirikul *et al.* (2013) reported a variable dry matter production in eight *C. purpureus* cultivars, which were fertilized with 375 kg ha^{-1} of N and harvested every 90 days. Their yields ranged from 27 t DM ha^{-1} year^{-1} to 58 t DM ha^{-1} year^{-1}. Their cellulose content varied between 35% and 47% and their hemicellulose content went from 19% to 26%, values attesting to this species' potential for cellulose production. These results substantiate harvesting biomass to obtain cellulose starting at 115 days of age and up until the maximum production age, established using parameter A of the Gompertz model (168-211 days). Figure 1b shows cultivars that grow or produce more cellulose without fertilization. These were the Maralfalfa, elephant grass, and Roxo cultivars (1600-2400 kg DM ha^{-1}). However, all cultivars, except for king grass (115 days), have an asymptote that starts at 168 days of regrowth, which is very close to their maximum production age as calculated with the Gompertz model.

The hemicellulose biomass curve of fertilized cultivars in Figure 2a presented a behavior similar to the cellulose biomass curves with Maralfalfa, elephant grass, and Taiwan cultivars. These cultivars produced between 3,000 kg DM ha^{-1} and 3,600 kg DM ha^{-1}, with an asymptote starting at a regrowth age of 168 days. Caballero-Gómez *et al.* (2016) obtained a higher hemicellulose production: 4,350 kg DM ha^{-1}. This was due to a higher biomass yield (15 t ha^{-1}) in the various *C. purpureus* cultivars, with 29% hemicellulose contents on a dry base. Figure 2b shows that the Maralfalfa cultivar grew exponentially and reached its maximum hemicellulose production in the first 60 days of regrowth. Meanwhile, king grass grew slower without fertilization with NPK, not reaching 1,000 kg DM ha^{-1} of hemicellulose at 168 days of age.

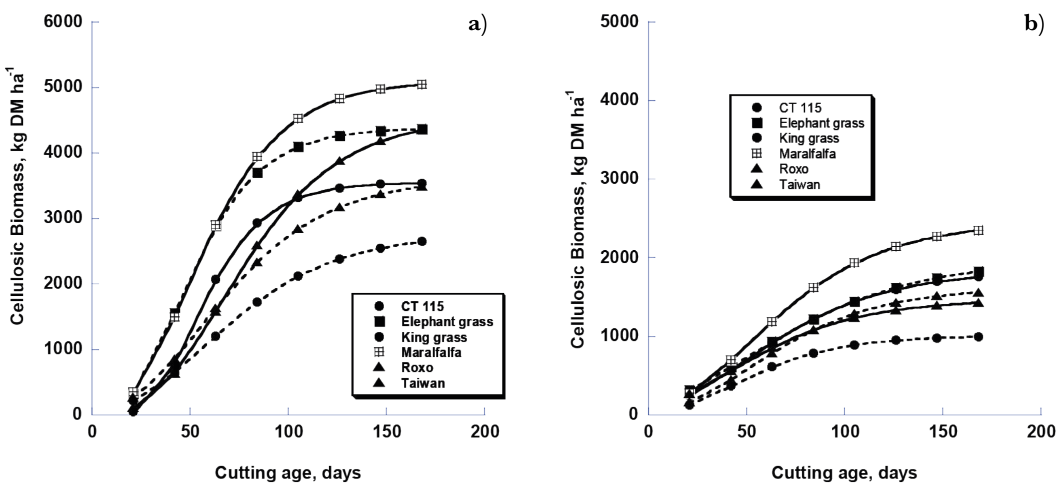

Figure 1. Cellulosic production curves: a) fertilized, b) not fertilized, in different *C. purpureus* cultivars harvested every 21 days in the tropics.

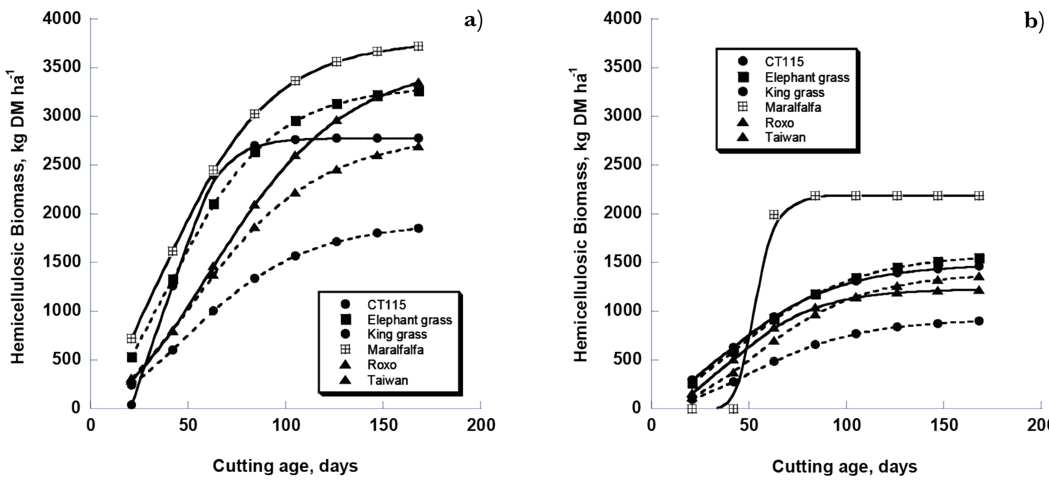

Figure 2. Hemicellulosic production curves: a) fertilized, b) not fertilized, in various *C. purpureus* cultivars, harvested every 21 days in the tropics.

Chemically and structurally, lignin represents a barrier to separating fiber. The association between lignin and polysaccharides determines plant material rigidity and structural resistance. Therefore, the paper industry requires materials with low concentrations of this compound since its elimination is an expensive process (Alvarez-Vasco *et al.*, 2017; Sun *et al.*, 2000). The Maralfalfa cultivar fertilized with NPK produces more than 1,100 kg DM ha^{-1} of lignin. Even though this occurs at 168 days of regrowth age, its asymptote starts at 115 days; it is advisable to harvest the forage starting at this age. We can observe a similar behavior in the elephant grass and CT-115 cultivars, but with lower lignin productions: 600 kg ha^{-1} and 800 kg ha^{-1}, respectively.

CONCLUSIONS

Fertilized Maralfalfa, elephant grass, and Taiwan cultivars are an alternative for the paper industry. They produce more than 4,000 kg ha^{-1} of cellulose, 3,000 kg ha^{-1} of hemicellulose, and just under 1,000 kg ha^{-1} of lignin during the rainy season in the tropics of Veracruz. Biomass cuts for paper production should start at 115 days and continue until a regrowth age of 168 days.

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AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Vegetative development and bean yield in magnetized nutrient solution in combination with variable pH

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ABSTRACT

Objective: Due to the importance of bean in human consumption, vegetative development and seed yield of Negro Veracruz beans were evaluated using a nutrient solution with different magnetization times in combination with different pH levels.

Design/Methodology/Approach: The experimental design used was completely randomized with a 4×6 factorial arrangement with three replications. 24 treatments of the combination of four magnetization times and six degrees of acidity of the nutrient solution were evaluated. The variables evaluated were vegetative development and yield components. The results were subjected to an analysis of variance and media separations were performed using Tukey's test ($\alpha \leq 0.05$).

Results: The magnetization and the degree of acidity of the nutrient solution significantly influenced the variables. The most outstanding treatments were 2 and 24 hours of magnetization in combination with pH values of 4 and 5, for most of the variables of vegetative development and evaluated yield components. The magnetization treatments of 2 h together with pH values of 4 and 5 increased height, biomass, days to flowering, number of pods, harvest index and seed yield.

Study Limitations/Implications: Care should be taken that exposure to the magnetic field at high intensities may produce adverse effects on growth and development.

Findings/Conclusions: It is concluded that an appropriate combination of magnetization time and degree of acidity of the nutrient solution improves vegetative development and yield.

Keywords: *Phaseolus vulgaris* L, magnetization, acidity, biomass, production.

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INTRODUCTION

Bean (*Phaseolus vulgaris* L.) is the most important legume for human consumption, rich in bioactive components such as enzyme inhibitors, lectins, phytates, oligosaccharides, phenolic, and anti-mutagenic and anti-proliferative properties (Suárez-Martínez

Image by PublicDomainPictures at Pixabay *et al.*, 2016). In Mexico, the per capita consumption of bean is approximately 9.8 kilograms (SAGARPA, 2015), and currently 82% of the needs are covered by domestic production while the rest comes from imports from the United States, Canada and China (SAGARPA, 2017). Likewise, studies by Gilani *et al.* (2017) indicate that there is a relationship between magnetized water and pH values, as well as the electric conductivity of water with pH adjustment. On the other hand, Hassan *et al.* (2019) mention that there are significant increments in the pH when the water is treated ionically with magnets in different intensities, forming alkaline molecules and decreasing the acidity. According to Amor *et al.* (2018), the pH increased slightly through time with the use of four magnets of different intensities, and after the magnets were withdrawn the initial pH was reestablished. The pH of water is varied, depending on the intensity of the magnetic field and the time of exposure; the changes in pH are electrochemical reactions that result from electric currents generated by the Lorentz force (Gatard, Deseure and Chatenet 2020; He *et al.*, 2022).Therefore, the objective of the study was to determine the vegetative development and the bean seed yield using nutritional Steiner solution subjected to different magnetization times in combination with different pH levels, under greenhouse conditions.

MATERIALS AND METHODS

The experiment was conducted in February 2019, in the greenhouse of Universidad Autónoma Agraria Antonio Narro Unidad Laguna, located in the city of Torreón, Coahuila, with geographical coordinates 25° 31' 11" North and 103° 25' 57" West, and 1123 masl. The climate according to Köppen modified by García (2004) is warm desert, type BWh, with maximum temperature of 40 °C and minimum of 6 °C, with mean rainfall of 250 mm.

Treatments and experimental design

Twenty-four (24) treatments were evaluated resulting from the combination of four magnetization times (Factor A: 0, 0.333, 2.0 and 24.0 hours per day) and six degrees of acidity (Factor B: pH of 3, 4, 5, 6, 7 and 8) of the nutritient solution in bean (Table 1). The experimental design was completely randomized with factorial arrangement 4×6 with three replications. These treatments began when the plants presented the simple unfolded leaves. The experimental unit consisted of one plant (López-Pérez *et al.*, 2019).

Development of the experiment

The variety Negro Veracruz from the INIFAP national bean program, liberated in 1980, was used. The seeds were disinfected with sodium hypochlorite at 4% (Espinosa-Palomeque *et al.*, 2019), submerging them for three minutes. Then, they were transplanted to pots with different treatments of the nutrient solutions in greenhouse conditions. Circular plastic containers with capacity of 5 liters were used. Aeriation was provided by a 120 W turbine, Hailea brand, model VB290G.

The chemical and physical properties of water, based on the laboratory analysis were the following: in microelements, Iron (Fe^3) 0, Zinc (Zn^+) 0.02, Copper (Cu^+) 0.03,

Table 1. Magnetization treatments and pH in the nutrient solution in black bean developed in a hydroponic system in greenhouse.

Factor A:	Factor B:	Treatments
Tm1: 0	pH1: 3	1 Tm1 pH1: 0 hours + pH de 3
		2 Tm1 pH2: 0 hours + pH de 4
Tm2: 0.333	pH2: 4	3 Tm1 pH3: 0 hours + pH de 5
		4 Tm1 pH4: 0 hours + pH de 6
Tm3: 2.0	pH3: 5	5 Tm1 pH5: 0 hours + pH de 7
		6 Tm1 pH6: 0 hours + pH de 8
Tm4: 24.0	pH4: 6	7 Tm2 pH1: 0.333 hours + pH de 3
		8 Tm2 pH2: 0.333 hours + pH de 4
	pH5: 7	9 Tm2 pH3: 0.333 hours + pH de 5
		10 Tm2 pH4: 0.333 hours + pH de 6
	pH6: 8	11 Tm2 pH5: 0.333 hours + pH de 7
		12 Tm2 pH6: 0.333 hours + pH de 8
		13 Tm3 pH1: 2.0 hours + pH de 3
		14 Tm3 pH2: 2.0 hours + pH de 4
		15 Tm3 pH3: 2.0 hours + pH de 5
		16 Tm3 pH4: 2.0 hours + pH de 6
		17 Tm3 pH5: 2.0 hours + pH de 7
		18 Tm3 pH6: 2.0 hours + pH de 8
		19 Tm4 pH1: 24.0 hours + pH de 3
		20 Tm4 pH2: 24.0 hours + pH de 4
		21 Tm4 pH3: 24.0 hours + pH de 5
		22 Tm4 pH4: 24.0 hours + pH de 6
		23 Tm4 pH5: 24.0 hours + pH de 7
		24 Tm4 pH6: 24.0 hours + pH de 8

Factor A: magnetization time in hours per day (Tm); Factor B: degree of acidity (pH).

Manganese (Mn^+) 0.01, Boron (B^+) 0.99; in cations (+), Sodium (Na^+) 120, Potassium (K^+) 13.0, Calcium (Ca^+) 288, Magnesium (Mg^+) 29.0; in anions (−), nitrates (NO_3) 23.03, Phosphorus (phosphates) 0.080, Dihydrogen phosphorus (H_2PO_4) 0.250, Sulfate (SO_4) 643.6, Carbonates (CO_3) 0, Bicarbonates (HCO_5) 170.83, Chlorides (Cl) 198.52; physical parameters, pH 7.80, electric conductivity (mS/cm) 2.21, rate of sodium absorption 1.80, and interchangeable sodium percentage 0.38%.

A potentiometer brand Hanna HI98130 was used for the pH readings. Magnets were used that were 2.5 cm long, 2.0 cm diameter, cylindrical, made from an alloy of neodymium, Iron and Boron, with lining of Chromium, Nickel and Copper, to magnetize the water, of the Obi brand, model 255590. The magnets were placed with an upwards direction (extreme North) and downwards (extreme South) (extreme Sur) (Zhang *et al.*, 2017), which presented an intensity of 0.380 Tesla. To measure the magnetic field of the magnets, a portable Gauss meter and biomagnetism polarity detector were used (Zhang *et al.*, 2017).

Variables evaluated

The phenological variables were recorded since sowing, using the methodology proposed by Escalante and Kohashi (2015): total biomass (TB, obtained when harvesting and determining the dry weight of the roots and the aerial biomass of plants); days to flowering (FL, number of days at 50% of flowering since sowing); height (PH, when measuring the total length of the main shoot on the surface); number of leaves (NL, obtained when counting all the leaves that are completely expanded and exposed with photosynthetic activity, including the two primary leaves and the trifoliate one), number of normal pods (NP, considering as a normal pod the one that had at least one normal seed with the size of the characteristic black color of the Veracruz genotype), number of grains per pod (NG, average number of seeds produced in each of the pods and which reached full development), seed yield (SY, determined by weighing all the normal seeds produced by the plant) and harvest index (HI in %, calculated when dividing the seed yield by the final aerial biomass) ($HI=SY/BM\times 100$).

Statistical analyses

The data of the variables evaluated were processed through the statistical package SAS, version 9.0 (SAS, 1999), with an analysis of variance conducted under a completely randomized design with factorial arrangement 4×6 and three replications. When significant statistical differences were detected in the variables, Tukey's honestly significant difference was used for means comparison (HSD, P=0.05).

RESULTS AND DISCUSSION

Vegetative development

The values of plant height were significantly different between magnetization times, pH and their interaction. The plants grew more when the nutrient solution was magnetized during 24 hours and its pH was 4 and 5. Regarding the effect of the interaction magnetization time and pH on plant height, the combinations of 24 hours with pH of 4 and 5, as well as 2 hours and pH of 4 resulted in higher plants, with increments of 48.4, 70.5 y 23.4%, respectively, compared to the control and same values of pH (Table 3). This agrees with Alattar *et al.* (2021) who mention that in *Zea mays* L. plants the length of the plants is significantly influenced by magnetized water.

The variable number of leaves per plant (NL) showed significant differences between magnetization times and pH, but not for their interaction. Magnetization times of 0.333, 2.0 and 24 hours presented higher number of leaves, being statistically similar between one another, but different from the control. The pH values of 3 to 6 showed higher numbers of leaves per plant and pH levels of 7 and 8 were lower, being statistically similar between one another (Table 2). The total biomass per plant (TB) showed significant differences between magnetization times, pH and their interaction. The exposition time to magnetism of 2.0 hours presented plants with higher weight, followed by the control and 24 hours, while a time of 0.333 hours showed the plants with lowest weight (Table 2).

For their part, Vashisth and Joshi (2017) indicated that increases in the number of leaves and plant height were due to times of exposure. In terms of the effect of the interaction between magnetization time and pH on plant biomass, the combinations of 2 hours and pH of 4, 2 hours and pH of 5, 24 hours with pH of 4 and 5 presented the plants with highest weight, corresponding to increments of 8.4, 81.6, 7.2 and 37.2%, respectively (Table 3).

Table 2. Main effects of different magnetization times and pH of the nutrient solution on plant height, number of leaves per plant, biomass, and days to flowering of black bean under greenhouse conditions.

Treatments	AP (cm)	NH	BT (g)	FL (d)
Magnetization time (hours)				
0	18.00 b	17.0 b	7.10 b*	67.28 ab
0.333	20.10 ab	17.9 ab	4.95 c	63.56 b
2.0	17.40 b	18.8 ab	8.72 a	76.83 a
24.0	23.10 a	19.6 a	7.61 b	70.83 ab
P>F	0.0010	0.0070	< 0.0001	0.0075
pH				
3	11.70 d	18.9 ab	3.14 c	58.33 b
4	30.10 a	19.4 a	10.25 a	63.83 b
5	26.00 ab	20.9 a	10.70 a	68.08 ab
6	21.00 bc	18.6 ab	6.40 b	70.00 ab
7	15.80 dc	16.6 bc	6.71 b	78.50 a
8	13.30 d	15.5 c	5.40 b	79.00 a
P>F	<0.0001	<0.0001	<0.0001	0.0002

AP: plant height (centimeters), NH: Number of leaves per plant, BT: Total biomass per plant (grams), FL: Days to flowering. * Means with different letters in a column are statistically different (Tukey 0.05).

The time of exposure to magnetism of 0.333 hours presented a shorter flowering period, while a time of 2.0 hours recorded the longest period to flowering. Concerning the effect of the interaction between magnetization time and pH on number of days to flowering, the combinations of 0 hours and pH of 5, as well as 0.333 hours and pH of 3 showed the shortest flowering periods, while 0 hours and pH of 7 and 8, 0.333 and pH of 7, 2.0 hours and pH of 5 and 8, as well as 24 hours and pH of 5 presented the longest periods to flowering (Table 3).

Yield components

The variable number of pods per plant showed significant differences between magnetization times, pH and their interaction. In relation to the effect of the interaction between magnetization time and pH on number of pods per plant, all the magnetization treatments with pH values of 4 to 7 showed high production of pods, whose increments varied from 47.4 to 168.6%. A similar behavior to the production of pods was observed on the variable number of grains per pod, since magnetization of the nutrient solution during 0.333 to 24 hours significantly increased the number of grains per pod compared to the control without magnetizing (Table 4).

Regarding the interaction between magnetization time and pH, no significant differences were detected between treatments (Table 5). The plants had higher seed yields when the nutrient solution was magnetized during 2 and 24 hours, which represent increments of 21.4 and 16.1% compared to the control. In relation to the

Table 3. Effects of the interactions between different magnetization times and pH levels of nutrient solution on plant height, number of leaves per plant, biomass and days to flowering of black bean under greenhouse conditions.

TEH	pH SN	PH (cm)	NLP	TBP (g)	DF (d)
0	pH3	11.88 gf	15.7 bc	2.50 i	57.67 abc
	pH4	24.53 abcdef	19.8 abc	10.73 cb	56.00 abc
	pH5	20.23 cdegf	18.3 abc	8.53 bcde	49.33 bc
	pH6	20.32 cdegf	17.3 abc	7.73 cdef	71.33 abc
	pH7	18.78 cdegf	14.9 bc	7.97 cdef	84.67 ab
	pH8	12.43 gf	16.0 abc	5.17 efghi	84.67 ab
0.333	pH3	10.60 g	20.2 abc	2.49 i	41.33 c
	pH4	29.10 abc	18.2 abc	7.13 defg	62.67 abc
	pH5	28.80 abcd	21.1 ab	7.10 defg	60.33 abc
	pH6	26.50 abcde	19.5 abc	5.47 efghi	64.33 abc
	pH7	11.10 gf	13.6 c	3.67 hi	82.00 ab
	pH8	14.20 egf	15.0 bc	3.83 ghi	70.67 abc
2.0	pH3	12.30 gf	19.5 abc	3.87 ghi	77.33 ab
	pH4	30.30 abc	19.5 abc	11.63 b	69.00 abc
	pH5	20.70 cdegf	21.6 ab	15.49 a	80.67 ab
	pH6	15.30 defg	17.8 abc	5.81 efghi	74.67 abc
	pH7	14.20 efg	17.8 abc	10.47bcd	70.00 abc
	pH8	11.50 fg	16.5 abc	5.12 fghi	89.33 a
24.0	pH3	11.88 fg	20.2 abc	3.70 hi	57.00 abc
	pH4	36.42 a	20.1 abc	11.50 b	67.67 abc
	pH5	34.50 ab	22.8 a	11.70 b	82.00 ab
	pH6	21.85 bcdefg	20.0 abc	6.60 efgh	69.67 abc
	pH7	19.17 cdefg	20.2 abc	4.70 fghi	77.33 ab
	pH8	14.97 efg	14.6 bc	7.50 cdef	71.33 abc

PH: plant height (cm), NLP: Number of leaves per plant, TBP: Total biomass per plant (g), DF: Days to flowering. *Means with different letters in a column are statistically different (Tukey 0.05).

effect of the interaction between magnetization time and pH on seed yield per plant, the best combinations were 24 hours and pH 4, as well as 24 hours and pH 5, with yields of 3.80 and 3.20 g per plant, equivalent to increments of 46.2 and 45.4%, compared to the non-magnetized solution and same acidity. The combinations of 2.0 hours and pH of 4 also stood out, as well as 2.0 hours and pH of 5, which had yields of 3.43 and 3.13 g per plant, whose increments were 31.9 and 42.3%, compared to the non-magnetized solution and same acidity (Table 5). Regarding the effect of acidity on the soil, Tosquy *et al.* (2020) reported that a pH decreases the yield in the bean crop. The variable harvest index (HI) showed significant differences between pH values and for the interaction between magnetization time and pH, but there were no significant differences for the magnetization time. The plants showed higher harvest indexes (26.8 to 32.5%) in the nutrient solution with pH values of 4 to 7. On the contrary, the harvest indexes were

Table 4. Main effects of different magnetization times and pH levels of the nutrient solution on number of pods, number of grains, seed yield and harvest index of black bean in greenhouse conditions.

Treatments	NNP	NG	SY (g)	HI (%)
Magnetization time (h)				
0	5.56 c	3.83 b	1.68 c	27.43 a
0.333	8.17 b	4.50 a	1.00 d	24.72 a
2.0	8.17 b	4.67 a	2.04 a	26.25 a
24.0	10.67 a	5.06 a	1.95 b	27.88 a
P>F	<0.0001	<0.0001	<0.0001	0.1760
pH				
3	2.75 c	2.00 d	0.47 f	19.33 c
4	11.00 a	5.42 ab	2.86 a	32.45 a
5	12.17 a	5.67 a	2.46 b	28.10 ab
6	11.08 a	5.08 ab	1.52 d	28.35 ab
7	9.25 b	4.75 bc	1.62 c	26.79 b
8	2.58 c	4.17 c	1.10 e	24.40 bc
P>F	<0.0001	<0.0001	<0.0001	<0.0001

NNP: Number of normal pods per plant, NG: Number of grain in normal pods, IC: Harvest index %, SY: Seed yield (g). * Means with different letters in a column are statistically different (Tukey 0.05).

low (19.3 and 24.4%) when the nutrient solution was very acid and alkaline (pH of 3 and 8) (Table 4). In relation to the effect of the interaction between magnetization time and pH on the harvest index, the best treatments were: 24 hours and pH of 4 to 6 (HI of 32.3 to 38.5%), as well as 2.0 hours and pH of 4 (HI of 34.8%); meanwhile, the combinations of 0, 2 and 24 hours with pH of 3.0 showed the lowest harvest indexes (12.3 to 18.5%) (Table 5). In the review by Nyakane *et al.* (2019) about the effects of magnetic fields on plants reported during 20 years and the beneficial effects of magnetized irrigation water, in the appropriate combination of intensity of the magnetic field and time of. Ospina-Salazar *et al.* (2018), when subjecting water to a magnetic field found that biomass and fruit yield of Tabasco chili pepper plants were higher, fresh weight and number of fruits of 27 and 13%, respectively. El-Ssawy (2020) reports that the integration of the NFT (nutrient film technique) hydroponic system with magnetized water at a high intensity (level 3) fostered a significant increase in the nutrient concentrations (N, P and K) and total soluble solids, although the pH decreased in a nutritious solution in lettuce. Zareei *et al.* (2019) determined that magnetized water and nutrient solutions stimulate biosynthesis. When it comes to forest species, Liu *et al.* (2019) indicated that irrigation with magnetized water promoted the growth of seedlings, root development, photosynthesis, and mineral nutrient contents of *Populus* × *euramericana*, and that saline water was improved with magnetized water, allowing its use for irrigation.

Table 5. Effects of interactions of different magnetization times and pH levels of the nutrient solution on number of pods, number of grains, seed yield, and harvest index of black bean in greenhouse conditions.

TEH	pH SN	NNP	NG	SY (g)	HI (%)
0	pH3	4.33 ijk	1.33 e	0.40 k	18.54 cde
	pH4	6.33 hij	4.67 abc	2.60 d	30.58 abcd
	pH5	7.67 fghi	4.67 abc	2.20 e	31.46 abcd
	pH6	7.00 ghi	4.67 abc	1.60 gf	24.26 abcde
	pH7	5.00 ijk	4.33 abcd	2.08 e	31.53 abcd
	pH8	3.00 jk	3.33 bcde	1.20 hi	28.21 abcd
0.333	pH3	1.67 k	1.67 e	0.49 jk	28.17 abcd
	pH4	9.33 efgh	5.33 ab	1.63 gf	25.97 abcde
	pH5	14.00 abc	6.00 a	1.27 h	24.88 abcde
	pH6	13.00 bcd	5.00 ab	1.37 gh	28.71 abcd
	pH7	9.00befgh	4.67 abc	0.60 jk	19.89 cde
	pH8	2.00 k	4.33 abcd	0.67 j	20.70 bcde
2.0	pH3	3.00 jk	2.33 de	0.42 k	12.28 e
	pH4	11.33 cde	5.67 a	3.43 b	34.80 ab
	pH5	12.00 bcde	6.00 a	3.13 c	23.20 bcde
	pH6	10.67 cdef	5.00 ab	1.26 h	28.15 abcd
	pH7	10.00 efgh	4.67 abc	2.75 d	30.27 abcd
	pH8	2.00 k	4.33 abcd	1.26 h	28.78 abcd
24.0	pH3	2.0 k	2.70 cde	0.60 jk	18.3 de
	pH4	17.0 a	6.00 a	3.80 a	38.5 a
	pH5	15.0 ab	6.00 a	3.20 bc	32.9 abc
	pH6	13.7 abc	5.70 a	1.80 f	32.3 abcd
	pH7	13.0 bcd	5.30 ab	1.00 i	25.5 abcde
	pH8	3.3 jk	4.70 abc	1.30 h	19.9 cde

NNP: Number of normal pods per plant, NG: Number of grain in normal pods, HI: Harvest index %, SY: Seed yield (g). * Means with different letters in a column are statistically different (Tukey 0.05).

CONCLUSIONS

The effect with the highest increment in treatments of 2 and 24 hours of magnetization, with pH of 4 and 5, was found on the variables mentioned before, where the most outstanding treatments were 2 and 24 hours of magnetization in combination with pH values of 4 and 5, for most of the variables of vegetative development and of yield components evaluated. The treatments of 24 hours of magnetization together with pH values of 4 and 5 increased height, biomass, days to flowering, number of pods, harvest index and seed yield.

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AGRO PRODUCTIVIDAD

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Effect of artificial light on alfalfa (*Medicago sativa* L.) production

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ABSTRACT

Objective: To study the effects of different sources of artificial light on the growth of alfalfa plants (*Medicago sativa* L.).

Design/Methodology/Approach: The experiment was established on four shelves with a height of 2.50 m with three divisions each, each division 80×60 cm long and wide, respectively. The sun's rays were allowed to shine on three of the upper divisions, and in the remaining nine divisions three different sources of artificial light were placed (LED, incandescent and fluorescent), with three divisions for each light source at a density of four lamps per division. The energy expenditure per lamp, the intensity of photons, and the production of dry matter were quantified.

Results: The data indicated that the incandescent lamp had energy expenditure 8 times higher than the LED lamp and 3.5 times higher than the fluorescent, although the light intensity emitted is 3 and 2 times higher in the LED lamp *vs.* incandescent and fluorescent, respectively. The highest production of dry matter was found with sunlight, obtaining values of 391 g m^{-2}, and the lowest production of 17 g m^{-2} was seen with the incandescent lamp.

Study Limitations/Implications: It is necessary to continue conducting research work on fodder production with artificial light, to increase biomass yields.

Findings/Conclusions: With the data obtained, it is concluded that LED light can be a viable alternative in the future to produce food for animal consumption.

Keywords: Dry matter, LED, fluorescent, incandescent.

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INTRODUCTION

Light plays a central role in plant physiology and ecology. Plants use light as a resource, through photosynthesis, and as a source of information (Bennie *et al.*, 2016), light is one of the most important environmental cues that affect the developing plant and regulate its behavior (Whitelam and Halliday, 2007). In commercial practice, greenhouse plants

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are supplied with supplemental light for a maximum of 16-20 h per day and the light intensity ranges between 100 and 200 μmol·m^{-2}·s^{-1} (Paradiso *et al.*, 2011), although lower levels are used for shade-adapted species. For example, according to Ouzounis *et al.* (2015) between 300-500 μmol·m^{-2}·s^{-1} is supplied for tomato (*Lycopersicum esculentum*) in commercial facilities in Scandinavia.

The photosynthesis process can be generated by artificial light sources including incandescent lamps (IL), fluorescent lamps (FL) and light emitting diodes (LED) (Massa *et al.*, 2008). However, the different types of artificial light sources have different light qualities for plant growth. For example, ILs are used mainly to extend the lighting time during seasons with a short daylight period. These convert only 15% of the electrical energy used into light for plant photosynthesis, while the remaining 85% is converted into heat that is not useful and can be harmful to plants (Massa *et al.*, 2008).

LED technology has not yet been fully integrated into the greenhouse control system and must be optimized in terms of light output and distribution, while the cost of LED lamps must be reduced to achieve sustainable and economically viable production (Morrow, 2008).

Light from FLs has been commercially applied in vegetable cultivation, uses less electricity and provides better plant growth than ILs (Shoji *et al.*, 2013). In Japan, about 60% of industrial plant farms use FLs as a light source (Shoji *et al.*, 2013). Many studies revealed that different types of LEDs could affect plant growth in terms of quantity and quality (Ruangrak and Khummueng, 2019); however, no study was found indicating the effect of artificial light on fodder production intended for animal consumption.

Therefore, this study aimed to research the effects of LED, incandescent and fluorescent artificial light sources on the growth of alfalfa (*Medicago sativa* L.) plants.

MATERIALS AND METHODS

The research work was carried out in the experimental greenhouse 3 ALA-2 SEC-2 of the Botany area of Colegio de Postgraduados, Montecillo, Texcoco, Estado de México, located at 19° 29' latitude North, 98° 53' longitude West, and 2240 masl.

The experiment was established on four shelves with a height of 2.50 m with three divisions, each division with dimensions of 80 cm long by 60 cm wide. On each division, a wood base with 20 cm height was placed where 70% sandy loam soil was added, with pH 8.2 and 4.1% of organic matter extracted from the experimental area known as new plot and 30% of compost of sheep and goat feces extracted from the CCIT experimental field of Colegio de Postgraduados. Seeds of the Jupiter variety were sown in all divisions at a density of 8 g m^{-2}, which were previously treated at 50%, generating an actual density of 4 g m^{-2}.

The sun's rays were allowed to shine on three of the upper divisions, and three different sources of artificial light (LED, incandescent and fluorescent) were placed on the remaining nine divisions, three divisions for each light source, at a density of four lamps per division, which were distributed at a height of 10 cm, with respect to the first leaf of the plant. They were provided with 18 h of artificial light per day and the shelves were covered with a black cloth that prevented sunlight from entering.

After planting, potable water was supplied every third day and continuous weeding was carried out every third day to prevent weed growth until day 150 when the first sampling was made. After 150 days, a ground level cutting was carried out to determine yield, then regrowth was allowed and a cutting was done at 28 days for three continuous periods. The mean of the four cuts represents the values reported as dry matter yield.

Dry matter yield

The dry matter (DM) yield of the aerial part was obtained by cutting the fodder at ground level. The biomass of each replicate was deposited in Ziploc plastic bags that were previously labeled, to later determine the partial moisture at the fodder laboratory of Colegio de Postgraduados, Montecillo Campus, as well as the residual moisture at the Animal Nutrition Laboratory of the Zootechnics Department of Universidad Autónoma Chapingo.

To quantify partial dry matter (pDM), the fresh sample was placed in a #8 paper bag and placed in a forced air furnace for 72 h at 55 °C. Once the time was over, the bags were removed from the oven and subsequently weighed on a Dibatec scale with a capacity of 600 g, and the pDM was calculated with the following formula.

$$\%pDM = (dry\ matter\ weight\ /\ fresh\ matter\ weight) * 100$$

To determine the total dry matter (tDM), the partially dried samples were placed in a furnace at 105 °C for 12 h (method 7.003, AOAC, 1980), using the following equation:

$$\%\ of\ tDM = (\%\ DM\ at\ 55\ °C) * (\%\ of\ DM\ at\ 105\ °C)\ /100$$

Intensity of light emitted and power consumption

To quantify the intensity of light emitted, a linear ceptometer, model LP-80 (DECAGON DEVICES INC.) manufactured in the United States of America, was used, in which an adapter was used to measure μmol of photons $m^{-2}\ s^{-1}$ at a specific site.

Commercial lamps were used and the energy consumption was obtained from the specifications of these lamps.

Photosynthetic rate

Twenty alfalfa plants of the Jupiter variety were grown in black perforated plastic bags measuring 20×20 cm, and their net photosynthesis rate (μmol CO_2 $m^{-2}\ s^{-1}$) was measured 100 days after sowing at the fodder laboratory of Colegio de Postgraduados.

Readings were taken with a previously calibrated portable photosynthesis meter system IRGA (Infra Red Gases Analyzer, USA), placing 5 leaves per repetition to make the measurement, which allowed to deduce the photosynthesis in alfalfa plants with different light sources. The photosynthesis rate (μmol CO_2 $m^{-2}\ s^{-1}$) was measured by placing a fully expanded leaf inside the assimilation chamber of the IRGA, where measurements are based on the differences in CO_2 concentration entering and exiting a closed chamber where the exposed leaf is found.

Statistical analysis

An analysis of variance was performed using the PROC GLM procedure of the SAS 9.0 statistical software (Statistical Analysis System version 2002), with a completely randomized design, in order to evaluate the relationship between the variables studied in the experiment. The means comparison was performed using Tukey's adjusted test (P=0.05).

RESULTS AND DISCUSSION

Intensity of light emitted and electricity expenditure per artificial light source

Table 1 shows the watts h^{-1} expenditure of the different artificial light sources; the energy expenditure was 8 times higher with the IL than with the LED and 3.5 times higher than with the FL. The intensity of the light emitted is 3 and 2 times higher in LED *vs.* IL and FL respectively. According to Ouzounis *et al.*, (2015) the use of LED lamps has the potential to generate significant energy savings for greenhouse producers who use artificial light sources because of the low energy expenditure. Nelson and Bugbee (2014) state that there are economic benefits when using LEDs, primarily produced in the United States, compared against other light sources.

In addition, there is a negative relationship between the distance from the artificial light source and the intensity, this trend agrees with that reported by Bennie *et al.* (2016), who mention that the greater the distance from the source, the lower the light intensity on a surface as it is scattered over a larger area.

With these results it can be inferred that in order to generate a high photosynthesis rate with an artificial light source, it is necessary to have a shorter distance with respect to the plant, otherwise the light intensity will not be sufficient to allow the photosynthesis process (Bennie *et al.*, 2016).

Table 1 shows the efficiency of the lamps, in which the LED artificial light emits more μmol m^{-2} s $watt^{-1}$ consumed, with values of 120 *vs.* 26.61 and 6.95 of the FL and IL, respectively. The efficiency data do not agree with those published by Ouzounis *et al.* (2015) who reported values close to 2 μmol m^{-2} s $watt^{-1}$, this difference with respect to the 120 μmol m^{-2} s $watt^{-1}$ found in this study may be due to the distance of the lamps with regards to the leaves, since the lamps were placed at a greater distance in the study by Ouzounis *et al.* (2015). Similarly, Van Leperen and Trouwborst (2008) report efficiencies of 1.9 μmol-m^{-2}-$watt^{-1}$ in high-pressure sodium lamps.

Likewise, the low efficiency of the IL to produce μmol m^{-2} s is related to the heat production it generates. According to Massa *et al.* (2008) and Etae *et al.* (2020) IL provides only 15% of illumination and 85% of the remaining energy is transformed into heat, which

Table 1. Consumption, intensity, and efficiency of artificial light sources.

Sources	Consumption watts/ lamp	Intensity μmol m^{-2} s^{-1} a 1 cm	Intensity μmol m^{-2} s^{-1} a 10 cm	Intensity μmol m^{-2} s^{-1} a 20 cm	Efficiency μmol m^{-2} s^{-1} $watt^{-1}$ a 10 cm
Incandescent light	60	417 [c]	97 [c]	27 [b]	6.95
Fluorescent light	23	612 [b]	149 [b]	22 [b]	26.61
LED ligth	10	1200 [a]	420 [a]	127 [a]	120.00

Different literals in the same column represent a significant difference with P<0.05.

justifies the high temperature presented by the ILs when they are illuminating, this being a negative effect of this source because they reached temperatures above 60 °C, when the room temperature fluctuated at 21 °C, which can cause negative effects on the crop if the ILs are near, contrary to what happened with LED and FL, which increased 1 °C with respect to the room temperature.

Table 2 shows alfalfa DM production with different photon sources; the highest production was observed with sunlight with values of 391 g m^{-2}, while the lowest production resulted from IL with 17 g m^{-2} ($P<0.05$). These results compared with those reported by Rivas et al. (2020), who observed production yields with open sunlit environment of 489 g m^{-2} DM for the winter season and 976 g m^{-2} for the summer season, data that are higher than those found in this study. With the above, it can be concluded that there is still much research to be done in alfalfa production in closed systems with artificial light sources.

On the other hand, the result of the division between dry matter production and intensity of light emitted can predict the efficiency of each light source to produce DM. According to the results, the light coming from LED is the most efficient with a value of 0.5 and the least efficient is from IL; however, it is important to emphasize that the value of sunlight intensity was the highest only throughout the day and sunlight intensity is not constant, so that the value of sunlight efficiency is overestimated.

The photosynthetic rate was determined by using the potted alfalfa plants. The highest value was observed with sunlight, presenting 28 μmol CO_2 m^{-2} s^{-1}, while with IL the value was the lowest at a distance of 1 cm (Table 3); however, it can be seen that as the plant is furthest from the light source the photosynthesis rate will be lower. The photosynthetic rate data of plants that received LED lighting at 10 cm 21 (μmol CO_2 m^{-2} s^{-1}) are statistically lower than those found with solar illumination at 13:00 hours of the day, when there was greater light intensity; however, the sunlight intensity is not constant and therefore this value is not permanent throughout the hours of sunlight. Thus, the values presented with LED lighting generate some expectation in the use of lamps in the future. Luna *et al.* (2020) reported values of photosynthetic rate of alfalfa lower than 20 μmol CO_2 m^{-2} s^{-1}, a value similar to that found in this study with the use of LED lighting.

The photosynthesis rate decreased as the distance between the light source and the plant increased, which may be attributed to the fact that the farther away the light source, the lower the photon intensity per surface area (Table 1).

Table 2. Dry matter production of alfalfa under different artificial light sources.

Light source	μmol m^{-2} s^{-1} 10 cm	Production of MS g m^{-2}	Production in g de MS μmol m^{-2} s^{-1}
Incandescent light	97 [c]	17 [d]	0.18
Fluorescent light	149 [c]	63 [c]	0.42
Led light	420 [b]	211 [b]	0.50
Sunlight	1680 [a]	391 [a]	0.23

The dry matter data are the average of four cutting periods, the first at 150 days after planting and three subsequent cuts from the sprouts with a rest of 28 days between cuts. Different literals in the same column represent a significant difference with $P<0.05$.

Table 3. Photosynthetic rate of alfalfa with different light source.

Light source	Photosynthesis rate (μmol CO_2 m^{-2} s^{-1}) to 1 cm	Photosynthesis rate (μmol CO_2 m^{-2} s^{-1}) to 10 cm	Photosynthesis rate (μmol CO_2 m^{-2} s^{-1}) to 20 cm
Incandescent light	13 [d]	4 [c]	2 [b]
Fluorescent light	17 [c]	5 [c]	2 [b]
Led light	24 [b]	21 [b]	4 [b]
Sunlight	28 [a]	28 [a]	28 [a]

Different literals in the same column represent a significant difference with $P<0.05$.

CONCLUSIONS

LED light can be a viable alternative in the future to produce food destined for animal consumption, despite having a lower biomass production compared to sunlight; it is more efficient compared to other artificial sources.

It is necessary to continue research work on fodder production with artificial light to obtain higher yields, which will allow us to prepare for an uncertain future.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Forage accumulation, morphological composition and height of *Panicum maximum* cv. Tanzania with organic and chemical fertilization

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ABSTRACT

Objective: To evaluate the dry matter accumulation, morphological composition and height in the *Panicum maximum* cv. Tanzania grass in order to determine the optimal cutting point under four fertilization schemes.

Methodology: Tanzania grass was evaluated with fertilizations: T1) chemical (120-60-00 NPK), T2) vermicompost (10 t ha^{-1}), T3) compost (10 t ha^{-1}), and T4) compost+leachate. Cuts were made every 14 days where dry matter (DM), morphological composition and height were measured. A randomized complete block design was used, with an arrangement of measures repeated over time.

Results: The maximum accumulation of DM in the T1, T2 and T3 treatments was at 80 days after cutting, the maximum height was with T1 at 80 days after cutting with 206.2 cm. Fertilization with biological products such as vermicompost, compost+leachate and compost presented a higher proportion and conservation of leaves over time.

Study Limitations/Implications: Grasslands are not seen as a crop so in most cases they are not fertilized; when they are, it is done with chemical fertilization so there is little information about organic fertilization in tropical fodders.

Conclusions: The optimal cutting point is from 50 DAC for chemical treatment, compost+leachate and vermicompost. Fertilization with vermicompost or compost+leachate can be an inexpensive and affordable option for producers to fertilize their meadows.

Keywords: *Urochloa maxima*, *Megathyrsus maxima*, compost, vermicompost, leachate.

INTRODUCTION

In tropical zones of Mexico, there is important diversity in grasses which are the most important source of feed in bovine and ovine livestock production; however, in most cases there is not an adequate management of grasslands, which is why it is difficult to apply

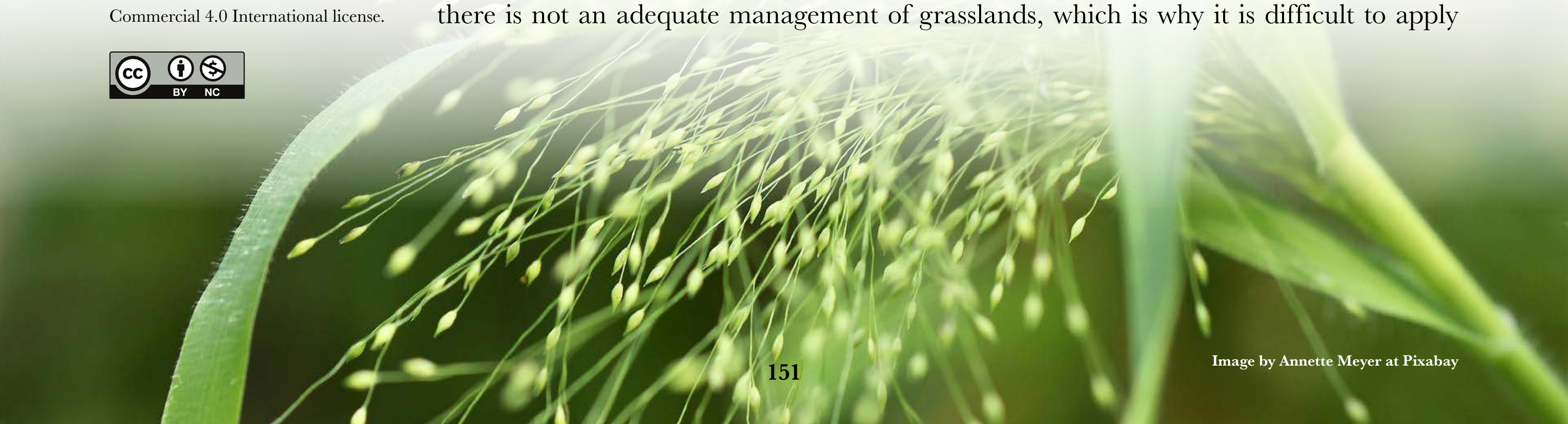

Image by Annette Meyer at Pixabay

nutrients, causing for the yield and the nutritional value of fodders to be low throughout the year (Suttie, 2003).

The grasslands on the Costa Chica are hardly considered to be a crop, which is why they are not fertilized, irrigated or given management regarding the entry and exit of livestock to allow a good establishment, density and quality. On the contrary, extensive grazing is carried out in the grasslands causing overexploitation of resources, and in general only the fire method is used to regenerate them, causing grave damage to the soil and the ecosystem (Rojas *et al.*, 2005). Because of this, it is necessary to generate information about the genotypes in their different growth stages, biomass accumulation, since it is documented that fodder quality can be determined indirectly with various analyses such as morphological composition and plant height, which can be used as indicators of the nutritional value of the fodder harvested (Araya and Boschini, 2005).

Organic fertilization in grasslands represents an opportunity to improve the grass quality, in addition to producers being able to compost animal manure and apply it, accompanied by leaf fertilization with leachates; however, there is scarce information about the adequate doses for grasslands, and only in some grasses (Sánchez-Santillán *et al.*, 2021; Jiménez *et al.*, 2010).

The *Panicum maximum* cultivar Tanzania grass is reported as an option in animal feed due to its high dry matter yield and quality (Andrade-Solórzano *et al.*, 2020), although there is not enough information about the response to organic fertilization. This is the reason why the objective was to evaluate the accumulation of dry matter, morphological composition, and height of the *Panicum maximum* cv. Tanzania grass under four fertilization plans, to determine the optimal cutting point.

MATERIALS AND METHODS

Experimental site

The study was performed in the Costa Chica region of the state of Guerrero in the municipality of San Luis Acatlán, at the *Campus* of Universidad Autónoma Chapingo (16° 51' 28.99" N and 98° 43' 26.13" W, at 311 masl). The region's climate is tropical with summer rains, with mean annual precipitation of 1395 mm (García, 2004), and data were also taken from the station nearest to the study zone (Figure 1). Soil analysis was conducted prior to sowing, where a sandy clay loam texture was found, with pH of 5.95, 4.22% organic matter, electric conductivity of 0.18 dSm^{-1}, apparent density of 1.30 g cm^3, free of total carbonates (0.01%), and moderately low in phosphorus (10.6 ppm).

Plant material and establishment

The land was prepared with two rake passes, on a surface of 5000 m^2. The establishment was carried out in August 2017, and the plant material was *Panicum maximum* cv. Tanzania grass. Three separate strips were established at 1 m between strip, and four lines in each strip separated by 20 cm. The density used was 6 kg ha^{-1} of pure sprouting seed. Weed management was performed manually. The evaluations were made after one year of grass establishment.

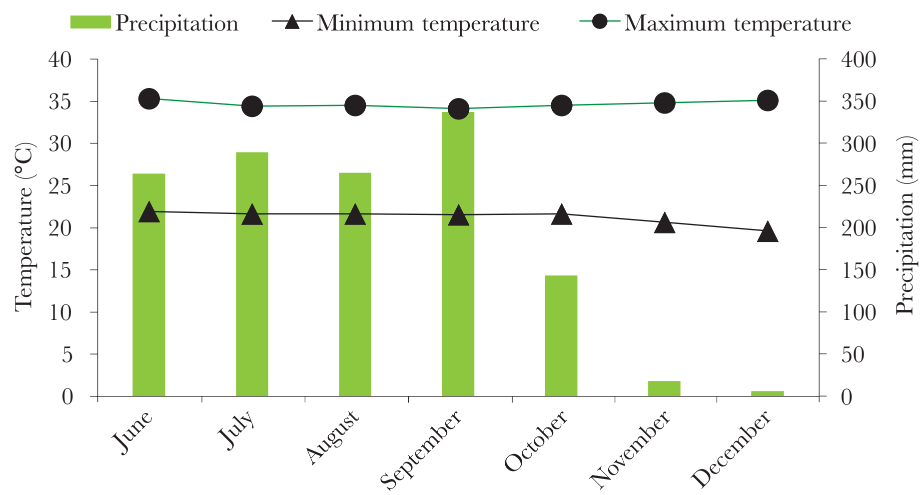

Figure 1. Maximum and minimum monthly mean temperature and accumulated rainfall during the study period. Source: Meteorological station located in El Carmen, San Luis Acatlán, Guerrero, Mexico.

Treatments

The grassland had one year of establishment when the homogenization cutting of the grass was made. The treatments were applied 15 days after the homogenization cut (DAC). Four fertilization plans were established: T1) chemical 120-60-00 NPK, where the sources of nitrogen and phosphorus were urea and DAP, respectively; T2) a dose of 10 t ha^{-1} vermicompost was applied; T3) consisted in 10 t ha^{-1} of compost; and T4) was 10 t of compost plus three applications of leachate to the fodder at intervals of 7 days, at a dose of 20%, applied with a manual aspersion backpack and an adjustable nozzle at 40 psi.

Variables

Sampling was done every fourteen days, with a total of six, which began at twenty days after homogenization cutting. Each sample was taken randomly, throwing a rod frame of 0.5 m $\times$ 0.5 m (0.25 m^2), from which all the fodder was cut at a height of 10 cm. The sample was weighed green with an electric scale (Truper, 40 kg); later, the grass was homogenized and a representative sub-sample was taken of 100 g, which was separated into leaf, stem, spike and dead material; it was placed in a paper bag and introduced into a forced air furnace (APSA) at a temperature of 55 °C until constant weight, which was done with the 100 g separated into the components. The dry matter yield per hectare and the percentage of the four morphological components were estimated (Cruz *et al*., 2011). The height of each experimental unit was taken with a ruler with ten repetitions, and the average was obtained. The samplings were made at 20, 35, 50, 65, 80 and 105 DAC of homogenization.

Statistical analysis

A completely randomized block design was established, with repeated measurements arranged in time, three blocks were traced perpendicularly to the slope, and an analysis of variance was conducted with the SAS (Statistical Analysis System Version 9.0 for Windows

2011) Proc Mixed procedure, as well as Tukey's means comparison ($p \leq 0.05$). The Curve Expert Professional 2.0 software (Curve Expert Computer Software. Vers 2.0 N.p. D.d. Web) was used to adjust the growth curves according to the most adequate model with its respective model and coefficient for each type of fertilization evaluated.

RESULTS AND DISCUSSION

The fodder accumulation curve with base treatment of vermicompost was adjusted to an exponential growth model (R^2=96%). The maximum dry matter accumulation was obtained with T2 (vermicompost), which happened at 80 d, with a total of 25,288.2 kg DM ha^{-1} (Figure 2); however, there was no difference compared to the other treatments in the same sampling date ($p \leq 0.05$). Likewise, for the case of the dates 20 and 35 DAC there was no difference ($p \leq 0.05$), although these two dates showed difference with the dates 50, 65, 80 and 95 DAC ($p \leq 0.05$).

The date that presented a maximum leaf accumulation was 50 DAC, with 14,830 kg ha^{-1}, which represents a leaf-stem rate of 2.9. After this date, the amount of leaf decreased, while the amount of leaf, inflorescence and dead material increased the same as all the treatments except the treatment with compost, where most of the leaf accumulation was found at 80 DAC.

The results obtained in this study are similar to those reported by Cornejo *et al.* (2019), who report that the Tanzania grass presented a yield of 3.21 t DM ha^{-1} at 30 DAC; however, in that study no type of fertilization is reported.

T1 (chemical) showed a maximum dry matter accumulation at 80 DAC with a yield of 24,516 kg DM ha^{-1}; however, this yield was equal to the cuts from dates 50, 65 and 95 DAC ($p \leq 0.05$) (Figure 3). The cutting date when the most leaf accumulated was at 50 DAC with 11,043 kg DM ha^{-1}. The dry matter accumulation curve with the chemical fertilization treatment was adjusted to an exponential model (R^2=95%), since it is equal ($p \leq 0.05$) in the first two dates of sampling, from 20 to 35 DAC, although the dry matter increases. However, from date 35 DAC to 50 DAC an exponential increase is seen, from 6,532 to 20,743 kg DM ha^{-1}, which later remains similar until the end of the curve, since

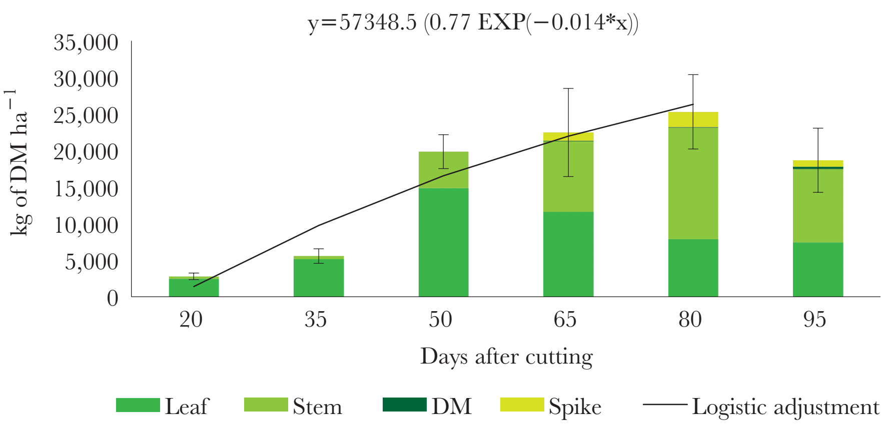

Figure 2. Dry matter accumulation and morphological composition of the Tanzania grass under a fertilization system with vermicompost.

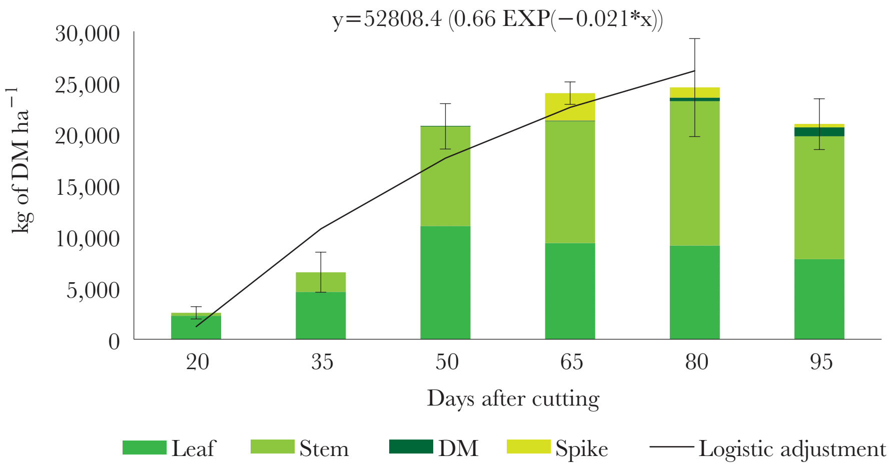

Figure 3. Dry matter accumulation and morphological composition of Tanzania grass under a chemical fertilization system.

there were no differences from 50 to 95 DAC. Likewise, an increase in stem production was seen from date 50 to 80 DAC, until a decrease of all morphological components in the last date of sampling.

The values obtained in this experiment are higher than those reported by Fortes *et al.* (2014), who report a yield of 3.94 kg of DM ha^{-1} at 90 DAC with a treatment of 50 kg of N ha^{-1}; this situation is possibly because the authors indicate that the experiment was developed in the dry season, which made the development of grass difficult.

T4 (compost+leachate) showed a constant increase of dry matter accumulation, since the maximum yield was found in the last date of sampling at 95 DAC with 23,963 kg DM ha^{-1}, contrary to what happened with the other treatments where the maximum yield was at 80 DAC. However, the maximum accumulation of the leaf component also happened at 50 DAC with 10,165 kg DM ha^{-1}. The first two dates of sampling behaved equally ($p \leq 0.05$), although different from the subsequent four dates (Figure 4). The dry matter accumulation curve was adjusted with an exponential model (R^2=98%).

The treatment that had lowest dry matter accumulation was T3 (compost), since at 80 DAC a yield of 20,833 kg DM ha^{-1} was reached, and the same happened with leaf accumulation which was 8,472 kg ha^{-1}, although compared to the other treatments where the maximum amount of leaf happened at 50 DAC. With fertilization based on compost, it happened at 80 DAC, which coincides with the maximum accumulation of the totality of the morphological components (Figure 5). The best adjustment for dry matter accumulation was the exponential model (R^2=99%).

Regarding the comparison of treatments between the same dates of sampling, no differences were observed ($p \leq 0.05$). This indicates that all the treatments can be an option to have some fodder available in the production systems (Luna *et al.*, 2015).

The height variable presented differences only in the cuts at 50 and 65 DAC, where T1 showed differences with the organic fertilization treatments ($p \leq 0.05$); however, in the dates 80 and 95 DAC there were no differences between any of the treatments (Table 1).

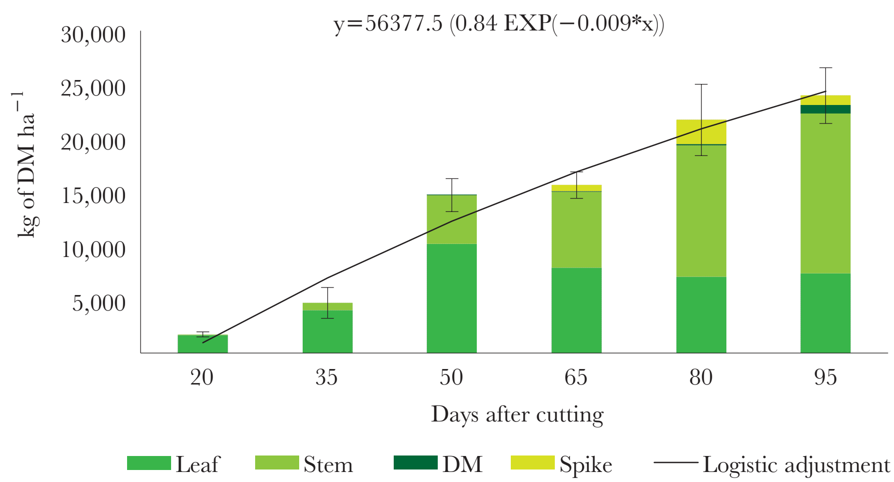

Figure 4. Dry matter accumulation and morphological composition of Tanzania grass under a fertilization system with compost+leachate.

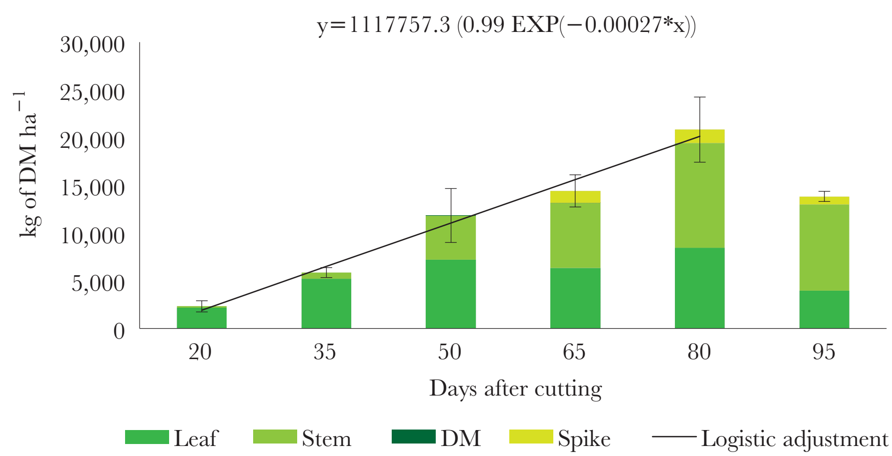

Figure 5. Dry matter accumulation and morphological composition of Tanzania grass under a fertilization system with compost.

Table 1. Tanzania grass heights (cm) in different cutting stages under different fertilization plans.

Treatment	Days after cutting					
	20	35	50	65	80	95
T3: Compost	66[a]	102a	143a	183[a]	187a	186a
T2: Vermicompost	67[a]	107a	152a	188[a]	193a	192a
T4: C+L	59[a]	99a	146a	189[a]	197a	195a
T1: Chemical	67[a]	115a	185b	204.7b	206.2a	199a

Different literals within each column indicate difference ($p \leq 0.05$).
C+L=compost+leachate

The results from this study are similar to those obtained by Sánchez-Hernández *et al.*, (2019), who report a height of 214.5 cm at 85 DAC with chemical fertilization, suggesting that this type of grass adapts well in tropical climates and that it is an option both for cutting and for grazing.

Nitrogenous fertilization in grasses affects the growth and quality of fodders, since when it is available, the development of plants will be faster with the more that this element is available. However, chemical fertilizers can alter the chemical properties of soils, generating environmental damages; therefore, organic fertilization is presented as an agroecological alternative to improve grass quality, increasing the availability of soil nutrients in the long term and conserving its physical and chemical properties, in addition to protecting the soil's fauna (Álvarez *et al*., 2016).

CONCLUSIONS

The organic treatments such as compost, vermicompost and leachate represent an option to fertilize the grasslands with Tanzania grass; likewise, the optimal cutting point is from 50 DAC when fertilized with chemical fertilizer, compost plus leachate, and vermicompost, although it can be until 80 DAC when it is fertilized with compost, since the quality is maintained until that date by the amount of leaf that there is in relation to the stem, although with lower dry matter yields than the other three treatments.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Meat production and quality of rabbits fed different diets and a biological activator

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ABSTRACT

Objective: To evaluate the effect of four diets on the productive performance of rabbits, carcass yields, meat color and pH.

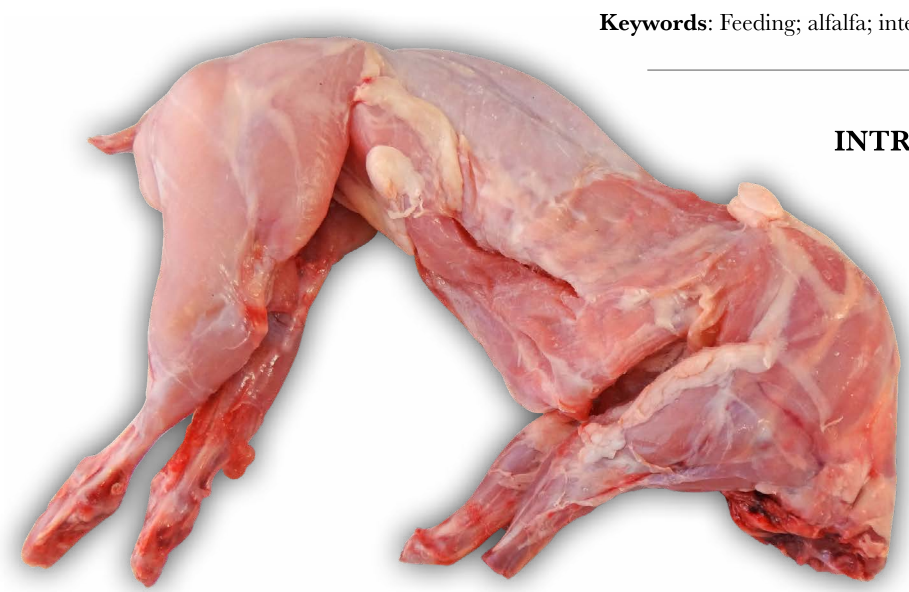
Design/Methodology/Approach: Thirty-two California breed rabbits were randomly assigned to each of the following diets: commercial feed alone (CF); commercial feed + biological-activator (CF + BA); alfalfa fodder + biological activator (AF + BA), and integral feed + biological-activator (IF + BA). The following were evaluated in the animals: daily weight gain (DWG), feed conversion (FC), daily feed intake (DFI), body weight (BW), empty body weight (EBW) and carcass yields (CY). The following were determined in the meat: pH, luminosity (L^*), red color (a^*) and yellow color (b^*). Means were compared using Duncan's test ($\alpha=0.05$).

Results: The commercial feed diets showed ($P<0.05$) higher values of DWG, DFI, BW and EBW than the other diets. The "commercial feed + biological-activator" diet produced ($P<0.05$) in general higher carcass weights and yields than the alfalfa-based diet, which produced ($P<0.05$) a higher pH and yellow color in the meat than the "commercial feed alone" diet.

Study Limitations/Implications: Feeding the rabbits by adding the biological-activator improves performance, meat color and pH.

Findings/Conclusions: Adding the biological-activator to the commercial feed improves the yield of empty body weight; adding it to the alfalfa fodder and comparing it with the commercial feed (alone or with activator) improves both the meat color (making it more yellow) and the pH (making it higher).

Keywords: Feeding; alfalfa; integral diet; weight gain; California breed.



INTRODUCTION

The livestock production sector in Mexico has been transformed at an unprecedented pace in recent decades, which is why the growing demand for foods of animal origin has been satisfied primarily by commercial livestock production and its associated chains. At the same time, millions of people in rural areas still breed livestock through traditional production systems, on which they base their means of subsistence and their family food security (García-Martínez, 2015). On the other hand, domestication of the rabbit has allowed its production in the livestock production sector and is considered, until today, a modest sector without growth at the national level (Del Toro *et al.*, 2016). However, rabbit production is characterized by the benefits that it represents, from which the following can be obtained: meat, skin, hair, urine, excrement and limbs. In addition, because of their short reproduction cycle, they require small spaces and have excellent productivity (Garcia 2012). These arguments are reinforced by Méndez-Zamora *et al.* (2016), who mention that rabbit meat is considered healthy because it has low cholesterol levels, similar to those of chicken and turkey meat. It also has high protein content, low content of sodium, fat and cholesterol, and characteristics that are apt for production at the small and medium scale (Selim *et al.*, 2021). Because of all these advantages, rabbit production is considered an alternative to satisfy the present and future dietary needs of sectors of the population that are economically disadvantaged, both in rural and urban areas (Nieves *et al.*, 2002). Therefore, the identification of adequate feeds is important, since it is not feasible to use similar ingredients in different geographical areas (Nieves *et al.*, 2009). Because of this, and with the purpose of reducing the indiscriminate use of antibiotics in animal production, studies have explored the use of various alternatives, including probiotics, which represent a potentially significant and safe therapeutic advance (Castro and Rodríguez, 2005). For example, vitafer is a biological product composed of bacteria, yeasts and their metabolites, which in addition contains vitamins and enzymes (Rodríguez *et al.*, 2013; Lazo-Pérez *et al.*, 2017). Therefore, the objective of this study was to determine the effects of four isoproteic diets on the productive behavior of rabbit, carcass yields, meat color and pH.

MATERIALS AND METHODS

The study was conducted in two phases: 1) Growth and fattening stage of the animals, which was carried out in the rabbit production unit of Instituto Tecnológico del Valle de Oaxaca, located in Ex-Hacienda de Nazareno, Xoxocotlán, Oaxaca. 2) Laboratory analysis of the products of animal origin in the FMVZ of Universidad Autónoma Benito Juárez de Oaxaca with address: Avenida Universidad S/N. Ex-Hacienda 5 Señores, Oaxaca, Mexico. This institute is located in the region Valles Centrales of the state of Oaxaca, at coordinates 17° 01' 16" latitude North and 96° 45' 51" longitude West, where Vertisol soils predominate (INEGI, 2010); with average temperature of 20.4 °C, maximum of 23.01 in the month of May, minimum of 17.51 °C in the months of December and January; with an average annual precipitation of 669.9 mm, the month of September is the one with most rainfall of 142.8 mm (CONAGUA, 2019). Thirty-two (32) rabbits of the California breed were used, of 35 d^{-1} of age, which were evaluated during 58 d^{-1} of fattening in the period of April to June 2016. The management of the animals was done intensively in wire cages (1.5×1.5 m), adapted with PVC troughs and water dispensers with plastic containers elaborated manually. Four diets were evaluated: commercial feed alone (CF); commercial feed + biological-activator (CF+BA); alfalfa fodder + biological-activator (AF+BA); and integral feed + biological-activator (IF+BA). The treatments were distributed in a completely randomized design, with 8 repetitions. The biological activator was elaborated previously based on: molasses at 10%, urea 0.5%, sulfates 0.3%, mineral salts 0.3%, soy paste 4%, corn 4%, lactobacillus 2%, and water 12%. The source of lactobacillus was Yakult (Ingredients: water, sugar, 3.6% powdered skim milk, glucose, artificial flavoring, *Lactobacillos casei* Shirota 108 Colony Forming Units (CFU/ml) (which means that the product contains 100 million bacteria) and two types of sweeteners: sugarcane and glucose. The activator was added directly to the feed at a rate of 15 ml per kg of LW. The concentrated meal in pellet form used was of commercial brand Malta Cleyton with 16% of protein. The alfalfa fodder was chopped into small particles of 2 to 3 cm and tedded; then, the activator was added and it was supplied directly in the feeding troughs. The treatment with integral diet was previously balanced at a level of protein inclusion of 16%, with the following ingredients: corn, soy paste, alfalfa, grass (Maralfalfa), mineral salts, and molasses.

The daily portion of feed that was supplied was 150 g of commercial feed or its equivalent of alfalfa fodder, and of the integral diet, at different hours of the day: 75 g in the morning and 75 g in the afternoon. The feed offered during the first week was 50% of the total portion, to later offer 100% of the total daily portion until the experiment ended.

The variables determined were: daily feed intake (DFI, obtained from the division of the total feed consumed by the live weight kilograms), daily weight gain (DWG, quantified weekly through the difference of final weight minus the initial weight by the number of days), and feed conversion (FC, obtained from daily feed intake over daily weight gain).

The sacrifice was done through the conventional methodology (NOM-033-ZOO-1995). The non-meat components (skin, legs, head, green and red entrails, gastrointestinal content) and the warm carcass were weighed after the sacrifice. The weighing of the cold carcass was done 24 h *post-mortem*. The variables quantified were: live weight, empty body weight and yield (EBWY, the empty body refers to the clean carcass without entrails and skin), warm (WCWY) and cold (CCWY) carcass weight and yield based on the weight of the empty body at 24 h *post-mortem* when the weighing of the cold carcass was done and the following variables were recorded on the *Longissimmus dorsi* muscle: intensity of luminosity (L*), of red color (a*) and of yellow color (b*), and the pH of the meat was also measured. A portable spectrophotometer brand Konica Minolta model CM-600D-Japan was used, which was previously calibrated to white. The measurement was performed three times per each meat sample in order to obtain an average and to have more accurate information. Likewise, the pH was measured three times with a general pH-501 potentiometer to obtain the average.

The data were subjected to an analysis of variance (ANOVA) and when statistical differences were found between treatments, Duncan's test was used for the multiple means comparison. All the statistical analyses were conducted with SAS (2016) using a significance level of 0.05.

RESULTS AND DISCUSSION

Productive behavior: The differences in daily weight gain (DWG) were highly significant ($p<0.01$) between treatments, as was the variable FC ($p<0.05$). Regarding the daily feed intake (DFI) there were also significant differences ($p<0.01$). The calculations of the coefficient of variation were found to be in a range of 2 to 15%, which indicates that the tests can be considered reliable (Coyac *et al*., 2013).

The variables DWG, FC and DFI showed significant differences between treatments (Table 1) and it was documented that the best response for the variables DWG and FC (20.75 and 4.6 g/g) was presented by the rabbits fed with the treatment based on meal with the addition of the biological activator. The DWI was lower in the treatment that represented the integral diet with the activator (129 g/d).

The results were higher compared to those reported by Nieves *et al*. (2002) who found 19.11 g for the DWG variable in fattening rabbits with a dietary mixture in form of *Leucaena leucocephala* flour; the difference in the better response of this variable in this study can be attributed to the efficiency of the use of the biological activator in the rabbits' diet. For the DFI the same author reported 58.57 g, value that is below what was found in this study, results that can be attributed to the amount of fiber from Leucaena, which could indicate the immediate sensation of fullness, although not because of this reason did it contain the necessary nutrients for the productive parameters. On the other hand, Ponce de León *et al*. (2002) found values between 16 and 20 g of daily weight gain for the breeds Chinchilla, California and New Zealand. Regarding the FC, the results exceed numerically the 3.5 g/g reported by Peniche *et al*. (2010) using balanced meal and 3.4 g/g with tulip and breadnut fodder.

Diet evaluations in rabbits that have been conducted previously with the inclusion of hydroponic green oats fodder in different percentages have shown values of 29.10, 26.18, 24.40, 23.49 and 16.35 g of daily weight gain per animal (Fuentes-Carmona *et al*., 2011). Other studies have found DWG of 26.69, 23.46, 22.00, 19.98 g with the inclusion of the Creole mango fruit in different percentages (Palma and Hurtado, 2010), as well as the daily weight gain of 29.49, 21.85 and 26.00 g with the inclusion of fodder shrub legumes of river tamarind (*Leucaena leucocephala*), naranjillo (*Trichanchera gigantea*) and mulberry (*Morus alba*) (Nieves *et al*., 2009). Other studies have reported 20, 19 and 18 g of daily weight gain with the inclusion of flour from fruits and leaves of the breadfruit tree (*Artocarpus altilis*) (Leyva *et al*., 2012). As can be observed there is great variation in the daily weight gain using fodder in rabbits' diet. The variations can be attributed to the different levels of protein used, among other experimental conditions.

Weight and yield in carcass: It was found that commercial feed alone, commercial feed + biological activator, presented higher averages ($p<0.05$) of live weight (LW), empty

Table 1. Averages of the variables of productive behavior of California breed rabbits with four types of diets.

Variable	Diets			
	CF	CF + BA	AF + BA	IF + BA
DWG, g	$19.6^{a} \pm 3.7$	$20.7^{a} \pm 1.94$	$11.1^{c} \pm 2.5$	$15.2^{b} \pm 2.5$
FC	$3.7^{b} \pm 48$	$4.6^{b} \pm 33$	$3.8^{a} \pm 29$	$4.0^{b} \pm 62$
DFI, g	$138^{a} \pm 2.5$	$136^{a} \pm 3.8$	$130^{b} \pm 1.6$	$129^{b} \pm 2.4$

CF: Commercial feed, BA: Biological activator, AF: Alfalfa fodder, IF: Integral feed, DWG: Daily weight gain; FC: Feed conversion, DFI: Daily feed intake. [abc] Different letters in rows indicate statistical difference (Duncan $p<0.05$).

body weight (EBW), warm carcass weight (WCW) and cold carcass weight (CCW); while alfalfa fodder + biological activator and integral feed + biological activator had the lowest averages of these variables (Table 2). These effects can be attributed to the last two diets having a higher proportion of fodder. In this regard, Fuentes-Carmona *et al.* (2011) indicated that the time of fattening with this type of diet was more prolonged. The averages of the empty body weight yield (EBWY) were affected ($p<0.05$) by the type of diet, since the animals that consumed commercial feed + biological activator presented the highest average, compared to the other three diets. This result can be attributed to the other three diets forming higher gastrointestinal content which caused a decrease in the yield. The warm carcass yield based on the empty body weight (WCYEBW) was lower ($p<0.05$) compared to the three remaining diets. The cold carcass yield based on the empty body weight (CCYEBW) was not affected by the type of diet, presenting a general average of 52.27%.

Regarding the values obtained in this study (WCYEBW, Table 2), the carcass yields obtained by Vásquez *et al.* (2007) in New Zealand and Chinchilla rabbits were slightly higher, 55.40%. On the other hand, for this variable, Pascual *et al.* (2005) found values of 54.31 and 54.14% in rabbits selected due to their growth speed, while Barrón *et al.* (2004) found higher values (56.7 and 55.6%) for the California breed.

Variables of pH and color: It can be seen (Table 3) that the variables pH and b* were significantly different ($p<0.05$) between diets, while the variables L* and a* were not different ($p>0.05$). Table 3 shows the pH and the intensities L*, a* and b* of the meat (*Longissimus dorsi* muscle) of the rabbits under study. The pH of the meat was higher ($p<0.05$) with the diet of alfalfa fodder + biological activator than with the diet of commercial feed alone. However, the four treatments were found within the normal range, considering an adequate pH for quality meat to be within a range of 5.6 to 5.9. The intensities L* and a* did not change ($p>0.05$) between diets. The intensity of yellow was higher ($p<0.05$) with the diet of alfalfa fodder + biological activator than with the diets based on commercial feed (Table 3).

Table 2. Averages of weight and yield of the California breed rabbit carcasses subjected to four types of diets.

Variables	Diets			
	CF	CF + BA	AF + BA	IF + BA
LW, g	2097.4^{a} ± 72.2	2106.4^{a} ± 86.3	1630.0^{b} ± 93.2	1773.0^{b} ± 80.7
EBW, g	1873.2^{a} ± 69.1	1903.1^{a} ± 83.4	1393.3^{b} ± 90.1	1525.5^{b} ± 78.1
EBWY, %	89.3^{a} ± 0.1	90.2^{b} ± 0.9	85.2^{a} ± 0.9	85.8^{a} ± 0.8
WCW, g	911.2^{ab} ± 14.9	947.5^{a} ± 17.5	867.4^{b} ± 20.5	884.6^{b} ± 16.1
WCYEBW, %	52.8^{ab} ± 0.7	53.8^{a} ± 0.9	51.6^{b} ± 0.9	52.8^{ab} ± 0.8
CCW, g	906^{ab} ± 17.6	941.32^{a} ± 20.7	848.97^{b} ± 24.2	877.76^{b} ± 19.2
CCYEBW, %	52.6^{a} ± 0.9	53.4^{a} ± 1.1	50.3^{a} ± 1.3	52.5^{a} ± 1.0

^{abc} Different letters in rows indicate statistical difference ($p<0,05$). CF: Commercial feed, BA: Biological activator, AF: Alfalfa fodder, IF: Integral feed, LW: Live weight, EBW: Empty body weight, EBWY: Empty body weight yield; WCW: Warm carcass weight, WCYEBW: Warm carcass yield based on the empty body weight, CCW: Cold carcass weight, CCYEBW: Cold carcass yield based on the empty body weight.

Table 3. Means of pH and color (L* a* b*) of the California breed rabbit carcass with four types of diets.

Variables	Diets			
	CF	**CF + BA**	**AF + BA**	**IF + BA**
pH	$5.8^{b} \pm 0.11$	$5.9^{ab} \pm 0.14$	$6.0^{a} \pm 0.06$	$5.8^{ab} \pm 0.19$
L*	$61.5^{a} \pm 2.8$	$61.4^{a} \pm 2.41$	$64.5^{a} \pm 3.41$	$64.4^{a} \pm 3.57$
a*	$-2.7^{a} \pm 0.37$	$-2.5^{a} \pm 0.96$	$-1.2^{a} \pm 2.02$	$-1.69^{a} \pm 2.05$
b*	$9.7^{b} \pm 1.19$	$9.6^{b} \pm 1.18$	$11.8^{a} \pm 2.09$	$10.8^{ab} \pm 1.52$

CF: Commercial feed, BA: Biological activator, AF: Alfalfa fodder, IF: Integral feed, L*: Luminosity, a*: green-red and b*: blue-yellow. abc Different letters in rows indicate statistical difference (Duncan $p<0.05$).

The results found in this experiment differ from what was found by Hernández *et al.* (2015), who reported a pH value of 6.27 for the California breed, in the *Longissimus lumborum* muscle, and a pH of 6.26 for the *Bíceps femoris* muscle. Another study carried out by Volek *et al.* (2014) found pH values of 5.61 and 5.58 in rabbit meat with two population densities. As for the results obtained in this study (Table 3), the pH values were approximate to those mentioned by García *et al.* (2012), with values of 5.66 and 5.64. On the other hand, Simonova *et al.* (2010) found values of 5.61-5.71, Bianospino *et al.* (2006) 5.57-5.61, Simitzis *et al.* (2014) 5.53, 5.52 and 5.53, with the addition of different percentages of herperisine in the rabbits' diet.

The intensities of color are similar to those found in a study carried out by Hernández *et al.* (2015) who measured these characteristics in different rabbit genotypes, finding values of L* (58.80, 63.21, 61.70); a* (0.11, 1.76, 1.29); b* (6.72, 12.61. 10.75). Simitzis *et al.* (2014) reported similar values to those of this study: L*52.3, 51.8, 52.1 and a* 3.55, 3.38, 3.41; and different in this variable: b* 9.44, 9.78, 9.56. On the other hand, Simonova *et al.* (2010) reported value ranges lower than those in this study, with values of L* 48.17-51.07; a* 1.49- 4.20; b* 8.45-9.07.

CONCLUSIONS

The treatment commercial feed + biological activator presented higher yield in the empty body weight. Therefore, the addition of the biological activator to the feed allowed a better use of the nutrients by the rabbits. Adding the biological activator to the commercial feed improves the empty body yield, adding it to the alfalfa fodder and comparing it with the commercial feed (alone or with activator), improves the color of the meat by making it more yellow, and improves the pH making it higher.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Vegetative propagation of bird's-foot trefoil (*Lotus corniculatus* L.) using different rooting agents

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ABSTRACT

Objective: To evaluate the growth dynamics based on the dry matter production of *Lotus corniculatus* L. variety 202700 and its morphological composition.

Design/Methodology/Approach: The study was carried out in greenhouses conditions, from December 2020 to May 2021. The effect of indole butyric acid (IBA) growth promoter in solid Radix 1500 and liquid Radix T 3000 presentation (S+RSolid and S+RLiquid) and a control (Substrate) was evaluated. A completely randomized design with three replications was used, each with twenty pots as replications. 1300 pots were planted, of which 20 were taken monthly from each treatment (30, 60, 90, 120 and 150 days) for subsequent data recording.

Results: S+RSolid was the one that presented the highest values followed by S+RLiquid and Control (S) respectively.

Study Limitations/Implications: Destructive sampling of less than 30 days or greater than 150 days was not considered.

Findings/Conclusions: The plants with the application of 1500 ppm of IBA registered the greatest response in terms of variables: number of leaves, number of stems, leaves (g), stems (g), roots (g), root height (cm), root volume (cm^3), greenness index, leaf area (cm^2/g) and plant height (cm), with respect to the rest of the treatments.

Keywords: *Lotus corniculatus* L., *Medicago sativa* L., *Trifolium repens* L., *Trifolium pratense* L.

Image by beauty of nature at Pixabay

INTRODUCTION

Bird's-foot trefoil (*Lotus corniculatus* L.,) is a Fabaceae plant, with relevant fodder potential (De los Santos and Steiner, 2003); it represents 90% of the area sown within the genus worldwide, mainly United States, Australia, Chile and Argentina (Escaray *et al.*, 2012). It presents adaptive qualities to diverse soil textures (Garcia, 2011), it also tolerates flooding, drought, saline soils, acidity, low fertility and phosphorus, and in associations it improves nitrogen availability (Striker *et al.*, 2005). It thrives under conditions where alfalfa (*Medicago sativa* L.), white clover (*Trifolium repens* L.) and red clover (*Trifolium pratense* L.) are unlikely to establish (Grant, 2009). Kirkbride (1999) mentions that Bird's-foot trefoil is a very variable species (it is taxonomically classified in different ways) and the most widely distributed of the *Lotus* varieties in the world. In terms of nutritional quality, it is mentioned that it is similar to the most common temperate legumes such as alfalfa, white clover and red clover for its excellent quality protein, which is 17.7 to 21.6% DM (Marley *et al.*, 2006). It contains condensed tannins that prevent timpanism in ruminants and protect protein degradation in the rumen (Ayala and Carámbula, 2009). *L. corniculatus* is an important option for livestock feed, it grows best in cold and humid climates, although also in tropical and subtropical climates. Like all fodders, it has marked disadvantages, such as slow initial establishment and low persistence (Ixtaina and Mujica, 2010).

Productivity and morphological and physiological characteristics of crops are modified by nutritional, genetic or specific factors such as regulators of growth development (Cortes *et al.*, 2019).

In the particular case of Mexico, there is little or no information about the behavior of different fodder ecotypes and the application of growth regulators. Growth regulators applied as rooting agents are used to induce and stimulate root development and stem thickening, through the content of nutrients and phytohormones that intervene in specific physiological processes, which, if not present in the plant make it difficult to achieve satisfactory development (Garay *et al.*, 2008). Indoleacetic acid and tryptophan (hormone and precursor) promote root cell division and plant growth (Chilon, E. and Chilon, J., 2015). Castrillon *et al.* (2008) studied the effects of indole-3-butyric acid (IBA), indole-3-acetic acid (IAA) and 1-naphthaleneacetic acid (NAA) on the survival of *Pushgay* cuttings in soil + peat and peat only, at different concentrations and showed that IBA at 200 mg/L in soil + peat presented the longest survival time, with 43 days; meanwhile, without regulator the cuttings survived only 21 days. Therefore, the objective of this study was to evaluate the effect of indolbutyric acid in liquid and solid form (RADIX®) on the rooting of bird's-foot trefoil stems in greenhouses.

MATERIALS AND METHODS

Location and date

The research was carried out in a glass greenhouse with passive ventilation by means of manually opened lateral vents at Colegio de Postgraduados, Campus Montecillo, Estado de México, located at an altitude of 2,250 masl. According to the Köppen's climate classification modified by García (2004), the region is C (W0) (W) b (i´) g, which corresponds to a temperate subhumid climate with summer rains and dry season in the winter, with low thermal fluctuation, mean annual rainfall of 686 mm and a mean annual temperature of 15.9 °C, with the month of May as the warmest and January as the coldest.

Plant matter

Original seed of genotype 202700 bird's-foot trefoil germplasm of erect habit originally from Uruguay was used, which was obtained through the Plant Genetic Resources Management Program at Colegio de Postgraduados, from the United States Department of Agriculture (USDA-ARS), Beltsville, Maryland, USA; this was evaluated for the first time since 1997, in an adaptability trial (García *et al.*, 2015; Álvarez *et al.*, 2018), but because the plants sown in the field have not generated seed they continue to be propagated by cuttings taken from the crown of those plants.

Treatments and experimental unit

The experiment was established in a completely randomized design (CRD), with three treatments, each with twenty pots as replications, every 30 days for 150 days.

Handling of potted plants

The substrate used was a mixture of agrolite (35.48%), peat moss (35.48%), vermiculite (3.22%), and soil (25.80%); a 17×17 cm bag was used (400 caliber with a capacity of 0.95 liters), which was filled with the mixture of substrates, irrigated at field capacity. The transplant began manually immediately, impregnating the rooting agent at 3 cm from the base two stems with the corresponding concentration and presentation; they were planted 4 cm deep in the pot, the distance between pots was separated 1 cm by treatments, watering was done weekly, and Captan® 50 was used to avoid root disease problems while no fertilization was done.

The treatments applied were the control, which consisted of substrate (S) without rooting agent; treatment two was substrate + solid rooting agent RADIX® 1500 (S+RSolid); and treatment three was substrate + liquid rooting agent RADIX® T 3000 (S+RLiquid). The experiment was established in December 2020 to April 2021. In the experiment, 1300 pots were used (to have enough repetitions for the experiment) in which cuttings from crowns of mother plants established in the field were transplanted (Garcia *et al.*, 2015; Alvarez *et al.*, 2018). The evaluations were carried out for 5 months, applying the destructive method, to observe the growth dynamics of the root and aerial part of the bird's-foot trefoil. The first sampling was performed at 30 days and, subsequently, cuttings were scheduled every 30 days, until reaching the age of 150 days (5 months).

Variables evaluated

Morphological composition

To determine the morphological composition, fodder harvested from the pots was separated into its components: leaves, stems, roots and dead material. They were placed in brown Kraft paper bags and weighed individually in a digital scale with a capacity of 500 g and an approximation of 0.01 g. They were then placed in a forced air furnace at a temperature of 55 °C for 72 hours to obtain the dry weight.

Plant height (PH)

A graduated ruler of 50 cm length and 1 mm of precision was used. The plant was separated from the pot and the ruler was placed from the root to the base of the stem and then from the base of the stem to the last leaf on the stem (top of the leaflets).

Leaf:stem ratio

The data originated from the morphological composition (leaf and stem) of the trefoil plants were used to estimate the leaf:stem ratio, which was calculated using the following formula:

$$L{:}\ S=LS$$

Where: L: S=Leaf: stem ratio. H=Dry weight of leaf component (g DM pot^{-1}); divided by T=Dry weight of stem component (g Ms pot^{-1}).

Greenness index (GI)

Chlorophyll readings were taken with the Minolta SPAD 502, designed for fast non-destructive determination, which evaluates quantitatively the intensity of leaf greenness (650 to 940 nm), obtaining averages (3 measurements of three upper leaves) of 20 plants per treatment. Chlorophyll concentration (μmol of chlorophyll per m^2 of leaf and in SPAD or CCI units) is an indirect indication of plant health and condition, and non-destructive sampling allows monitoring chlorophyll concentration in plants during the experiment (Sainz and Echeverría, 1998).

Root volume (RV)

The values were recorded by water volume difference with a 250 ml graduated cylinder which was filled to its capacity and the roots of 20 previously washed and dried plants were introduced.

Leaf area (LA)

In each treatment, trefoil stems were cut at pot level, separated into stem and leaves, which were placed on acetates and immediately taken to the CID, Inc. leaf area integrator, model CI-202 scanner, from which leaf area readings were obtained in cm^2.

Statistical analysis

An analysis of variance was performed with the GLM procedure of the Statistical Analysis System (SAS, 1999), under a completely randomized design with 3 treatments and 20 replications, the means comparison for each dependent variable with increasing age was performed using Tukey's test ($P<0.05$) and a regression analysis for each variable in order to describe the trend, coefficient of determination and significance.

Greenhouse temperature

The temperature inside the greenhouse was obtained with a digital maximum and minimum thermometer (BioTemp) placed at 1.60 meters from ground level next to the plant pots. The monthly maximum temperature ranged between 22 °C and 45 °C and the monthly minimum temperature ranged between 8 °C and 14 °C.

RESULTS AND DISCUSSION

Morphological composition

When analyzing the behavior of the variables evaluated during the establishment (Table 1), it was found that there were significant differences between treatments. The means comparison in number of leaves (NLeaves), number of stems (NStems), leaves, stems and roots of *Lotus corniculatus* L. at different ages, showed that the variables increased steadily and had statistical differences ($p<0.05$). The S+RSolid at 150 days of age showed the highest value of these variables, followed by the Control (S) and S+RLiquid treatments with no differences between them. This shows that in the case of the regulators, the best results were obtained with Radix 1500 (Solid), since it favored the number of stems, number of leaves, and weight of roots, stems and leaves (Table 1 and Figure 1) from the beginning to the end of the experiment. This could be due to the difference in the impregnation of the products, with powder being more favorable as it is released gradually on contact with the moisture of the substrate than in liquid and the latter being more concentrated. The regression models and coefficients of determination were high ($R^2>0.86$) for the variables evaluated; the best fitting model was the polynomial model (Figures 1 and 2) for the treatments.

Table 1. Statistical significance of means for morphological variables in *Lotus corniculatus* L.

DDTE (days)	Treatment	NLeaves	NStems	Leaves (g pot^{-1})	Stems (g pot^{-1})	Roots (g pot^{-1})
30	Control (S)	38 b	3 c	0.01 c	0.02 b	0.08 b
	S+ESolid	66 a	6 a	0.04 a	0.06 a	0.26 a
	S+ELiquid	45 b	4 b	0.03 b	0.05 a	0.10 b
60	Control (S)	125 ab	3 b	0.08 b	0.05 b	0.12 b
	S+ESolid	152 a	4 a	0.13 a	0.10 a	0.33 a
	S+ELiquid	116 b	3 b	0.08 b	0.06 b	0.15 b
90	Control (S)	171 b	6 b	0.13 b	0.07 b	0.18 b
	S+ESolid	334 a	9 a	0.35 a	0.21 a	0.46 a
	S+ELiquid	132 b	4 c	0.12 b	0.07 b	0.14 b
120	Control (S)	371 b	10 b	0.27 b	0.17 b	0.31 b
	S+ESolid	811 a	19 a	0.61 a	0.41 a	0.66 a
	S+ELiquid	390 b	9 c	0.30 b	0.17 b	0.28 b
150	Control (S)	581 c	13 c	0.51 c	0.26 c	0.42 c
	S+ESolid	1867 a	30 a	1.43 a	1.15 a	1.73 a
	S+ELiquid	858 b	15 b	0.74 b	0.50 b	0.76 b
	DMS	148	2	0.12	0.09	0.12

*a, b, c.=Different letters in the same column are statistically different. Tukey at 0.05. NLeaves=Number of leaves; NStems=Number of stems; Control (S)=Substrate without rooter; S+ESolid=Substrate + solid rooter; S+ELiquid=Substrate + liquid rooter DDTE=Days after cutting transplant; DMS: Minimum significant difference.

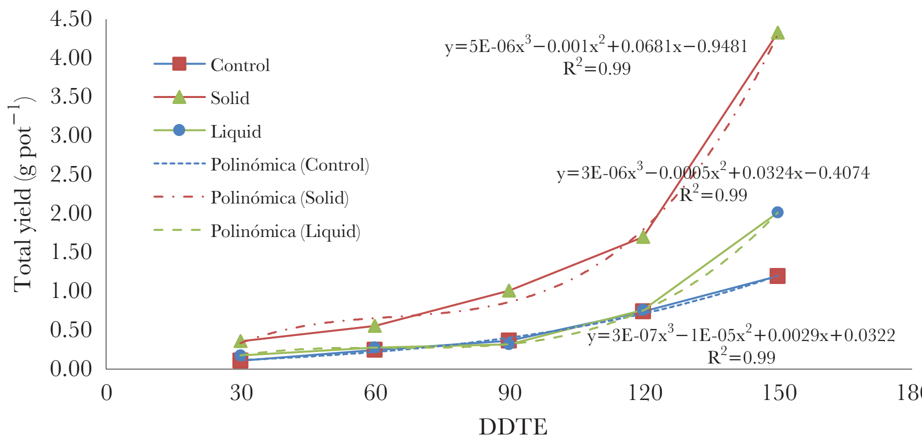

Figure 1. Average yield (g pot^{-1}) of *Lotus corniculatus* variety 202700, in plants 30 and 150 days after cutting transplant (DACT).

Plant height

Table 2 and Figure 2 show that there were differences between treatments, being S+RSolid with 29 cm the one that presented the highest values, followed by S+RLiquid with 22 cm and Control (S) with 20 cm, respectively, at 150 days. Garcia *et al.* (2015)

Table 2. Statistical significance of means for the variables leaf:stem ratio, root height, root volume, greenness index, leaf area and plant height in *Lotus corniculatus* L.

DDTE (days)	Treatment	H: T (g pot^{-1})	LR (cm)	Vol. (cm^3)	IV (μmol m^2)	AFE (cm^2/g)	AP (cm)
30	Control (S)	0.10 a	5.03 b	0.37 b	21.41 b	2.18 c	7.96 c
	S+ESolid	0.97 a	6.56 b	1.54 a	29.95 a	8.00 a	12.40 a
	S+ELiquid	0.86 a	10.26 a	1.57 a	24.55 b	5.32 b	10.52 b
60	Control (S)	1.80 a	18.09 a	0.94 b	30.47 ab	17.21 c	14.13 b
	S+ESolid	1.50 a	21.33 a	2.11 a	32.13 a	50.75 a	18.68 a
	S+ELiquid	1.33 a	17.17 a	1.94 a	26.13 b	43.24 b	14.64 b
90	Control (S)	2.27 a	22.81 b	2.66 b	28.26 c	22.70 b	15.24 b
	S+ESolid	1.69 a	32.29 a	5.84 a	38.47 a	61.35 a	19.93 a
	S+ELiquid	2.12 a	24.75 b	2.69 b	32.11 b	20.74 b	15.88 b
120	Control (S)	1.62 ab	33.81 b	6.13 b	27.85 b	38.03 b	16.28 c
	S+ESolid	1.50 b	38.60 a	14.18 a	34.75 a	90.02 a	21.81 a
	S+ELiquid	1.80 a	33.97 b	7.51 b	32.43 ab	45.31 b	17.51 b
150	Control (S)	1.97 a	30.27 b	9.19 c	41.19 b	46.53 c	20.22 c
	S+ESolid	1.24 c	37.47 a	27.71 a	45.79 a	252.65 a	29.49 a
	S+ELiquid	1.48 b	34.62 ab	15.69 b	41.51 b	115.97 b	21.85 b
	DMS	0.23	4.08	2.46	2.71	18.41	1.54

*a, b, c.=Different letters in the same column are statistically different. Tukey at 0.05. H: T=Leaf-Stem Relationship; LR=root length; Vol.=Root volume; Green index=Chlorophyll; AFE=Specific leaf area; AP=Plant height; Control S=Substrate without rooter; S+E Solid=Substrate + solid rooter; S+E Liquid= Substrate + liquid rooter; DDTE=Days after cutting transplant; DMS: Minimum significant difference.

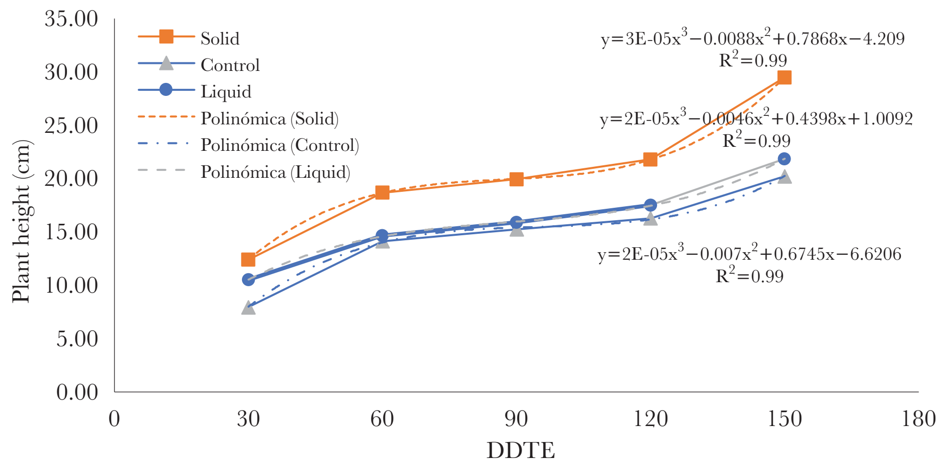

Figure 2. Average plant height (cm) of *Lotus corniculatus* variety 202700, in plants 30 and 150 days after cutting transplant (DACT).

mention that in the case of *Lotus corniculatus*, yield and height have also been related to erect and prostrate growth habits.

Leaf:stem ratio

When treatments were compared for the average leaf:stem ratio, no significant difference was found during the first 120 days, and it was not until 150 days when the Control (S) obtained the highest values, followed by S+RLiquid and S+RSolid (Table 2). The leaf:stem ratio values found were similar to those found in other studies. Berroterán (1989) obtained 2.02 in *Andropogon gayanus* and 0.61 in *Digitaria swazilandensis*; Calzada *et al.* (2014) obtained on average 0.73 in Maralfalfa grass (*Pennisetum* sp.), and the authors also mention that the greater the plant height, the greater the proportion of shaded leaves. Plant height presents a negative correlation with leaf biomass as plant height increases.

Greenness index

Regarding leaf greenness measured with the SPAD, the S+RSolid presented higher values than the S+RLiquid and Control (S) (Table 2). Wolfe *et al.* (1988) and Dwyer and Houwing (1991) mention that the chlorophyll content in the corn leaf is closely and positively related to the concentration of N in the leaf, and therefore, it reflects the nitrogenous state of the crop and the availability of N.

Root volume

Regarding root volume, it can be seen that it increased as the days of growth progressed (30-150 days) with the highest values for S+RSolid being 14 cm^3 followed by S+RLiquid 8 cm^3 and 6 cm^3 for control (S) at 120 days; and 28 cm^3 S+RSolid, 16 cm^3 S+RLiquid and, finally, control (S) 9 cm^3 at 150 days (Table 2). Rose *et al.* (1991) mention that root volume correlates positively with survival and growth, since these can prevail to transplanting stress, due to a greater capacity for water and nutrient hydration. Alzugaray *et al.* (2004)

reported that in plantations of the same species with greater volume, they presented a greater response to water stress by showing high concentrations of nutrients in the leaves, as well as a greater general growth of the plant.

The following researchers have shown that indole-3-butyric acid concentrations with a higher concentration (0.3%) provide the crop with better results in terms of root number, for example in *Dracaena deremensis* (Angulo, 2011). Rájala and Peltonen Sainio (2001) mention that applying growth regulators leads to an increase in root growth (increase in length and volume) and an increase in the root:stem ratio under field conditions. Likewise, Perez and Vertel (2010) found that macro, micro and trace elements in the rooting agent lead to this increase in plant growth.

In bird's-foot trefoil at 120 days it reached a length of 38 cm with Radix 1500 followed by Radix T 3000 with 34 cm and the control with 33 cm respectively.

The 0.15 (S+RSolid) and 0.3 % (S+RLiquid) AIB were applied as growth promoter and using soil + peat as substrates. Castrillón *et al.* (2008) report that AIB applications resulted in higher survival of cuttings in soil + peat substrate. Maldonado *et al.* (2017) in nanche *Malpighia mexicana* A., and *Byrsonima crassifolia* (L.) using Indolbutyric Acid (IBA) as rooting promoter in concentrations of 1000, 3000 and 10 000 ppm, and a control without rooting agent, planted in peat with sand, found that the two species of nanche presented low survival and sprouting, and concluded that the use of rooting promoters is necessary to obtain roots, and the propagation should be done under shade.

Leaf area (LA)

Leaf area began to increase from 30 days of age as the dry weight of leaves and the plant maturity increased; the maximum LA of 253 cm^2/g was presented at 150 days of age in S+RSolid, followed by S+RLiquid with 116 cm^2/g and control (S) with 46 cm^2/g, respectively. Bultynck *et al.* (1999) mention that LA is one of the main variables that can affect plant growth, by modifying the leaf area and the photosynthetic efficiency with respect to nitrogen use.

In the greenhouse where the experiment was carried out, the average temperature found was 8-43 °C (minimum and maximum, respectively). Ecke *et al.* (2004) found in studies carried out with Pascuas (*Euphorbia pulcherrima* Will. ex Klotzsch) that the ideal temperatures for rooting range between 22-24 °C if it propagates in a temperature higher than 26 °C, and its development is much slower and stops when the temperature is higher than 30 °C. Leakey and Mesén (1991) indicate experiences with other tropical species, where they show that the optimum air temperature that favors rooting is 20 to 25 °C; these data could have modified the results of the experiment in *Lotus corniculatus* L. since the plant height remained constant when temperatures were low.

CONCLUSIONS

The application of the rooting agent produced significant differences in the variables under study. The powdered rooting agent was the one that generated the most constant values, followed by the liquid and the control. The highest yields in green fodder and dry matter were achieved with the powdered rooting agent as the plant age increased from 30 to 150 days. Regarding the dynamics of root and aerial growth, the highest amount of DM accumulation occurred at four and five months. In addition, the use of rooting promoters is necessary to obtain a greater amount of roots, which help the plants to have an optimal development, reflected in the growth dynamics.

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AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Yield, intercepted radiation, and morphology of crotalaria (*Crotalaria juncea* L.) at different densities

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ABSTRACT

Objective: The objective of this study was to evaluate the yield, intercepted radiation, and morphology of crotalaria (*Crotalaria juncea* L.) at different planting densities, in the dry tropics of the state of Guerrero, Mexico.

Design/Methodology/Approach: The treatments were four planting densities: 400,000, 200,000, 100,000 plants ha^{-1} and overseed. Additionally, a growth analysis was carried out at 30, 38, 45, 52, 60, 68, and 75 days of growth, after the pod was fully developed. The following variables were evaluated: dry matter yield, intercepted radiation, and morphology composition.

Results: Regardless of the cutting age, the dry matter was recorded in the following descending order: 400,000 > overseed > 200,000 > 100,000 plants ha^{-1} planting densities, with 19,837, 17,918, 8,786, and 4,074 kg DM ha^{-1}, respectively.

Study Limitations/Implications: In order to improve livestock feeding in the tropics, the perspective of the producers about the use of pulses as forage must be broadened.

Findings/Conclusions: A 400,000 crotalaria plants ha^{-1} planting density and cuts after 45 days of growth are recommended. During this period, the meadow reaches its optimal structural characteristics, while the intercepted radiation reaches 95%.

Keywords: Pulses, intercepted radiation, yield, plant density.

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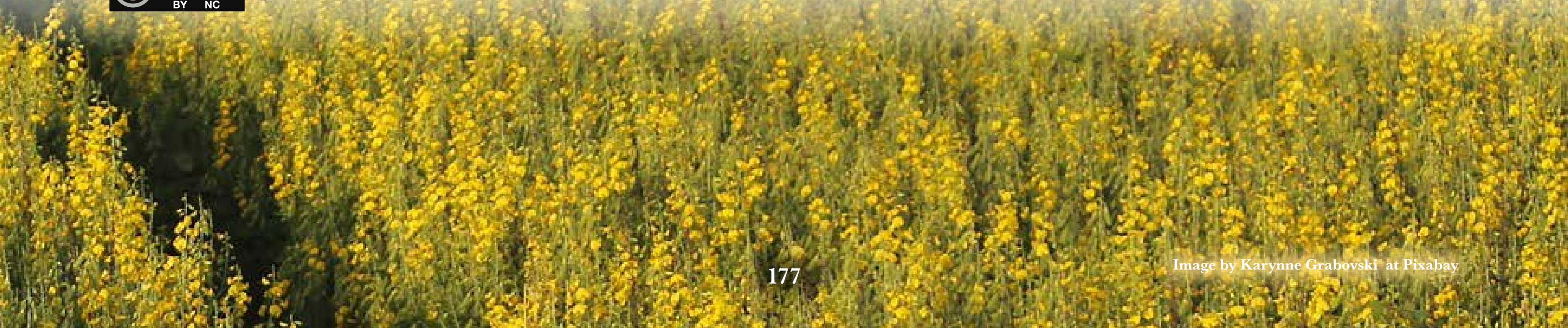
INTRODUCTION

In the livestock-raising regions of the state of Guerrero, ruminant production is carried out under an extensive system and feeding is based on stubble and native grasses with little nutrient content. This type of feeding reduces the weight and number of animals and, consequently, the profits of the producers. In view of this situation, the option is to use cutting and annual species, which can be transformed into flour or silage and can be used as supplement during the dry season (Tui *et al.*, 2015; Castro *et al.*, 2017). Including pulses in the ruminant diets increases the nutrition value of the feed; additionally, this



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type of plants has been proven to be beneficial to the environment (Douxchamps *et al.*, 2014). For instance, they are used in the recovery of degraded soil (as coverage, green manure, or environmental nitrogen fixators), reducing greenhouse gasses (McSorley, 2001; Prudhomme *et al.*, 2017).

Crotolaria (*Crotalaria juncea* L.) is an annual species, native to India, which belongs to the family Fabaceae. Genus *Crotalaria* is made up of 350 species; they develop and grow in humid and dry tropics (Santana and Ascencio, 2011). Several studies about the production condition of this species mention that it produces high dry matter yields (5.0-12.5 t ha^{-1}) (Akanvou *et al.*, 2001; Jiménez *et al.*, 2005; Li and Stoffellia, 2002). Additionally, this pulse is an excellent green manure and vegetal coverage (Almeida-Santos *et al.*, 2019). Its presence in the soil increases the nutrient content and yield of different species (mainly grasses) which grow in association or rotation (Lemaire *et al.*, 2014; Muraoka *et al.*, 2002). There is a relationship between the phenological state and the physical quality and nutrition parameters in vegetable species. If they produce a large quantity of leaves, they have a higher quality. However, when the size and number of stems increase, the quality diminishes (Lemaire, 2001). Yield can be the opposite of quality. In late stages, yield is higher, but quality is lower. Determining the seasonal distribution of yield involves a search for balance between quantity and quality of forage species (Castro *et al.*, 2012; Matthew *et al.*, 2001). Therefore, the objective of this study was to evaluate the yield, intercepted radiation, and morphology of crotalaria (*Crotalaria juncea* L.) at different planting densities and age of the plant, in the dry tropics of the state of Guerrero, Mexico.

MATERIALS AND METHODS

Experimental plot location

The study was carried out from July to October 2020, in an experimental plot in Tecuescontitlán, Tepecuacuilco de Trujano, Guerrero, Mexico (18° 08' N and −99° 33' W, a 782 m.a.s.l). The climate is subhumid warm with summer rains (790 mm annual average rainfall) and a 26 °C average temperature (García, 2004). The soil has a 7.3 pH, 0.3 dS m^{-1}, and 2.1 % organic matter. Figure 1 shows the maximum, medium, and minimum temperatures, as well as the weekly rainfall accumulation during the study period. The data were obtained from the weather station 12,092, located in Tonalapa del Sur, 51 km away from the experimental plot.

Plot management

The experimental plot was established on July 30, 2020, during the rainy season. The land was prepared with traditional techniques (fallow, two harrows, and furrow). Sixteen experimental units made up of 5×5 m plots —randomly distributed and with 3 repetitions— were used. Four planting densities (treatments) were evaluated. The seeds were sown by hand, placing them in the furrows, at a depth that doubled the size of the seed. The distance between furrows was 50 cm and the distance between plants was 5, 10, and 20 cm, resulting in 400,000, 200,000, 100,000 plants ha^{-1} per each distance. For its part, control was overseed (approximately 380,000 plants ha^{-1}). No irrigation or fertilizers were used, and weed was controlled by hand. From the 30 days after the emergence,

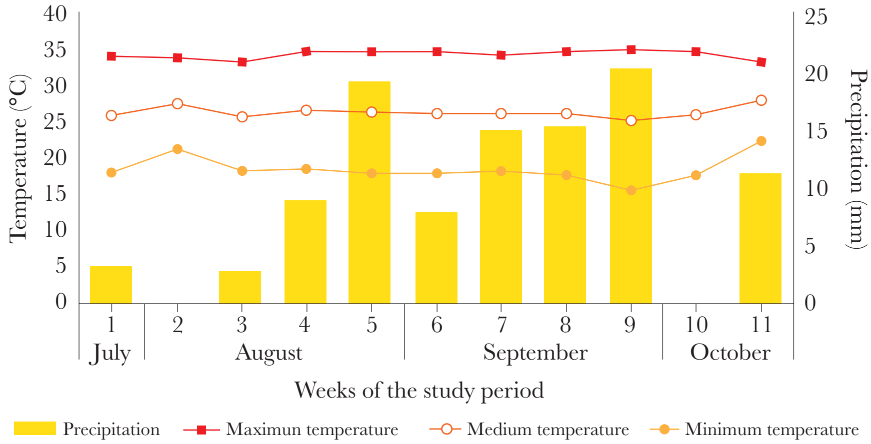

Figure 1. Maximum, medium, and minimum temperatures and weekly rainfall accumulation recorded during the experiment.

samplings were carried out at 8-day intervals, until the plants reached their reproductive stage and had fully developed seeds.

Evaluated variables

Dry matter yield

The 1-m linear method was used to carry out randomly destructive samplings on the experimental units and control in order to establish dry matter (DM) forage (kg ha^{-1}) yield. Forage was harvested 10 cm above ground level. Subsequently, it was weighted and put in paper bags. It was dried at 60 °C in a forced-air electrical stove until it reached a constant weight.

Intercepted radiation

In order to measure intercepted radiation, a day before the cutting, five random instant readings of each repetition were made. The length (cm) of shadow cast by the canopy were measured using a 100 cm rule. It was placed on the surface of the soil, between the furrows (under the canopy), at approximately 1:00 pm.

Morphology composition

In order to determine the morphology composition, a ≈20% subsample was taken from the forage yield sample. This subsample was divided into its morphology components: stem, leaf, flower, and pods (leaflet + seeds). Each component was weighted, put in paper bags, and dried in an electric stove at 60 °C, until they reached a constant weight.

Statistical analysis

The data was analyzed using a completely randomized design with an arrangement comprised of divided plots and four repetitions. The PROC GLM of SAS 9.2 (2009) procedure was used and the means comparison was carried out with a Tukey test (α=0.05). Simple polynomic regressions were carried out in order to compare intercepted radiation and the leaf component. The significance correlation coefficients ($p<0.05$) were calculated, and an analysis of variance and a means comparison test (Tukey $p<0.05$) were also carried out.

RESULTS AND DISCUSSION

Dry matter yield

Table 1 shows the dry matter yield of crotalaria when the planting density and the cutting age change. Regardless of the cutting age, the average behavior of this variable was recorded in the following descending order: 400,000 > overseed > 200,000 > 100,000 plants ha^{-1} planting densities, with 19,837, 17,918, 8,786, and 4,074 kg DM ha^{-1}, respectively ($p<0.05$). Regarding the cutting age, the highest dry matter yield (28,363 kg ha^{-1}) was obtained at 75 days, while the lowest was obtained at 30 days (527 kg ha^{-1}); planting densities ($p<0.05$) were not considered.

In this and other studies, dry matter (DM) yield varies at different planting densities and depends on the interspecific competition (mainly for nutrients and light). Santos *et al.* (2011) and Mosjidis *et al.* (2013) determined that crotalaria has a 15,831-10,000 kg ha^{-1} yield. Jiménez *et al.* (2005) pointed out that the yield depends on the sowing season, the management, the planting densities, and climatic conditions (mainly rainfall and temperature).

Intercepted radiation

Intercepted radiation is a measure studied in grasses and pulses, which establishes the optimal harvest time when a 95% radiation is obtained. Therefore, during the evaluation of pulses (specifically crotalaria), the highest values of intercepted radiation (>93%) were observed at 45 days in the 400,000, 200,000 plants ha^{-1}, and overseed planting densities ($p<0.05$) (Table 2). A lower intercepted radiation ($p<0.05$) was obtained at the ages of 30, 38, 52, 60, 68, and 75, in all the planting densities evaluated.

Maldonado-Peralta *et al.* (2019) and Rojas-García *et al.* (2018) pointed out that the optimal harvesting moment is when the meadow has a 95% intercepted radiation. Rojas-García *et al.* (2017) and Rojas *et al.* (2016) confirmed that this is the appropriate value for harvesting or grazing pulses by themselves or associated pulses, respectively. This is the result of the quality and quantity attributes of the forage. The development of crotalaria

Table 1. Forage yield (kg ha^{-1}) of crotalaria, at different planting densities and cutting age.

Density (plants ha^{-1})	Age at cut (days after the emergency)							Average
	30	38	45	52	60	68	75	
400,000	680 ^{Ag}	3,424 ^{Af}	7,762 ^{Be}	16,411 ^{Ad}	29,497 ^{Ac}	37,810 ^{Ab}	43,278 ^{Aa}	19,837 ^{A}
200,000	482 ^{BCf}	2,016 ^{Bef}	3,551 ^{Ce}	7,448 ^{Bd}	11,603 ^{Cc}	16,086 ^{Cb}	20,317 ^{Ba}	8,786 ^{C}
100,000	399 ^{Ce}	1,052 ^{Cde}	2,162 ^{Dcd}	3,354 ^{Cc}	4,982 ^{Db}	7,718 ^{Da}	8,856 ^{Ca}	4,074 ^{D}
Overseed	549 ^{Bg}	3,686 ^{Af}	8,992 ^{Ae}	16,354 ^{Ad}	22,449 ^{Bc}	32,393 ^{Bb}	41,004 ^{Aa}	17,918 ^{B}
Average	527 ^{g}	2,544 ^{f}	5,616 ^{e}	10,871 ^{d}	17,132 ^{c}	23,501 ^{b}	28,363 ^{a}	

Means with the same lower-case letter in the same row (^{abcd}) and capital letters (^{ABCD}) in the same column are not statistically different (Tukey: $\alpha=0.05$).

Table 2. Intercepted radiation (%) of crotalaria, at different planting densities and cutting age.

Density (plants ha^{-1})	Age at cut (days after the emergency)							Average
	30	38	45	52	60	68	75	
400,000	24 ^{Be}	55 ^{Ad}	95 ^{Aa}	82 ^{Ab}	69 ^{Ac}	58 ^{Ad}	58 ^{ABd}	63 ^{A}
200,000	29 ^{Af}	49 ^{Be}	93 ^{Aa}	76 ^{ABb}	69 ^{Ac}	60 ^{Ad}	58 ^{ABd}	62 ^{B}
100,000	27 ^{ABe}	49 ^{Bd}	88 ^{Ba}	74 ^{Bb}	69 ^{Ab}	59 ^{Ac}	59 ^{Ac}	60 ^{D}
Overseed	24 ^{Be}	53 ^{ABd}	93 ^{Aa}	80 ^{ABb}	70 ^{Ac}	53 ^{Bd}	54 ^{Bd}	61 ^{C}
Average	26 ^{e}	52 ^{d}	92 ^{a}	78 ^{b}	69 ^{c}	58 ^{d}	57 ^{d}	

Means with the same lower-case letter in the same row (^{abcd}) and capital letters (^{ABCD}) in the same column are not statistically different (Tukey: α=0.05).

crops at 45 days had a better coverage. This was an efficient crop, because it spreads its canopy during the initial growth, increasing the coverage and the interception of light. In this study, the 400,000, 200,000 plants ha^{-1} and overseed planting densities obtained 95, 93, and 93% radiation, respectively. However, this was not the case with the 100,000 plants ha^{-1} density, which only reached 88% radiation. These results do not match the results of Jiménez *et al.* (2005), who evaluated a 172,000 plants ha^{-1} planting density and found 97% intercepted radiation.

Morphology composition

Table 3 shows the morphology composition of crotalaria in different planting densities and cutting age. A higher proportion of leaves was found during the first days of the evaluation. Regardless of the planting density, the proportion was higher at day 30 (62%) and lower at day 75 (15%) after the emergence ($p>0.05$). At day 30, 100,000 and 200,000 plants ha^{-1} planting densities recorded the highest number of leaves at the beginning of the research (66% and 62%, respectively). However, at day 75, both planting densities also had the lowest number of leaves during the last cut (10% and 14%, respectively). Regardless of the densities, the highest stem percentage was recorded at day 52, obtaining a 75% average. The lowest percentage was recorded at day 30 of their development, obtaining a 37% stem average ($p<0.05$). Flowers appeared after 52 days, in the 200,000 plants ha^{-1} (3.1%) and the overseed (0.6%) planting densities; these percentages increased up to day 75 (4.8 and 3.9%, respectively). However, the 400,000 and 100,000 plants ha^{-1} planting densities recorded the highest flower percentage ($p<0.05$) (7.2 and 6.6%, respectively) at the cutting (75 days). Meanwhile, crotalaria pods appeared 14 days after the flowers (day 68). The highest percentage of pods (22.1%) was recorded at day 75 ($p<0.05$) in the 200,000 plants ha^{-1} density. On this regard, Oliveira *et al.* (2020) mentioned that planting density is a determining factor of the morphology composition of crotalaria. They also pointed out that if density increases, the biomass increases, but pod production diminishes. This phenomenon was not observed in this research, because pod production started 75 days after the emergence. Therefore, statistical differences between the highest and the lowest evaluated densities were not recorded. In this regard, Abdul-Baki *et al.* (2001) pointed out that the morphology composition is determined by the vegetative development cycle, because, at the beginning of the development, leaves have a higher percentage. This percentage diminishes over time, as stems, flowers, and pods start to grow, as we reported in this research.

Intercepted radiation regression (%) of leaves (%)

Figure 2 shows the regression coefficients (R^2) between the intercepted radiation (%) and the leaves (%), when the different planting densities change. Overall, all the regressions had a polynomial trend and a high relationship. The highest relationship took place in the 400,000 plants ha^{-1} density with a 0.898 ($p<0.001$) R^2, obtaining 95% of intercepted radiation, 45 days after the emergence, with 32% of leaves. However, overseed planting density recorded the lowest relationship with a 0.629 ($p<0.05$) R^2, reaching a maximum intercepted radiation of 93%, 45 days after the emergence, with 28% of leaves. Meanwhile, in a research about alfalfa, Teixeira *et al.* (2007) evaluated the frequency and intensity of grazing and reported a 95% maximum intercepted radiation, as a result of a greater leaf area (3.6).

Table 3. Morphology composition (%) of crotalaria, at different planting densities and cutting age.

Density (plants ha^{-1})	Age at cut (days after the emergency)							Average
	30	**38**	**45**	**52**	**60**	**68**	**75**	
				Leaf				
400,000	56 ^{Ba}	45 ^{Ab}	32 ^{ABc}	30 ^{Ac}	21 ^{Bd}	20 ^{Bd}	18 ^{Ad}	32 ^{B}
200,000	62 ^{ABa}	44 ^{Ab}	30 ^{ABc}	25 ^{Bc}	24 ^{Ac}	23 ^{Ac}	14 ^{ABd}	32 ^{B}
100,000	66 ^{Aa}	42 ^{ABb}	40 ^{Abc}	31 ^{Acd}	23 ^{ABde}	20 ^{Be}	10 ^{Bf}	33 ^{A}
Overseed	64 ^{Aa}	40 ^{Bb}	28 ^{Bc}	26 ^{Bcd}	24 ^{Acd}	23 ^{Acd}	18 ^{Ad}	32 ^{B}
Average	62 ^{a}	43 ^{b}	33 ^{c}	28 ^{d}	23 ^{e}	22 ^{e}	15 ^{f}	
				Stem				
400,000	43 ^{Ad}	54 ^{Bc}	67 ^{ABb}	76 ^{Aa}	76 ^{Aa}	75 ^{Aa}	68 ^{Aab}	65 ^{A}
200,000	37 ^{ABc}	55 ^{Bb}	69 ^{ABa}	74 ^{Ba}	74 ^{Ba}	71 ^{Ba}	58 ^{Bb}	62 ^{B}
100,000	33 ^{Be}	57 ^{ABd}	59 ^{Bcd}	75 ^{Aab}	75 ^{Aab}	77 ^{Aa}	76 ^{Aa}	64 ^{AB}
Overseed	35 ^{Bc}	59 ^{Ab}	71 ^{Aa}	74 ^{Ba}	74 ^{Ba}	72 ^{Ba}	68 ^{Aab}	65 ^{A}
Average	37 ^{d}	56 ^{c}	67 ^{b}	75 ^{a}	75 ^{a}	74 ^{a}	68 ^{b}	
				Flower				
400,000	--	--	--	1.4 ^{Bc}	1.4 ^{Bc}	3.3 ^{Bb}	7.2 ^{Aa}	4.0 ^{A}
200,000	--	--	--	1.0 ^{Cc}	1.0 ^{Cc}	5.2 ^{Aa}	4.8 ^{BCab}	3.5 ^{B}
100,000	--	--	--	1.8 ^{Ab}	1.8 ^{Ab}	1.6 ^{Cb}	6.6 ^{Aba}	3.3 ^{B}
Overseed	--	--	--	0.9 ^{Cb}	0.9 ^{Cb}	3.7 ^{Ba}	3.9 ^{Ca}	2.3 ^{C}
Average	--	--	--	1.4 ^{c}	1.4 ^{c}	3.5 ^{b}	5.6 ^{a}	
				Sheath				
400,000	--	--	--	--	--	0.2 ^{Ab}	5.3 ^{Ba}	2.8 ^{D}
200,000	--	--	--	--	--	0.1 ^{Ab}	22.1 ^{Aa}	11.1 ^{A}
100,000	--	--	--	--	--	--	6.3 ^{Ba}	6.3 ^{B}
Overseed	--	--	--	--	--	0.1 ^{Ab}	8.8 ^{Ba}	4.5 ^{C}
Average	--	--	--	--	--	0.1 ^{b}	10.6 ^{a}	

Means with the same lower-case letter in the same row (^{abcd}) and capital letters (^{ABCD}) in the same column are not statistically different (Tukey: $\alpha=0.05$).

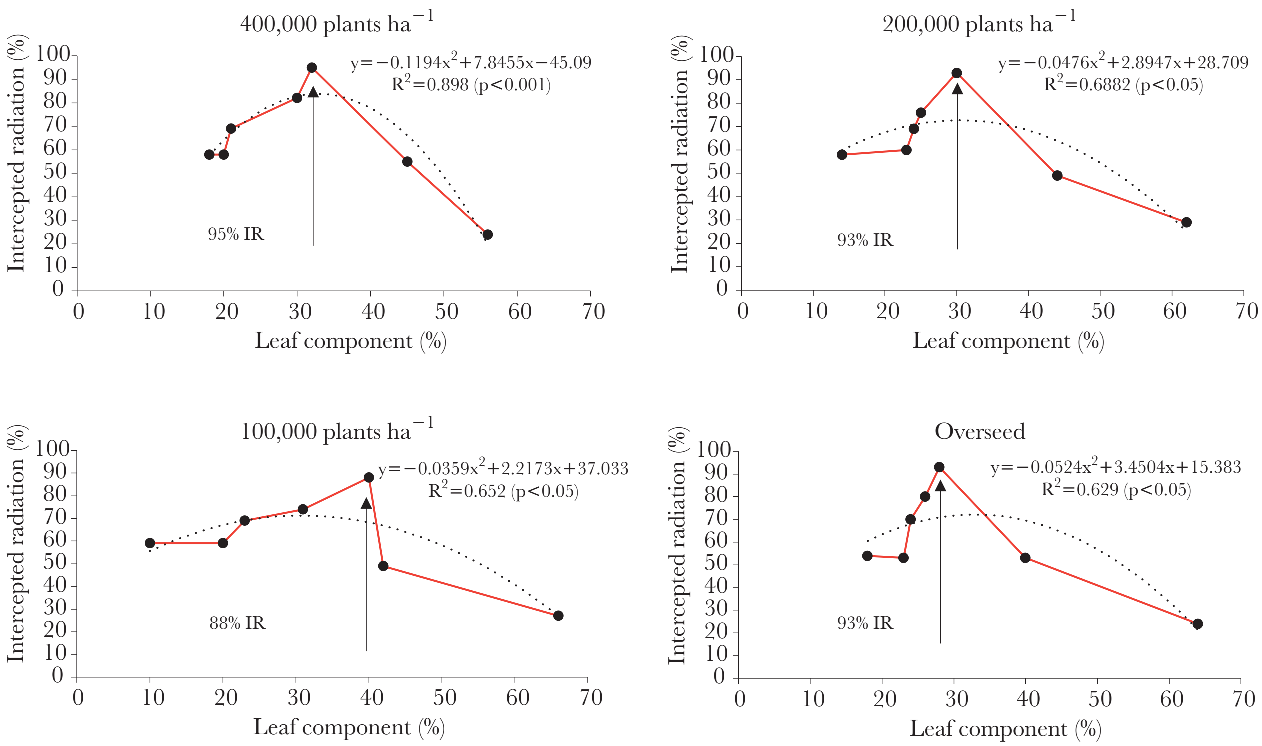

Figure 2. Regression coefficient between intercepted radiation (%) and leaves (%) of crotalaria, at different planting densities and cutting age.

Finally, in their research about white clover pulse, associated to orchard grass and perennial ryegrass, Rojas *et al.* (2016) found a high relationship between the intercepted radiation and the growth rate in all the associations, with 0.87 ($p<0.001$) R^2 in average. They also pointed out that a higher growth rate is linked with a higher intercepted radiation rate and *vice versa*.

CONCLUSION

Crotalaria obtained a higher yield with a 400,000 plants ha^{-1} planting density. The best structural characteristics of the meadow and 95% of intercepted radiation can be obtained 45 days after the emergence. However, a 200,000 plants ha^{-1} planting density is more conducive to a higher pod production.

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AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Effect of breed, breeding season, eCG dose, and eCG application time on the estrous cycle of hair ewe lambs

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ABSTRACT

Objective: To evaluate the effect of breed, breeding season, dose and application time of equine chorionic gonadotropin (eCG) on the estrous cycle and ovarian activity in hair ewe lambs.

Design/methodology/approach: We studied 216 hair ewe lambs (62 Dorper, 69 Katahdin, and 85 Pelibuey) —91 in high breeding season and 125 in low breeding season—, who were synchronized with intravaginal sponges containing 20 mg of fluorogestone acetate (FGA), and intramuscular equine chorionic gonadotropin (eCG; 200 and 300 IU). The treatments are breed, breeding season, eCG dose, and eCG application time. We analyzed the presence of estrus using a logistic regression model, while for the interval to estrus and the ovulation rate we applied an analysis of variance, using a completely randomized design with a 2×2×2×3 factorial arrangement with the PROC LOGISTIC and PROC GLM procedures.

Results: The breed was a factor ($P<0.01$) in the presence of estrus: Dorper ewe lambs presented 9.74 times more possibilities than Pelibuey. The interval to estrus was shorter ($P<0.05$) in Dorper (29.5 ± 0.9 h) and Katahdin (29.1 ± 0.9 h) than in Pelibuey (34.8 ± 0.9 h). The interval to estrus was lower ($P<0.05$) when we applied 200 or 300 IU of eCG 24 h before the end of the protocol, than when we applied 200 IU of eCG at the time of progestogen withdrawal. The ovulation rate was only affected by breed ($P<0.05$): it was higher in Pelibuey (2.4 ± 0.1) than in Dorper (2.0 ± 0.1) and Katahdin (1.9 ± 0.1).

Study limitations/implications: Conducting a second study would be advisable to complement this research. This would include the gestation stage of females and relate it to the ovulation rate, while also measuring the ovarian structures by means of ultrasound.

Findings/conclusions: The main influencing factor on estrus and ovarian activity in hair ewe lambs synchronized with progestogens is breed.

Key words: Ewes, Hair breeds, Estrous synchronization, Ovulation rate.

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INTRODUCTION

The application of exogenous hormones in ewes is a reproductive tool used in artificial insemination programs to synchronize or induce estrus. These hormonal treatments are essential to make lamb production more efficient throughout the year. There are various protocols for the synchronization and/or induction of estrus; however, protocols based on progestogens and eCG result in better estrous activity and fertility (Barrett *et al.*, 2004; Ali, 2007). These protocols consist of administering a progestogen to the ewe for 9-14 days to simulate the luteal phase of the estrous cycle. After this period, the progestogen is withdrawn, and the follicular growth and ovulation rate are stimulated by administering eCG (equine chorionic gonadotropin) (Cline *et al.*, 2001). Although these protocols have been successful in accomplishing estrous synchronization, the response varies according to genetic and environmental factors (Arroyo *et al.*, 2006; Estrada *et al.*, 2006). Moeini *et al.* (2007), for instance, reported different responses to estrus between Iranian Sanjabi and Lori wool ewes treated with FGA and 400 IU of eCG. The observed variation was of 52.2% of Sanjabi ewes in estrus as compared to 91.1% among the Lori breed. Prolificacy and fecundity in Dorper ewes increased up to 20% when the eCG was applied 24 hours before the end of the synchronization protocol, as compared to when it was applied at the end of said protocol (Zeleke *et al.*, 2005). Other factors that affect the response of wool ewes to these hormonal treatments with progestogens are the dose of eCG (Kridli *et al.*, 2006), the time of year (Langford *et al.*, 1983; Rosa and Bryant, 2003), the body condition (Esen, 2001), and the geographic region (Fenton *et al.*, 1997). There are few studies focusing on the factors that influence the reproductive response of ewes subjected to synchronization protocols in Mexico's climatic conditions. Martínez-Tinajero *et al.* (2007) found that the eCG application time affects the presence of estrus in Blackbelly ewes under tropical conditions. Similarly, Macías (2007) reports that the eCG dose, eCG application time, breed, and time of year affect the response to estrus and its onset time under the dry tropical conditions of northeastern Mexico. Therefore, the objective of this research was to evaluate the effect of breed, breeding season, dose, and application time of equine chorionic gonadotropin (eCG) on the estrous cycle and ovarian activity in hair ewe lambs.

MATERIALS AND METHODS

Study area description

This research was conducted from February 23 to September 27, 2018, at the University Ranch of the Universidad Autónoma de Ciudad Juárez, Ciudad Juárez, Chihuahua. The region is located at 1100 masl; the climate is hot (BWh) and cold desert (BWK); the average annual temperature is 16-18 °C; and the average annual precipitation is 244 mm (INEGI, 2007).

Experimental design and animals

We conducted four estrous synchronization programs during the year, using 216 hair ewe lambs (62 Dorper, 69 Katahdin, and 85 Pelibuey). The ewe lambs were 8 months old, had a live weight of 25-30 kg, and a body condition score (BC) of 2.5-3.5, using the 1-5 scale (Thompson and Meyer, 1994).

The first program was conducted from February 23 to March 14 with 66 ewe lambs (19 Dorper, 20 Katahdin, and 27 Pelibuey); the second from April 30 to May 20 with 59 ewe lambs (17 Dorper, 19 Katahdin, and 23 Pelibuey); the third from July 15 to August 4 with 63 ewe lambs (17 Dorper, 20 Katahdin, and 26 Pelibuey); and the fourth from September 6 to 27 with 28 ewe lambs (9 Dorper, 10 Katahdin, and 9 Pelibuey).

In order to assess the effect of the breed, we defined the following treatments:

T1) Dorper (62 ewe lambs)
T2) Katahdin (69 ewe lambs)
T3) Pelibuey (85 ewe lambs)

Before starting each synchronization program, we randomly distributed the ewe lambs into four groups to apply the following treatments:

T1) 200 IU of eCG 24 h before sponge withdrawal;
T2) 200 IU of eCG at the time of sponge withdrawal;
T3) 300 IU of eCG 24 h before sponge withdrawal, and
T4) 300 IU of eCG at the time of sponge withdrawal.

These treatments were applied in each of the four estrous synchronization programs conducted during the year.

We defined the high breeding season as the time of year when more than 70% of the ewes show estrus and ovulation behavior, and the low breeding season as the time of year when this percentage is less than 50%.

In order to evaluate the breeding seasons, we studied 216 ewe lambs: for the high season, n=91 (28 Dorper, 33 Katahdin, and 30 Pelibuey); and for the low season, n=125 (34 Dorper, 36 Katahdin, and 55 Pelibuey). The treatments were as follows:

T1) High breeding season
T2) Low breeding season

Females were kept in the barn. They were fed twice a day (8 am and 5 pm) with fresh orange pulp (9.6%; CP) on a dry basis; 300 g head^{-1} day^{-1} of supplement (14% CP) on a dry basis, and 2.85 Mcal of ME, made with ground sorghum (70%), wheat bran (7%), soybean meal (12%), chopped buffel grass hay (7%), molasses (3%), and mineral salts (1%). This supplement was offered daily at 8 am, and water was offered *ad libitum*.

Synchronization program and management

The estrous synchronization program consisted of intravaginally applying a sponge impregnated with FGA (20 mg; Chronogest CR®, MSD, Animal Health) to each ewe lamb for 12 d; the eCG treatments (GonActive® eCG, Virbac) were applied before sponge withdrawal. The incidence and distribution of estrus was determined 24 h after sponge withdrawal by introducing marker males provided with an anti-mating apron. The ewe lambs detected in estrus were registered and separated to facilitate estrus in the remaining ewe lambs. The ovulation rate was determined by direct observation and counting of *corpus luteum* on the surface of the ewe lambs' ovaries through laparoscopy. This was performed 8 d after sponge withdrawal using a rigid laparoscope (Karl Storz Endoscope; Storz).

Study variables

Three variables were determined in each treatment: presence of estrus (ewe lambs that presented estrus after sponge withdrawal); interval to estrus (time interval between sponge withdrawal and presence of estrus), and ovulation rate (number of *corpus luteum* per lamb presenting estrus).

Statistical analysis

The presence of estrus was analyzed under a logistic regression model that included the effects of the eCG dose (200 or 300 IU), eCG application time (−24 h and 0 h), breeding season (high and low), and breed (Dorper, Katahdin, and Pelibuey). To analyze the interval to estrus and the ovulation rate, we applied an analysis of variance using a completely randomized design with a 2×2×2×3 factorial arrangement. The factors included in the analysis of variance were the same as in the logistic regression model, in addition to the possible interactions between factors. Means were compared with the t-student test at $P<0.05$. Trends were established when the analysis indicated $0.05 \geq P \leq 0.10$. All analyses were performed using the PROC LOGISTIC and PROC GLM procedures of the SAS software (SAS, 2004).

RESULTS AND DISCUSSION

Presence of estrus

Table 1 shows the results of the logistic regression analysis on factors that influence the presence of estrus. Breeding season, PMSG dose and application time were factors that did not affect ($P>0.05$) the presence of estrus in ewe lambs synchronized with FGA. On the contrary, breed was indeed a factor of influence ($P<0.01$): Dorper ewe lambs presented 23.9% better estrous behavior than Pelibuey ewe lambs; however, only a 6.8% difference ($P>0.05$) was observed between Katahdin and Pelibuey as a result of the synchronization protocol.

Among the factors studied, only breed affected the response to the presence of estrus (OE), which coincides with the results found in wool ewes, as reported by Emsen and Yaprak (2006), and Moeini *et al.* (2007). Both studies reported variations between breeds (Awassi *v.* Red Karaman and Iranian Sanjabi *v.* Lori) in the percentage of ewes presenting estrus after being synchronized with a progestogen and eCG. Meanwhile, after applying a synchronization program similar to the one used in this research, Macías (2007) found a higher percentage of estrus in Pelibuey Canelo ewes than in those of the Pelibuey Blanco, Blackbelly, and Dorper breeds. However, other studies did not find any differences in the presence of estrus between wool breeds (Romano *et al.*, 2000; Boscos *et al.*, 2002). Moeini *et al.* (2007) mention that breeds that have been improved to increase prolificacy are more sensitive and likely to respond to hormonal treatments for estrus synchronization

Table 1. Logistic regression on factors that affect the presence of estrus (OE) in hair ewe lambs treated with FGA.

Treatment/variable	N	PE % (n)	Odd Ratio	Confidence interval 95%	$P>X^2$
Breed					
Dorper	62	96.8 (60)	9.74	1.93-49.19	0.0059
Katahdin	69	79.7 (55)	2.15	0.81-5.69	0.1226
Pelibuey	85	72.9 (62)			
Doses of eCG					
200	104	80.8 (84)	0.73	0.33-1.60	0.4314
300	112	83.0 (93)			
Time application of eCG					
0 h	100	78.0 (78)	0.57	0.26-1.26	0.1648
-24 h	116	85.3 (99)			
Reproductive season					
high	91	74.7 (68)	0.48	0.19-1.26	0.1358
low	125	87.2 (109)			

FGA: Fluorogestone acetate; eCG: Equine chorionic gonadotropin.

and induction. García-Guerra *et al.* (2018) mention that there is occurrence of follicular codominance in ewes. This results in multiple ovulations and therefore increases the response to estrus and ovulation. Hence, multiple ovulation is due to genetic (breed), dietary, and hormonal influence on follicular development and growth, as well as on oocyte-granulosa cell interaction (cumulus oophorus complex).

This might explain the variations found in the presence of estrus among the breeds studied. It is worth mentioning that the Dorper ewe lambs showed a higher increase in the presence of estrus than the Pelibuey. A higher percentage of females of both breeds (and Katahdin) presented estrus between 24 and 36 h after the end of the synchronization protocol. Hashemi *et al.* (2006) and Kridli *et al.* (2006) reported the presence of estrus between 24 and 36 h after progestogen withdrawal in ewes of different breeds synchronized with FGA and eCG (500 and 600 IU). In this sense, Martínez-Tinajero *et al.* (2006) —using the same protocol, with 150 and 300 IU of eCG— found 80% of ewes in estrus between 24 and 48 h after finishing the hormonal treatment. These results suggest that the time it takes for ewes treated with FGA and eCG to present estrus after finishing the synchronization protocol does not depend on the breed, but rather on other factors, such as nutrition and environmental aspects.

Interval to estrus and ovulation rate

Table 2 shows the results of the effect of breed, breeding season, and interaction between eCG dose and application time on the interval to estrus and ovulation rate in ewe lambs synchronized with FGA. The interval to estrus was shorter ($P<0.05$) in Dorper (29.5 ± 0.9 h) and Katahdin (29.1 ± 0.9 h) ewe lambs than in Pelibuey (34.8 ± 0.9 h). The interaction between eCG dose and application time also affected the interval to estrus,

Table 2. Effect of breed, breeding season, and eCG application on interval to estrus and ovulation rate of hair ewes synchronized with FGA.

Variable	n	Interval to estro ±SE	Ovulation rate ±SE
Breed			
Dorper	60	29.5 ± 0.9a	2.0 ± 0.1b
Katahdin	55	29.1 ± 0.9a	1.9 ± 0.1b
Pelibuey	62	34.8 ± 0.9b	2.4 ± 0.1a
Reproductive season			
high	68	29.0 ± 0.8a	2.0 ± 0.1a
low	109	32.6 ± 0.8a	2.2 ± 0.1a
Dose × Time of application of eCG			
200 UI 0 h	34	34.6 ± 1.0a	1.8 ± 0.2a
200 UI −24 h	50	30.4 ± 1.0b	2.1 ± 0.2a
300 UI 0 h	45	31.5 ± 1.0b	2.1 ± 0.2a
300 UI −24 h	48	30.8 ± 1.0b	2.2 ± 0.2a

[a, b] Different superscripts within the same column indicate a significant difference (P<0.05); FGA: Fluorogestone acetate; eCG: Equine chorionic gonadotropin.

which was longer (P<0.05) in ewe lambs treated with 200 IU at progestogen withdrawal (34.6±1.0 h) than in ewe lambs with other treatments (mean=30.9±1.0 h). When 200 and 300 IU of eCG were applied at −24 h, and 300 IU at 0 h, the interval to estrus was similar (P>0.05). The variation observed in the ovulation rate was due to the effect of breed (P<0.05). Pelibuey had a higher (P<0.03) ovulation rate (2.4±0.1 *corpus luteum*) than Dorper (2.0±0.1 *corpus luteum*) and Katahdin (1.9±0.1 *corpus luteum*).

In this research, we observed that Pelibuey ewe lambs took longer (5.5 h) to present estrus after progestogen withdrawal; however, they had a better ovulation rate than other breeds (2.4±0.1 *v.* 1.95±0.1 *corpus luteum* on average). In this regard, the increase in the ovulation rate in Pelibuey ewe lambs may be due to the better prolificacy that this breed presents naturally in relation to the Dorper and Katahdin breeds (Bartlewski *et al.*, 1999).

The FSH affinity of the follicles depends exclusively on the receptors presented by the follicles during their growth and is related to the number of receptors. Hence, when there are more receptors, the dominant follicle or follicles tend to manifest in growth and, therefore, to be dominant and/or ovulatory. The ovulation rate increases for this reason. However, this ovulation rate should go hand in hand with an increase in estrogen levels, since the larger amount of dominant follicles would be expected to produce a greater amount of estrogens. This would result in an interval to estrus similar or shorter than the one found in the other two breeds. In this regard, Rekik *et al.* (2002) mention that the response of ewe lambs to hormonal treatments that seek to induce or synchronize estrus varies due to immaturity of the reproductive system, environmental factors such as nutrition, live weight, season of birth, among others (Martínez *et al.*, 2001; Madani *et al.*, 2009). In an estrous synchronization program with FGA and eCG in Pelibuey Blanco, Pelibuey Canelo, Dorper, and Katahdin ewes, Macías (2007) found that the interval to estrus did not vary between breeds (average of 28.8 h). This value was similar to that observed in this research for Dorper and Katahdin ewe lambs. Romano *et al.* (2000) compared the interval to estrus between Suffolk, Hampshire, and White Face breeds of wool ewes, and did not find variations derived from breed. These results are different from those found in the present research, probably due to the type of ewes used (adults).

The interaction between eCG dose and application time affected the interval to estrus ($P<0.05$), but not the ovulation rate. This is probably due to the fact that modifying the eCG dose and administration time generates changes in the pattern of follicular development (Ali, 2007), which may be favorable or unfavorable for the acceleration of follicular development and growth, and for the elevation of plasma estrogen levels (Ustuner *et al.*, 2007). These results demonstrate the importance of the eCG hormone to increase the degree of synchrony between reproductive events and the presence of estrus when ewes are treated with progestogens. Based on our findings, the onset time of estrus is the same when applying doses of 200 or 300 IU of eCG 24 h before withdrawing the intravaginal device and when applying one dose of 300 IU of eCG at the time of withdrawal. In general, these results coincide with those reported in other studies (Ali, 2007; Macías, 2007; Ustuner *et al.*, 2007).

CONCLUSIONS

The main influencing factor on estrous cycle and ovarian activity in hair ewe lambs synchronized with progestogen is breed. When a more predictable and compact estrus is preferred, the eCG should be administered prior to sponge withdrawal (preferably 24 hours).

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Physical characteristics of eggs from Mexican Creole, Hy-Line Brown and Rhode Island Red hens in intensive production

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ABSTRACT

Objective: To characterize the egg from three hen genotypes: Mexican Creole (MC), Hy-Line Brown (HLB) and Rhode Island Red (RIR).

Design/Methodology/Approach: Three groups of each genotype were formed using 75 hens (30 MC, 30 HLB and 15 RIR), 20 weeks old. Daily, for 84 days, two eggs were chosen randomly from each group to determine: weight (w, g), length (L, cm), width (Wth, cm), shape index (SI), volume (VOL, cm^3), area (AR, cm^2), shell color (SCo), yolk color (YCo), white weight (WW, g), yolk weight (YW, g), shell weight (SW, g), white proportion (WProp), yolk proportion (YProp), and shell proportion (SProp). The means were compared with Tukey's test, $P<0.05$, using the SAS software.

Results: The genotype HLB was superior ($P<0.05$) in W, Wth, SI, VOL, AR, SCo, WW, and WProp (61.220 g, 4.400 cm, 0.801, 55.890 cm^3, 71.723 cm^2, 6.834, 38.030 g and 0.621, respectively). There were no differences between genotypes ($P>0.05$) in L (5.383 to 5.490 cm). The MC hens were superior ($P<0.05$) in YCo, YW and SProp (6.738, 15.923 g and 0.132, respectively). The SW differed ($P<0.05$) between genotypes: HLB (7.550 g), MC (6.661 g) and RIR (6.205 g). MC and RIR had higher ($P<0.05$) YProp (0.314 and 0.304, respectively) than HLB (0.250).

Study Limitation/Implications: The study contemplated only one part of the production period of the birds.

Findings/Conclusions: Each genotype produced egg with particular physical characteristics, with Creole hens standing out due to their high values of yolk color and proportions of yolk and shell.

Keywords: Dish egg, physical characteristics, Mexican Creole, Rhode Island Red, Hy-Line Brown.

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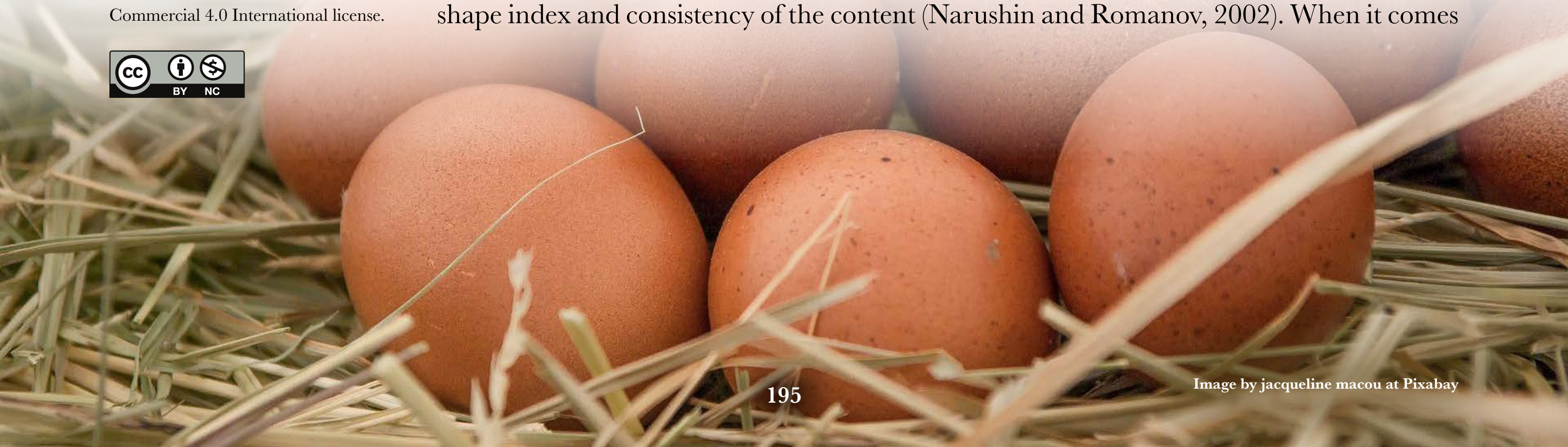
INTRODUCTION

The physical characteristics of egg are important from the biological and economic point of view (Mine and Kovasc, 2004; Moulo *et al.*, 2010; Alkan *et al.*, 2013), and some of the most important are the following: weight, thickness and porosity of the shell, length, shape index and consistency of the content (Narushin and Romanov, 2002). When it comes



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to the consumer, egg quality is determined by additional characteristics to those already mentioned: cleanliness, freshness, surface area, mass, volume, packaging coefficient, and shell quality (Narushin, 1997; Duman *et al.* 2015). Likewise, egg quality includes aspects related to the shell, the albumin and the yolk (Ahmadi and Rahimi, 2011; Yang *et al.*, 2014; Duman *et al.*, 2015).

Many laboratory techniques have been developed to determine the egg quality in hens of commercial varieties (Abadia *et al.*, 1998). On the contrary, little is known about the egg quality of Creole hens in Mexico (Juárez-Caratachea, 2010). Researching Creole hens takes on great scientific, social and economic importance, given the current interest in the conservation of zoogenetic resources (VillacisVillacís, 2014; Andrade *et al.*, 2015). In a study conducted by Andrade *et al.* (2015) about the physical characteristics of eggs from Campera and Creole hens found that eggs from Campera hens showed the best results in terms of weight, width and length (55.4 g, 41.9 and 54.9 mm, respectively). Although the study concludes that Campera hens outperform Creole hens in the variables studied, it would be important to conserve Creole hens as a genetic resource because aspects about their productive performance are still unknown as are characteristics of the egg and meat they produce. In a study conducted in an intensive system, Segura-Correa *et al.* (2007) reported an average weight of the first egg from Creole hens of 45.3 g. Cuca-García *et al.* (2015) found that the average weight of eggs collected in some localities of Estado de México, Morelos and Tlaxcala was 50 g, and the average size of width and length (5.7 cm and 4.0 cm, respectively) in backyard conditions.

Based on this, it is clear that little is known about the physical characteristics of the egg (weight, length, width, shape index, volume, area, shell color, yolk color, white weight, yolk weight, shell weight, white proportion, yolk proportion, and shell proportion) of Mexican Creole hens, and likewise it is unknown whether these characteristics differ from other genotypes of hens available in Mexico such as Rhode Island Red and Hy-Line Brown.

Based on the background described, the objectives of this study were to characterize the eggs from Mexican Creole (MC), Rhode Island Red (RIR) and Hy-Line Brown (HLB) hens in terms of different physical properties of the egg, and to understand the differences between those genotypes.

MATERIALS AND METHODS

Location and period

This study was conducted from September to November, 2021, with duration of 84 d, in the Experimental Poultry Farm of the Zoology Department of Universidad Autónoma Chapingo, located on km 18.5 of the Los Reyes-Lechería, Texcoco highway, Estado de México. The place is located on coordinates: 19° 29' 13.1" latitude North and 98° 53' 47.2" longitude West and the region's climate is classified as C(w2)(w)b(i')g, which corresponds to a temperate sub-humid climate with summer rains, according to García (2004).

Bird management

The birds were placed in a shed with natural environment, with lateral mobile shutters and North-South orientation. Seventy-five (75) hens were used (30 Mexican Creole, 30 Hy-

Line Brown and 15 Rhode Island Red), 20 weeks old. The birds were housed in individual cages, and the dimensions of each cage were 30 cm wide, 45 cm deep, 36 cm tall on the superior part and 41 cm tall on the frontal part. The cages are pyramidal modules of two levels (5 cages per level and 20 cages per module). Each cage had 30 cm of metal sheet feeding trough and a cup-type automatic water dispenser. A lighting program of 16 hours of light and eight hours of darkness was used. Water and feed were offered with unrestricted access. The diet used was proposed based on the nutritional needs recommended for laying birds (NRC, 1994) (Table 1).

Genotypes and variables

The birds were housed in individual cages. Three groups were formed with 10 birds of each genotype, MC and HLB, and three groups of five birds of the RIR genotype. For the 84 d of the experiment, two eggs were collected daily and randomly from each group, for as long as the bird production would allow it. Likewise, daily, the following were

Table 1. Composition (%) of the experimental diet for Mexican Creole, Rhode Island Red, and Hy-Line Brown hens.

Ingredient	%
Corn	36.72
Soybean meal	31.67
Calcium carbonate 38%	11.05
Vegetable oil	17.44
Calcium phosphate 21/17*	2.04
Sodium chloride	0.38
Vitamin premix	0.30
Methionine 99%	0.35
L-Threonine	0.05
Total	100.00
Calculated nutrient content	
Metabolizable energy (kcal/kg)	2880
Crude protein (%)	18.230
Digestible arginine (%)	1.126
Digestible lysine (%)	0.905
Digestible methionine + cystine digestible aves (%)	0.800
Digestible tryptophan (%)	0.202
Digestible threonine (%)	0.620
Digestible isoleucine (%)	0.694
Digestible valine (%)	0.738
Linoleic acid (%)	9.512
Calcium (%)	4.510
Non-phytic phosphorus (%)	0.530
Sodium (%)	0.190
Chloride (%)	0.233

*21% calcium, 17% phosphorus.

measured in the eggs selected: weight (W, g), length (L, mm), width (Wth, mm), shape index (SI), volume (VOL, cm^3), area (AR, cm^2), shell color (SCo), yolk color (YCo), white weight (WW, g), yolk weight (YW, g), shell weight (SW, g), white proportion (WProp), yolk proportion (YProp), and shell proportion (SProp). During the entire experimental phase, a total of 1465 eggs were evaluated (504, 502 and 459 of Mexican Creole, Hy-Line Brown and Rhode Island Red, respectively). The variable W was determined with an electronic scale of 500 g capacity and 0.01 g precision (Model MH-200, Brand MKS TOOLS). The L and Wth of each egg were measured with a Vernier (Model HER-411, STEREN) with a measurement range of 0 to 150 mm and 0.1 mm of resolution. The L was determined on the longitudinal axis of the egg and the Wth on the transversal axis at the half height of the longitudinal axis. The SI was calculated using the following formula by Duman *et al.* (2016):

$$SI=(Wth/L)\times 100$$

The variables VOL and AR were calculated with the expressions

$$VOL=0.913\times W$$

and

$$AR=0.558\times P\ 0.67$$

respectively, according to Etches (1996): in both expressions W refers to the egg weight. The SCo was determined based on the ZIMPRO® range of colors with a scale of nine tonalities. The YCo of each egg was determined with the Ovocolor BASF® color range with a scale of 15 colors. The variables WW, YW and SW were obtained with an electronic scale of 500 g of capacity and 0.01 g of precision (Model MH-200, Brand MKS TOOLS), and for that purpose the weight of each whole egg was recorded, then it was broken and with the support of an egg white separator each component of the egg was separated to record their weight. The values WProp, YProp and SProp were calculated with regards to the weight of the whole egg.

Statistical analysis

The design was completely random, where the hen genotype was the only factor. The values of the variables from each pair of eggs were averaged and considered as the experimental unit. The data were analyzed with the MIXED procedure of the SAS statistical software (SAS Institute Inc., 2011) under the general linear model and the means were compared using Tukey's test ($P<0.05$).

RESULTS AND DISCUSSION

The egg from Hy-Line Brown hens produced higher values ($P<0.05$) of the variables W, Wth, SI, VOL and AR (61.220 g, 4.400 cm, 0.801, 55.890 cm^3 and 71.723 cm^2, respectively) compared to the egg from Creole Mexican or Rhode Island Red hens (Table 2), and no differences were detected (P>0.05) for those variables between these two genotypes. Likewise, differences were not detected (P>0.05) in egg L of the three genotypes (5.383 to 5.490 cm). The variables SCo, WW and WProp were different between genotypes (P<0.05) with higher values for the egg from Hy-Line Brown hens (6.834, 38.030 g and 0.621, respectively), followed by Rhode Island Red (5.124, 29.122 g and 0.579, respectively), and with lower egg values from Creole Mexican hens (2.688, 28.023 g and 0.553, respectively). In contrast (Table 2), the egg from Mexican Creole hens was superior (P<0.05) than the egg from Rhode Island Red and Hy-Line Brown hens, in terms of YCo, YW and SProp (6.738, 15.923 g and 0.132, respectively). Finally, differences were also observed between genotypes (P<0.05), for the variable SW: the egg from Hy-Line Brown hens had the highest value (7.750 g), followed by Mexican Creole (6.661 g) and Rhode Island Red (6.205 g). Regarding YProp, the egg from Mexican Creole and Rhode Island Red hens (0.314 and 0.304, respectively) had a higher value compared to Hy-Line Brown (0.250).

The eggs from genotype Hy-Line Brown had higher values in the variables W, Wth, SI, VOL, AR, SW, WW, and SProp. These results can be due in large part to this genotype being a commercial line that has been improved through time for particular physical characteristics, in contrast with the Creole birds. Rodríguez and Bravo (2019) studied egg weight in laying hens of the Hy-Line Brown line in the first laying phase and found average weights of 56.64 g, and a mean of 61.220 ± 0.242 was obtained in this study. The

Table 2. Adjusted means (± SE) of physical characteristics of egg from Mexican Creole with number of birds=30, number of eggs=504; Rhode Island Red with 15 birds and 459 eggs, and Hy-Line Brown with 30 birds and 502 eggs in intensive production.

Variable	Mexican Creole	Rhode Island Red	Hy-Line Brown
P (g)	50.650 ± 0.242↾	50.266 ± 0.253↾	61.220 ± 0.242↾
L (cm)	5.383 ± 0.078	5.407 ± 0.821	5.490 ± 0.786
A (cm)	4.005 ± 0.048↾	3.980 ± 0.050↾	4.400 ± 0.048↾
IF	0.757 ± 0.009↾	0.750 ± 0.009↾	0.801 ± 0.009↾
VOL (cm^3)	46.243 ± 0.221↾	45.893 ± 0.231↾	55.890 ± 0.221↾
AR (cm^2)	63.146 ± 0.198↾	62.796 ± 0.210↾	71.723 ± 0.199↾
CoCas	2.688 ± 0.050↾	5.124 ± 0.053↾	6.834 ± 0.050↾
CoYema	6.738 ± 0.060↾	6.390 ± 0.061↾	5.439 ± 0.058↾
PClara (g)	28.023 ± 0.186↾	29.122 ± 0.194↾	38.030 ± 0.186↾
PYema (g)	15.923 ± 0.182↾	15.220 ± 0.191↾	15.249 ± 0.182↾
PCasc (g)	6.661 ± 0.042↾	6.205 ± 0.044↾	7.750 ± 0.042↾
PropClara	0.553 ± 0.002↾	0.579 ± 0.002↾	0.621 ± 0.002↾
PropYema	0.314 ± 0.004↾	0.304 ± 0.004↾	0.250 ± 0.004↾
PropCasc	0.132 ± 0.000↾	0.124 ± 0.000↾	0.130 ± 0.000↾

a,b,c Means with different letter within each row are different (P<0.05). EE: standard error. P: egg weight, L: egg length, A: egg width, IF: shape index, VOL: volume, AR: area, CoCas: eggshell color, CoYema: yolk color, PClara: white weight, PYema: yolk weight, PCasc: eggshell weight, PropClara: white ratio, PropYema: yolk ratio, PropCasc: eggshell ratio. N: number of birds, n: number of eggs per genotype.

longitudinal and transversal diameters are associated directly with the egg's weight; that is, the heavier eggs have diameters that are also larger and vice versa. Regarding the egg weight, North and Bell (1998) and Andrade *et al.* (2015) point out that it depends mainly on the bird's age, size of the yolk, and environment of production and of the diet.

The shape index has a very significant effect on the resistance to squashing (Anderson *et al.*, 2004). Therefore, characteristics such as shape index and shell thickness avoid the risk of producing broken eggs and, this way, eggs of better quality are obtained. Duman *et al.* (2015) found a statistically significant positive correlation between the egg's shape index and the egg's superficial area ($P<0.005$). Eggs could be ordered from higher to lower in terms of the superficial area as round, standard and defined. These results agree with the findings by Alkan *et al.* (2013). According to Nordstrom and Ousterhout (1982), the shell weight is significantly and positively influenced by the egg weight. These authors found that 47% of the variation in the weight of the egg shell was due to the egg weight; therefore, this explains the result that was obtained in the variable shell weight (SW) on the Hy-Line Brown genotype. The proportions change, particularly in function of the egg size and indicate that the large ones contain less proportion of yolk than the small ones, which agrees with Delpech (1980). This is why the size of the eggs from the Hy-Line Brown genotype is closely correlated with the proportion of white, as well as with the other variables: weight, width, shape index, volume, area, white weight, shell weight, and white proportion.

The yolk color is determined by the hen breed and does not have anything to do with its quality, nutritional value or flavor (Suárez-Diéguez, 2021). Finally, the trend towards presenting a lighter yolk color (less content of xanthophyll) agrees with what was mentioned by Barrantes *et al.* (2006), who state that commercial eggs present lower color in comparison to the eggs obtained in a grazing system. That is, Creole hens probably produce more yolk color, since they are bred in the backyard, so their eggs present a similar yolk tone to those of grazing hens.

Abudabos *et al.* (2017) mention that the eggs from Creole birds can vary in weight and size depending on the age of the hens. Juárez-Caratachea *et al.* (2010) report that the average size of the egg from Creole hens is lower than from hens of commercial lines, while Jerez (1999) reports in trials with artificial incubation of Creole egg, that from the total of non-incubating egg, 8.97% was selected because it was small egg (65 g). In a study conducted by Segura-Correa *et al.* (2007), the average weight of the first egg from Creole hens was 45.3 g and increased with age until reaching 60.7 g at 39 weeks. The lower weight of the first egg and of the egg during the laying period from Creole hens, in comparison to commercial hens, is because the first were not selected for a larger size of the egg.

The genotype of the Creole Mexican hens has some particularities that are considered important. The Creole hens have statistically higher results in the variables YCo, YW, YProp and SProp. The yolk color is a variable that has been considered in recent years as a quality factor of the egg. This indicates that the yolk color depends on natural or artificial pigments in the feed consumed by the birds (Mikova *et al.*, 2014). A very intense color is rather demanded in the market, which is why the darker yolk is more pleasant when the egg is cooked or fried (RCAN, 2008). The color of a food continues to be one of the organoleptic factors of greatest importance for the consumer. Food color can indicate quality and freshness (Manguregui, 2020). Danilov (2000) and Islam and Dutta (2010) point out that the ratio between weight, length and width of the eggs, and the proportion of yolk, albumin and shell increased with the egg weight, and this increase is in relation with the age of the hen, reaching a plateau at the end of the laying cycle. In addition, the embryonic development of the hen egg depends on variables similar to those already mentioned, particularly of the yolk and the genetic line (Finkler *et al.*, 1998; Onagbesan *et al.*, 2007).

The internal characteristics of the egg quality, such as yolk weight and albumin weight, are very important from the nutritional point of view (Bain, 2005; Islam and Dutta, 2010). Although knowledge of the proportions of the white and the yolk have low interest for consumers, due to their relation with egg breakage, they have great importance for the poultry and dietary industries. These proportions change, particularly in function of the egg size: the largest have less proportion of yolk than the smallest (Delpech, 1980). This information explains the result that was obtained with the variable yolk proportion, whose mean was 0.314 ± 0.004. It should be highlighted that in this study, the Mexican Creole genotype produced smaller eggs compared to the Hy-Line Brown genotype. The Rhode Island Red genotype produced few significantly high values in most of the variables studied. Only the variable YProp, from the Creole and from this genotype, was higher than that of the Hy-Line Brown genotype. The result obtained is directly related to the egg weight. As has been mentioned, the yolk weight depends directly on the egg weight. It is important to highlight that all the physical and morphological characteristics are closely correlated with the egg weight. Hanusová *et al.* (2015) obtained an egg weight that is affected significantly ($P \leq 0.01$) by the breed. The eggs from the Oravka breed hens were heavier (60.96 ± 0.56 g) than those from the Rhode Island Red breed (57.60 ± 0.76 g). In this study, the egg weight from Rhode Island Red hens was 50.266 ± 0.253 g. This type of results depends on many factors, primarily genetic and dietary. The egg weight of the Raza Rhode Island Red breed found by Monira *et al.* (2003) was 57.20 g.

In addition, in the study by Hanusová *et al.* (2015) the weight of the white was significantly higher ($P \leq 0.05$) in the Oravka breed (34.96 ± 0.58 g) compared to the Rhode Island Red breed (32.78 ± 0.73 g). These results are similar to those obtained in this study, where the Rhode Island Red genotype resulted in a value of 29.122 ± 0.194 g, followed by Hy-Line Brown (38.030 ± 0.186 g). These weights of whites were higher ($P < 0.05$) than the value obtained with the Creole genotype (28.023 ± 0.186).

CONCLUSIONS

Eggs from the genotypes of the birds studied showed particular physical characteristics that distinguish them. The egg from Hy-Line Brown hens had higher values in some characteristics of commercial importance, among which the egg weight and the white proportion stand out. However, the egg from Mexican Creole hens was characterized by having better values in other characteristics that are also important for the consumer, such as yolk color, or for egg handling, such as shell proportion. The egg from Rhode Island Red hens showed similar values to those of two other genotypes in different characteristics. It is advisable to conduct a larger study to improve the egg from Creole Mexican hens.

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AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Forage evaluation based on oat on scenarios of intercrop and organic nutrition

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ABSTRACT

Objective: To evaluate the behavior of yield with different varieties of oat under monoculture and association conditions, applying different sources of nutrition.

Design/Methodology/Approach: Oat varieties were sown under monoculture conditions and 50% association, applying three sources of plant nutrition and a control, in the autumn-winter cycle. A completely randomized design with a factorial arrangement (3×3×4) was used, with the factors being the varieties of oats (chihuahua, turquesa, karma), the associations (monoculture, triticale and vetch) and the sources of nutrition (*Glomus fasciculatum* mycorrhiza, liquid bat guano, combination and control).

Results: Chihuahua stood out in dry matter (DM) yield, productivity index, leaf: stem ratio, harvest index and leaf area index, the karma variety stood out in botanical composition, Land Equivalent Ratio (LER), height and number of leaves. The association with triticale stood out in DM yield, productivity index and botanical composition. The vetch stood out in LER, leaf: stem ratio, harvest index and leaf area index. The monoculture stood out in the height of plants and number of leaves. The guano highlighted the harvest index, maintaining statistical equality with the mycorrhiza in LER.

Study Limitations/Implications: The results are based on the interaction of the factors with an irrigation regime in the temperate climate of the Valles Centrales of Oaxaca, Mexico.

Findings/Conclusions: The variety that stood out the most was the karma variety; however, the quality of the chihuahua variety can be discussed when comparing the relationships of the variables. The crop association that generated the best results was vetch, while triticale generated higher yields. The nutrition that generated the best results was guano, and there were a large number of statistical equalities with the control.

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INTRODUCTION

Oat (*Avena sativa* L.) is a fodder crop from temperate climates, of agronomic interest in Mexico, since a growth of 1.28% has been estimated for annual production between the year 2016 and 2030 according to data from SAGARPA (2017). It is a plant that stands out due to its use in livestock feed, because of its nutritional wealth that can be attributed to avenanthramides that are present in different amounts according to the variety of oat, as mentioned by Raguindin *et al.* (2021) and Ortiz-Robledo *et al.* (2013).

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When it comes to yield, Mendoza-Pedroza *et al.* (2021) mentions that the chihuahua variety present yields of note throughout its productive life. In this regard, Flores-Juárez *et al.* (2019) indicate that the varieties turquesa and chihuahua do not present significant difference in the production of dry matter (DM).

Crop association in oat production is a technique used to improve yield and resources, and in this regard, Colque-Romero (2002) conducted trials with triticale, associated with oat and vetch, obtaining results that are statistically equal in terms of DM yield. However, Lithourgidis *et al.* (2006) mention that oat height decreases in association, compared to its monoculture.

In contrast, in the oat-vetch association yields have been obtained of 16.6 t ha^{-1} (Flores-Nájera *et al.*, 2016). In this sense, the association of oat and vetch has been described as a technique to generate higher yields of green fodder, DM and fodder quality, compared to the oat monoculture, according to Espinoza-Montes *et al.* (2018). Vetch has been shown to reduce loss of fertile soil (Rodrigo-Comino *et al.*, 2020), that is, it contributes to soil mechanics. The quality of vetch as a nutritional element has been proven when used as a substitute for soy flour (25%) for lamb feed (Gül *et al.*, 2005); however, it has been noted that its use in monogastric animals is not favorable (Huang *et al.*, 2017).

Organic nutrition, when considering the systematic processes of soil and its microbiology, such as the application of beneficial microorganisms, has shown favorable improvements in the rhizosphere of crops (Li *et al.*, 2020; Trujano *et al.*, 2008). The application of biofertilizers like bat guano improves the amounts of organic matter, C, N, Ca and Mg, and increases the soil microflora (Sridhar *et al.*, 2006). As complement, the mycorrhiza *Glomus fasiculatum* facilitates the absorption of P, Ca, Fe, Mg, Zn, N (Rodrigues & Rodrigues, 2020), causing an increase in the development of the oat's leaf area and DM yield, without affecting the leaf:stem rate and plant height (Flores-Juárez *et al.*, 2019). About this, Torres *et al.* (2016) and Santana-Espinoza *et al.* (2020) mention that in oat production, results can be obtained that are statistically equal in 100% inorganic nutrition and 100% organic nutrition, which is opposite to that found by Montaño-Carrasco *et al.* (2017) who recommend the use of organic fertilizers to improve the yield and quality in the production of fodder oat.

The objective of the study was to evaluate the yield of different oat varieties in conditions of monoculture and association, applying different sources of nutrition.

MATERIALS AND METHODS

The study was conducted in the autumn-winter cycle (November-March) at the facilities of Instituto Tecnológico del Valle de Oaxaca, with address Ex Hacienda de Nazareno Sn Agencia de Policía Nazareno, Centro, 71230 Santa Cruz Xoxocotlán, Oaxaca (17° 01' 07.4" N and 96° 45' 51.5" W) at 1558 masl. The crop was maintained under irrigation conditions.

A completely randomized design was used with factorial arrangement (3×3×4), where the factors were the oat varieties (chihuahua, turquesa and karma), the crop association in 50:50 proportion (monoculture, triticale and vetch), and the organic nutrition (control, mycorrhiza *Glomus fasiculatum*, liquid bat guano, and a combination of the two), for a total of 36 treatments established in plots of 16.67m^2.

Plowing tasks and broadcasting sowing with a density of 120 kg ha^{-1} for each treatment were carried out; sowing was done under equal conditions, since all the seeds were found without alterations at the time. Depending on the treatment, the following were applied weekly: 4 l ha^{-1} of leaf bat guano, 6 kg ha^{-1} of mycorrhiza *Glomus fasciculatum* via soil (on the soil) diluted in water (20 spores per gram of soil, with purity of 85-98%), and the combination which had an application of 50% guano and 50% mycorrhiza; in every case a spray pump with capacity of 20 l was used.

The DM yield was obtained by cutting fodder (120 days) in four samples of 0.25 m^2 for each treatment, making use of a metallic square of 0.5 m per side, which was thrown randomly, and collecting only the plants that sprouted within the sampling area; then the total weighing of each sample was carried out in a digital scale of 20 kg capacity, obtaining the estimation of green matter yield. A subsample was obtained which was subjected to 6 days in a drying chamber at 70 °C to calculate the percentage of DM and thus estimate the DM yield (kg ha^{-1}). The ratio between yield in DM and the days to harvest, which in every case was 90 days, was calculated in order to obtain the productivity index. The botanical composition was calculated for each sample obtained, in a scale of 0 to 1, based on the ratio between the oat weight in the sample and the total weight of the sample. With the objective of defining whether there was a benefit in the oat yield compared to its monoculture, the Land Equivalent Ratio (LER; Mead & Willey, 1980) was calculated, using the following formula:

$$LER = Y_{ai}/Y_a$$

where Y_{ai}=oat yield in association and Y_a=oat yield in monoculture.

The leaf:stem ratio was calculated based on the subsamples, which were separated into their morphological components and weighed after drying, through the calculation of weight (leaf)/weight (stem). The harvest index was calculated from the ratio of weight of the leaf subsample and total weight of the subsample. The leaf area index was calculated from the weight of 1 cm^2 of leaf DM (per species), making use of the digital Vernier and an electronic gram scale; then, the leaf DM yield per treatment was calculated from data obtained from the harvest index and DM yield, and next the relation between this 1cm^2 and the leaf DM yield was calculated for each species and for each treatment.

Non-destructive sampling of five plants selected randomly was conducted at the time of cutting, where the distance between the ground and the spike (or the maximum point) of the plant was measured with a tape measure. A non-destructive count of the number of leaves from five samples per treatment was performed; vetch has compound leaves, so these were considered for the count.

The corresponding data were recorded in a spreadsheet and analyzed through the statistical software SAS On demand version, performing analysis of variance and means comparison with Tukey's test (P≤0.05).

RESULTS AND DISCUSSION

As Table 1 shows, the chihuahua variety presented the highest yield, which outperformed the turquesa and karma varieties by 14 and 77%, respectively, which agrees with what was reported by Mendoza-Pedroza *et al.* (2021), presenting different results from those mentioned by Flores-Juárez *et al.* (2019). Regarding the variables of interspecific competition, the karma oat stood out in botanical composition, since it allowed higher coexistence with its associations, and since it is the variety with highest LER, it can be noted that these associations increased the productivity yield, compared to its monoculture, which has a LER value of 1.4629; therefore, it is interpreted that the associations generated 46% more of karma oat production compared to the monoculture.

The association with triticale presented higher yields in fodder and botanical composition, meaning that having a better response with the oat allowed for both the oat and the triticale to produce sufficient DM, compared to the association with vetch and the monoculture, having different results from those reported by Colque-Romero (2002). However, the association with vetch stands out in LER, so that due to its value being 1.7754, it can be interpreted that it increases oat production by 77.54%, although since its contribution is minimal in botanical composition, since oat covered 96.35%, it can be understood that the values of yield and productivity index are statistically equal to monoculture, result that differs from what was described by Espinoza-Montes *et al.* (2018) and Flores-Nájera *et al.* (2016).

The application of bat guano and the mycorrhiza *Glomus fasciculatum* did not present a difference in the yield variables with the control, which is different from what was reported by Flores-Juárez *et al.* (2019), who reported higher yields with the use of mycorrhiza. However, Li *et al.* (2020), Montaño-Carrasco *et al.* (2017) and Sridhar *et al.* (2006) mentioned that when observing both the mycorrhiza and the guano, the latter in kind, they were a factor that contributed to oat being able to stand out in LER, since the application of

Table 1. Means comparison of the variables of fodder yield and interspecific competition.

Factor	Level	Yield		Interspecific competition	
		Yield kg DM ha^{-1}	Productivity index	Botanical composition	LER*
Variety	Chihuahua	8720.75 a	96.897 a	0.9757 c	1.1759 b
	Turquesa	7620.64 b	84.674 b	0.9444 b	1.2660 b
	Karma	4929.17 c	54.769 c	0.9105 a	1.4629 a
Association	Monoculture	6647.76 b	73.864 b	1.0000 c	1.0000 b
	Triticale	7991.65 a	88.796 a	0.8671 a	1.1294 b
	Veza	6631.14 b	73.679 b	0.9635 b	1.7754 a
Nutrition	Witness	7173.64 a	79.707 a	0.9554 a	1.0110 c
	Combined	7048.70 a	78.319 a	0.9427 a	1.2167 bc
	Micorriza	7054.06 a	78.379 a	0.9399 a	1.4359 ab
	Guano	7084.33 a	78.715 a	0.9361 a	1.5429 a

*Earth equivalent ratio.
Treatments with different letters in the column are statistically different (Tukey $P \leq 0.05$).

these nutrients allowed the oat in association to have higher production compared to the monoculture.

Table 2 shows that the chihuahua variety presented higher values in the variables of leaf production, being that the leaf:stem ratio was equal to the karma variety. About the associations, vetch stood out in terms of the leaf production in every case. Regarding the nutrition, guano was constant in its influence for leaf production, being that the variables of leaf:stem ratio and harvest index did not have significant difference with the control. The mycorrhiza favored the leaf area index, which agrees with what was reported by Flores-Juárez *et al.* (2019), although this variable did not have a significant difference with guano or with the combination of nutrients.

Table 3 shows that the karma variety was the one that generated a higher number of leaves, both for itself as oat and for its associations, since the greater heights were for this

Table 2. Means comparison of the variables related with the fodder leaf.

Factor	Level	Leaf:stem ratio	Harvest index (leaf)	Leaf área index
Variety	Chihuahua	0.739 a	0.3487 a	3.9458 a
	Turquesa	0.507 b	0.2335 c	1.9127 b
	Karma	0.688 a	0.2744 b	2.2441 b
Association	Monoculture	0.581 b	0.2576 b	1.922 b
	Triticale	0.483 c	0.234 b	2.133 b
	Veza	0.804 a	0.3649 a	4.046 a
Nutrition	Witness	0.7056 a	0.309 a	2.322 b
	Combined	0.5947 b	0.271 b	2.743 ab
	Micorriza	0.6067 ab	0.273 b	3.039 a
	Guano	0.6735 ab	0.288 ab	2.698 ab

Treatments with different letters in the column are statistically different (Tukey $P \leq 0.05$).

Table 3. Means comparison of the growth variables per species.

Factor	Level	Oats		Triticale		Vetch	
		Plant height cm	Number of sheets	Plant height cm	Number of sheets	Plant height cm	Number of sheets
Variety	Chihuahua	94.11 b	7.61 b	109.29 b	5.85 a	33.075 c	8.90 c
	Turquesa	101.10 a	8.18 a	123.45 a	5.25 a	40.340 b	10.55 b
	Karma	106.12 a	8.28 a	95.83 c	5.00 a	57.705 a	14.75 a
Association	Monoculture	115.50 a	8.95 a				
	Triticale	103.29 b	7.58 b				
	Veza	82.54 c	7.55 b				
Nutrition	Witness	102.48 a	8.42 a	117.82 a	5.66 a	40.16 b	11.33 a
	Combined	102.30 a	8.04 ab	103.92 ab	5.80 a	36.68 b	9.00 b
	Micorriza	92.90 b	7.62 b	102.27 b	5.06 a	53.55 a	12.93 a
	Guano	104.08 a	8.02 ab	114.08 ab	4.93 a	42.42 b	12.33 a

Treatments with different letters in the column are statistically different (Tukey $P \leq 0.05$).

variety of oat and for its association with vetch. However, statistically, the monoculture presented a greater height and number of leaves than its associations, datum that agrees with what was mentioned by Lithourgidis *et al.* (2006). Regarding nutrition, statistical equality can be seen between the guano and the control, being that the predominant values are mostly those of the control.

CONCLUSIONS

The chihuahua variety presents a similar behavior in leaf:stem rate to the karma variety, which is the variety of smallest size and with the least number of leaves. The dry matter yield is higher indicating that the number of leaves per ha will be higher than the karma variety can produce. The karma variety was more benefitted with the associations. Triticale and vetch were benefitted with the karma variety, with the highest number of leaves found with this variety. The botanical composition was predominantly oat, and although the dry matter yield of vetch is similar to that of the monoculture, it was generated with just half of the oat seed. In the growth variables, the mycorrhiza generated better results in the associations with vetch, not so in the monoculture or in the association with triticale. The guano and the mycorrhiza stood out as factors that allowed the oat to increase LER and botanical composition in the associations.

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AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Production of corn and sunflower fodder, and its preference as silage among ewes in Mineral de la Reforma, Hidalgo

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ABSTRACT

Objective: To assess the forage production of corn and corn with sunflower forage, as well as ewes' preference for this forage as silage.

Design/methodology/approach: A completely randomized block design with three replications was used in the field, while a completely randomized design (Tukey α=0.05) was preferred for work in laboratory. Sowing was done in spring-summer 2020 under rainfed conditions. The treatments were as follows: 100% corn; 90% corn + 10% sunflower; and 80% corn + 20% sunflower. Forage production was assessed at 126 days of sowing. Once ensiled, forage was assessed again through a bromatological analysis. Silage preference was evaluated for 20 days with 10 pregnant Hampshire × Suffolk ewes with a live weight of 44.8 kg.

Results: The combination of 80% corn + 20% sunflower delivered a higher fresh forage yield ($P<0.001$; 28 t ha^{-1}), a higher percentage of soluble protein ($P<0.01$), and a higher percentage of lignin ($P<0.001$; 4.6%). The ewes preferred the 100% corn silage, since it contained a lower percentage of non-fiber carbohydrates (22.2%), a lower percentage of acid detergent fiber (35.3%), and a lower percentage of neutral detergent fiber (59.4%).

Study Limitations/Implications: Sunflower should be established in soils with low amounts of broadleaf weed seeds, since chemical control cannot be applied to the said weeds.

Findings/Conclusions: A greater amount of forage was produced per surface unit when 80% corn was combined with 20% sunflower. Ewes preferred the 100% corn silage due to its lower percentage of lignin and a higher *in vitro* digestibility of neutral detergent fiber.

Key words: Alternative forage, Nutritional quality of silage, Silage preference.

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INTRODUCTION

Feed for ruminant cattle in production is based on forage crops and grazing forage. However, the use of silages is important to maintain the animals' body condition during the dry season without reducing livestock efficiency (Echavarría, 2007).

The sunflower is native to northern Mexico and the southeastern United States. Its fresh forage yield is approximately 8-12 t ha^{-1} (Fassio *et al.*, 2001). The crude protein (CP) content in the bud, flowering, and physiological maturity stages is 16, 11, and 9%, respectively. Meanwhile, digestibility of its organic matter is 75 (bud), 72 (flowering), and 63% (physiological maturity). In this regard, after assessing 13 sunflower genotypes, Tomich *et al.* (2003) reported yields of 12.1-29.1 t ha^{-1} on a wet basis. Velázquez-Martínez et al. (2018) reported yields of fresh corn forage in the semiarid rainfed conditions of the State of San Luis Potosí: 4.8 t ha^{-1} in Charcas and 28.0 t ha^{-1} in Matehuala. This indicates variability between the types of corn used by local producers under different soil and moisture conditions. Similarly, after assessing several combinations of corn and sunflower (100% corn, 75% corn + 25% sunflower, 50% corn + 50% sunflower, 25% corn + 75% sunflower, and 100% sunflower) in Almoloya de Juárez, Estado de México, Aragadvay-Yungán *et al.* (2015) concluded that sunflower silage could be an alternative to substitute up to 25% of corn silage. Sunflower provides a similar level of protein and energy supply than monoculture corn silage. However, water demand (consumptive use) is different for each crop: 467 mm for corn and 390 mm for sunflower (Villanueva *et al.*, 2001). This is an important factor for places with scarce and badly distributed rainfall.

When describing ewes' selection of forage material, Anderson *et al.* (2010) mention that ewes prefer forage with higher protein contents, low fiber carbohydrate levels, and a high fodder value. Therefore, we must keep in mind that, as plants get older, grass quality decreases due to lignin aggregation (Velázquez-Martínez *et al.*, 2022). The organic matter digestibility of sunflower decreases with physiological maturity, as a consequence of a higher lignin content, as well as of high oil contents, which affect the metabolism of ruminal microbiota (Fassio *et al.*, 2001).

Consequently, the combined cultivation of corn and sunflower in different proportions for silage during the wet season can improve the nutritional quality of silage. Sheep will normally select the silage with the highest percentage of protein, highest digestibility, and lowest lignin levels. Since this phenomenon has not been documented, the objective of our study was to assess different combinations of forage production (100% corn, 90% corn + 10% sunflower, 80% corn + 20% sunflower), to ensile them, to conduct a bromatological analysis in a certified laboratory, and, finally, to determine which of the three silages sheep prefer as feed.

MATERIALS AND METHODS

Study site

The study was conducted at La Pila, Mineral de la Reforma, State of Hidalgo, Mexico, during the 2020 spring-summer agricultural cycle. The site is located at 20° 07' 06.22" N and 98° 40' 29" W, at an altitude of 2,510 m. The climate is temperate semi-arid, with an average annual temperature of 15 °C and 540 mm of rainfall. The type of soil is Vertisol with a clayey texture (Ramírez-Bautista *et al.*, 2017). We used corn seeds of the Asgrow brand, Faisán variety, which is a three-way cross that flowers 95 days after sowing. The sown sunflower (*Helianthus annuus*) was of the Sunspot variety, which reaches the milk-dough stage at 126 days. The ewes used to select the silage were 18-month-old Suffolk×Hampshire crossbreeds with a 4-month pregnancy and an average live weight of 44.85 kg. The study comprised soil preparation, sowing, first and second weeding, cutting, and silage of forage materials (June-November, 2020), as well as ewe conditioning and the selection of silage by the ewes (December, 2020-January, 2021).

Soil preparation was carried out on May 26, 2020 and comprised a fallowing and two harrowings. Sowing was carried out on June 6, 2020 on soil at field capacity, at a depth of 15 cm, with a manual grain drill and without fertilization. Forage materials proportions (treatments) were 100% corn, 90% corn + 10% sunflower, and 80% corn + 20% sunflower, all of which were sown in three complete randomized blocks with three replications. Following Escalante-Estrada *et al.* (2008), we sowed 75,000 plants ha^{-1}. When combining corn and sunflower, seeds were mixed according to each company's information on purity and viability. Subsequently, seeds were weighed on a Truper® scale No. 15161 (5.0 kg; México).

The experimental unit consisted of two 6-m long furrows separated by 0.80 m. The forage was cut 10 cm above the soil surface and weighed on a Torino® dial hanging scale (Morelia, Michoacán, Mexico). Forage materials were harvested at 126 days, as indicated by Aragadvay-Yungán *et al.* (2015). The dry matter sample was determined by weighing all fresh forage in each experimental unit, and then weighing 25% of the said forage, mincing it, and laying it out on paper in a ventilated greenhouse for 15 days. Afterwards, each treatment's materials were put in previously labelled paper bags and arranged in a Ciderta® air forced stove (Huevla, Spain) at 55 °C during 5 hours, and then they were weighed. To ensile the forage, we minced it manually (<1 inch), placed it inside 100-L plastic barrels in duplicate, compacted it gradually, and hermetically sealed the barrels. After 45 days, the barrels were opened and the dry matter was determined. When three replications of the dry matter sample reached a weight of 2.0 kg in on a Truper© digital scale No. 15161, the silages were placed in a greenhouse environment with air flow for two weeks. Afterwards, they remained in an air forced stove at 55 °C for 5 h and were weighed. To conduct the analyses, 0.5-kg samples were taken per treatment on a dry basis in duplicate and sent to the Agro Lab de México S.A. de C.V. certified laboratory, in Gómez Palacio, Durango, Mexico. The following data were determined in the said lab: crude protein percentage (CP; %), soluble protein (%), acid detergent fiber (ADF; %), neutral detergent fiber (NDF; %), non-fiber carbohydrates (NFC; %), fat (%), total digestible nutrients (TDN; %), organic matter digestibility at 30 h (%), *in vitro* dry matter digestibility (%), and *in vitro* NDF digestibility (%). In addition, the following data were determined in Mcal kg^{-1}: net energy for lactation (NE_l), net energy for maintenance (NE_m), net energy gain (NE_g), and metabolic energy (ME). With these results, we were able to start the ewes' silage selection test.

Animal conditioning and experimental management

Before the silage selection process, ewes were dewormed with 0.1% subcutaneous ivermectin at doses of 20 μL kg^{-1} body weight and oral albendazole at doses of 200 μL kg^{-1} body weight and revaccinated with subcutaneous one-shot Biobac 7-way bacterin at doses of 20 μL kg^{-1} body weight. Afterwards, ewes were placed for their adaptation in individual shaded corrals (1.5×1.7 m) for nine days from 13:00 to 17:00 h. They were provided rye grass hay and water *ad libitum*. The ewes' silage selection was evaluated for 20 days. Ewes remained together from 08:00 to 12:00 h and were offered 700 g animal^{-1} rye grass hay and water *ad libitum*. The selection of the three silages took place between 13:00 and 17:00 h in the individual corrals with water *ad libitum*. In each corral, we placed a trough with three separate compartments holding 510 g of each silage: 1) corn silage; 2) 90% corn + 10% sunflower silage; and 3) 80% corn + 20% sunflower silage. After 17:00 h, all animals remained together for the night.

The assessed variables were: production of fresh forage and dry matter (t ha^{-1}); bromatological analysis in the laboratory; and which of the three silages the ewes preferred (g animal^{-1}). In addition, ewes were weighed before and after the experiment with a 200-kg Torino© hanging crane scale (Mexico) with a 0.5 kg interval.

Data analysis

Data were subjected to an analysis of variance using the statistical software SAS/STAT (2010) and means were compared using Tukey's test (α=0.05), before conducting Bartlett's test for homogeneity of variance. The forage production model was as follows:

$$Y_{ij}=\mu\, Treat_i+Block_j+e_{ij}, \quad (1)$$

where Y_{ij}=response variable in treatment i, replication j; μ=overall mean; $Treat_i$=effect of treatment i, where i=1, 2, and 3; $Block_j$=effect of block j; e_{ij}=random error.

The model used to analyze the consumption preference data was as follows:

$$Y_{ijk}=\mu+Sup_i+Day_j+(Sup_i\times Day_j)+e_{ijk} \quad (2)$$

where Y_{ijk}=observed response in the sampling time in the *j-th* subsample, *i-th* sunflower inclusion level, in the *k-th* replication; μ=overall media; Sup_i=effect of the type of supplement i, where i=1, 2, and 3; Day_j=effect of the sample on time j, where j=1... 20; $Sup_i\times Day_j$=effect of the interaction between the *i-th* type of supplement and the *j-th* sampling day; e_{ijk}=experimental error associated with all observations (Y_{ijk}).

RESULTS AND DISCUSSION

We observed a difference between the fresh forage yield (P<0.001) and dry forage (P<0.01; Table 1): 80% corn + 20% sunflower was 1.15 times higher than corn monoculture, contrary to the findings of Warren (1980), who observed a higher amount of dry matter in corn monoculture (11.2 *v.* 9.3 t ha^{-1}). A comparison between the production of fresh

Table 1. Production in rainfed conditions of fresh corn forage and dry corn fodder, and corn + sunflower fodder in Mineral de la Reforma, State of Hidalgo, Mexico.

Proportion of the seeds sowed	FM (t ha^{-1})	DM (t ha^{-1})
Corn 100%	22.4 c †	7.0 b
Corn 90% + sunflower 10%	24.8 b	7.4 ab
Corn 80% + sunflower 20%	28.0 a	8.0 a
Average	25.1	7.4
Significancy	***	*
SEM	0.32	0.15

† Different lower-case letters in the same column are statistically different averages; ***P<0.001, *P<0.05. FM=Fresh matter, DM=Dry matter. SEM=Standard error of the mean.

corn forage obtained in this study and the results of Velázquez-Martínez *et al.* (2018) for Charcas (4.8 t ha^{-1}) and Matehuala (28 t ha^{-1}) shows that production in Mineral de la Reforma, Hidalgo, is within the range of corns cultivated under rainfed conditions. A higher proportion of sunflower resulted in a higher forage yield (P<0.01). However, growing sunflower is difficult, since its development in soils with a history of weed seedbanks will be lower, because selective herbicides for broadleaf weeds cannot be applied.

The bromatological analysis of silages based on corn monoculture (90% corn + 10% sunflower, and 80% corn + 20% sunflower) did not show any differences (P>0.05) regarding CP and total digestible nutrients (TDN) (Table 2). However, soluble protein was higher in 90% corn + 10% sunflower (P<0.01), which matched a higher score in the production of volatile fatty acids. We can therefore assume that microorganisms make better use of forage protein for multiplication, which results in a higher production of metabolic protein and volatile fatty acids (Velázquez-Martínez *et al.*, 2022). In this regard, Okoruwa and Igene (2014) mention that a higher digestibility of neuter detergent fiber (NDF) in rumen results in a higher production of volatile fatty acids in the following order: acetate, propionate, and butyrate. This phenomenon was observed in the monoculture silage (P<0.01). The metabolic energy of forage depends on the digestibility and concentration of protein, fat, fiber and non-fiber carbohydrates, as well as on carbohydrate type and digestibility (Núñez *et al.*, 2014). All these elements affect the ruminants' consumption of dry matter.

We observed differences (P<0.001; Figure 1) when testing which silages ewes preferred to consume; however, there were no differences for time (P=0.0784) or interaction (P=0.8781). The average silage consumption was: 355 g animal^{-1} d^{-1} for 100% corn; 237 g animal^{-1} d^{-1} for 90% corn + 10% sunflower; and 235 g animal^{-1} d^{-1} for 80% corn + 20% sunflower. Corn monoculture was 1.5 times higher than both combinations. The means comparison test showed that ewes preferred the monoculture silage over the corn and sunflower silage (P<0.01) and that no differences were observed between silages with sunflower (P>0.05; Figure 1). This could be explained by the lab results, which show that the corn silage contained less lignin (P<0.001; Table 2). In addition, the content of non-fiber carbohydrates was higher in the corn silage (P<0.01), another reason for ewes to like it better. This phenomenon was recorded by Anderson *et al.* (2010), who mention that

Table 2. Chemical composition of three silages produced under rainfed conditions in Mineral de la Reforma, Hidalgo, Mexico, and used for selection by ewes.

Variable	Corn 100%	Corn 90% - sunflower 10%	Corn 80% - sunflower 20%	Significancy	SEM
Crude protein (%)	10.4	10.2	10	NS	0.07
Soluble protein (%)	59.0 c †	72.5 a	66.0 b	0.01	0.86
ADF (%)	35.4 b	35.9 a	35.8 a	0.01	0.043
NDF (%)	59.3 b	61.1a	61.9 a	0.01	0.18
Lignin (%)	3.2 c	3.6 b	4.6 a	0.001	0.043
DIV 30 h (%)	80.5 a	77.5 b	78.1ab	0.05	0.41
DIV NDF 30 h (%)	67.3 a	65.2 b	64.1b	0.01	0.17
NFC (%)	22.3 a	20.5 c	21.6 b	0.01	0.05
Fat (%)	2.9 a	2.3 b	2.2 b	0.01	0.04
TDN (%)	58.9	59.3	57.5	NS	0.33
NE_l (Mcal kg^{-1})	1.19 a	1.16 ab	1.12 b	0.05	0.007
NE_m (Mcal kg^{-1})	1.18 a	1.17 a	1.12 b	0.01	0.005
NE_g (Mcal kg^{-1})	0.62 a	0.61a	0.55 b	0.01	0.004
ME (Mcal kg^{-1})	2.38 a	2.29 b	2.22 c	0.01	0.01
VFA value	6.27 b	7.16 a	5.48 c	0.001	0.007

† Means with the same letter in the same line are not statistically different (Tukey α=0.05). SEM=Standard error of the mean. NS=Not significant (P>0.05). ADF=Acid detergent fiber. NDF=Neutral detergent fiber. IVD=*In vitro* digestibility. NFC=Non-fiber carbohydrates. NE_l=Net energy for lactation. NE_m=Net energy for maintenance. NE_g=Net energy for gain. ME=Metabolic energy. VFA=Volatile fatty acids.

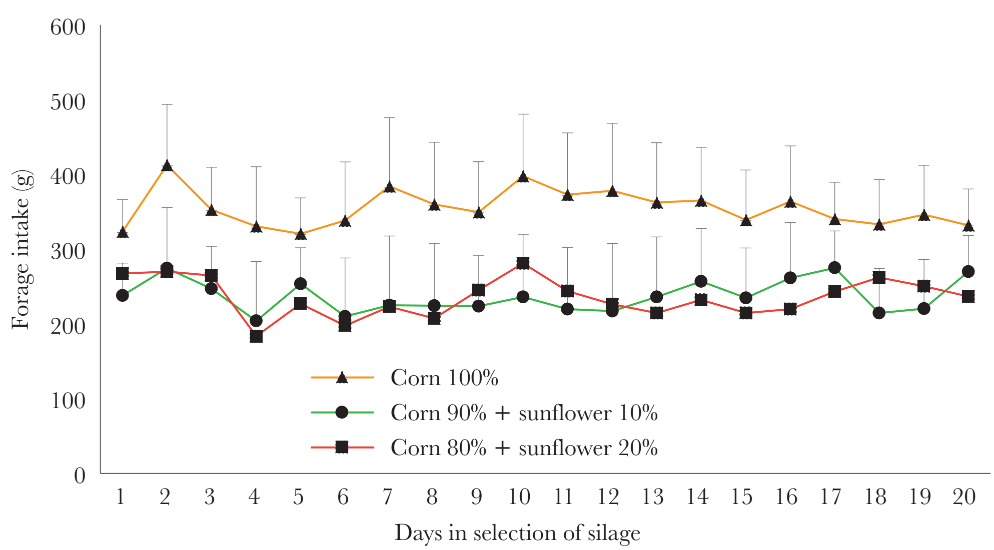

Figure 1. Ewes' silage consumption preference in Mineral de la Reforma, Hidalgo, Mexico.

ewes select their diet based on the crude protein, digestible and non-digestible fiber, and lower pubescence of forage materials; therefore, silages with sunflower are less likely to be preferred. The ewes' final average weight after 20 days of silage selection was 49.77 kg. The daily weight gain was 0.258 g.

CONCLUSIONS

When more forage per surface unit must be produced, the best combination for the site under rainfed conditions is 80% corn + 20% sunflower. However, ewes preferred the 100% corn silage as a result of its lower percentage of neutral detergent fiber and its lower lignin content, which improves digestibility.

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Chemical composition of Taiwan grass (*Pennisetum purpureum* Schum.) at different harvesting intervals

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ABSTRACT

Objective: To evaluate the effect of the harvesting interval on the quality of Taiwan grass (*Pennisetum purpureum* Schum.).

Design/Methodology/Approximation: Crude protein (CP), *in vitro* dry matter digestibility (IVDMD), crude fiber (CF), neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin, ether extract, and ashes were determined. Samples were collected from the Papaloapan experimental site of the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, Isla, State of Veracruz (18° 01' 45" N, 95° 31' 35" W). Treatments consisted of five harvesting intervals (30, 60, 90, 120, and 150 days). Data were analyzed under the general linear model and means were separated using Tukey's test ($P<0.05$).

Results: The nutritional value decreased ($P<0.05$) as the harvesting interval increased from 30 to 150 days. The following elements decreased: CP (leaves, from 12.3 to 3.7%; stems, from 8.9 to 2.1%), IVDMD (leaves, from 66.5 to 43.5%; stems, from 62.7 to 32.5%), ether extract (leaves, from 2.4 to 1.4%; stems, from 1.4 to 0.6%), and ashes (leaves, from 10.3 to 6.1%; stems, from 10.9 to 2.9%). On the contrary, the following elements increased: CF (leaves, from 28.4 to 41.1%; stems, from 33.4 to 44.5%), NDF (leaves, from 60.4 to 72.5%; stems, from 63.8 to 74.3%), ADF (leaves, from 36.7 to 46.8%; stems, from 34.6 to 50.7%), and lignin (leaves, from 9.7 to 15.3%; stems, from 11.0 to 18.3%).

Study Limitations/Implications: Neither 30 days harvesting intervals nor yields (tons) per hectare were taken into account.

Findings/Conclusions: Taiwan grass should be harvested at 60 days, when its nutritional value has not decreased too much.

Keywords: Taiwan grass, nutritional value, harvesting interval, chemical composition.

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INTRODUCTION

The nutritional composition of grasses is very important in tropical regions (Araya and Boschini, 2005). High-quality and high-biomass annual production forages are a significant alternative for those regions (Singh *et al.*, 2013; Maldonado-Peralta *et al.*, 2019). On recent years, grasses (particularly, Taiwan grass) with high potential for cutting and grazing systems have been introduced to Mexico. Bohnert *et al.* (2011) and Norton *et al.* (2016) determined that *Pennisetum purpureum* Schum. is adapting to the tropical regions of Mexico (Madera *et al.*, 2013). Tropical forages are C_4 plants with a high photosynthetic rate, which provides them with a high capacity for biomass production (Bemhaja, 2000). These plants were selected as a result of their high biomass production (Na *et al.*, 2013). However, these plants have a lower digestibility (Bohnert *et al.*, 2011) and have a lower protein content (Barbehenn *et al.*, 2004; Sosa-Montes *et al.*, 2021) than C_3 plants. They develop in geographical regions where solar radiation and environmental temperature allow them to grow all year long (Singh *et al.*, 2013), as long as enough humidity is available.

An appropriate management of Taiwan grass could improve its nutritional value. Gómez-Gurrola *et al.* (2015) reported that the Taiwan grass' protein diminishes as the cutting interval increases. Santana *et al.* (2010) recorded over 14% of protein at 18 days of regrowth. Ramos-Juárez *et al.* (2018) proved that the protein content of grass increases with a 0.5-1% urea supplement.

Currently, there are few studies about the chemical composition and digestibility of Taiwan grass (*Pennisetum purpureum* Schum.). Therefore, the objective of this study was to evaluate the chemical composition and *in vitro* dry matter digestibility (IVDMD) of Taiwan grass, at different harvest intervals (30, 60, 90, 120, and 150 days).

MATERIALS AND METHODS

Forage samples were obtained from the Papaloapan experimental site of the Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias, Isla, State of Veracruz (18° 01' 45" N and 95° 31' 35" W, at 65 m.a.s.l). The climate is subhumid warm, with summer rains, a 25.7 °C annual average temperature, and a 1,000 mm average precipitation (García, 2004).

Sampling collection

In order to obtain the samples, *Pennisetum purpureum* were cut from a 15 m long furrow, with 0.5 m of separation between furrows. The cutting was made up of three replications. The plants were initially fertilized with 75-33-10 kg ha^{-1} N-P-K. The same elements were used for the 150-66-20 kg ha^{-1} annual maintenance fertilization. Both applications were carried out during the rainy season. After the standardization cutting, plants were harvested at 30-, 60-, 90-, 120-, and 150-day intervals. The leaves and the stems were separated from each sample. The samples were then put in paper bags and dried in a forced-air stove at 55 °C, until they reached constant weight. Partial dry matter content was determined for each morphology component of the samples. Subsequently, the samples were crushed in a mill with a 1 mm crible.

Chemical analysis

Crude protein (N×6.25), neutral detergent fiber, acid detergent fiber, lignin, ether extract, and ashes percentages were determined using the AOAC method (1980). The analysis was carried out in the Sección de Nutrición Animal lab, Departamento de Zootecnia, Universidad Autónoma de Chapingo.

In vitro dry matter digestibility

A 0.3 g sample was subjected to a digestion (39 °C, 48 h) with ruminal fluid, using McDougal's artificial saliva as buffer solution. The residue was digested with neutral detergent; the digestibility percentage was calculated subtracting the non-digested cell wall percentage from 100% (Van Soest, 1994). The ruminal fluid of a Holstein bull with a fistula was used in all the samples. The bull was fed maize and alfalfa silage.

Statistical analysis

The experimental unit consisted of the plants from a 15-m long furrow. Three furrows, whose plants were harvested at 30, 60, 90, 120, and 150 days after the standardization cutting, were established. During the experimental period, leaves and stems were subject to fifteen observations. Each individual morphology component was statistically analyzed and the harvest ages were considered as treatments. The SAS 9.3 statistical package was used to analyze the data. The PROC GLM (lineal general model) was used to carry out the analysis of variance. Tukey's test was used to compare the means between treatments. The significance level was 5%.

RESULTS AND DISCUSSION

Crude protein

Figure 1 shows the crude protein content and the harvest interval for stem and leaf. Significative differences ($P<0.05$) were found between some of the intervals. The protein percentage diminished as the age of the plant increased ($P<0.05$). The stem recorded

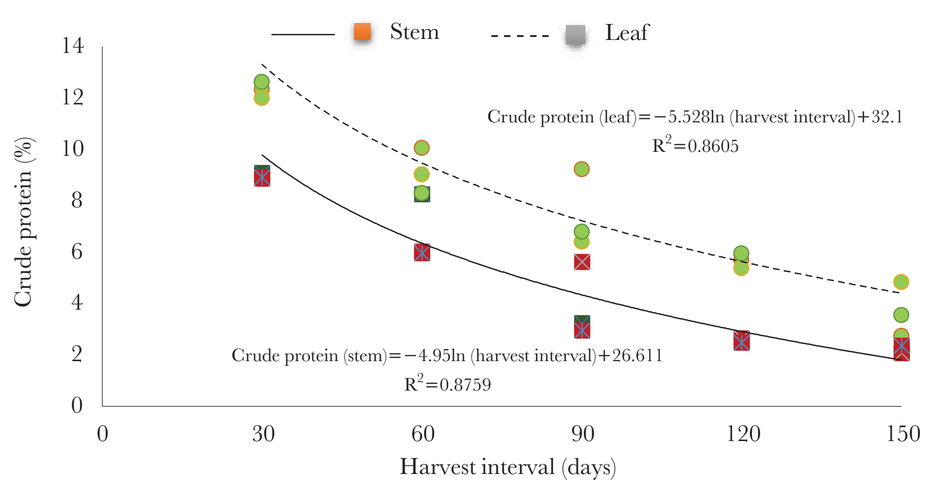

Figure 1. Leaf and stem crude protein content of Taiwan grass (*Pennisetum purpureum* Schum.), at different harvesting intervals.

the following values: 8.9, 6.7, 3.9, 2.5, and 2.1% for the 30-, 60-, 90-, 120-, and 150-day intervals, respectively. Without taking into account the 120 and 150 intervals —which were similar—, all other intervals had a different stem protein concentration. Leaf recorded the following values: 12.3, 8.1, 7.4, 5.6, and 3.7% at 30, 60, 90, 120, and 150 days, respectively. All the values are statistically different among themselves ($P<0.05$). Both stem and leaf recorded their maximum protein value at 30 days: 8.9 and 12.3%, respectively.

Araya and Boschini (2005) recorded 10.8, 7.1, 5.2, and 5.8% (stem) and 14.5, 14.3, 12.1, and 11.6% (leaf) values, at 70, 98, 126, and 140 days, respectively. These findings are higher than those found in this work. Bemhaja (2000) reported a 15.5% (13.9-17.1%) average protein in different *Pennisetum purpureum* Schum. cultivars, whose height reached 1.20 m. These results are higher than those obtained in this research at 30 days. The crude protein content and quality of the Taiwan grass tend to change and depend on different factors such as: harvesting interval, grazing frequency, soil fertility (Bemhaja, 2000), and leaf:stem ratio (Na *et al.*, 2015).

In vitro dry matter digestibility

Figure 2 shows the *in vitro* dry matter digestibility (IVDMD) percentage of Taiwan grass, according to the harvesting interval. The following values were recorded: 62.7, 53.3, 43.0, 35.6, and 32.5% (stem) and 66.5, 62.7, 55.0, 47.9, and 43.5% (leaf) at 30, 60, 90, 120, and 150 days, respectively. All the values are statistically different among themselves ($P<0.05$). Santana *et al.* (2010) pointed out that protein diminishes as the fiber percentage increases. The protein percentage of Taiwan grass depends on nitrogen fertilization (Singh *et al.*, 2013).

Madera *et al.* (2013) conducted IVDMD studies of Taiwan grass in Yucatan. Their equations were used to estimate the following results: 64.6, 61.3, 58.0, 54.7, 51.4, and 48.0% (leaf) and 61.8, 55.6, 49.4, 43.2, 37.0, and 30.8% (stem), at 45, 60, 75, 90, 105, and 120 harvesting days, respectively. They proved that, as the harvesting interval increases, IVDMD diminishes, as a result of lignification and fiber increase in Taiwan grass (Santana

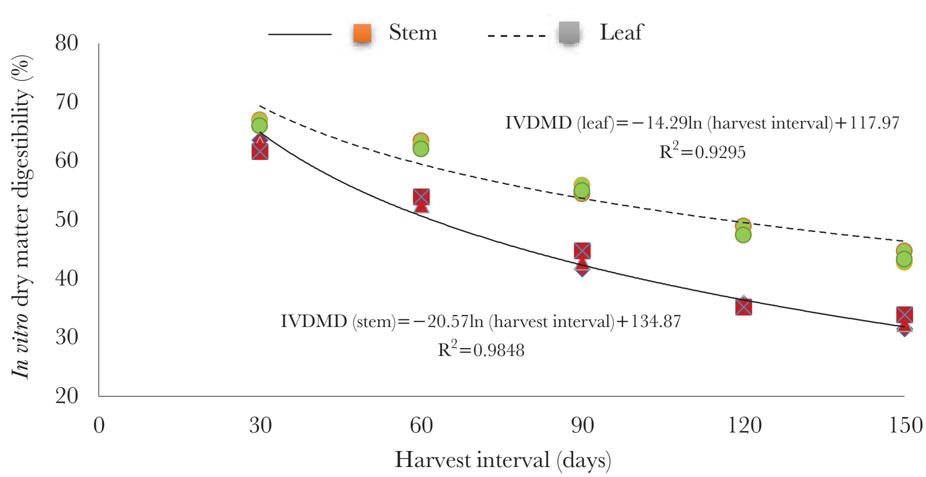

Figure 2. *In vitro* dry matter digestibility (IVDMD) percentage of the leaves and stems of Taiwan grass (*Pennisetum purpureum* Schum.), at different harvesting intervals.

et al., 2010). Chacón-Hernández and Vargas-Rodríguez (2009) reported the following IVDMD percentages 58.7, 55.9, and 52.0%, at 60, 75, and 90 harvesting days, respectively. Madera *et al.* (2013) obtained similar results, while Chacón-Hernández and Vargas-Rodríguez (2009) obtained lower percentages than those obtained in this research.

Lignin

Figure 3 shows lignin content at different harvesting intervals. Both in leaf and in stem, lignin showed a low content increase ($P<0.05$) after 30 days (11% leaf and 21% stem) and after 60 days (9.7% leaf and 11.0% stem). In Costa Rica, Chacón-Hernández and Vargas-Rodríguez (2009) reported different lignin content in whole plants: 12.2, 13.3, and 13.6%, at 60, 75, and 90 days, respectively. The lignin percentage obtained in that study at 60 days matches those found in this study, in which lignin percentage kept increasing as plants got older, until it reached 15.3% (leaf) and 18.3% (stem), at 120 days.

An increase in lignin content (Figure 3) entails a diminishing of forage digestibility, as a result of the formation of the lignin-carbohydrate complex, which embeds in cellulose and physically prevents the glucosidase enzyme action (Van Soest, 1983). Therefore, in this study, when lignin (Figure 3) increased, the IVDMD diminished (Figure 2).

Crude fiber

Figure 4 shows the crude fiber (CF) content of Taiwan grass, at different harvesting intervals. Leaf and stem had significative differences ($P<0.05$) between intervals. The following CF results were recorded: 33.4, 35.9, 38.5, 42.7, and 44.5% (stem) and 28.4, 32.9, 36.2, 38.9, and 41.1% (leaf), at 30, 60, 90, 120, and 150 harvesting days ($P<0.05$). Nevertheless, between days 120 and 150, no CF differences were recorded ($P>0.05$) in stem.

Both morphology components showed a CF increase as their age increased. Santana *et al.* (2010) reported 31.3 and 35.4% CF values at 30 and 60 days after the regrowth. These values are similar to those obtained in this study. Guerra-Medina *et al.* (2021) recorded

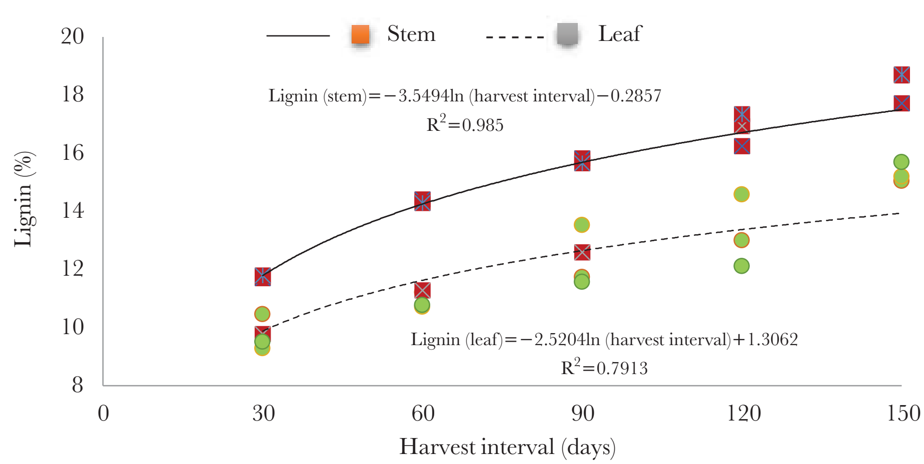

Figure 3. Lignin percentage of leaf and stem of Taiwan grass (*Pennisetum purpureum* Schum.), at different harvesting intervals.

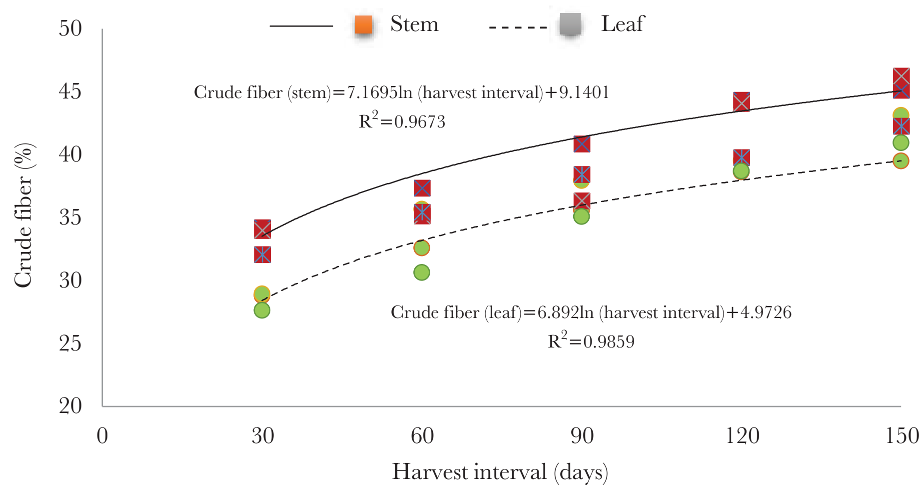

Figure 4. Crude fiber percentage of leaf and stem of Taiwan grass (*Pennisetum purpureum* Schum.), at different harvesting intervals.

38.2, 37.5, and 37.3% CF values in Taiwan grass, with and without fertilization, at 45 days after regrowth. These values are slightly higher than those found in this study.

Neutral detergent fiber

Figure 5 shows the neutral detergent fiber (NDF) content of Taiwan grass, at different harvesting intervals. Stem and leaf had significant differences ($P<0.05$). The following NDF contents were recorded in the stem: 63.8, 67.1, 69.5, 72.8, and 74.3%, at 30, 60, 90, 120, and 150 days of age, respectively. No significant differences were found with cuttings at 60 and 90 days, as well as 120 and 150 days ($P>0.05$). The following results were recorded for leaves: 60.4, 64.3, 67.3, 70.4, and 72.5%, at 30, 60, 90, 120, and 150 days of harvest. All the values are different among themselves.

Vivas-Quila *et al.* (2019) reported 58.7, 57.8, 60.6, 59.3, and 62.8% NDF content, at 50, 60, 70, 80, and 90 harvesting days, respectively. These values are slightly lower than those

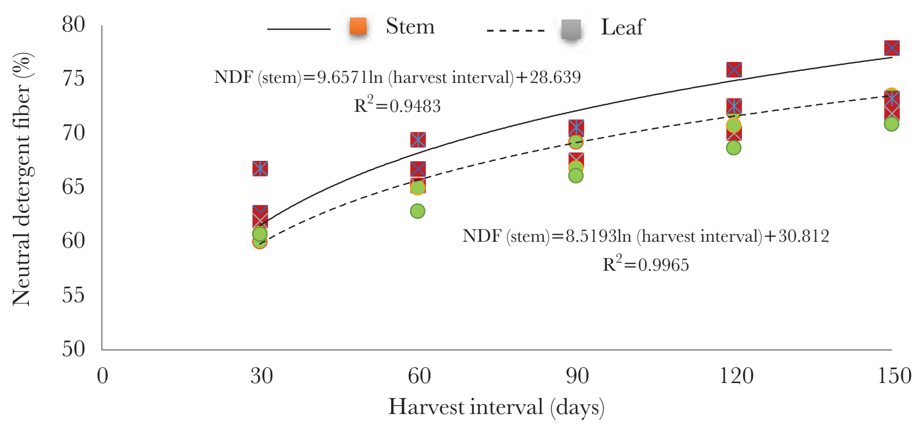

Figure 5. Neutral detergent fiber percentages of leaf and stem of Taiwan grass (*Pennisetum purpureum* Schum.), at different harvesting intervals.

obtained in this study. Gómez-Gurrola *et al.* (2015) recorded the following NDF content: 63.4, 69.0, 75.1, and 77.6%, at 30, 60, 90, and 120 harvesting days, respectively. The NDF data obtained by these authors increased as the harvesting interval increased. Their results match the findings of this study.

Acid detergent fiber

Figure 6 shows the acid detergent fiber (ADF) content. Leaf and stem recorded significant differences ($P<0.05$) and the ADF content increased as the harvesting interval increased.

The following ADF values were recorded: 34.6, 43.2, 46.2, 48.4, and 50.7% (stem) and 36.7, 40.4, 42.5, 44.2, and 46.8% (leaf), at 30, 60, 90, 120, and 150 harvesting days, respectively. ADF values were higher in the stem than in the leaves. Vivas-Quila *et al.* (2019) reported the following ADF content: 39.3, 41.7, 43.0, 44.3, and 44.9%, at 50, 60, 70, 80, and 90 harvesting days, respectively. Those results are similar to those obtained in this research. Guerra-Medina *et al.* (2021) recorded the following ADF values in Taiwan grass, 45 days after the regrowth: 44.1 and 50.2% (with fertilization) and 43.4, 44.1, and 50.2% (without fertilization). The values obtained without fertilization are similar to those obtained in this study, after 60 days (stem, 43.2%; leaf, 40.4%).

Ether extract

Figure 7 shows the ether extract (EE) content. Leaf and stem recorded significative differences ($P<0.05$) and the EE content diminished as the harvesting age increased. The following EE values were recorded: 1.4, 1.3, 1.1, 0.8, and 0.6% (stem) and 2.4, 2.1, 1.7, 1.5, and 1.4% (leaf), at 30, 60, 90, 120, and 150 harvesting days, respectively. EE values were higher in leaves than in stems.

Chacón-Hernández and Vargas-Rodríguez (2009) recorded similar values —1.4, 1.4, and 1.3%, at 60, 75, and 90 harvesting days, respectively— to those obtained in this study. Guerra-Medina *et al.* (2021) reported 1.0% EE at 45 days of age; this value is lower than

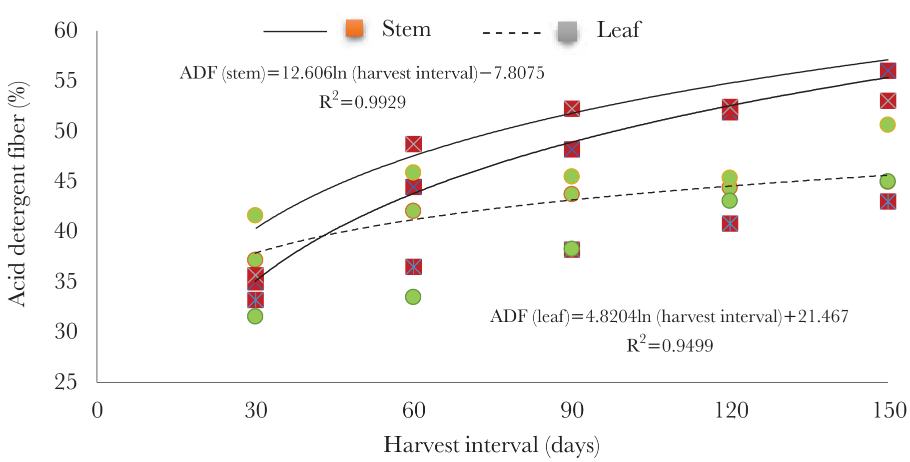

Figure 6. Acid detergent fiber percentage of leaf and stem of Taiwan grass (*Pennisetum purpureum* Schum.), at different harvesting intervals.

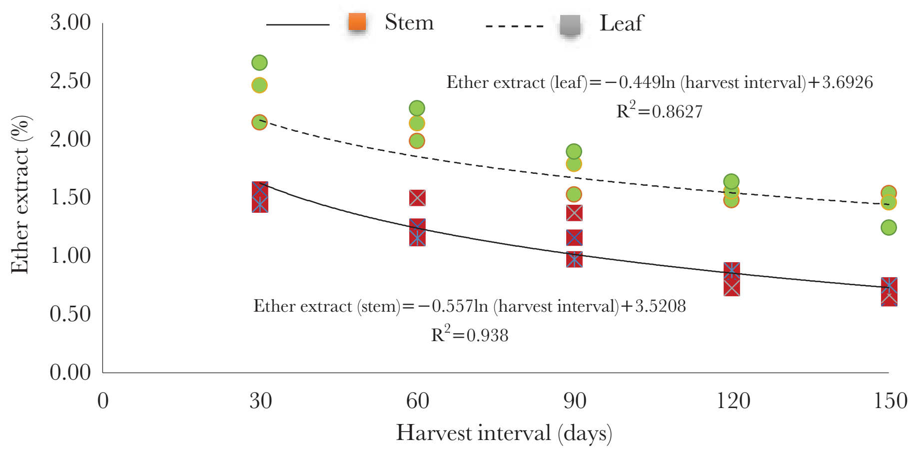

Figure 7. Ether extract percentage of leaf and stem of Taiwan grass (*Pennisetum purpureum* Schum.), at different harvesting intervals.

those found in this study. Umami *et al.* (2020) reported 3.3 and 3.5% EE values in three *Pennisetum purpureum* cultivars. These values are higher than those recorded in this study. Therefore, lipid concentration in forages tend to diminish, as the proportion of leaves or the leaf:stem ratio diminishes, which takes place when the plants grow older (Madera *et al.*, 2013).

Ashes

Figure 8 shows the ash content. Leaf and stem recorded significative differences ($P<0.05$) and ash content diminished, as the age of the plant increased. The following ash content was recorded: 10.9, 9.0, 6.9, 4.5, and 2.9% (stem) and 10.3, 9.2, 7.4, 6.3, and 6.1% (leaf), at 30, 60, 90, 120, and 150 harvesting days, respectively. Ash values were similar in leaves and stems.

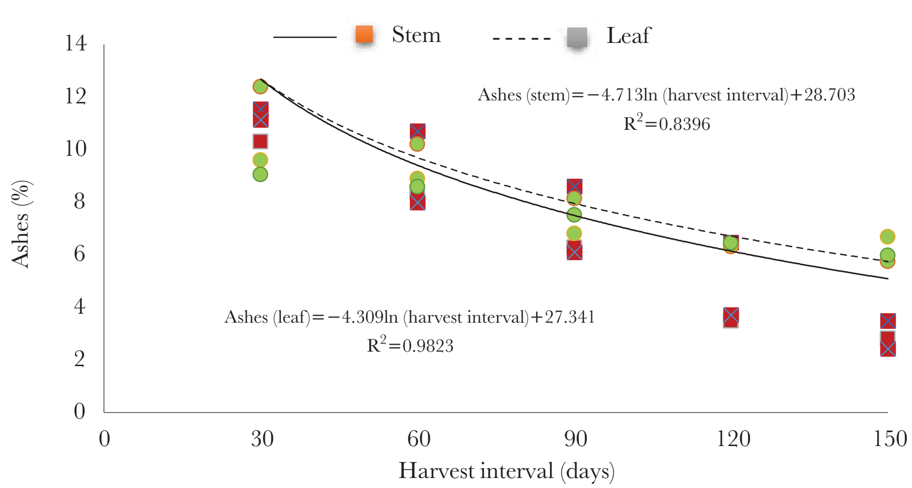

Figure 8. Ash content percentage of leaf and stem of Taiwan grass (*Pennisetum purpureum* Schum.), at different harvesting intervals.

Chacón-Hernández and Vargas-Rodríguez (2009) recorded 14.5, 13.9, and 13.6% ash values, at 60, 75, and 90 harvesting days. These values are higher than those obtained in this study. Gómez-Gurrola *et al.* (2015) reported the following values: 15.4, 11.9, 11.7, and 8.8% ash content, at 30, 60, 90, and 120 days after the cutting. Guerra-Medina *et al.* (2021) recorded 21.3% ash value, at 45 days of age. These values far exceed those found in this study. Umami *et al.* (2020) recorded 10.8% ash values in three *Pennisetum purpureum* cultivars. These values are similar to those obtained in this study at 90 days.

Overall discussion

Taking into account all nutrients, the CP, IVDMD, EE, and ash contents diminished and the CF, NDF, ADF, and lignin fiber contents increased, as the age of the plants increased. Consequently, the quality of the grass also diminishes; however, plant (Madera *et al.*, 2013) and hectare (Gómez-Gurrola *et al.*, 2015) yields increase as the age of the plant increases. Therefore, quality diminishes with a high yield of *Pennisetum purpureum* and vice versa. Using the eight logarithmic equations shown in Figures 1-8, the concentration per nutrient can be calculated, at any harvesting interval.

CONCLUSIONS

Regarding stem and leaf, protein, digestibility, ether extract, and ash content diminished, while the fiber components (crude fiber, neutral detergent fiber, acid detergent fiber, and lignin) increases, as the age of the plant increases. Consequently, the nutrient value of Taiwan grass (*Pennisetum purpureum* Schum.) also diminishes. Therefore, the recommendation is to harvest Taiwan grass at 60 days, when its yield is not so low and its nutrient value has not diminished too much.

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