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Colegio de Postgraduados

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Production and quality of meat from hair sheep grazing on Tanzania grass and supplemented with different protein levels

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ABSTRACT

Objective: To evaluate the effect of a supplementary feeding (with different crude protein (CP) levels) in the yield and growth performance and meat characteristics of hair lambs grazed on Tanzania grass.

Design/Methodology/Approach: A 120-d^{-1} experiment was conducted; it included four treatments and seven replications in a completely randomized design. Twenty-eight hair lambs (22.6$\pm$1.6 kg LW) were allowed to graze on Tanzania grass (*Panicum maximum*) and were provided concentrate feeds (with 10, 12, 14, and 16% crude protein). The aim was to assess the effects of the latter food on growth, carcass characteristics, and meat quality.

Results: Compared with the lambs fed with 10, 12, and 14% CP, the heaviest carcasses ($P<0.05$) were obtained from lambs fed with 16% CP. The percentage of crude protein and fat of the biceps femoris linearly increased ($P<0.05$) as the CP percentage increased in the concentrate feed. Meat color, water retention capacity, and cutting force were not impacted by the CP percentage of the concentrate feeds. Compared with the lambs fed with 10, 12, and 14%, the lowest palmitic acid percentage and the highest oleic acid percentage were found in the meat of lambs fed with 16% CP.

Study Limitations/Implications: A high area was selected to avoid excessive rain.

Findings/Conclusions: Compared with the lambs fed with 10, 12, and 14% CP, the lambs grazed on Tanzania grass and supplemented with 16% CP grew more, recorded a higher carcass yield, and their meat had a better unsaturated fatty acids ratio.

Keywords: Growth, Carcass, Pelibuey, Crude Protein, *Panicum maximum*.

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INTRODUCTION

Sheep farming in Mexico mainly takes place in the central, northern-central, and southern regions of the country. Southern Mexico is characterized by a tropical, warm, and humid weather, with quantitative and qualitative seasonal fluctuations regarding food supply (Gómez-Vázquez *et al.*, 2011). Therefore, the main limitations of this sheep breeding system include low nutrient forages that do not meet the food requitements of the animals (Gómez-Vázquez *et al.*, 2011; Amendola-Massiotti *et al.*, 2018). However, grazing Pelibuey production is expanding in the tropical region of Mexico. Consequently, the main products are <8 month-young lambs with a live weight of 20 kg. These lambs are purchased by commercial breeders, who finalize them with diets rich in grains, until the lambs reach a 30-40 kg live weight (Gómez-Vázquez *et al.*, 2011). This yield-growth rate is more difficult to obtain with Pelibuey sheep than with other breeds used for meat production (*e.g.*, Dorper and Katahdin). These breeds have been crossed with Pelibuey to improve the growth rate of lambs (Gómez-Vázquez *et al.*, 2011; Herrera-Corredor *et al.*, 2021). In the tropics, grazing Pelibuey and its hair sheep crosses have recorded <75 g^{-1} d^{-1} weight gains; however, when they are completely confined or when their diet is supplied with concentrates, they reach a 191-218 g^{-1} d^{-1} average weight gain (Galina *et al.*, 2007; Piñeiro-Vázquez *et al.*, 2009). Consequently, if animal yield is to reach its maximum value, supplementation is essential for a successful grazing sheep production (Ramírez *et al.*, 1995; Gómez-Vázquez *et al.*, 2011). In addition, supplementing with proteins increased weight gain (WG) in pure breed sheep under a tropical grazing system (Habib *et al.*, 2001; Becholie *et al.*, 2005; Mayren-Mendoza *et al.*, 2018). The best tropical green forage supply is 300 g^{-1} d^{-1}, rather than 150 and 600 g^{-1} d^{-1} (Archiméde *et al.*, 2008). Nevertheless, the proteins in supplements, growth yield, and meat quality of hair sheep crosses that graze on tropical grasses have been the evaluated in a limited number of researches. Therefore, the objective of this study was to evaluate the effect of including supplementary feeding and different crude protein (CP) levels on growth yield and meat characteristics of hair sheep grazed on Tanzania grass.

MATERIALS AND METHODS

The growth behavioral test was developed in a commercial sheep farm in Jalapa, State of Tabasco, Mexico. The area has a 26 °C average temperature and a mean relative humidity of 80%. The experiment consisted of four treatments and 7 replications. The experimental units (EU) were 28 hair lambs (22.6±1.6 kg body weight; 9-month-old), grazing in Tanzania grass (*Panicum maximum*). The EU were assigned in a completely randomized experimental design (CDR). The treatments consisted of four concentrate feeds (Table 1) with different CP levels: 10,12, 14, and 16%, in a dry basis. Therefore, a quantitative factor with equally spaced levels was studied. One and a half hectares were divided into four pastures. A single group of lambs grazed in each area from 06:00 am to 06:00 pm, under a 30-day rotation arrangement. Subsequently, the lambs were housed in 2.5×1 m individual pens, inside a stable, where each lamb was fed 300 g of concentrate feed. Lambs had free access to fresh water.

The period of adaptation to the pens and to the supplementary feedings lasted 15 d-1. The growth performance test lasted 120 d-1. Twice a month, before the supplementation, samples of the supplementary feedings were taken directly from the feeders; the composite sample lasted 120 d-1. Five-hundred grams of the composite samples were dried in a

Table 1. Ingredients and proximal analysis of the supplementary feedings.

	Tanzania grass	**Concentrate feed (CP % DM)**			
		10	**12**	**14**	**16**
Ingredient, % MS					
Grain of corn		25.0	25.0	25.0	25.0
Soy Flour 48% PC		0.0	2.5	7.1	11.7
Wheat flour		15.0	15.0	15.0	15.0
Rice polishing		15.0	15.0	15.0	15.0
MG5 Grass Hay		27.2	24.5	19.9	15.3
Coconut flour		5.0	5.0	5.0	5.0
Sugar cane molasses		10.0	10.0	10.0	10.0
Mineral Premix		2.0	2.0	2.0	2.0
Salt		0.5	0.5	0.5	0.5
Calcium carbonate		0.3	0.3	0.3	0.3
Urea		0.0	0.2	0.2	0.2
Composition					
Dry matter, %	78.0	88.1	88.3	88.2	88.6
Crude protein, % DM	11	10.3	12.1	14.2	16.1
Neutral detergent fiber, % DM	70	35.41	30.99	30.01	28.22
Acid detergent fiber, % DM	38	20.16	17.17	16.81	15.30
Ashes, %	12	28.51	26.51	23.12	19.73
EM	1.17	2.54	2.57	2.65	2.72

CP: crude protein; DM: dry matter; High bioavailability mineral compound: phosphorus 5%, calcium: 13%, sodium: 16%, chlorine: 24%, magnesium: 0.60%, sulphur: 0.18%, inorganic zinc: 3,000 ppm, organic zinc: 250 ppm, manganese: 1,100 ppm, cobalt: 125 ppm, iodine: 40 ppm, selenium: 5 ppm, vitamin A: 275,000 UI/kg, vitamin D3: 12,500 UI/kg, vitamin E: 500 UI/kg. MG5 grass hay: Tanzania grass (*Panicum maximum* cv. Tanzania) in a dry basis.

convection oven, at 90 °C for 48 h, in order to determine dry matter (DM), crude protein (CP), and ashes (AOAC, 2005), as well as neutral and acid detergent fiber (Van Soest *et al.*, 1991). Body weights were recorded at the beginning of the experiment and, subsequently, every 14 d^{-1}.

Grass samples (0.5 m^2) were taken from the prairies, at the beginning and at the end of each grazing period; the soil was cut 8 cm above ground level. Every day, for five days (115-120), each lamb was given 3-g capsules of chromium oxide. Stool samples (20-30 g) were taken from each animal during five days (115-120 days) and, afterwards, the samples were compared per animal. Grass consumption was estimated according to the method described by Geerken *et al.* (1987). Feed conversion was calculated as the ratio between daily intake (grass+300 g of concentrate, in a dry matter basis) and daily weight gain, from day 115 to day 120.

The lambs were slaughtered when the 120 d^{-1} test period was completed. The weight of the carcass was recorded right after the slaughter (warm carcass) and once the carcass was refrigerated (frozen carcass), at −20 °C, for 24 h in an authorized slaughterhouse.

One day after the slaughter, 200 g of bicep femoris were taken from the left hind leg; the sample was wrapped in kitchen foil and frozen at −20 °C for its subsequent analysis. The color of the meat (*a**: red intensity; *b**: yellow intensity; and *L**: luminosity) was measured using a Minolta CM-2002 chroma meter (Minolta Co., LTD, Japan). Afterwards, the Chroma value $\left(Chroma = a*2 + b*2\right)$ and the Hue value $\left(Hue = \left[\tan{-1}\left(b*/a*\right)\right]\right)$ were also determined. The pH of the meat was calculated using a HI 99163 digital pH meter (Hanna Instruments, Inc., USA). The tenderness of the meat was measured using the Warner Bratzler method, with a TAX-T2 texture analyzer (Texture Technologies Corp, Scarsdale, NY, USA). Water retention capacity was determined using the gravimetric method proposed by Honikel (1998). A water bath was used to find out water cooking loss: 4 g of meat were placed in hermetic bags and cooked at 75 °C for 35 minutes. The total percentage of evaporative and drip losses was calculated according to Obuz *et al.* (2003), using the following formula:

$$\left[\left(raw\ material - weight\ after\ cooking\right) / raw\ weight\right] \times 100$$

The moisture, fat, crude protein (CP), and ash content of the meat were determined following the procedures of the AOAC (2005). Fifty grams of the meat samples were homogenized and lyophilized (Lyph-Lock 6, Labconco Co., MO, USA.) and one gram was extracted using 2:1 (v/v) chloroform-methanol, following the indications of Folch *et al.* (1957). Stratified samples were analyzed using a HP 6890 gas chromatograph (Hewlett-Packard Co., DE, USA.), in order to establish fatty acid content.

To determine the importance of the quantitative factor (CP %), each one of the evaluated variables was subjected to an analysis of variance, using the CDR method. For some of the variables, it included the repeated measurements factor (measurement days). The initial live weight (LW) was used as a covariable; however, it was excluded from the model (P>0.05). The lamb was considered as a random element in the model and the repeated measurement was analyzed inside the animal. Feed conversion and meat characteristics were analyzed without the repeated measurement. In order to evaluate the effect of the supplement (CP %), a Tukey's multiple comparison test was carried out. Additionally, once the quantitative factor reached a significative level, lineal, quadratic, and cubic octagonal polynomials were used to determine the polynomial degree that accurately describes the average response of the variable in question. The data were processed using the pro mixed procedure and the SAS v 9.4 statistical software (2017).

RESULTS AND DISCUSSION

The final weight, total weight, and daily weight gain, as well as the weight of the carcass composition, were quadratically impacted (P<0.05) by the increase of the CP percentage in the concentrate feed. However, the feed conversion index was not impacted by the different protein levels of the concentrate feed (Table 2). Consequently, the heaviest lambs and carcasses (P<0.05) were those fed with concentrate + 16% CP, while those fed with concentrate feed + 10, 12, and 14% CP obtained lower results (Table 2).

Table 2. Growth behavior of hair lambs grazing on Tanzania grass (*Panicum maximum* cv. Tanzania) and supplemented with a concentrate feed with different CP percentages.

	Concentrate feed (CP % DM)					P-Value	
	10	**12**	**14**	**16**	**SEM**	**L**	**Q**
Initial live weight, kg	22.7	22.5	22.7	22.8	1.59	0.12	0.13
Final live weight, kg	30.3	30.5	32.2	35.7	1.93	0.01	0.49
Total weight gain, kg	7.6	8.0	9.5	12.9	1.31	0.01	0.19
DWG, g	65.0	65.0	79.1	107.5	5.93	0.01	0.04
Total DMC, kg/d	0.69[a]	0.68[a]	0.77[b]	0.99[c]	0.11	0.01	0.04
Grass consumption, kg/d	0.38	0.39	0.47	0.70	0.19	0.01	0.32
Feed conversion	10.72	10.4	9.7	9.23	0.84	0.01	0.43
Warm carcass yield, kg	13.7	13.9	14.6	17.0	1.57	0.01	0.45
Frozen carcass yield, kg	12.9	13.0	13.6	16.0	1.71	0.01	0.25
WCY, %	45.21[a]	45.57[a]	45.34[a]	47.62[b]	3.11	0.32	0.01
FCY, %	42.57[a]	42.62[a]	42.24[a]	44.82[b]	2.09	0.21	0.01

CP: crude protein; DM: dry matter; SEM: standard error of the mean; DWG: daily weight gain; DMC: dry matter consumption; WCY: warm carcass yield; FCY: frozen carcass yield.
[a, b, c] Superscripts in the same line are different ($P<0.05$).
Tanzania grass: *Panicum maximum* cv. Tanzania.

Kashani and Bahari (2017) have proved that males reach a greater weight gain, with a lower amount of fat, than females; they are also better fed and have a better food conversion and daily weight gain. Gomes *et al.* (2012) have reported that animals with low intake of residual feed have a lower support metabolizable energy requirement. Therefore, they have more energy available for production —*i.e.*, the animals with high residual gain use less energy in their physiological support processes— and, consequently, they have more energy available for the deposition of tissues (Gomes *et al.*, 2012). In addition, Montelli *et al.* (2021) and Nascimento *et al.* (2020) reported that lambs with high residual weight gain use food more efficiently. This phenomenon could be associated with lower rumination rates. Consequently, the difference between the various parameters could be the result of the different protein balance, rather than of the different energy balance.

Meanwhile, the total dry matter consumption (DMC) recorded differences between the concentrate feeds with 14 and 16% CP and the concentrate feeds with 10 and 12% CP; however, the highest difference percentage was obtained by the concentrate feed with 16%. Meanwhile, a lineal growth was observed in grazing as the CP percentage increased in the concentrate feed. The highest value was obtained once again by the concentrate feed + 16% CP (Table 2). There were significative differences ($P<0.05$) between the yield of the warm and frozen carcasses. Compared with the concentrate feed with 10, 12, and 14% CP, the highest yield was obtained by the concentrate feed with 16% CP.

Cooking did not cause a loss of moisture content, ash percentage, color measurements, cutting force, water retention capacity, and water loss in the treatments of this experiment. However, the CP percentage and the biceps femoris recorded a linear increase ($P<0.05$) as the CP percentage increased in the supplementary feeding. The concentrate feed +

14 and 16% CP obtained better results than the supplementary feedings + 10 and 12% CP (Table 3).

The ash content of the chemical composition of the biceps femoris did not differ between the protein levels of the supplementary feeding. This phenomenon can be the result of the mineral proportion on the muscle tissue, which remains constant between hair sheep (Fidelis *et al.*, 2017). Meanwhile, Giraldez *et al.* (2021), in their study about Assaf sheep, and Fidelis *et al.* (2017), in their study about Nellore bulls, did not find differences in the ash content of the longissimus muscle and they also found similarities in the meat moisture content of the animals. Both results match the finding of this experiment.

There were no differences in the pH of the biceps femoris between the different CP levels of the concentrate feeds in this study. Other studies (Giráldez *et al.*, 2021; Montelli *et al.*, 2021) reported a similar pH behavior in sheep meat. These results also match the findings of Gomes *et al.* (2012) and Fidelis *et al.* (2017), who reported a similar pH in Nellore livestock, 24 h after they were slaughtered. Almeida *et al.* (2017) mentioned that the pH recommended for a good quality meat in small ruminants must fluctuate between 5.5 and 5.8; a similar value was recorded in this study for the feed with 10 and 16% CP for the 5.5 and 5.8 pH, respectively.

Regarding the color of the meat of the lambs, there were no differences in the L^*, a^*, and b^* parameters, between the different protein levels of the concentrate feed; these results match the findings of Arce-Recinos *et al.* (2022). The main factors that influence meat color include: pH, quantity and chemical state of myoglobin in the muscle, and intramuscular fat (Corazzin *et al.*, 2019). The color of the meat could be potentially explained by the lack of pH differences between the different protein levels of the concentrate feed. Montelli *et al.* (2021) reported lower L^* values (33.13) than the results obtained in this study. To be considered acceptable for consumption, the values of fresh lamb meat must be higher than L^* 34 and a^* 9.5 (Khliji *et al.*, 2010). In this regard, the average color values (L^* 38.6, a^* 19.7, and b^* 6.5) are close to the acceptable parameters for meat.

The level of fat in the meat plays an important role in its tenderness. Therefore, high fat level meat requires less cutting force than low fat level meat (Sañudo *et al.*, 2000). In this study, there were no difference in the cutting force required to cut cooked meat between the different protein levels of the concentrate feed. This phenomenon could be the result of the lack of differences, 24 h after the slaughter, in the intramuscular ether extract content and the pH, among the different protein levels of the supplementary feeding. Meanwhile, Montelli *et al.* (2021) recorded similar results with lambs. They observed that feed efficiency did not have an impact on the tenderness of the meat. However, efficient lambs can produce meat that requires a stronger cutting force, 3 d^{-1} after they are slaughtered (Giráldez *et al.*, 2021). Nevertheless, other authors have reported a similar cutting force (Gomes *et al.*, 2012; Fidelis *et al.*, 2017). Cooking loss is another meat parameter measured 24 h *post mortem*.

Meat has a high water content (approximately 75%). Most of this water is found inside the myofibrils, between the myofibrils and the cell membrane (sarcolemma), between the muscle cells, and between the muscle fascias. A small proportion of the water in the muscle is also attached to the proteins (Huff-Lonergan and Lonergan, 2005). Water loss is related

Table 3. Chemical composition and characteristics of the biceps femoris of hair lambs that grazed on Tanzania grass and were supplemented with concentrate feed, with different levels of crude protein.

	Concentrate feed (CP % DM)					P-Value	
	10	**12**	**14**	**16**	**SEM**	**L**	**Q**
Humidity, %	73.1	72.8	71.9	72.8	0.28	0.11	0.12
Crude protein, %	17.1[a]	19.5[b]	21.2[c]	21.5[c]	0.32	0.01	0.08
Fat, %	14.2[a]	16.5[b]	18.6[c]	18.9[c]	0.31	0.01	0.06
Ashes, %	1.1	1.0	1.0	1.0	0.01	0.20	0.32
Final pH	5.6	5.7	5.7	5.8	0.04	0.21	0.49
Color[1]							
L* (luminosity)	40.0	38.7	37.5	38.5	1.70	0.27	0.49
a* (reddening)	20.7	19.4	18.4	20.6	1.00	0.25	0.45
b* (yellowing)	7.0	6.2	6.0	6.8	0.80	0.43	0.19
Croma, $a^{*2}+b^{*2}$	27.4	25.6	25.3	27.4	1.30	0.32	0.43
Hue angle, $\tan^{-1}$(b*/a*)	32.6	31.7	31.1	31.7	3.30	0.12	0.25
Cutting force, kg/cm^2	2.7	3.1	3.0	2.8	0.22	0.42	0.21
WRC, mL	19.7	19. 8	19.5	19.7	0.20	0.46	0.48
WCL, mL	30.8	31.1	31.2	30.5	0.45	0.42	0.49

CP: crude protein; DM: dry matter; SEM: standard error of the mean; Lineal effect of the protein percentage in the concentrate feed ($P<0.05$).

*L** values are a luminosity measure (a higher value indicates a lighter color): *a** values are a reddening measure (a higher value indicates a redder color); and *b** values are a yellowing measure (a higher value indicates a yellower color).

WRC: water retention capacity, WCL: water cooking loss.

[a, b, c] Superscripts in the same line are different ($P<0.05$).

to the temperature used during cooking; this process can induce the denaturalization and contraction of myofibrils and collagen proteins between 40 and 60 °C. Consequently, the loss of water impacts the quality of meat, which becomes more resistant and firmer (Suleman *et al.*, 2020). These results match the findings of Giráldez *et al.* (2021), who reported a higher cooking loss during the first measurement (0 d) with efficient lambs (26.0%) than with inefficient lambs (22.6%). Nevertheless, their results differ from those reported by Montelli *et al.* (2021) with Dorper × Santa Inés male lambs, whose low, medium, and high classes had similar cooking loss percentages.

Most of the fatty acids were not impacted by the treatments; however, as the CP % increase in the concentrate feed, the highest molar ratio ($P<0.05$) of palmitic (C16:0) and oleic (C18:1, cis-9) acids in the biceps femoris had a quadratic impact ($P<0.05$) (Table 4). Compared with the meat of the lambs fed with a concentrate feed with 10, 12, and 14% CP, the lowest percentages of palmitic acid and the highest percentages of oleic acid were found in the meat of the lambs fed with a concentrate feed with 16% CP. Likewise, the highest percentage of unsaturated fatty acids ($P>0.05$) and the lowest percentage of saturated fatty acids ($P<0.05$) were found in the meat of lambs fed with a concentrate feed + 16% CP.

Table 4. Fatty acids ratio in the bicep femoris of lambs supplemented with concentrate feeds.

	Concentrate feed (CP % DM)					P-Value	
	10	**12**	**14**	**16**	**SEM**	**L**	**Q**
Lauric C12:0	0.3	0.3	0.4	0.3	0.09	0.42	0.46
Myristic C14:0	3.3	3.3	3.8	3.1	0.9	0.21	0.42
Pentadecanoic C15:0	0.6	0.4	0.5	0.4	0.8	0.12	0.11
Palmitic C16:0	24.9	25.4	24.7	23.5	3.1	0.19	0.21
Heptadecanoic C17:0	1.2	1.0	1.1	1.0	0.3	0.37	0.29
Stearic C18:0	19.7	19.3	18.5	18.0	3.9	0.45	0.65
Palmitoleic C16:1	1.8	2.3	2.4	2.0	0.5	0.13	0.37
Oleic C18:1 cis-9	41.1[b]	41.1[b]	41.3[b]	44.5[a]	4.3	0.01	0.04
Elaidic C18:1 trans-9	1.8	1.7	2.0	1.7	0.3	0.17	0.36
Linolenic C18:3n-3	0.3	0.2	0.3	0.2	0.1	0.19	0.54
Linoleic C18:2n-6	4.5	4.6	4.5	4.8	1.8	0.44	0.44
CLA cis-9, trans-11	0.5	0.4	0.5	0.5	0.07	0.13	0.47
Saturated	50	49.7	49	46.3	7.9	0.19	0.32
Unsaturated	50	50.3	51	53.7	7.1	0.42	0.46

CP: crude protein; DM: dry matter; SEM: standard error of the mean.
[a, b] Superscripts in the same line are different ($P<0.05$).

Oleic acid (18:1n-9) is beneficial to human health. According to Calder (2015), monounsaturated oleic acid (part of the Omega 9 family) reduces cholesterol and blood pressure and can improve glucose control and insulin sensitivity. Meanwhile, Schwingshacki and Hoffmann (2014) conducted a systematic review and a meta-analysis, proving that a higher oleic acid consumption is connected to a lower risk of coronary heart disease, cardiovascular events, and cardiovascular mortality. Therefore, the fatty acid (FA) composition has a significant influence on the attributes of meat and its nutritional value (Wood and Enser, 2017). The data about the FA composition of lamb meat are diverse. On the one hand, Camacho *et al.* (2017) reported that hair female lambs had more monounsaturated FA (mainly oleic acids) than male lambs. On the other hand, Erasmus *et al.* (2017) observed a similar behavior, reporting that female lambs had a higher α-linolenic acid (C18:3n-3) and polyunsaturated FA content in the subcutaneous fat than male lambs. In this study, the meat of male lambs recorded a higher fatty acid content (oleic C18:1 cis-9), while the rest of the meat did not report FA differences. Currently, there is a growing interest in manipulating the carcass quality and the FA composition of livestock meat, since consumers would find a higher polyunsaturated FA content more appealing (Vahmani *et al.*, 2020).

CONCLUSIONS

The meat of hair lambs that grazed on Tanzania grass and were supplemented with a concentrate feed + 16% CP recorded higher growth performance and carcass yields, as well as a better unsaturated fatty acid ratio, than the meat of lambs supplemented with a concentrate feed + 10, 12, and 14% CP.

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Colegio de Postgraduados

Biological control of *Spodoptera frugiperda* J. E. Smith and *Schistocerca piceifrons piceifrons* Walker using entomopathogenic fungi

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ABSTRACT

Objective: Evaluate the biological effectiveness of native entomopathogenic fungi to control *Spodoptera frugiperda* and *Schistocerca piceifrons piceifrons*, as well as the natural incidence of parasitoids in *S. frugiperda*.

Design/Methodology/Approach: Six strains of *Metarhizium anisopliae* (A1, A2, A3, A4, A5, and A6) and three strains of *Isaria fumosorosea* (B4, B5, and B6) were collected. *S. frugiperda* larvae were inoculated with all the isolated strains of *Metarhizium* and *Isaria*; while, the nymphs of *S. p. piceifrons* were inoculated with strains A1, A5, A6, B4, and B5. Mortality (%) and natural incidence of parasitoids in *S. frugiperda* larvae were evaluated. Mortality was analyzed using a one-way ANOVA and a comparison of means (Duncan; α=0.05) in the INFOSTAT 2021 software. Parasitism was reported with descriptive statistics (%).

Results: Strains A1, A6, and B6 caused the highest mortality (86.6-90.0 %) in *S. frugiperda* larvae. Strains A1, A5, A6, and B6 caused the highest mortality in nymphs of *S. p. piceifrons* (90-100%). Two families of parasitoids were recorded: Tachinidae (Diptera; 7.8 %) and Braconidae (Hymenoptera). Wasps of the genus *Meteorus* sp. account for 92.2% of the latter family.

Study Limitations/Implications: The biological effectiveness evaluations of the entomopathogenic fungi were carried out under laboratory conditions. The results must still be validated on the field.

Findings/Conclusions: Strains A1, A5, and A6 showed a good control of the *S. p. piceifrons* nymphs. Strain B6 is a biological control alternative for *S. p. piceifrons* and *S. frugiperda*, since it recorded the highest mortality for both species.

Keywords: Fall armyworm, Central American Locust, control alternative, *Meteorus*.

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INTRODUCTION

The fall armyworm [*Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae)] and the Central America locust [*Schistocerca piceifrons piceifrons* Walker (Orthoptera: Acrididae)] are key pests that attack cotton (*Gossypium hirsutum* L.), rice (*Oryza sativa* L.), sugarcane (*Saccharum officinarum* L.), corn (*Zea mays* L.), sorgum (*Sorghum vulgare* Moench), and soybean (*Glycine max* L.) (Fotso-Kuate *et al.*, 2019; Overton *et al.*, 2021). In tropical regions, they can cause of up to 100% losses in production; consequently, in several African and

Image by Alejandro Sánchez

Central American countries, they are considered a threat to food security (Barrientos-Lozano *et al*., 2021; Servín and Mendoza, 2022).

S. frugiperda is native to Central America and it is widely known throughout the continent (Jing *et al*., 2021). In recent years, it has become an invasive pest in Africa, Asia, and Australia (Paredes-Sánchez *et al*., 2021), where it is considered corn's main pest. It has a great number of hosts, a wide migratory capacity, high fertility, and a rapid development of resistance against chemical and biological pesticides, such as *Bacillus thuringiensis* Berliner (Jing *et al*., 2021; De Souza-Ribas *et al*., 2022). Just in twelve producing countries in Africa, grain yield losses range from 8.3 to 20.6 million of tons per year, which accounts for 2.5-6.2 billion of dollars in economic losses (Day, 2017). *S. p. piceifrons* has two stages: the solitary stage, in which it does not damage crops, and the gregarious stage, in which they group in swarms of millions of individuals. Their great migratory capacity allows them to move from one country to another, causing economic losses, both in agriculture and cattle raising (Le Gall *et al*., 2019; Pérez-Ramírez *et al*., 2019; Barrientos-Lozano *et al*., 2021).

S. frugiperda and *S. p. piceifrons* control is mainly based on chemical management, given the high efficiency of molecules and the availability of the products. Depending on the pest species that needs to be controlled, pesticides are made up of mixed or individual formulations using active ingredients from the following chemical families: avermectins, benzoylphenyl ureas, carbamates, diamides, spinosyns, phenylpyrazoles, neonicotinoids, organophosphate, and pyrethroids (Paredes-Sánchez *et al*., 2021; Kulye *et al*., 2021; Birkhan *et al*., 2023).

Currently, there are different environmentally friendly alternatives for pest control, including crops Bt (*Bacillus thuringiensis*), natural enemies (parasitoids and predators) and entomopathogenic fungi. Gutiérrez-Ramírez *et al*. (2015) and Ordóñez-García *et al*. (2015) point out that *S. frugiperda* can be attacked by parasitoids from various families of the orders Hymenoptera (Braconidae, Ichneumonidae, Platygastridae, and Trichogrammatidae) and Diptera (Tachinidae), whose parasitism rate ranges from 3 to 42%. In this regard, local parasitoids from these families have already been reported in Mexico and in several African countries; consequently, these parasitoids can help to keep this pest under control in the Old World (Koffi *et al*., 2020). Regarding entomopathogenic fungi, *Metarhizium anisopliae* (Mechnikov) Sorokin can cause a 43-100% mortality in third-instar larvae of *S. frugiperda* (Ullah *et al*., 2022b; Munywoki *et al*., 2022) and a 70-100% mortality in nymphae and adults of *S. p. piceifrons*. Additionally, it can cause mortality in others species of the Acrididae family, including *Melanoplus sanguinipes* Fabricius (Barrientos-Lozano *et al*., 2021; Dakhel *et al*., 2019). Genus *Metarhizium* is widespread in natural ecosystems and can be used (along with *Beauveria bassiana* (Bals.-Criv.) Vuill) to control the Central America locust (Brunner-Mendoza *et al*., 2019; Barrientos-Lozano *et al*., 2021).

Altinok *et al*. (2019) and Gandarilla-Pacheco *et al*. (2021) have reported that *Isaria fumosorosea* (Wize) Kepler, B. Shrestha & Spatafora is efficient to control Lepidoptera. This species can cause a 29-100% mortality among *S. frugiperda* and *S. litura* Fabricius larvae (Lei *et al*., 2020; Ullah *et al*., 2022a). However, there are no reports about the effect of *I. fumosorosea* on *S. p. piceifrons*. Consequently, in view of the high economic cost and the irreparable environmental damage caused against beneficial fauna, soils, and water bodies by the excessive application of the pesticides used to control *S. frugiperda* and *S. p. piceifrons*, the objective of this study was to evaluate the biological efficiency of native entomopathogenic fungi for the control of the said species, as well as the natural incidence of parasitoids on *S. frugiperda*. The results will help to implement efficient and accessible control measures that will minimize the risks posed to human health and the environment.

MATERIALS AND METHODS

The research was carried out in 2022, in the Laboratorio de Toxicología of the Campo Experimental Las Huastecas (CEHUAS), Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP); located at 22° 33' 58.56" N and 98° 9' 49.91" W, in the municipality of Altamira, Tamaulipas, México.

Origin of the entomopathogenic fungi

The entomopathogenic fungi strains of *M. anisopliae* e *I. fumosorosea* were collected from the soils of the González, Llera, and Mante Tamaulipas municipalities, in June 2022 (Table 1). They were isolated using the insect tramp technique (Zimmermann, 1986, modified by Sánchez-Peña, 2011). The isolated strains were purified through the direct transfer of conidia from larvae with mycosis to a Potato Dextrose Agar (PDA) culture medium (Hayek *et al.*, 2012). Conidia production was carried out in solid substrate (commercial rice), at the Laboratorio de Botánica of the Unidad Académica Multidisciplinaria Mante (UAMM), Universidad Autónoma de Tamaulipas. The Humber taxonomic keys were used to identify the microscopic (conidia, phialides, and hyphae) and macroscopic (growth and color) morphology of the entomopathogenic fungi. The strains were preserved at the UAMM lab, using the inclined tube technique with mineral oil (Sharma and Smith, 1999).

Biological effectiveness test of *Metarhizium anisopliae* and *Isaria fumosorosea* for the control of *Spodoptera frugiperda*

Three-hundred *S. frugiperda* larvae in their 4th and 5th instars were collected from *Urochloa fasciculata* (Sw.) R. Webster grass, in an experimental plot of the CEHUAS. In the Laboratorio de Toxicología, the larvae were kept in an expanded polystyrene icebox until the evaluation was carried out. To prevent cannibalism, they were fed abundant grass.

To conduct the biological effectiveness test, the *S. frugiperda* larvae were inoculated with six *M. anisopliae* strains (A1, A2, A3, A4, A5, and A6) (Table 1), three *I. fumosorosea* strains (B4, B5, and B6), and a control treatment (distilled water + Tween 20 at 0.03%). The conidia suspensions were carried out in a distilled water solution plus Tween 20 at 0.03% and with conidia produced in 12 g of rice as solid production medium.

The conidia suspension of the entomopathogenic fungi was adjusted to a 1.5×10^8 and a 5.1×10^8 conidia/mL concentrations for the *M. anisopliae* and *I. fumosorosea* strains, respectively. These concentrations were standardized according to *Metarhizium* A2 and *Isaria* B5, the entomopathogenic fungi strains that produced the lowest number of conidia in 12 g of rice. Groups of 30 larvae of *S. frugiperda* were inoculated for five seconds, using the immersion technique; subsequently, they were placed in absorbent paper to remove the conidia suspension excess. Each of the *M. anisopliae* and *I. fumosorosea* strains were

Table 1. Geographic data of the entomopathogenic fungi used in the bioassays.

Entomopathogenic fungus	Code	Ejido or community	Municipality	Geographical coordinates
Metarhizium anisopliae	A1	Ejido El Ébano	Llera	23° 53' 98,99" N 99° 03' 44,25" O
Metarhizium anisopliae	A2	Rancho Nuevo del Norte	Llera	23° 41' 12,15" N 98° 98' 37,34" O
Metarhizium anisopliae	A3	Mante	Mante	22° 71' 65,67" N 98° 96' 24,33" O
Metarhizium anisopliae	A4	González	González	22° 81' 64,01" N 98° 43' 15,37" O
Metarhizium anisopliae	A5	El Chaparral	González	22° 51' 46,57" N 98° 44' 45,42" O
Metarhizium anisopliae	A6	González	González	22° 68' 47,13" N 98° 52' 77,15" O
Isaria fumosorosea	B4	González	González	22° 81' 64,01" N 98° 43' 15,37" O
Isaria fumosorosea	B5	El Chaparral	González	22° 51' 46,57" N 98° 44' 45,42" O
Isaria fumosorosea	B6	González	González	22° 41' 05.00" N 98° 31' 39.80" O

evaluated separately. After the inoculation of the entomopathogenic fungi, the larvae were placed separately in 30 mL plastic containers. They were provided five *U. fasciculata* leaves as a food source; the leaves were changed every 24 h. The treatments were incubated for five days, at 27±1 °C and with a 85% relative humidity. Each treatment consisted of five replicates of six larvae each. Mortality was evaluated every 24 h; a larva was considered dead when it did not respond to the stimulus of a brush. Mortality was analyzed using a one-way ANOVA and Duncan's multiple range test ($\alpha=0.05$) was used to compare the means, in the INFOSTAT 2021 software (Di Rienzo *et al.*, 2020).

Natural incidence of parasitoids on the fall armyworm (*Spodoptera frugiperda*)

The emergence of parasitoids during the evaluation of the biological effectiveness of the entomopathogenic fungi against *S. frugiperda* led to the decision of evaluating natural incidence and identify the species. One-hundred thirty-three larvae from the 4th and 5th instars (size: 1-2 cm) were collected for this experiment. The larvae were placed in an icebox with plenty of grass and, subsequently, they were taken to the Laboratorio de Toxicología of CEHUAS. In the laboratory, larvae were placed individual in 30 mL plastic containers, with five *U. fasciculata* leaves (10 cm^2) as food source; the leaves were changed every 24 h. The natural parasitism was evaluated for 15 days, while the number of pupae and the emergence of the parasitoids were recorded every 24 h. The first 30 parasitoids that emerged were placed in 96% ethanol, in 2 mL Eppendorf tubes; they were subsequently identified using the taxonomic keys described by Wharton *et al.* (1997). The natural parasitism of *S. frugiperda* was reported using descriptive statistics (parasitism percentage).

Biological effectiveness test of *Metarhizium anisopliae* and *Isaria fumosorosea* for the control of Central America locust (*Schistocerca piceifrons piceifrons*)

Three hundred nymphae of Central America locust (*S. p. piceifrons*) (size: 10-18 mm) were collected from the *U. fasciculata* grass of an experimental plot at the CEHUAS. The locust nymphae were taken to the Laboratorio de Toxicología of CEHUAS in an icebox. Their antennal segments were counted to determine the nymphal instar, using a Motic stereoscopic microscope. The results established that the nymphs were in their 2^{th} and 3^{th} nymphal instars (20-22 antennal segments).

Three strains of *M. anisopliae* (A1, A5, and A6), two of *I. fumosorosea* (B4 and B5), and a control treatment (distilled water plus Tween 20 at 0.03%) were evaluated. The conidia suspension was similar to the experiment carried out with *S. frugiperda*. The conidia concentration was adjusted to 5×10^8 conidia/mL. Groups of 50 Central America locust nymphae were separately inoculated, using the spraying technique; seven 0.3 mL sprayings were carried out with an atomizer, 20 cm away from the nymphae. Subsequently, groups of 10 specimen were placed in a 250 mL container. They were provided 20 *U. fasciculata* leaves as a food source; these leaves were changed every 24 h. The treatments were incubated for four days, at a temperature of 27 ± 1 °C and with an 85% relative humidity. Five replicates with 10 nymphae each were evaluated. Mortality was evaluated every 24 h, during four days. A nymphae was considered dead when it did not respond to the stimulus of a brush. The biological effectiveness for the control of *S. frugiperda* and *S. p. piceifrons* were carried out under laboratory conditions. The experiments were carried out using a completely randomized design, with five replicates, consisting of groups of 10 *S. p. piceifrons* nymphae per treatment. Mortality was analyzed similar to the bioassay carried out for *S. frugiperda*.

RESULTS AND DISCUSSION

Biological effectiveness test of *Metarhizium anisopliae* and *Isaria fumosorosea* for the control of *Spodoptera frugiperda*

The mortality of the *S. frugiperda* larvae caused by *M. anisopliae* recorded significative differences between treatments, 48 to 120 h after the application of the treatment ($p<0.05$). *M. anisopliae* caused 10-20% mortality, 48 h after the start of the treatment. From 72 to 120 h after the application of the treatment, *Metarhizium* A1 and A6 caused the highest mortality, reaching 90 % (Table 2). Meanwhile, the *Metarhizium* strains A2-A5 caused the lowest mortality: 63.3 to 86.6% during the same evaluation period (Table 2).

Under laboratory conditions, *M. anisopliae* applications with a 1×10^8 conidia/mL concentration can cause a 72-100% mortality in 3^{rd} instar larvae of *S. frugiperda* (Ullah *et al.*, 2022b). These results match the findings of this study: a 73-90% mortality among *S. frugiperda* larvae of the 4^{th} and 5^{th} instars can be obtained, depending on the *M. anisopliae* native strain applied. Native strains induce natural epizootics or high rates of insect mortality, as a result of the high population of pests and the high pathogenic and virulence of the entomopathogenic fungi, as well as the regional climatic conditions to which this type of fungi is adapted (Dufau *et al.*, 2021). Munywoki *et al.* (2022) and other authors have reported that a 1×10^9 conidia/mL concentration of *M. anisopliae* cause a low mortality (43%); the above is possibly due to the fact that the strain used for that study was not appropriate for Lepidoptera. Some populations of genus *Metarhizium* can affect specific types of insects, such as *S. p. piceifrons* and *Locusta migratoria manilensis* Meyen (Brunner-Mendoza *et al*., 2019; Barrientos-Lozano *et al*., 2021).

Regarding *I. fumosorosea*, significant statistical differences were recorded in the mortality of *S. frugiperda* larvae between treatments, from 72-120 h after the application ($p<0.001$). The strain B6 of *I. fumosorosea* induced the highest mortality percentages: it can cause an 86.6% and 100% mortality, 72 h and 120 h, respectively, after the application (Table 2). Meanwhile, strain *Isaria* B4 recorded the lowest mortality (76.6%), 120 h after the application of a 5.1×10^8 conidia/mL concentration.

I. fumosorosea is widely used to control Hemiptera; however, several authors have pointed out the high efficiency of this species for the control of *S. frugiperda*, *Spodoptera littoralis* Boisduval, and *Spodoptera exigua* Hübner (Altinok *et al*., 2019; Gandarilla-Pacheco *et al*., 2021). Therefore, it can be a biological alternative for the control of this type of pests.

Currently, there is scarce information about the effectiveness of *I. fumosorosea* against *S. frugiperda*. In this regard, Lei *et al*. (2020) pointed out that a 1×10^9 conidia/mL concentration of *I. fumosorosea* can cause 50-100% mortality in larvae of 1^{st}-4^{th} instars. In that study, the highest mortality rates were found in the lowest larvae instars of *S. frugiperda*. Several studies have reported that a 1×10^7 conidia/mL concentration of *I. fumosorosea* can cause a 20-50% mortality rate among the larvae of *Spodoptera litura*. Additionally, the effectiveness increased during the early larvae instars (Ullah *et al*., 2022a).

Table 2. Biological effectiveness test of entomopathogenic fungi used for the control of fall armyworm (*Spodoptera frugiperda*).

Treatments	Mortality (%)				
	24	48	72	96	120
	Time after application (h)				
Control	0.0	6.6 ab	10.0 b	23.3 c	33.3 c
Metarhizium A1	0.0	0.0 b	73.2 a	86.6 a	90.0 a
Metarhizium A2	0.0	20.0 a	56.6 a	73.3 ab	73.3 ab
Metarhizium A3	0.0	0.0 b	23.3 b	49.9 b	63.3 b
Metarhizium A4	0.0	20.0 a	56.3 a	73.3 ab	83.3 ab
Metarhizium A5	0.0	10.0 ab	60.0 a	83.3 a	86.6 ab
Metarhizium A6	0.0	20.0 a	66.6 a	80.0 a	90.0 a
P-Value	-	0.0445	<0.0001	0.0001	0.0001
Control	0.0	6.6 a	10.0 c	23.3 c	33.3 c
Isaria B4	0.0	23.3 a	56.6 b	63.3 b	76.6 b
Isaria B5	0.0	26.6 a	69.9 ab	76.6 ab	90.0 ab
Isaria B6	0.0	26.6 a	86.6 a	93.3 a	100.0 a
P-Value	-	0.1908	<0.0001	0.0001	<0.0001

Different letters (a, b, c) within each species indicate a significative statistical difference between treatments (Duncan; $\alpha=0.05$).

According to the biological effectiveness test, *M. anisopliae* and *I. fumosorosea* did not have an impact on the emergence of local parasitoids. From the 48 h to the 120 h after the application, a high percentage of parasitized larvae was observed (Figure 1A), from which parasitoid larvae emerged, which pupated and these were observed attached to a type of pedicel attached to the leaves of *U. fasciculata* or to the plastic container. The 33.6% of the 300 larvae used for the biological effectiveness test of the entomopathogenic fungi were parasitized. The emergence of adult parasitoids was observed after 5 or 6 days in the laboratory. The parasitoids were identified as wasps from the genus *Meteorus* (Braconidae). The species of this genus are polyphagous endoparasitoids of the larvae of several Coleoptera and Lepidoptera, and have been widely reported in the literature (Fujie *et al.*, 2019). Aguirre *et al.* (2015) mentioned that genus *Meteorus* prefers to parasitize larvae of the Arctiinae, Megalopygidae, Noctuidae, Nymphalidae, and Pyralidae families. These results match the findings of this study, where it parasitized larvae of *S. frugiperda* (Noctuidae).

Natural incidence of parasitoids of the fall armyworm (*Spodoptera frugiperda*)

Out of the 133 larvae collected, 48.4% (n=64 parasitoids) showed parasitism 192 h after the collection. Parasitism increased from 12.0 to 48.4%, in proportion to the incubation period of the larvae in the laboratory (Figure 1B). Of the 64 parasitoids found, two families were identified: Tachinidae (Diptera) (7.81%, n=5), of which only the pupae were observed because the parasitoids did not emerge, and wasps of family Braconidae (Hymenoptera)of the genus *Meteorus* (92.2%, n=59). According to Gutiérrez-Ramírez *et al.* (2015), Ordóñez-García *et al.* (2015), and Serrano-Domínguez *et al.* (2019), in Mexico, *S. frugiperda* presents a high parasitism rate (22-42%) by several species of Hymenoptera (Braconidae, Cheloninae, Eulophidae, Ichneumonidae, Platygastridae, Trichogrammatidae, and Rogadinae) and Diptera (Tachinidae). These findings match the results of this study. In the Mexican states of Durango, Sinaloa, Michoacán, and Tamaulipas, the parasitism of *S. frugiperda* by *Meteorus* ranges from 3 to 22% (Villegas-Mendoza *et al.*, 2015).

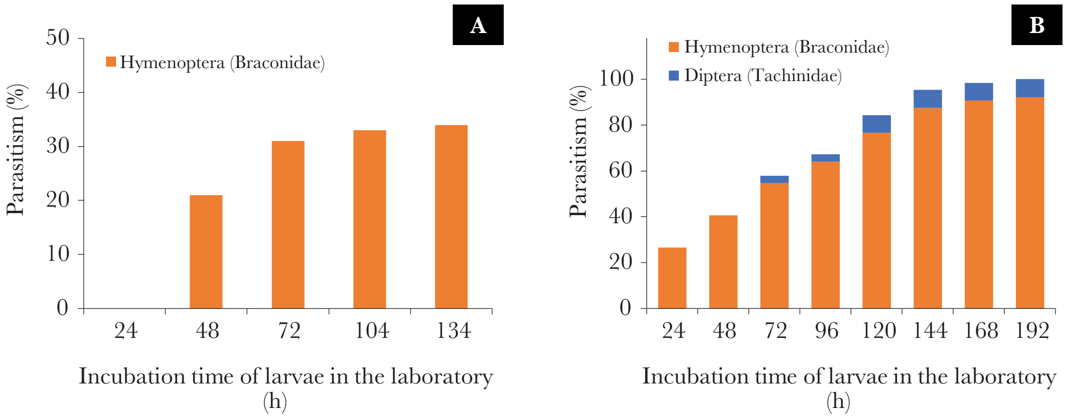

Figure 1. Incidence of natural parasitism on *Spodoptera frugiperda* larvae, with (A) and without (B) entomopathogenic fungi applications in Altamira, Tamaulipas.

In this study, the *Meteorus* sp. larvae that emerged from *S. frugiperda* caused a round wound (generally in the eighth abdominal segment), while Tachinidae caused a similar wound on the 4^{th} and 5^{th} abdominal segment. Both wounds had a white color that turned darker two hours after the emergence of the parasitoid. Likewise, it was observed that the larvae of *S. frugiperda* can survive 24 h after the emergence of the parasitoid larvae. This result matches the findings of Villegas-Mendoza *et al.* (2015). The formation process of the pupae of the parasitoid was not observed; however, Villegas-Mendoza *et al.* (2015) pointed out that this stage can take 40 minutes. Overall, the pupae of the parasitoid started to emerge from 24 h up to168 h after the collection. In addition, the adults emerged from the pupae at 6.3±1.5 days in the laboratory. In this regard, Villegas-Mendoza *et al.* (2015) reported that *Meteorus* sp. can emerge from the pupae, after 7.2-7.5 days at 24±1 °C. Perhaps the wasps of this study emerged 1.05 days sooner than the number of days reported by these authors, because the incubation process of this research was carried out at 27±1 °C.

Biological effectiveness test of *Metarhizium anisopliae* and *Isaria fumosorosea* for the control of Central American locust (*Schistocerca piceifrons piceifrons*)

Significant statistical differences between treatments were reported for the mortality of *S. p. piceifrons* nymphae caused by *M. anisopliae* and *I. fumosorosea*, 24 to 96 h after the application ($p<0.05$) a 5×10^8 conidia/mL concentration (Table 3). *Metarhizium* strains caused a higher mortality among the nymphae of Central America locust respect to *I. fumosorosea*, perhaps as a result of the closeness of the *M. anisopliae* strain used in this research to *M. anisopliae* var. acridum (a very specific species which is widely used to control locust in different countries) (Kamga *et al.*, 2022). Brunner-Mendoza *et al.* (2019) and Barrientos-Lozano *et al.* (2021) have reported that *Metarhizium* sp. is an entomopathogenic fungi used to control highly specific pests. This phenomenon was also observed in this research: the three *Metarhizium* strains under evaluation caused a 98-100% mortality among Central America locusts, during the 4-day long experiment.

Table 3. Biological effectiveness tests of *Metarhizium anisopliae* and *Isaria fumosorosea* for the control of Central America locust (*Schistocerca piceifrons piceifrons*).

Treatments	Mortality (%)			
	24	48	72	96
	Time after application (h)			
Control	0.0 b	2.0 C	4.0 b	6.0 c
Metarhizium A1	22.0 a	92.0 a	100.0 a	100.0 a
Metarhizium A5	0.0 a	68.0 ab	98.0 a	100.0 a
Metarhizium A6	8.0 ab	70.0 ab	90.0 a	98.0 a
Isaria B4	4.0 b	12.0 c	12.0 b	22.0 b
Isaria B6	14.0 ab	56.0 b	88.0 a	96.0 a
P-Value	0.0478	0.0001	<.0001	<.0001

Different letters (a, b, c) indicate a significative statistical difference between treatments (Duncan; $\alpha=0.05$).

At 24 h, the highest number of dead nymphs was observed in the *Metarhizium* A1 treatment, compared to the other treatments evaluated. From 72 to 96 h, the *Metarhizium* strains A1, A5, and A6 caused the highest mortality (90-100%); additionally, in statistical terms, the three local strains can cause a similar mortality. *Metarhizium* species are widely used to control pests of the Cercopidae and Acrididae families. This entomopathogenic fungi genus is very abundant in natural ecosystems and has a higher effectiveness than other entomopathogenic fungi (Brunner-Mendoza *et al.*, 2019). Currently, *Metarhizium* is the entomopathogenic fungi most used to control Central America locust and it is still subject of study, given its very specific action range against certain pests. Additionally, it can be easily reproduced (Barrientos-Lozano *et al.*, 2021). In field evaluations, *M. anisopliae* var. acridum caused a mortality of up to 90% among *S. p. piceifrons* adults in southeastern Mexico (Barrientos-Lozano *et al.*, 2021). Dakhel *et al.* (2019), report to *M. anisopliae* caused a 77% mortality among *Melanoplus sanguinipes* (Orthoptera: Acrididae), under greenhouse conditions.

Regarding *I. fumosorosea*, the strain B6 reached its peak mortality (96%) among *S. p. piceifrons* nymphae at 96 h, while the B4 population only reached a 22% mortality, in the same evaluation period. There are no reports about the effect of *I. fumosorosea* on *S. p. piceifrons*; this entomopathogenic fungus is generally associated with Hemiptera and Lepidoptera pests (Zimmermann, 2008; Sani *et al.*, 2023).

CONCLUSIONS

M. anisopliae strains A1, A5, and A6 showed good control of *S. p. piceifrons* nymphs under laboratory conditions in a short period, under the concentration used. Regarding *I. fumosorosea*, strain B6 represents a biological alternative for *S. p. piceifrons* and S. frugiperda because they caused because a high mortality in both species.

M. anisopliae and *I. fumosorosea* did not affect the emergence of local parasitoids of the Braconidae family, because a high presence of parasitism was observed under laboratory conditions, likewise the genus *Meteorus* can be considered and studied in future works to be used as a biological controller of *S. frugiperda* larvae in southern Tamaulipas.

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Selection and chemical composition of plant species consumed by goats under drought conditions in three microregions of the Comarca Lagunera, Mexico

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ABSTRACT

Objective: To evaluate the plant selection of the diet of goats during the dry season and the chemical composition of the said plants in three microregions of the Comarca Lagunera, Mexico.

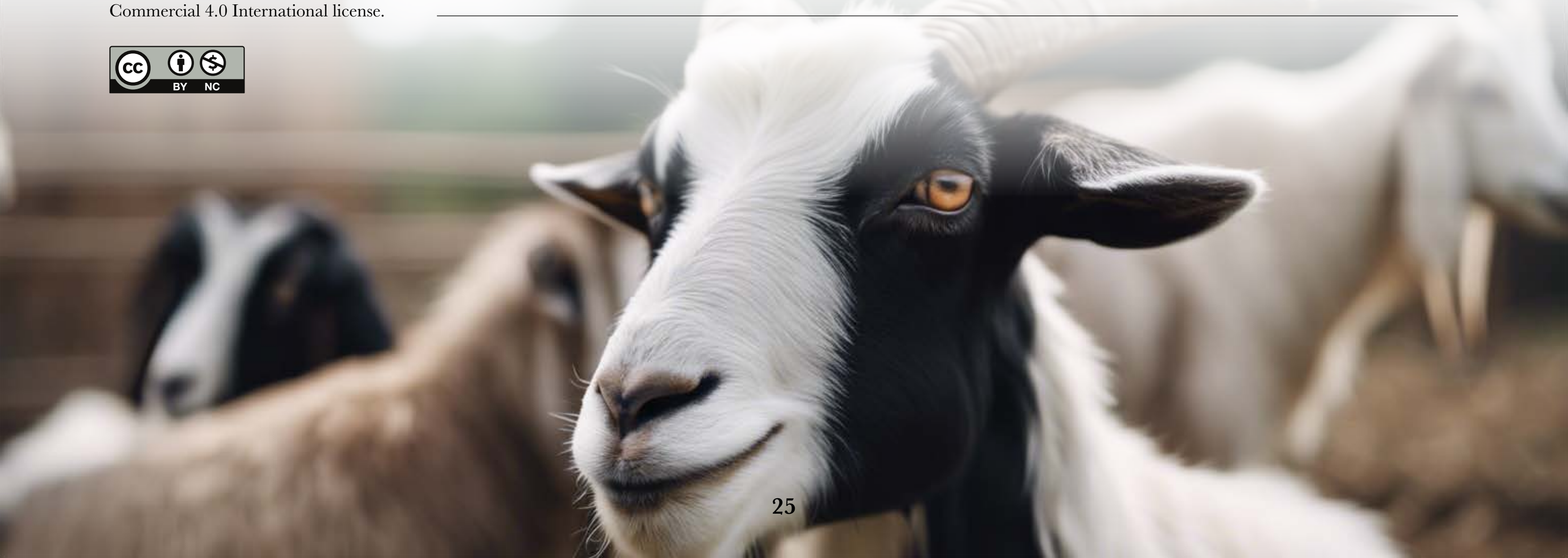
Design/Methodology/Approach: Three microregions were identified within the Comarca Lagunera: 1) mountain slope, 2) plain, and 3) cultivation areas. The selection of the goat diet, the availability of forage, and the nutritional composition (CP, EE, DNF, AFD, NFC, TDN, and NEl) of plant species and strata were evaluated in the three microregions.

Results: The shrubs and herbaceous in the Comarca Lagunera had the highest forage availability and a better nutritional profile during winter. On the one hand, these plant strata had an adequate protein content. On the other hand, the overall energy content reached <1 Mcal kg^{-1} DM.

Study Limitations/Implications: The plant strata have an adequate protein content; however, the energy content is not enough and limits growth. Consequently, supplementation programs for grazing dairy goats in the Comarca Lagunera must include sources of energy.

Findings/Conclusions: Regardless of the microregion, the energy content of the species consumed by grazing dairy goats in the Comarca Lagunera is low.

Keywords: Ruminant nutrition, kid production, forage evaluation.



INTRODUCTION

Mexico and Brazil are the main breeders of goats in the Americas. In these two countries, this economically marginal activity is carried out using native or local goats (Escareño *et al.*, 2012). Nevertheless, the sector has a high social importance, as a result of the income source and the high-quality protein it generates for the low-income population in the rural areas (Ortiz-Morales *et al.*, 2022).

Goat exploitation can be classified according to production (meat or milk) or the feeding system (grazing or confined) (Escareño *et al.*, 2013). In this regard, Alejandre-Ortiz *et al.* (2016) point out that meat production is the most important production system in Latin America and the Caribbean. Large extensions of communal lands are used for this purpose. In Mexico, goat production is carried out in arid and semiarid regions of the country and kids are its main product. These systems are based on native plants grazing (Maldonado-Jáquez *et al.*, 2018). Consequently, increasing the number of births within the herd is the most effective way to improve the production system (Alexandre and Mandonnet, 2005). The number of born, weaning, and survivor kids determines the economic feasibility of a production unit (Casey and Webb, 2010). In this regard, some of the problems that limit productivity in extensive production systems include excessive time to produce the first offspring, low kid productivity, long intervals between births, reduced prolificity, low milk production (as a result of a short lactation period), and a marked reproductive seasonality (Mellado, 2008).

Reproduction is the main factor that limits goat production in Mexico (Andrade-Montemayor *et al.*, 2017). Therefore, reproduction is closely related to goat nutrition, particularly, during gestation. When the nutritional requirements of a goat are not fulfilled, miscarriage becomes a very likely event which has an impact on the number of births (Terrazas *et al.*, 2012). In northern Mexico, the mortality rate can reach up to 50% in winter (dry season) and it can diminish down to 10% in summer (rainy season). This phenomenon is the consequence of a combination of reproductive seasonality, low temperatures and, above all, an inefficient feeding during this period (Mellado *et al.*, 2000).

Consequently, the implementation of supplementation strategies aimed to increase goat milk production is necessary to increase the survival probabilities of the kid. Therefore, information about the nutritional quality of the plant species consumed by goats in the pasture, during the dry season, is required to develop more efficient supplementation strategies (Pinos-Rodríguez *et al.*, 2007; Ramírez-Orduña *et al.*, 2008; Velderrain-Algara *et al.*, 2010). Additionally, producers must consider changes in the availability and the variety of foraging species per microregion (Mellado *et al.*, 2012).

The Comarca Lagunera is a region located between the Mexican states of Durango and Coahuila. This region is the major dairy basin, both for goats and cattle (SIAP, 2011). This region includes sixteen municipalities: five from the state of Coahuila (Francisco I. Madero, Matamoros, San Pedro de las Colonias, Torreón, and Viesca) and eleven from the state of Durango (General Simón Bolívar, Gómez Palacio, Lerdo, Mapimí, Nazas, Rodeo, San Juan de Guadalupe, San Luis del Cordero, San Pedro del Gallo, Tlahualilo, and Cuencamé). It has a mountain slope and a plain and it covers approximately 4,788,750 ha (SEMARNAT, 2010). Consequently, the microregions include plants with different characteristics. The objective of this study was to determine the plant selection of the diet of goats during the dry season and its chemical composition, in three microregions of the Comarca Lagunera, Mexico.

MATERIALS AND METHODS

Study area

The study was carried out in the states of Coahuila and Durango, Mexico, in a region known as the Comarca Lagunera, which is located between 24° 22' N and 102° 22' W, at 1,139 m.a.s.l. According to the Köppen climate classification, this region is classified as BWhw (very dry or desertic climate). The weather is semi-warm, with cold winters. The mean annual precipitation reaches 240 mm. The mean annual temperature is 25 °C in the shade, ranging from −1 °C in winter to 44 °C in summer (García, 2004).

The study began one day after the winter solstice (December 22) of 2021 and was completed on February 22, 2023. A monthly sampling was carried out on December 22, January 22, and February 22. The sampling was conducted in three microregions within the Comarca Lagunera, where goat production activities take place. The first microregion was the mountain slope. This region is characterized by its higher humidity percentage (because of water runoffs) and its higher content of clay in the soil. These factors promote a higher vegetation growth (Villanueva *et al.*, 2011). The second microregion was the plain. It is characterized by its sandy soil with low humidity, as well as its low diversity of plant species (Villanueva *et al.*, 2011). The third region consisted of the cultivation areas. After the harvests, these areas are used by goat producers, as a result of the humidity residues left behind by the irrigation system. This phenomenon increases the abundance and availability of plant species, especially herbaceous (Salinas- González *et al.*, 2015).

Diet selection

Seven multiparous adult goats were selected from each microregion. They were followed during their grazing routes (from 5 to 10 km), with the aim of identifying the plants they selected for their diet. In order to prevent the observers from influencing the behavior of the goats, they were followed by two groups of people, who took notes and collected samples from the species consumed by the goats. Four ≈300-g samples were taken from the plant species consumed by most of the goats (N>75%); the samples were taken at the same height at which the animals fed. The samples included only the leaves of these species. The samples were placed in paper bags for their transportation to the proximal chemical analysis lab. The specimens chosen by the animals were adult plants, with green foliage, which had reached their physiological maturity (Toyes-Vargas *et al.*, 2013).

Forage availability

Forage availability was evaluated in nine permanent plots of 20×20 m (three plots per microregion). The plots were randomly chosen from a map of the study area (100×100 m grid), where the plots located in each of the apexes of the squares could be potentially chosen. Forage availability was determined by species and plant strata (shrubs, cactus, herbaceous, and grass). Shrub availability was estimated following the Adelaide method:

establishing a 20×20 m plot and 3 sub plots of 1×1 m within the first plot (Andrew *et al*., 1979). Subsequently, a tree and shrub sampling was conducted in the larger plot, counting the branches of each tree or shrub. All the leaves from three or shrubs branches were collected. Additionally, a sampling from the herbs and grasses of the 1×1 m sub plots was carried out, cutting the plants at ground level. Dead grasses or grasses with high lignification were excluded from the process since the goats did not find them appealing.

Lab analysis

In order to determine the dry matter (DM) content, the fresh samples were weighted and, subsequently, dried in a forced air oven at 50 °C, until they reached a constant weight (approximately at 72 h). Additionally, the content (g kg^{-1}) of crude protein (CP), non-fiber carbohydrates (NFC), and ethereal extract (EE) were determined (AOAC, 2019). The neutral detergent fiber (NDF) and acid detergent fiber (ADF) content was likewise established (Van Soest *et al*., 1991). The values of net energy (Mcal kg^{-1}) for lactation (NEl) were estimated using the Agricultural and Food Research Council model (AFRC, 1993). The total digestive nutrient (TDN) was estimated using the following equation:

$$TDN\,(\%) = \big(digestible\ crude\ protein + digestible\ non\text{-}structural\ carbohydratres + digestible\ neutral\ detergent\ fiber\ corrected\ for\ protein + 2.25 \times digestible\ ethereal\ extract\big)/100$$

(Pond *et al*., 2002).

Statistical analysis

An analysis of variance was carried out, considering the plant species as the variation source for the DM, CP, NFC, EE, NDF, ADF, ENl, and TDN variables. Regarding the forage availability variable, the source of variation was the plant strata. The statistical design was completely random, and the means were compare using Tukey's test (α=0.05) in the SAS package (2002). In order to comply with the assumptions of the analysis of variance, the data shown in g/100 g were previously transformed using arcsine.

RESULTS AND DISCUSSIONS

Goats chose to consume the following shrubs: *Larrea tridentata*, *Mimosa monancistra*, *Celtis pallida*, and *Prosopis* spp. Regarding cacti, the goats mainly chose *Opuntia* spp. Meanwhile, goats preferred such herbaceous species as *Amaranthus hybridus*, *Verbena canescens*, *Solanum elaeagnifolium*, and *Tithonia tubaeformis*. Grasses such as *Chloris gayana*, *Bouteloua* spp., and *Cenchrus ciliaris* were the least appealing to goats (Table 1).

These results match the findings of Pinos-Rodríguez *et al*. (2007), Ramírez-Orduña *et al*. (2008), Velderrain-Algara *et al*. (2010), and Mellado *et al*. (2012). These authors evaluated the consumption and diets of goats in northeastern Mexico. The availability of foraging species in the pastures is lower during the droughts than in other seasons; consequently, goats have to adapt to these conditions.

Table 1. Chemical composition of the plant species selected by goats in three microregions of the Comarca Lagunera, Mexico.

Plant	CP	EE	NDF	ADF	NFC	TDN	ENl
	g kg^{-1}						Mcal kg^{-1}
Mountain Slope							
Shrubs							
Larrea tridentata	104.2 c	22.8 a	504.3 c	407.3 b	195.1 a	409.3 b	2.1 a
Mimosa monancistra	121.2 b	24.1 a	531.2 b	296.6 c	163.8 b	477.5 a	1.2 b
Celtis pallida	206.3 a	8.6 b	641.6 a	534.1 a	111.6 c	485.3 a	0.8 c
P-Value	0.001	0.017	0.0013	<.0001	<.0001	0.0285	0.0191
Cacti							
Opuntia spp.	43.2	29.1	302.4	215.8	60.1	524.6	1.8
P-Value	---	---	---	---	---	---	---
Herbacaous							
Verbena canescens	128.1	19.3	567.8	474.1	242	463.5	1.1
Chenopodium album	136.8	20.6	536.7	379.8	236.8	501.7	0.9
P-Value	<.0001	0.011	<.0001	<.0001	0.019	<.0001	0.0014
Grasses							
Cynodon dactylon	79.4	5.3	770.8	470.3	84.7	397.4	0.7
Cenchrus ciliaris	44.3	6.2	806.1	517.3	74.3	416.2	0.8
P-Value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0261
Plain							
Shrubs							
Prosopis spp.	212.4	6.3	391.3	276.6	187.4	467.8	1.1
P-Value	---	---	---	---	---	---	---
Herbaceous							
Amaranthus hybridus	179.2	6.4	478.5	364.9	193.7	427.8	1.1
P-Value	---	---	---	---	---	---	---
Grasses							
Chloris virgata	92.6	3.7	665.3	344.8	69.8	413.8	0.5
Bouteloua gracilis	54.1	2.9	722.6	435.4	149.2	410.5	0.6
P-Value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Cultivation areas							
Shrubs							
Prosopis spp.	189.3	4.2	401.7	312.6	173.8	453.9	1.0
Larrea tridentata	117.6	17.5	518.4	421.3	188.4	433.2	1.2
P-Value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Cacti							
Myrtillocactus geometrizans	63.3	14.6	387.6	271.9	71.2	545.8	1.3
P-Value	---	---	---	---	---	---	---
Herbaceous							
Amaranthus hybridus	236.4 a	7.3 a	467.3 a	378.5 a	187.4 a	453.6 b	1.0 b
Solanum elaeagnifolium	138.9 c	4.7 b	417.8 c	289.6 c	173.6 b	487.3 a	1.0 a
Tithonia tubaeformis	173.3 b	4.1 b	443.6 b	319.8 b	163.2 c	439.5 c	0.9 c
P-Value	<.0001	<.0001	0.0052	<.0001	0.002	0.0403	0.0037
Grasses							
Bouteloua spp.	63.2	1.7	718.3	465.4	136.7	407.9	0.4
Cenchrus ciliaris	47.8	2.6	783.1	567.3	93.8	417.6	0.5
P-Value	<.0001	<.0001	0.0018	<.0001	<.0001	0.0232	<.0001

CP: crude protein, EE: ethereal extract, NDF: neutral detergent fiber, ADF: acid detergent fiber, NFC: non-fiber carbohydrates, TDN: total digestible nutrients, NEl: net energy for lactation. Different letters within the column (a, b, c) indicate significative statistical difference (Tukey; α=0.05).

In this regard, goats have flexible, generalist, and opportunistic feeding strategies, since they consume a great variety of plants in grazing areas. Studies carried out in northeastern Mexico indicate that about 126 plant species make up the diet of grazing goats.

Overall, the forage distribution per plant strata is divided into 40% shrubs, 29% herbs, and 31% grasses (Mellado *et al.*, 2012; Haenlein and Ramírez 2007). However, this study confirms that less than ten plants constitute about 90% of the diet during the whole year (Mellado *et al.*, 2004), particularly during the droughts.

During most of the year, the diet of goats mainly consists of deciduous or perennial shrubs (>50%) (Mellado *et al.*, 2003). The leaves and fresh pods (especially from *Prosopis* spp.) are very nutritious forages (between 189 and 212 g kg^{-1} CP in DM; Table 1). They are staple foods (up to 25% of the diet) during autumn and winter, when the quality and the quantity of the forage are low (Mellado *et al.*, 2004). *Prosopis* spp. is one of the most abundant species in northeastern Mexico; its prevalence has increased in large areas of the Chihuahuan Desert, at the expense of the desert pastures (Fredrickson *et al.*, 2006). Goats consume its leaves with moderation (Mellado *et al.*, 2003). However, the pods of these plants are abundantly consumed by the goats, possibly as a result of their high nutritional content (Abdullah *et al.*, 2011).

Meanwhile, the goats in this study chose *Larrea tridentata*. This perennial xerophytic species was chosen by the goats, regardless of its cover and season of the year. This plant can account for up to 15% of the diet (Mellado *et al.*, 2011). The resistance of *Larrea tridentata* to drought —along with its moderate levels of protein (104-117 g kg^{-1} DM; Table 1) and its continuous metabolic activity under extreme drought conditions (Allen *et al.*, 2008)— seems to be the attribute that makes it appealing to goats.

Meanwhile, herb consumption is lower during winter, as a consequence of its low availability (Mellado *et al.*, 2004). This study confirmed the findings of Mellado *et al.* (2012), who reported that key herbs that the goats from northeastern Mexico prefer are *Amaranthus hybridus*, *Tithonia tubaeformis*, and *Solanum elaeagnifolium*. The goats do not find appealing *Solanum elaeagnifolium* during its flowering stage (Mellado *et al.*, 2008). However, as a result of its high nutrient content (139 g kg^{-1} CP in DM; Table 1), this plant can account for 30% of their diets during winter (Mellado *et al.*, 2014).

In the pastures of northeastern Mexico, goats eat less grasses and the consumption percentage seldom exceed 5% of their diet during any month of the year (Mellado *et al.*, 2004). The results of this study match the findings of Mellado *et al.* (2005), who reported that some of the most consumed grasses are *Bouteloua gracilis* and *Cenchrus ciliaris*. Biomass availability varied according to the plant strata and the microregion (Table 2).

The microregion with the highest biomass production was the mountain slope. In the plains, herbaceous were the plant strata with the highest availability. In the cultivation areas, herbaceous and grasses were the most available plants. There were no significant statistical differences regarding their availability in both microregions. In conclusion, the mountain slope microregion could stand a higher animal load, while the cultivation areas could stand a lower animal load. However, cultivation areas have a higher plant species diversity and the best nutrient profile. Consequently, goat production can reach higher yields in this microregion. Nevertheless, in order to prevent overgrazing, this area must

Table 2. Biomass availability per plant strata with forage potential, in three microregions of the Comarca Lagunera, Mexico.

Plant Strata	Mountain Slope	Plain	Cultivation Areas	P-Value
	t ha^{-1}			
Shrubs	3.67 a	0.30 b	0.02 c	<.0001
Cacti	6.00 a	0.00 c	0.02 b	<.0001
Herbaceous	0.31 b	0.86 a	0.37 b	0.0137
Grasses	0.67 a	0.24 c	0.46 b	0.0406

Different letters within the column (a, b, c) indicate significative statistical difference (Tukey; α=0.05).

have low animal loads. Shrubs and herbaceous are the plant strata with the best nutritional profile. The average CP content in shrubs and herbaceous reached 164 and 170.4 g kg^{-1} DM, respectively. Grasses had the lowest nutrient value (average: 66.3 g kg^{-1} DM) and recorded <1.0 NEl (Table 3).

According to the nutritional composition of the different plant strata, crude protein (CP) in shrubs and herbs is enough to meet the nutrient requirements of goats (approximately 16%). However, the energy available in the different strata is mostly low

Table 3. Chemical composition of plant strata with foraging potential, in three microregions of the Comarca Lagunera, Mexico.

Plant	CP	EE	NDF	ADF	NFC	TDN	ENl
	g kg^{-1}						Mcal kg^{-1}
Shrubs							
Mountain slope	151.7 b	16.9 a	556.8 a	418.3 a	148.7 b	454.3 b	1.3 a
Plain	187.4 a	7.9 c	403.6 c	295.1 c	183.2 a	462.8 a	1.1 b
Cultivation area	152.9 b	11 b	458.1 b	365.8 b	180.7 a	450.2 b	1.1 b
P-Value	<.0001	<.0001	<.0001	<.0001	0.035	0.024	0.0271
Cacti							
Mountain slope	38.7	30.3	317.1	224.7	58.3	514.2	1.7
Plains	---	---	---	---	---	---	---
Cultivation areas	53.2	17.9	368.4	263.5	72.2	534.1	1.3
P-Value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Shrubs							
Mountain slope	141.2 b	20.3 a	549.8 a	429.2 a	240.1 a	480.8 a	0.1 b
Plains	182.7 a	5.2 b	476.6 b	371.5 b	201.8 b	418.3 c	1.1 a
Cultivation areas	187.4 a	4.3 b	437.1 c	359.5 c	167.1 c	463.2 b	1.0 a
P-Value	0.041	0.016	<.0001	<.0001	<.0001	<.0001	
Grasses							
Mountain slope	61.3 b	4.2 a	718.4 b	503.2 a	79.3 c	423.5 a	0.6 a
Plains	71.4 a	4.6 a	691.8 c	423.7 b	89.1 b	412.6 a	0.6 a
Cultivation areas	53.6 c	2.3 b	756.3 a	498.1 a	99.5 a	401.2 b	0.57 b
P-Value	<.0001	<.0001	<.0001	<.0001	<.0001	0.018	0.0374

Different letters within the column (a, b, c) indicate significative statistical difference (Tukey; α=0.05).

(<1.0 Mcal kg^{-1} DM). This deficit intensifies, since goats start to lactate during this period; therefore, the consumption of plants with an appropriate energy content is very important (Granados-Rivera *et al.*, 2020). Consequently, the scarce energy content in northeastern Mexico limits the nutritional content of the diets of goats during winter (Maldonado-Jáquez *et al.*, 2017).

CONCLUSIONS

Based on the results obtained in this study, shrubs and herbaceous are the most abundant forage, regardless of the microregion. They also recorded the best nutritional profile during the drought season in the Comarca Lagunera. In addition, these plant strata have an appropriate protein content. However, the low energy content limits the nutritional value of the diet. Therefore, supplementation programs for grazing dairy goats in the Comarca Lagunera must include sources of energy.

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Colegio de Postgraduados

Rhizobacteria inoculation and its effect on the productive parameters of sorghum

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ABSTRACT

Objective: To evaluate the effect of the *Bacillus megaterium* and *Pseudomonas japonica* rhizobacteria on the productive parameters of sorghum.

Design/Methodology/Approach: The experiment was carried out in Padilla, Tamaulipas, where the effect of inoculating a sorghum crop with the *Bacillus megaterium* KN13 and *Pseudomonas japonica* KC14 strains on its productive parameters was evaluated. Both strains were used in two concentrations (10^6 and 10^7 CFU). A randomized block design was applied, consisting of five treatments (two strains × two concentrations, plus a control), with six and nine replications.

Results: The *B. megaterium* and *P. japonica* strains can fix nitrogen and produce siderophores. Inoculating these strains into the sorghum crop increases grain yield, plant height, panicle length, plant stem diameter, and aerial dry weight. Better results are recorded when the strains have a 10^7 CFU concentration.

Study Limitations/Implications: Each type of soil and crop has various microbiomes.

Findings/Conclusions: The use of an adequate concentration of rhizobacteria improves sorghum production; therefore, it is a sustainable alternative, both for the nutrition of the crop and the reduction of the use of synthetic fertilizers.

Keywords: PGPR, *Bacillus megaterium*, *Pseudomonas japonica*.

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INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) Moench] is one of the most productive species and plays a major role in global food security (Mareya *et al.*, 2019). In Mexico, sorghum is the third most produced grain. In 2022, 1.4 million ha were planted nationwide; 55.8% of those hectares were located in Tamaulipas, which ranks first among the states that produce this species (SIAP, 2023). Sorghum is mainly used to produce food for the livestock sector. Synthetic fertilizers are applied to increase sorghum yield (and the yield of many other crops); however, high doses pollute and consequently impact soil fertility (Mikhak *et al.*, 2017).

The use of rhizobacteria is an option to replace synthetic fertilizers, reducing their negative impact on the soil and the environment (Vocciante *et al.*, 2022). Rhizobacteria promote plant growth, since they facilitate the absorption of nutrients (*e.g.*, nitrogen, phosphorus, and iron), as a consequence of their nitrogen fixation, phosphate solubilization, and siderophore production capacities (Haiyambo *et al.*, 2015). The genera *Pseudomonas* (Zahid *et al.*, 2015; Moreira *et al.*, 2016), *Bacillus* (Breedt *et al.*, 2017; Mumtaz *et al.*, 2017), *Azospirillun* (Florio *et al.*, 2017), and *Enterobacter* (Ibort *et al.*, 2017) stand out among the most studied plant growth-promoting rhizobacteria (PGPR).

The use of PGPR in various crops has been extensively researched. However, the lack of enough information on sorghum crops makes further studies into this matter a necessity. The objective of this work was to evaluate the effect of the *Bacillus megaterium* and *Pseudomonas japonica* rhizobacteria on productive parameters of sorghum.

MATERIALS AND METHODS

The experiment was carried out in 2020, at the ejido El Tablero (24° 05' N, 99° 00' W, and 36 m.a.s.l.), Padilla, Tamaulipas, Mexico. The physical and chemical characteristics of the sampling soil are: clay loam texture, high calcareous nature, low salts, pH=8.4, OM=2.9%, N=12.2 ppm, P=48.4 ppm, and K=364 ppm. The sampling was carried out at a depth of 0-30 cm.

Biological material

Two strains were isolated from the corn rhizosphere and identified as *Bacillus megaterium* KN13 and *Pseudomonas japonica* KC14. These strains have been evaluated in previous trials and are known to promote plant growth.

Nitrogen fixation evaluation

The indirect ammonium ion method was used to evaluate the ability of the two bacterial strains to fix nitrogen, using the Berthelot colorimetric technique (phenol-hypochlorite), as described by Escobar *et al.* (2011). A spectrophotometer was used for the absorbance reading (632.9 nm), considering all strains with positive readings as fixative. The evaluation was performed in triplicate for each strain.

Siderophore production

CAS agar was used to determine if both strains produced siderophores (iron chelating compounds). After culturing the bacteria at 30 °C for 5 days, the presence of orange halos was detected around the colony, indicating siderophore activity. For each strain, the assay was performed in triplicate (Haiyambo *et al.*, 2015).

Field trial

Before sowing, the seeds of sorghum hybrid D47 were sterilized (5 min in 70% ethanol, 5 min in 10% NaClO, and three rinses with sterile distilled water) and kept at 4 °C for 24 h (Ibort *et al.*, 2017). The two strains were allowed to grown in LB for 24 h at 28 °C. They were stirred at 200 rpm. CFU were counted with a Neubauer chamber. The bacterial solutions were adjusted to a concentration of 10^6 and 10^7 CFU mL^{-1}. Subsequently, the seeds were inoculated via a 1-h immersion in each of the bacterial solutions. A second inoculation was performed 25 days after sowing, adding 2.5 mL of bacterial solution per plant. The same treatment was carried out on the control plants, using sterile distilled water instead of the bacterial solution (Angulo *et al.*, 2014). Agronomic management consisted of two relief irrigations, mechanical weed control, pest control (with chemical insecticides), and no fertilization. The harvest was carried out 135 days after sowing.

Experimental design

A completely randomized block design was used. The experiment consisted of five treatments, with six repetitions for the performance variables and nine repetitions for the morphometric variables. The treatments were called Bm7, Bm6, Pj7, and Pj6 (Bm: *Bacillus megaterium* KN13; Pj: *Pseudomonas japonica* KC14; 7: 10^7 CFU; and 6: 10^6 CFU). The variables evaluated were: furrow grain weight, grain yield (ha^{-1}), plant height, panicle length, plant stem diameter, and aerial dry weight. To calculate biomass, plants were dried in an oven at 70 °C for 48 h. Rows (7 m long, 0.8 m between rows) were established with a population density of 20 plants per linear m. To calculate the yield, 5 linear m of each repetition were harvested, projecting the production in kg ha^{-1}.

Statistical analysis

A general linear model analysis and multiple comparison of LSD means test (significance level: $P \leq 0.05$) were performed using the Rstudio statistical software, version 4.0.3.

RESULTS AND DISCUSSION

Table 1 shows the results of the biochemical tests. Both strains can fix nitrogen and produce siderophores; therefore, they can improve the availability of nitrogen and iron and suppress the effect of phytopathogens (Beneduzi *et al.*, 2012). In other words, they are rhizobacteria with the potential to promote plant growth (Haiyambo *et al.*, 2015). Specifically, *B. megaterium* has nitrogen fixation abilities (Yousuf *et al.*, 2017). The siderophores secreted by microorganisms are usually the source of iron for sorghum cultivation (Beneduzi *et al.*, 2012).

Figure 1 shows the results for sorghum grain yield, including a highly significant statistical difference between treatments regarding furrow grain weight ($F=60.02$, g.l.=4, $P<4.752e\text{-}09$***) and grain yield in kg ha^{-1} ($F=60.17$, g.l.=4, $P<4.668e\text{-}09$***). There

Table 1. Evaluation of the ability of rhizobacteria to nitrogen fixation and siderophore production.

Strains	Biochemical tests	
	Nitrogen fixation	Siderophore production
Bm KN13	+	+
Pj KC14	+	+

Bm KN14=*Bacillus megaterium*. Pj KC14=*Pseudomonas japonica*.

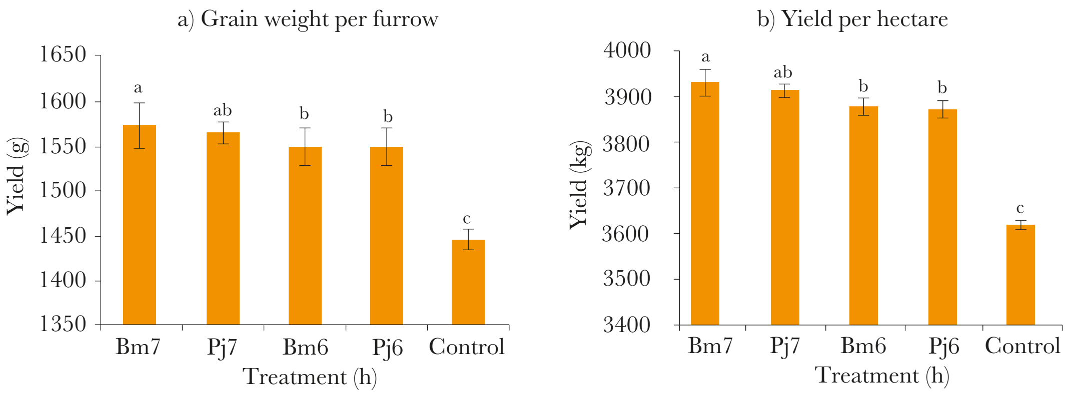

Figure 1. Effect of rhizobacteria inoculation on sorghum grain yield. Bm7=*B. megaterium* (10^7 CFU). Bm6=*B. megaterium* (10^6 CFU). Pj7=*P. japonica* (10^7 CFU). Pj6=*P. japonica* (10^6 CFU).

were no statistical differences between these two variables, neither for the block, nor for the block × treatment interaction. The treatment inoculated with Bm7 obtained the highest yield, while the control reported the lowest yield. Previous studies report increases in grain production in wheat (*Triticum* spp.) inoculated with *Bacillus* spp. (Upadhyay and Singh, 2015) and *Pseudomonas* spp. (Nadeem *et al.*, 2013). The same effect has been reported for corn inoculated with *Bacillus* and *Pseudomonas* spp. (Moreira *et al.*, 2016, Mumtaz *et al.*, 2017). The 10^7-concentration obtained higher results than the 10^6-concentration. João-Alves *et al.* (2021) reported that, in a study with corn and soybean, increasing the concentration of the bacterial solution increased production. In conclusion, a higher concentration of CFUs in these strains is necessary to obtain better results.

The results of the morphometric variables are presented in Table 2. Regarding the plant height, aerial dry weight, and stem diameter variables, only the following treatments recorded a statistical difference: F=31.1676, g.l.=4, P<3.465e-09***; F=67.74, g.l.=4, P<1.012e-112***; and F=5.2862, g.l.=4, P<0.003374**. The block and the interaction do not show a statistical difference. Regarding the panicle length variable, the following treatments had a statistical difference: F=70.2913, d.f.=4, P<6.75e-13*** and the block F=7.7873, d.f.=2, P<0.0002475***. The one exception was the block × treatment interaction.

Table 2. Effect of rhizobacteria inoculation on the morphometric variables of sorghum.

Treatment	Plant height (cm)	Panicle length (cm)	Aerial dry weight (g)	Stem diameter (cm)
Pj7	122±0a	22.8±0a	124±0a	24.5±0a
Bm7	121.22±1.86a	23.21±0.28a	124.78±2.86a	24.78±1.09a
Pj6	120.67±1.41a	22.98±0.186a	122.78±2.64a	24±1.22a
Bm6	120.56±1.67a	23±0.353a	123.11±2.80a	24.78±0.83a
Control	114±1.5b	21.48±0.399b	106.33±2b	23±0.86ab

Bm7=*B. megaterium* (10^7 CFU). Bm6=*B. megaterium* (10^6 CFU). Pj7=*P. japonica* (10^7 CFU). Pj6=*P. japonica* (10^6 CFU).

Compared to the control plants, the plants inoculated with both concentrations of the two strains had a positive effect on the evaluated parameters. Similar results have been reported in corn plants, where the use of *Bacillus* spp. and *Pseudomonas* spp. had a positive effect: it promoted growth and increased forage and grain production (Nadeem *et al.*, 2013; Zahid *et al.*, 2015; Mumtaz *et al.*, 2017). Positive effects have also been reported on the biomass production of wheat crops inoculated with *Bacillus* spp. and *Pseudomonas* spp. (Upadhyay *et al.*, 2011; Nadeem *et al.*, 2013; Upadhyay and Singh, 2015). This study only evaluated the capacity of the strains to fix nitrogen and produce siderophores; however, these two genera of bacteria can promote growth by other mechanisms, such as phytohormone production and the solubilization of phosphate, potassium, and zinc (Vaikuntapu *et al.*, 2014; Gandhi and Muralidharan, 2016; Mumtaz *et al.*, 2017), as well as the release of some micronutrients (Haiyambo *et al.*, 2015). The effect of *B. megaterium* and *P. japonica* on the growth promotion and production of sorghum may be influenced by a wide range of the mechanisms of action that are attributed to these bacteria.

CONCLUSIONS

Taking into consideration the trend towards the reduction in the use of synthetic fertilizers, the use of rhizobacteria to improve sorghum production is a feasible and environmentally acceptable alternative. The results show that a 10^7 CFU concentration of rhizobacteria improves sorghum production. Further studies are required to evaluate the optimal number of inoculations, as well as the specific concentration at which each strain records the best results.

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Colegio de Postgraduados

Use of Andromed® and OviXcell® diluents in the processing of sheep semen with the addition of HTF

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ABSTRACT

Objective: To assess the effect of two ovine semen diluents, Andromed® and OviXcell®, on the mass motility, vitality, and acrosomal integrity of spermatozoa processed at different temperatures and with the addition of post-thawed HTF (human tubal fluid).

Design/Methodology/Approach: Semen from 2 Dorper ram was used. Four ejaculations per male were collected and diluted with Andromed® and Ovixcell®. There were four treatments with Ovixcell®: 1) fresh Ovixcell®, 2) refrigerated Ovixcell®, 3) post-thawed Ovixcell®, and 4) post-thawed Ovixcell® + HTF. Four treaments with Andromed® were also carried out: 1) fresh Andromed®, 2) refrigerated Andromed®, 3) post-thawed Andromed®, and 4) post-thawed Andromed® + HTF. Mass motility, vitality, and acrosomal integrity were performed with a CASA computer system and statistically analyzed with the GLM procedure of the SAS software.

Results: When the effect of Ovixcell® mass motility was assessed, the following results were obtained, with differences ($p<0.05$) between treatments: 87% in fresh semen, 72% refrigerated semen, 55% in post-thawed semen, and 68% in post-thawed semen + HTF. A clear difference ($p<0.05$) was observed when HTF was added to post-thawed semen (13%). Andromed® behaved in the same way as Ovixcell® ($p<0.05$) and a 18% recovery was observed with the addition of HTF. A high percentage of live spermatozoa with intact acrosome was observed for fresh semen (97.8%), while it diminished ($p<0.05$) as the temperature of refrigerated and frozen semen gradually decreased.

Study Limitations/Implications: More experimental units should be used, despite the increase in maintenance costs per animal.

Findings/Conclusions: The use of Andromed® and OviXcell® diluents was satisfactory in relation to the mass motility, vitality, and acrosomal integrity in spermatozoa processed at different temperatures (fresh, refrigerated, and post-thawed). Furthermore, the addition of HTF increased post-thawed mass motility.

Keywords: Diluents, Andromed®, OviXcell®, HTF, sheep.

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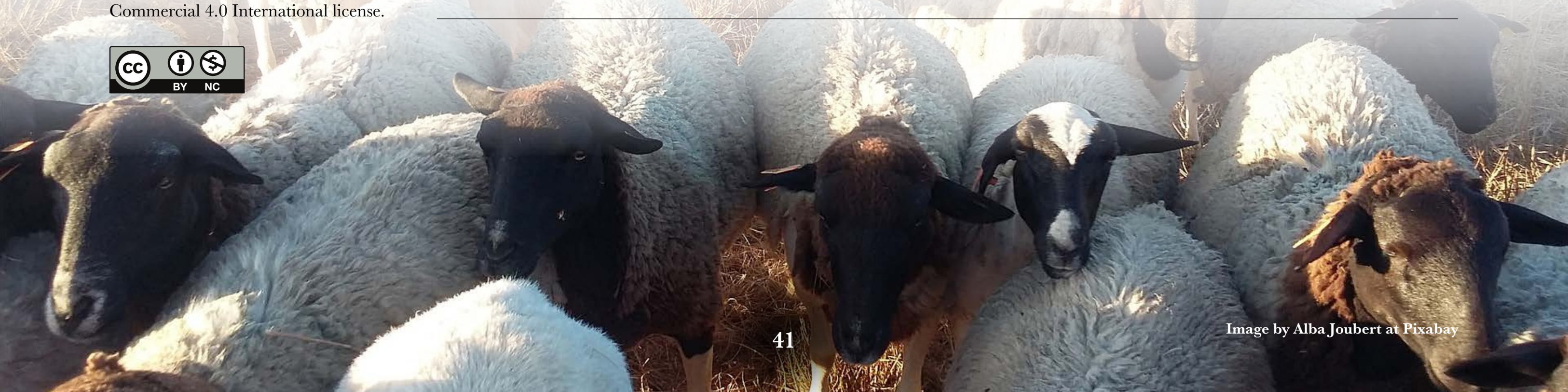

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INTRODUCTION

Semen conservation plays an important role in artificial insemination (AI) programs for sheep; however, its process faces several disadvantages. For example, pregnancy percentage ranges between 30-50% with refrigerated semen, as a consequence of the difficulties related to the sperm motility of the semen used (Yaniz *et al.*, 2005). Similarly, when frozen semen is used, the fertilization capacity of spermatozoa shows a significant decrease. This situation is related to damage to sperm cell caused by the use of diluents, which do not protect the spermatozoa during the preservation (refrigeration and freezing) processes (Canizales, 2013). In addition, the use of inadequate preservation methods caused a low fertilizing capacity of both refrigerated and frozen semen (Aitken and Clarkson, 1988). Therefore, choosing the most appropriate diluent is of great importance.

The addition of diluent is an important step in the semen preservation process, as it provides nutrients and volume to the semen sample (Gadea, 2003). Diluents should preserve and extend sperm life and guarantee an adequate nutrient environment for the spermatozoa, during storage and upon thawing for AI (Allai *et al.*, 2018).

The Andromed® and OviXcell® diluents pose no risk of microbiological contamination, because they are free of animal proteins, which increases fertility rates. These diluents contain soy lecithin, which, given its plant origin, has a greater protective effect during the cooling process (Fukui *et al.*, 2008), consequently improving sperm viability and fertility (Canizales, 2013).

Human Tubal Fluid (HTF) is a product used in the embryo maturation process and is an alternative that, used in conjunction with the diluent, increases semen motility during the cooling and post-thawing process. This product contains compounds such as sodium chloride, potassium chloride, calcium chloride dihydrate, and magnesium sulfate heptahydrate (Quinn *et al.*, 1985), which make it a highly nutrient-rich medium. In addition, HTF contains energy sources such as pyruvate, lactate, and glucose, as well as albumin as the main protein supplement (Gimeno *et al.*, 2006). HTF has been shown to improve embryonic development and gestation rate after *in vitro* fertilization and it can be used to process semen from all kind of domestic animals (Conaghan *et al.*, 1998). Therefore, the objective of this study was to assess the effect of two semen diluents, Andromed® and OviXcell®, on the mass motility, vitality, and acrosomal integrity of spermatozoa processed at different temperatures, with the addition of post-thaw HTF to protect sheep sperm cells.

MATERIALS AND METHODS

Description of the study area

The current study was conducted at the Rancho Universitario of the Universidad Autónoma de Ciudad Juárez, located in Ciudad Juárez, Chihuahua. This region has a predominantly hot desert (BWh) climate, with an average annual temperature of 16° to 18 °C, an average altitude of 1,100 m.a.s.l., and an average annual rainfall of 244 mm (INEGI, 2017).

Management of experimental units

The study was carried out with semen from 2 to 4-year old Dorper rams. During the reproductive season (from November to February), four samples from each male were collected by ejaculation in an artificial vagina. These ejaculates were subjected to four treatments with each diluent (Andromed® and Ovixcell®). The four Ovixcell® treatments were: 1) fresh Ovixcell®, 2) refrigerated Ovixcell®, 3) post-thawed Ovixcell®, and 4) post-thawed Ovixcell® + HTF. Meanwhile, the four Andromed® treaments were: 1) fresh Andromed®, 2) refrigerated Andromed®, 3) post-thawed Andromed®, and 4) post-thawed Andromed® + HTF. Dilution in all treatments was performed 1:5, following the recommendations of the manufacturer. After the diluents were added, mass motility (%), vitality (%), and acrosomal integrity (%) were assessed at the different treatment temperatures (fresh, frozen, and refrigerated). The tests were carried out by means of the CASA (Computer-Assisted Sperm Analysis) procedure (Amann and Wabersky, 2014).

Experimental design

The study lasted two months (November and December) and the experiment consisted of adding two types of nutrient media (diluents) and HTF to the ejaculates. Finally, differences between treatments were assessed for each diluent, based on the mass motility, vitality, and acrosomal integrity variables.

Semen sample collection

Seminal samples were obtained by means of the artificial vagina (Wulster-Radcliffe *et al.*, 2001) and electroejaculation (Morillo *et al.*, 2012) techniques.

Diluted fresh semen

Eight ejaculates from two males were used. One ejaculate was assigned to be diluted with a specific diluent and processed at a specific temperature (fresh, refrigerated, and frozen). Ten replicates were performed for each diluted ejaculate. In total, 80 observations were made among the 4 study treatments for each diluent, as described below.

The four treatments that used the Ovixcell® diluent were: fresh Ovixcell®, refrigerated Ovixcell®, post-thawed Ovixcell®, and post-thawed Ovixcell® + HTF. Meanwhile, 4 treatments used the Andromed® diluent: fresh Andromed®, refrigerated Andromed®, post-thawed Andromed®, and post-thawed Andromed® + HTF.

Semen evaluation

The mass motility, vitality, and acrosomal integrity seminal parameters were assessed for each treatment.

Mass motility: 5 μL of each replicate per treatment were placed on a slide at 35 °C on top of a thermal plate (Clemente *et al.*, 2012), for their observation under the microscope using CASA.

Vitality: To assess the sperm vitality, 5 μL of each replicate per treatment were placed in a counting chamber for their analysis at 35 °C. Subsequently, they were observed using CASA, to determine the number of live spermatozoa.

Acrosomal integrity: The same amount of semen was used to determine sperm viability. This parameter was observed with CASA, in which the acrosomal shape of the spermatozoa was analyzed, checking that it had an oval head, a straight neck, and a long and thin tail without folds (Clemente *et al.*, 2012).

Diluted refrigerated semen

Once the diluted fresh semen was analyzed, it was stored at 4 °C for 2 hours. Afterwards, mass motility, vitality, and acrosomal integrity were assessed, following the same procedure as in the fresh diluted semen.

Post-thawed semen

To assess its characteristics, post-thawed semen was processed after it had been refrigerated at 4 °C for 2 hours. Subsequently, 0.25-mL straws were filled with semen and the freezing process was initiated, following the liquid nitrogen vapour method described by Jerez *et al.* (2016). The straws were placed 7 cm above the level of the liquid nitrogen (LN, −140 °C) for 10 min; they were then directly immersed in the LN (−196 °C) and stored until further analysis. Semen was analyzed 24 hours later, for the post-thaw Ovixcell® and post-thaw Andromed® treatments, thawing the straw by immersion in water at 37 °C for 50 seconds. Similarly, once semen was thawed, HTF was added to both diluents (post-thawed Ovixcell® + HTF and post-thawed Andromed® + HTF), before the assessment of mass motility, vitality, and acrosomal integrity.

Statistical analysis

The mass motility, vitality, and acrosomal integrity variables were analyzed with the GLM procedure in SAS V9 (SAS, 2004), followed by Tukey's multiple comparison test (α=0.05).

RESULTS AND DISCUSSIONS

Mass motility

Mass motility (MM) recorded the following percentages when Ovixcell® was used. with some differences ($p<0.05$) between treatments: 87% (fresh semen), 72% (refrigerated semen), 55% (post-thawed semen), and 68% (post-thawed semen + HTF). Such behavior could be the result of the oxidative stress induced by cryopreservation, which has adverse effects on post-thawed semen quality (Jiménez-Aguilar *et al.*, 2021). Mass motility follows a downwards trend, according to the gradual decline in temperature (changing from fresh to refrigerated semen and subsequently frozen). A 13% increase in MM was also observed when HTF was added to post-frozen semen (Figure 1). For their part, Mata-Campuzano *et al.* (2015) mention that, at the time of cryopreservation, spermatozoa are exposed to physical and chemical impacts that hinder viability, decrease motility, damage the acrosome, and decrease fertility.

Results from the assessment of the effect of the Andromed® diluent on the quality of fresh, refrigerated, and post-thawed semen and of Andromed® + HTF on the quality of post-thawed fresh sperm indicate 92, 69, 53, and 71% MM, respectively, with differences ($p<0.05$) between fresh, refrigerated, and post-thawed semen.

Andromed® evidently induces excellent motility levels in fresh semen, although this characteristic diminishes considerably as the storage temperature decreases (refrigerated and frozen); however, an 18% increase in MM was observed when HTF was added at the thawing stage (Figure 1). Berkovich *et al.* (2013) mention that the addition of antioxidants to diluents during semen freezing improves post-thawing characteristics, since they protect the polyunsaturated fatty acids of the spermatozoa, consequently preventing oxidative stress damage.

Finally, during the assessment of differences between diluents, both Andromed® and Ovixcell® show the same effect on mass motility, at different storage temperatures and despite the addition of HTF ($p<0.05$).

Ovixcell® maintained the motility characteristics of spermatozoa that were processed in fresh, refrigerated, and frozen conditions, as well as their quality characteristics for AI. Combining the diluent with HTF improved MM; this result matches the findings of Khalifa and Lymberopoulos (2013), who used the Ovixcell® diluent and observed a higher sperm motility than with the use of an egg yolk and milk-based diluent.

This result may be caused by the combination of Ovixcell® and HTF; after semen has been subjected to cold temperatures, it maintains high motility, as a result of the large amount of nutrients that these two substances provide together. Values above 80% for MM in semen refrigerated at 4 °C are considered excellent; therefore, the results of the current study indicate that the assessed treatments meet the requirements for their use as diluents and, to a greater extent, for the use of the Ovixcell® + HTF combination.

When artificial insemination is performed, semen should have many live spermatozoa with high motility percentages (>50%), ensuring high pregnancy rates. According to the results of the current study, both diluents can be used in any of the treatments tested to process semen at refrigeration temperatures (4 °C).

The use of Andromed® + HTF improved the motility of post-thawed spermatozoa (71%); in comparison, the MM of spermatozoa post-thawed only recorded 53% motility ($p<0.05$) with Andromed®.

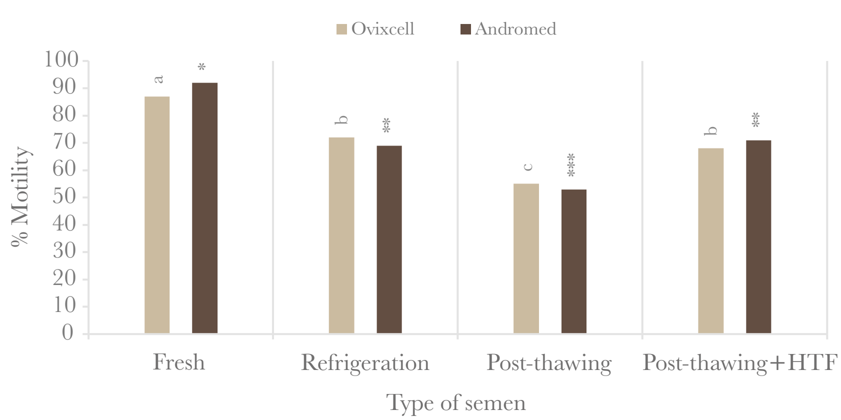

Figure 1. Mass motility (MM) of Dorper sheep spermatozoa processed at different temperatures (fresh, refrigerated, and post-thawed), with 2 diluents and post-thawed HTF.

Perhaps once semen has been subjected to cold temperatures with the dilution of Andromed® and HTF, it maintains a high motility given the large amount of nutrients provided by the combination of these two substances. El-Keraby *et al.* (2010) and Singh *et al.* (2012) determined that using Andromed® diluent increased the sperm motility of sheep semen stored at 4 °C (>5%). The effect of HTF on sperm cells is directly based on sperm capacitation and on changes in reactive oxygen species (ROS) levels, which have been studied in humans, but not yet in domestic species (Shih *et al.*, 2016; Hernández *et al.*, 2021).

According to Hernandez *et al.* (2014), the use of animal protein-free diluents (*e.g.*, Andromed®) show a better response, since their phospholipid content is derived from soybean extract and spermatozoids are not affected by the phospholipase used in other diluents.

Acrosome vitality and integrity

With the Andromed® diluent, a high percentage of live spermatozoa with intact acrosome was observed in fresh semen (97.8%), as well as a reduction ($p<0.05$) as temperature gradually increased (refrigerated and frozen semen). As for spermatozoa without acrosome, no differences were found between treatments ($p>0.05$), although differences in the percentage of dead spermatozoa were recorded ($p<0.05$).

The use of Andromed® preserved the motility, vitality, and acrosomal integrity of spermatozoa processed at different storage temperatures (fresh, refrigerated, and frozen), either alone or combined with HTF. These results match the findings of Arifiantini and Yusuf (2010), who reported viability values of 84.2% when semen was diluted with Andromed®. In this sense, Velez *et al.* (2014) asserted that the average (normal) vitality is >60%; therefore, the results found in this study are quite satisfactory in terms of vitality.

Table 1. Vitality and acrosomal integrity of Dorper sheep spermatozoa processed at different temperatures (fresh, refrigerated, and post-thawed), with Andromed® diluent and post-thawed HTF.

Treatment	Vitality and acrosome classification		
	Lsia (%)	**Lswa (%)**	**Dead sperm (%)**
Fresh semen	97.8a	1.3a	0.9a
Refrigerated semen	88.8b	0.7a	10.5b
Post-thawed semen	85.6b	1.1a	13.3b
Post-thawed + HFT	87.1b	1.3a	11.6b

Lsia: live sperm intact acrosome; Lswa: live sperm without acrosome. a,b Different literals between columns are different (Tukey; $\alpha=0.05$).

CONCLUSIONS

Similar efficient performance regarding the mass motility, vitality, and acrosomal integrity of sperm processed at different temperatures (fresh, refrigerated, and post-thawed) was observed with the use of the Andromed® and OviXcell® diluents. Finally, adding HTF to both diluents increased post-thawed mass motility with respect to post-thawed sperm motility.

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Colegio de Postgraduados

Year-long evaluation of total soluble proteins in the trunk of two pine species from northeastern Mexico

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ABSTRACT

Objective: To quantify the one-year total soluble protein (TSP) concentration in the trunk of two pine species.
Design/Methodology/Approach: The Bradford method (1976) was used to determine the TSP concentration in the two pine species. Statistical tests were subsequently performed with the IBM SPSS 18 Software, using a general linear model (GLM) univariate analysis.
Results: The TSP concentration was different for each month. The highest concentration was recorded in August (6.84 mg gMS^{-1} for *Pinus pinceana* Gordon and 6.82 mg gMS^{-1} for *Pinus cembroides* Zucc), and the lowest was registered in April (5.53 mg gMS^{-1} for *Pinus cembroides* Zucc) and February (6.64 mg gMS^{-1} for *Pinus pincenana* Gordon).
Study Limitations/implications: There is a lack of information regarding these two pine species and scarce studies explain the behavior of the TSP.
Conclusions: The concentration of total soluble proteins varies in each month of the year. Further studies that include more plant organs are required to obtain a broader protein profile for both species.

Keywords: vegetative storage, nutrients, ecophysiology, proteins.

INTRODUCTION

Pinus pinceana Gordon and *Pinus cembroides* Zucc are two species endemics to Mexico; they are distributed across the states of Coahuila, Nuevo León, Zacatecas, San Luis Potosí, and Querétaro (Villareal *et al.*, 2009). Both species have great economic importance, give the use of their timber as firewood and fuel and of their seeds as food (CONABIO, 2019). The stands of *Pinus pinceana* Gordon are small and its populations are restricted,

Image by Alejandro Sánchez

additionally, grazing goats and the collection of firewood and seeds have brought this species to the brink of extinction, consequently, it is subject to special protection according to NOM-059-ECOL-2001 and NOM-059-SEMARNAT-2010 (Quiroz *et al.*, 2017).

Nitrogen is abundant in plants and may be present as soluble proteins and vegetative storage proteins (El Zein *et al.*, 2011), which are crucial to the development of cellular, metabolic, and genetic coding structures. The lack of this element can lead to poor development or growth (Espino *et al.*, 2018). The increasing temperatures and droughts caused by climate change and human activities currently threaten the populations of the observed pines in more arid areas (Martiñón *et al.*, 2010). Studies about both woody species and the behavior of total soluble proteins are scarce, there are only reports for a few species: *Caladium bicolor* (Ortiz *et al.*, 2015), *Quercus petraea* and *Fagus sylvatica* L. (Valenzuela *et al.*, 2010), and *Carya illinoinensis* Koch (Núñez *et al.*, 2019, 2021). Furthermore, studies about the nitrogenous compound storage in the trunk and roots (El Zein *et al.*, 2011) and the mobilization of the said compounds into new growth tissues are very limited and scarce (Villar *et al.*, 2015). Despite the economic importance of their seeds, there is no management and conservation program for both pine species (Fuentes *et al.*, 2019). Our objective was to evaluate total soluble protein (TSP) concentration in *Pinus pinceana* Gordon and *Pinus cembroides* Zucc, specifically on the trunk, at a 1.3-m height, during an annual cycle.

MATERIALS AND METHODS

This study lasted one year (January-December 2022). It was conducted in the Jagüey de Ferniza ejido, in the south area of the municipality of Saltillo, Coahuila, Mexico (101° 02’ 17” N, 25° 14’ 47” W). Five individuals of each species were systematically selected according to the diameter of their trunks (≈60 cm). Monthly samples were taken from the trunk cores, 1.3 m above ground level using a Pressler drill (Haglof®, 15mm/0.200” diameter, 3 edges). The methodology reported by Núñez *et al.* (2021) was used for sampling and part of the sample processing. The samples were transported to the Departamento de Recursos Naturales Renovables of the Universidad Autónoma Agraria Antonio Narro, in Saltillo, Coahuila, where they were dehydrated at 50 °C for one week, using a HS45-AIA drying oven (Novatech). The samples where then analyzed using the Bradford method (1976). An analytical balance (Adventurer™ Pro) was used to weigh 10 mg of dry matter, which were placed in 2-mL microtubes. Subsequently, 2 mL of the extraction solution (KH_2PO_4, Na_2HPO_4, and polyvinylpyrrolidone) were added to each of the microtubes. The samples were stirred in a vortex mixer for 10 min and centrifuged for 15 min at 10,000 rpm. Afterwards, 500 μL of the resulting solution were poured into plastic cuvettes, to which 500 μL of the reagent were added (Quick Start™ Bradford Protein Assay). The cuvettes with the solution were stirred and left to settle for five minutes, before a sensitive UV spectrophotometer (JENWAY 6320D) was used to analyze the solution at a 595-nm absorbance.

Statistical analysis

The Kolmogorov-Smirnov test was performed to verify that the data met the normality and homoscedasticity assumptions. Once the assumptions were confirmed, the TSP concentration of each species was compared using a GLM univariate analysis, considering month and height as the main factors in the model. The statistical analyses were performed with the help of the IBM SPSS 18 software (significance level: $\alpha \leq 0.05$).

RESULTS AND DISCUSSION

The TSP concentration was statistically different for each month evaluated (F=13.335, g.l.=11,365, P=0.000). Figure 1 shows that August recorded the highest TSP concentration for both species (6.84 mg gMS^{-1} in *Pinus pinceana* Gordon and 6.82 mg gMS^{-1} in *Pinus cembroides* Zucc), while the lowest TSP concentrations occurred in April for *Pinus cembroides* Zucc (5.53 mg gMS^{-1}) and February for *Pinus pinceana* Gordon (6.64 mg gMS^{-1}).

Plants can mobilize nutrients that will subsequently contribute to their optimal development in each season of the year (Perdomo *et al.*, 2010). Therefore, *Pinus pinceana* Gordon and *Pinus cembroides* Zucc showed variations in TSP concentration.

TSP concentrations recorded the highest results for both species in August (6.84 mg gMS^{-1} in *Pinus pinceana* and 6.82 mg gMS^{-1} and *Pinus cembroides*). These results differ from the *Quercus robur* study conducted by Valenzuela *et al.* (2011), who reported the highest concentration in October (0.15±0.03 g·100g^{-1}) and a significant decrease of TSP in June. In this study, a decreasing TSP concentration could only be observed in Pinus cembroides in June (6.23 Mg gMS^{-1}), while the lowest record of these compounds in *Pinus pinceana* occurred in February (6.64 Mg gMS^{-1}).

The results also differ from the observations made by Muñoz *et al.* (1993), who reported an increase in N in the tissues of *Prunus persica* during autumn (August to November). However, this study failed to detect a fixed pattern in TSP concentrations. Núñez *et al.* (2021)

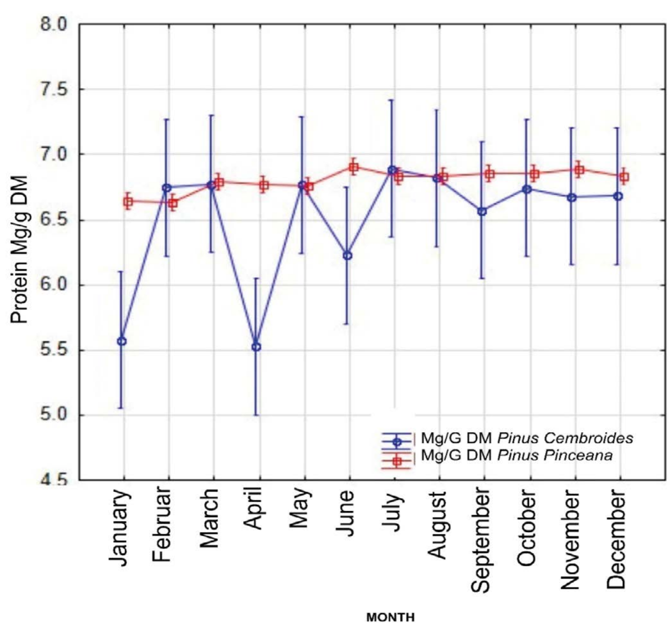

Figure 1. Monthly variation in TSP concentration in the trunk of two *Pinus* species over the course of one year.

Table 1. Average TSP concentration (mg protein/g DM) in two *Pinus* species.

Month	protein Mg /g DM *Cembroides*	Protein mg /g DM *Pinceana*
August	6.82 a	6.84 a
July	6.89 a	6.83 a
January	5.58 ab	6.64 bc
March	6.77 ab	6.79 a
May	6.77 ab	6.76 abc
December	6.69 abc	6.83 a
February	6.74 abc	6.64 b
June	6.23 abc	6.90 a
November	6.68 abc	6.89 a
October	6.74 abc	6.86 a
September	6.57 abc	6.85 a
April	5.53 c	6.77 abc

Milligrams of protein over grams of dry matter (protein mg /g DM).
$_z$Different lines between columns indicate significant differences (Tukey, $p<0.05$).

found a proportional relation between protein nitrogen concentration and temperature increase in *Carya illinoinensis* —*i.e.*, the percentage of protein nitrogen increases along with temperature. For their part, Taulavuori *et al.* (2014) reported that protein nitrogen content increased with high temperatures.

The variation in TSP concentration could be the result of differences between species and their development stages; one species could be more sensitive to biotic or abiotic variables (Tromp and Ovaa, 1973). It is worth highlighting that the concentration of protein compounds tends to increase or decrease in a matter of hours or days, due to the plasticity of plants, various species modify their behavior to survive in heterogeneous environmental conditions (Villar *et al.*, 2013). The plasticity of plants is defined as the ability of a particular genotype or population to express distinct phenotypes in the face of any biotic or abiotic variation, through changes in their morphology and physiology (Hernández *et al.*, 2015; Villamizar *et al.*, 2012).

The results of the present study show that TSP concentration in plants can fluctuate and that plants can retranslocate these compounds to meet their needs (Aerts and Chapin, 1999). The fluctuation of TSP may be impacted by different causes, such as environmental variables or the abiotic stress that the plant suffers (Upendra and Dagla, 2016). Tuberosa (2012) and Brunner *et al.* (2015) reported that, when plants experience high levels of drought stress, they activate protein-based mechanisms to protect themselves from cellular damage.

CONCLUSIONS

The concentration of total soluble proteins in the two observed species varies in each month of the year. An analysis of the complete proteic profile of both species is still required to characterize the mobilization dynamics of nitrogenous compounds, taking the effect of environmental variables into consideration.

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Colegio de Postgraduados

Reproductive and productive behavior of hair sheep under an intensive production system

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ABSTRACT

Objective: To evaluate the reproductive and productive behavior of four hair sheep breeds in an intensive production system.

Design/Methodology/Approach: Data recorded over 4 years (2016-2019) from a production unit that works with Blackbelly, Pelibuey, Dorper, and Katahdin breeds were analyzed. The following variables were evaluated: fertility, prolificacy, birth weight (BW), weaning weight (WW), weaned lamb yield (WLY), daily weight gain during lactation (DWGL), offspring born alive (OBA), male offspring (MO), and female offspring (FO). All information was analyzed using Chi-square tests and analysis of variance.

Results: The fertility, BW, WW, and WYL variables recorded different values between breeds ($P<0.05$). The greatest fertility and prolificacy were recorded by Blackbelly, while Dorper recorded the lowest values ($P<0.05$). BW and WW were highest in Dorper and lowest in Blackbelly ($P<0.05$). Finally, the sheep that recorded the highest WYL were the Blackbelly, while the Dorper registered the lowest values ($P<0.05$). BW and DWGL values reached maximum values ($P<0.05$) in Dorper rams, followed by Katahdin. The greatest number of OBA was observed in Blackbelly and Pelibuey ($P<0.05$).

Study Limitations/Implications: Further studies should be carried out to validate this research.

Findings/Conclusions: Blackbelly ewes produced more kilograms of weaned lambs per lambing ewe. The Dorper and Katahdin rams in the pre-weaning period recorded higher weight at weaning.

Keywords: reproductive efficiency, productivity, fertility, weight gain.

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INTRODUCTION

As a result of the high demand for sheep (*Ovis aries*) meat products in Mexico, special attention should be given to lamb production systems focused on supply, since the increase in demand for sheep products has forced Mexico to import live cattle for

slaughter (Bobadilla *et al.*, 2021). Since the annual production of sheep meat in Mexico amounts to 39,852 t and the *per capita* consumption per year is 0.6 kg, approximately 63,000 t year^{-1} must be imported, mainly from New Zealand, Australia, the United States of America, Chile, and Canada (SIAP, 2019). Consequently, sheep production should be redirected towards the production of lambs for supply (Calderón-Cabrera *et al.*, 2022).

Sheep farming in Mexico has incorporated hair breeds, as a result of their ability to adapt to hot, temperate, and dry climates (González-Garduño *et al.*, 2010). Additionally, Vicente *et al.* (2020) have reported that they do not have reproductive seasonality, a characteristic that allows the sheep industry to maintain a constant meat production throughout the year. Additionally, studies carried out in the arid and dry region of northwestern Mexico during the summer season have documented that high temperatures do not drastically reduce the productive capacity of the Pelibuey, Katahdin, and Dorper hair breed sheep, as well as their crosses (Macías *et al.*, 2013; Macías *et al.*, 2016). For their part, Chay *et al.* (2019) reported that the combination of prolificacy with the low mortality rate of lambs in the pre-weaning period is an essential factor that favors productivity in Pelibuey and Katahdin sheep breeds. Consequently, these animals have high productive and reproductive potential in any region of the country.

Two hair sheep breeds (Pelibuey and Blackbelly) are frequently used in production systems, due to their high reproductive efficiency and their level of biological adaptation to heat and high humidity conditions (Vicente *et al.*, 2020). Likewise, breed groups such as Katahdin and Dorper are used as parental breeds in some commercial herds (Oliva *et al.*, 2002; Nava *et al.*, 2006). However, Chay *et al.* (2019) mentioned that the birth weight and pre-weaning development of lambs of the Pelibuey breed are lower than in other breeds, such as Katahdin and Dorper (Macías *et al.*, 2012; Hinojosa *et al.*, 2015). Even though the Blackbelly and Pelibuey breeds have lower productive performance, it is necessary to evaluate their behavior in sheep production systems located in different regions of the country, both as a pure breed and in different crossbreeding systems with Dorper and Katahdin (Bores *et al.*, 2002). The birth weight and pre-weaning development of the lambs of hair breeds are low and inferior to wool or meat breeds —a situation that has led to the incorporation of rams from breeds specialized in meat production, such as Dorper and Katahdin (Macías *et al.*, 2010), into crossbreeding systems.

For their part, Dorper breed sheep have demonstrated a great growth rate: when they are crossed with Pelibuey females, lambs can gain up to 25% more weight per day than Pelibuey breed lambs (Cloete *et al.*, 2000; Hinojosa *et al.*, 2013; Mayren-Mendoza *et al.*, 2018). There is scarce information about the development of offspring of hair sheep breeds from birth to weaning, which makes it difficult to characterize the pre-weaning performance of each of these breeds. Therefore, generating this information would increase the efficiency of the sheep production systems of the country. Consequently, the objective of this research was to evaluate the reproductive and productive behavior of four hair sheep breeds in an intensive production system.

MATERIALS AND METHODS

Location of the study area

The study was carried out at the Rancho Universitario of the Universidad Autónoma de Ciudad Juárez, located 63 km northwest of the city, at 1,100 m.a.s.l. and with an average annual rainfall of 244 mm. According to the Köppen classification, modified by García (2004), the climate of the region is hot (BWh) and cold (BWK) desert.

Database

A database was generated from the reproductive and productive records of lambing ewes, mated with rams of the same breed, and their offspring. This information was collected over a 4-year period (2016-2019) and was the result of the implementation of synchronized mating programs, in which male and female hair sheep (Blackbelly, Pelibuey, Katahdin, and Dorper) were used. The records of 400 females and eight males were used to generate information about both the breeders and the offspring born in the study period. Exogenous hormones were used with females with synchronized estrus, which were subjected to controlled natural mating with rams of the same breed. The fertility (%), prolificacy, and weaned lamb yield (WLY; kg female^{-1}) of the ewes were recorded. In the case of the lambs, the following variables were recorded: birth weight (BW; kg), daily weight gain during lactation (DWGL; g), weaning weight (WW; kg), offspring born alive (OBA; n), male offspring (MO; %), and female offspring (FO; %). The information obtained from the offspring was used to evaluate the reproductive and productive performance of both ewes and rams.

Animals and management

The females and males were kept in an intensive production system (stabling and one birth every eight months). They were housed in pens equipped with feeders, waterers, and shades, and fed *ad libitum* with a maintenance diet [9% crude protein (CP) and 3.2 Mcal of metabolizable energy (ME kg^{-1})], made up of commonly used ingredients in the study region: Sudan grass (*Sorghum drumondii*) hay and alfalfa (*Medicago sativa*).

Regarding reproductive management, the females were integrated into groups of 25 individuals and were programmed to give birth every eight months (three births in two years). Each group of females was subjected to a synchronized mating program, which consisted of the use of a progestin for 12 days (intravaginal sponge; 20 mg of Cronolone; Chronogest CR®, MSD, Animal Health) and the intramuscularly injection of 250 IU of eCG (equine chorionic gonadotropin; GonActive® eCG, Virbac), 24 hours before the sponges were removed. Once the females showed estrus, a controlled mating program was used with two matings per female: one at the beginning of estrus and the second, 12 h later. Finally, the number of mated, pregnant, and lambing females was recorded to determine fertility and prolificacy. Likewise, during the lambing, lactation, and weaning season, all information regarding the lambs was recorded.

Study variables

The variables evaluated were: fertility, prolificacy, BW, WW, WYL, DWGL, OBA, MO, and FO.

Statistical analysis

All information was analyzed under a completely randomized design, using breed as treatment (Blackbelly, Pelibuey, Katahdin, and Dorper). The prolificacy, BW, WW, DWGL, OBA, and WYL variables were analyzed with the PROC GLM procedure. Fertility, FO, and MO were analyzed using a Chi-square test with the PROC FREQ procedure. Mean comparisons were performed with the t-student test at $P<0.05$. All statistical procedures were developed with the SAS statistical package version 9.12 for Windows (SAS, 2004).

RESULTS AND DISCUSSION

The results of the reproductive and productive evaluation of the hair sheep breed are presented in Table 1. The Blackbelly breed sheep had higher fertility (94%; $P<0.05$) than all the other breeds. Prolificacy was higher ($P<0.05$) in sheep of the Blackbelly, Pelibuey, and Katahdin breeds than in the Dorper breed, which recorded a 1.3 prolificacy (Table 1). Birth weights (BW) were higher ($P<0.05$) in lambs of the Dorper and Katahdin breeds, a behavior that was reflected in the weight at weaning ($P<0.05$; Table 1). Blackbelly showed the highest performance of weaned lamb ($P<0.05$) and surpassed the other breeds in the study by 17% (30.6 *vs.* 26.1 kg female^{-1}; Table 1).

In this study, fertility ranges from 89.6% to 94.1% and the maximum value was obtained by the Blackbelly breed. In this regard, Macías *et al.* (2009) report similar results in Pelibuey sheep for this variable, with a range of 81 to 93%. On the contrary, Gómez *et al.* (2006) reported lower fertility (78%).

The Blackbelly and Pelibuey breeds stand out in the prolificacy variable with respect to the other two breeds evaluated; this increased prolificacy results in a greater number of lambs born and therefore greater productivity. Regarding the offspring born alive variable, the Blackbelly and Pelibuey breeds stand out. In this sense, Rastogi (2001) and Knights *et al.* (2012) reported that the Blackbelly breed shows high prolificacy (1.77 and 2.0, respectively). Regarding the Pelibuey breed, Martínez *et al.* (2011) and Magaña *et al.* (2013) report prolificacies of 1.41 and 1.53, respectively. Similarly, Hernández-Montiel *et al.* (2020) stated that the prolificacy value in Pelibuey is ~1.5 lambs per birth; this value may be associated with the genetic makeup of the breed, since 57 SNPs associated with litter size have been reported. Some of the genes associated with litter size in Pelibuey include: CLSTN2, MTMR2, CCDC174, NOM1, ANKRD11, DLG1, ALPK3, ROBO2, CGA,

Table 1. Reproductive and productive evaluation of four hair sheep breeders.

Breed ewe	Fertility (%)	Prolificacy* Mean±SEM	Birth weight (kg) Mean±SEM	Weaning weight (kg) Media±SEM	Weaned lamb yield (kg ewe^{-1}) Mean±SEM
Blackbelly	94.1a	1.9±0.20a	2.9±0.20b	16.1±1.12b	30.6±2.3a
Pelibuey Canelo	89.6b	1.6±0.10a	3.1±0.14b	16.4±1.50b	26.2±1.7b
Katahdin	91.9b	1.5±0.13a	3.6±0.08a	17.5±0.08a	26.3±2.6b
Dorper	90.8b	1.3±0.18b	4.3±0.17a	19.8±0.86a	25.7±1.7b

a,b Values with different literal within columns, are different (p>0.05). * Lambs per ewe calved. ±SEM=Standar Error Means.

and KDM4A. Additionally, four SNPs (s37914.1 (Chr 4: 117, 719, 020 bp), s02969.1 (Chr 5: 184, 537 bp), OAR15_13905772.1 (Chr 15: 13, 872, 637 bp), and s15631.1 (Chr 19: 57.489.437 pb)) confirm that different underlying genetic mechanisms are involved in the prolificacy of Pelibuey sheep, such as candidate genes related to reproduction, seasonality, milk production, and weight. In the case of the Dorper breed, Gavojdian *et al.* (2013) reported low prolificacy (1.2); this value is similar to the result obtained in this study (1.3), which causes a lower number of lambs at birth.

Regarding the birth weight (BW) variable, similar results were obtained in the Blackbelly (2.9 kg) and the Pelibuey (3.2 kg) breeds; these results match the findings of Macías *et al.* (2009) who indicated a BW of 3.3, 2.9, and 3.1 kg for the Pelibuey, Katahdin, and Dorper breeds, respectively. However, in the present study, the Katahdin and Dorper breeds obtained similar birth weights (3.6 and 4.3 kg, respectively), which were higher than the Blackbelly and Pelibuey breeds. These results are similar to those reported in other studies, in which of 4.0 kg (Gavojdian *et al.*, 2013) and 3.8 kg (Mellado *et al.*, 2016) were obtained for the Dorper breed: the BW detected for Katahdin lambs in this study matches the results of Burque (2005) and Castillo *et al.* (2021), who reported a BW of 3.5 and 4.0 kg, respectively.

In the weaning weight (WW) variable, Macías *et al.* (2009) reported weights of 18.5 kg for Dorper and 14.5 kg for Pelibuey —values lower than those found in the present study (19.8 kg for Dorper). In a study carried out in the Mexican humid tropics, Chay *et al.* (2019) found weaning weights of 15.3 kg for Pelibuey and 16.0 kg for Katahdin —lower than the values obtained in this study.

There is a relationship between the number of lambs born and weaned per ewe, reflect in the efficiency with which Blackbelly sheep wean a greater number of kilograms per ewe, followed by Pelibuey and Katahdin. This has a favorable effect on the weaning productivity of hair ewes: ewes with two lambs (mainly Blackbelly and Pelibuey) record better results. The ability of an ewe to wean a greater number of lamb kilograms is conditioned by the size of the litter at birth, growth rate, and pre-weaning mortality (Macías-Cruz *et al.*, 2009, Macías-Cruz *et al.*, 2012). Ewes with two lambs were more productive at weaning, indicating that the combination of prolificacy with low lamb mortality rate in the pre-weaning period is an essential factor for the improvement of the productivity of hair ewes (Chay *et al.*, 2019). A better economic remuneration for sheep farmers would be achieved by combining the reproductive efficiency of Blackbelly with the productive efficiency of Dorper; therefore, greater WYL would be achieved with the direct crossing of the two aforementioned breeds.

Table 2 shows the results for the productive parameters of hair breed rams on their offspring. Birth weights (BW) are higher ($P<0.05$) in Dorper, followed by Katahdin, with a similar behavior ($P<0.05$) in the daily weight gain for the lactation (DWGL) variable. For the offspring born alive variable, a greater number of offspring was observed in the Blackbelly and Pelibuey breeds ($P<0.05$). Finally, for the female offspring and male offspring variables, no significant effect was observed between ram breeds and their offspring ($P>0.05$).

Table 2. Productivity of the offspring of the rams of four hair breeds.

Breed ram	Birth weight (kg) Mean±SEM	DWG during lactation (g) Mean±SEM	Live born pups (n) Mean±SEM	% Female offspring	% Male offspring
Blackbelly	2.5±0.20a	142±0.20a	54±1.7a	60	40
Pelibuey Canelo	3.1±0.14a	125±0.10a	48±1.1a	45	55
Katahdin	3.7±0.08b	209±0.13b	45±2.0a	54	46
Dorper	4.2±0.17b	259±0.18b	39±1.3b	50	50

a,b Values with different literal within columns, are different (α=0.05). ± SEM=Standar Error Means.
DWG: daily weight gain.

The birth weight (BW) values for Dorper breed lambs in this work were similar to those reported in other studies: 3.8 kg (Czismar *et al.*, 2013) and 4.4 kg (Castillo *et al.*, 2021). The BW obtained in Katahdin lambs matches the figures reported by Ehrhardt *et al.* (2018) and Vicente-Pérez *et al.* (2021): 3.7 and 3.9 kg, respectively. Although the BW values reported in this study were obtained using pure breeds, these results match the findings of López *et al.* (2021), who found a similar BW (3.8 kg), when Dorper or Katahdin parental breeds are used. Likewise, Macías *et al.* (2010) have reported that crosses of these paternal breeds with the maternal Pelibuey breed have the advantage of producing offspring for supply with acceptable growth rates.

The pre-weaning behavior of hair sheep breeds must be studied to determine their productive and economic efficiency, before deciding whether or not they should be incorporated into commercial farms. In this regard, Hinojosa *et al.* (2009) highlighted the pre-weaning productive efficiency of Dorper and Katahdin. These authors evaluated lambs on daily pre-weaning weight gain and established a better performance by these two breeds. These results match the findings of this work, where the same two breeds were superior to Blackbelly and Katahdin. This has a favorable impact on weaning weights, which is important in production systems where more kilograms of lamb can be weaned. Furthermore, crossing Blackbelly and Pelibuey breeds (as maternal base) and Dorper and Katahdin (as paternal base) would have a greater impact on the weight of the lambs at weaning and on the kilograms of lamb weaned per female.

CONCLUSIONS

The Katahdin and Dorper breeds are an alternative for the improvement of the weaning weight compared to the Blackbelly and Pelibuey breeds. However, the Blackbelly breed favors higher weaned lamb performance than the Pelibuey, Katahdin and Dorper breeds. The Blackbelly breed should be included in intensive production systems, as pure breeds or crossbred with other breeds, to increase the number of lambs born.

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Effect of cryopreservation of *in vitro* produced embryos on pregnancy rate of cows transferred at fixed time in the dry tropics

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ABSTRACT
The objective of this study was to determine the effect of cryopreservation (CP) of *in vitro* produced embryos (EIV) on the pregnancy rate (PR) of cows transferred at fixed time (FTET) in the dry tropics. The experimental design was completely randomized with a 2×2 factorial arrangement. The sample consisted of 280 embryos produced *in vitro*. The factors were the CP (vitrified and fresh) and the Rural Development District (DDR 01 and 02). The experiment consisted of 70 replicates per treatment: T1) fresh transferred embryos; T2) vitrified embryos; T3) DDR + fresh embryos; and 4) DDR + vitrified embryos. The pregnancy diagnosis was carried out 60 days after embryo transfer. The Xi^2 test was used to analyze PR which was the response variable per treatment. PR was higher in fresh-transferred embryos than in vitrified embryos (53.6±4.2 *vs.* 27.1±3.7; P<0.05), and in DDR02 than in DDR01 (47.1±4.2 *vs.* 33.6±3.9; P<0.05). Therefore, the transfer of fresh embryos and the DDR02 had a positive overall effect on PR (40.3%).

Keywords: Biotechnologies, Synchronization, Ovulation, Cows.

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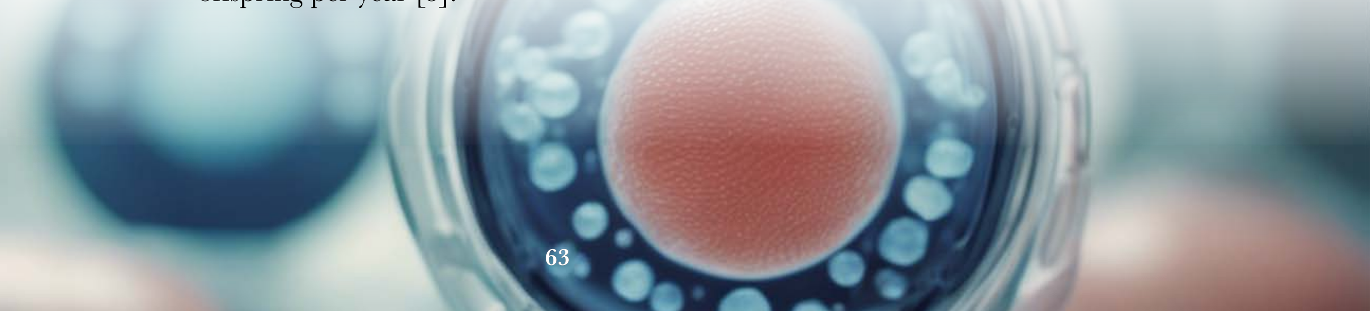
INTRODUCTION

The embryo transfer (ET) requires selection and physical and pharmacological handling of the receptor cows; the success of this biotechnological procedure depends on many factors [1]. *In vitro* fertilization and cryopreservation (CP) of embryos help to increase their mass production, their storing in germplasm banks, and their transportation around the world [2]; the ultimate aim of the producers is to use embryos in transfer programs. The in vitro production (IVP) of embryos is an assisted reproduction biological tool that speeds up the genetic progress of animals. This tool allows a genetically superior female to produce a greater number of embryos (and consequently offspring) during her reproductive life. Under natural mating or artificial insemination systems, a cow would give birth to only one offspring per year [3].



Non-transferred fresh embryos subjected to CP can remain available for an undetermined time, before they are used for transfer or for commercial purposes [4]. Vitrification (VT) is one of the methods used to cryopreserve cattle embryos. This method allows the change from liquid to solid without the formation of intracellular ice crystals [5]. VT is an alternative for embryo preservation with optimal viability percentage. Additionally, this is a simple, easily executed, cost-efficient technique, that results in 40-46% pregnancy rates for in vitro produced embryos transferred vitrified or fresh [6, 7]. In 2022, the International Embryo Technology Society (IETS) reported that 1,521,018 *in vitro* produced embryos were transferred all over the world in 2021. Out of this total, 32.6% were cryopreserved embryos, while 67.4% were fresh-transferred embryos. South America is the world leader in this biotechnology, with 378,114 transfers of fresh embryos and 279,291 transfers of frozen embryos. A total of 91,954 *in vitro* produced embryos derived from follicular aspiration was reported in Mexico; out of this total, 8.2% were transferred fresh, which accounted for a production increase of 244.6% compared with 2020 [8]. Therefore, the objective of this study was to determine the effect of cryopreservation of *in vitro* produced embryos on pregnancy rate of cows transferred at fixed time in the dry tropic.

MATERIALS AND METHODS

This study complied with the requirements of the Comission of Bioethics and Animal Welfare and the guidelines of the Internal Rules of Procedure of the Facultad de Medicina Veterinaria y Zootecnia of the Universidad Veracruzana (Title VII, Chapters I, II, III, IV, Articles 92-124) and the NOM-033-ZOO-1995, clause 6.1.b.

Lab location

The *in vitro* production of embryos was carried out in the Laboratorio RGA IN VITRO (Reproducción Genética Avanzada), located in Boca del Río, Veracruz.

Location of the livestock production units

The cows from which the oocytes were extracted —and which were used for the *in vitro* production of embryos— belonged to a commercial dairy herd (Rancho Fuentezuelas) located in Tequisquiapan, municipality of San Juan del Río, Queretaro (20° 31' N and 99° 53' W, at 1,870 m.a.s.l.). The transfer of the *in vitro* produced embryos was carried out in dual-purpose cattle ranches, located in the municipalities of Atoyac de Álvarez and Florencio Villareal, which belong to Rural Development Districts 01 and 02, respectively. These RDDs are located in the State of Guerrero. The municipality of Atoyac de Álvarez is located in the Costa Grande of Guerrero (17° 03' N and 100° 05' W, at 602 m.a.s.l). The municipality of Florencio Villarreal is located in the Costa Chica of Guerrero (16° 43' N and 99° 07' W and at 16 m.a.s.l).

Characteristics and management of the donors

Twenty non-lactating and non-pregnant Holstein cows were used. They were included in a multiple ovulation commercial program and kept in pens. All the cows were fed conserved forage and a balanced diet prepared at the farm with 16% CP (3.2 Mcal/kg) and mineral salts. Cows had *ad libitum* access to water. Based on the transrectal ultrasound carried out during the examination of the selected cows, their reproductive tracts showed no anomalies [9]. Body condition (BC) of the cows was 3.4±0.1 points (1 to 5 points scale: 1=extremely thin and 5=obese) [10]. The cows were apparently healthy and they complied with the local deworming and vaccination plans.

Characteristics and management of the recipients

Two-hundred eighty *Bos indicus*×*Bos taurus* recipient cows were used in the experiment. They were 4.5±1.2 years old and weighed 482.5±69.2 kg (live weight). At least 60 days had elapsed since parturition. The cows were apparently healthy. In order to discard pathological or anatomo-functional alterations, a transrectal palpation of their reproductive tract was carried out. Additionally, the selected recipients met the following requirements: 1) BC from 5 to 7 in the 1-9 dual-purpose cattle scoring (1=extremely thin and 9=obese) [11]; 2) not being under any pharmacological treatment; and 3) showing signs of reproductive activity, whether by the presence of a corpus luteum (CL) detected during transrectal palpation or by evidence of follicular activity detected through transrectal ultrasound of the ovaries (Aloka SSD500 portable ultrasound, with a 5.0 MHz transrectal transducer probe) [9, 12], following the recommendation of the IETS.

The feeding of the recipients was based on semi-extensive rotational grazing on Gamba grass (*Andropogon gayanus* Kunth), Mulato grass (CIAT 36061), and giant star grass (*Cynodon plectostachium*). In addition, they were supplemented with 2 kg cow^{-1} d^{-1} of a commercial concentrate feed with 19% CP (Lechero 20 CSA Malta®, Texo de México S.A de C.V) 30 days before and 30 days after the embryo transfer. They were also given *ad libitum* a mineral supplement with a higher bioavailability of phosphorus.

Health management included a 5-mL subcutaneous (SC) application of a bacterin-toxoid (Bobact 8® MSD, Mexico), in order to prevent pneumonic pasteurellosis (shipping fever), blackleg, malignant edema, gas gangrene, necrotic infectious hepatitis, and enterotoxaemia (pulpy kidney). In addition, they were vaccinated against IBR, BVD (types 1 and 2), Pl3, and BRSV; this vaccine includes the *Campylobacter fetus* bacterin, combined with inactive *Leptospira* (2 mL/SC, Bovi-Shield GOLD® FP® 5 VL5. Zoetis, Mexico). The cows were de-wormed with 1% doramectin (200 mcg/kg PV, SC, Dectomax® Zoetis, Mexico) to prevent lungworms and gastrointestinal parasites. They were given a bath every 15 days to prevent ticks, using 12.5% amitraz (Taktic®, MSD, México). Seven days before the start of the ovulation synchronization protocol, each recipient received via intramuscular (IM) 10 mL of phosphorus (Tonofosfán®, MSD, Mexico), 8 mL of selenium (Mu-Se®, MSD, Mexico), and 5 mL of vitamin A, D, and E (Vigantol®, Bayer, Mexico).

Ovulation synchronization program

On Day 0, ovulation was synchronized in all the recipients using a progesterone-releasing intravaginal device (CIDRB®; Zoetis, Mexico) together with the IM injection of 2 mg of estradiol benzoate (Benzoato de estradiol®; Zoetis, Mexico). On Day 5, cows received via IM 400 IU of equine chorionic gonadotropin (eCG; Novormon®; Zoetis, Mexico) and 25 mg of dinoprost tromethamine (Lutalyse; Zoetis, Mexico). On Day 8, the CIDR-B was removed and the recipients received via IM 1 mg of estradiol cypionate (E.C.P.®, Zoetis, Mexico) [13].

Characteristics of the transferred bovine embryos

Two-hundred eighty bovine embryos produced in vitro (140 fresh embryos and 140 embryos vitrified in Cryotop® devices) were used in the experiment. They were quality 1 compacted blastocysts. The ovum pick-up (OPU) technique was used to obtain the oocytes which originated the embryos; they came from Holstein donors that were subjected to in vitro fertilization with semen of registered Gyr bulls (JCVL215; Astro FIV Cabo Verde).

***In vitro* embryo production technique**

Preparation of the donors and follicular aspiration (OPU)

Before each procedure, the feces were removed from the rectum of the donors and the perineal region was washed with water and 70% ethanol. Epidural anesthesia (100 mg lidocaine; Lidocaína®, Lab. Intervet, Mexico) was applied before each OPU session, in order to reduce intestinal peristalsis and the pain from the procedure. The transducer was inserted via transvaginal and the ovary was paired with it through transrectal manipulation to conduct the aspiration of all the visible follicles. Follicular aspiration was performed by a single trained technician using a B-mode real-time ultrasound scanner (Mindray 2200®; Mindray Bio-Medical Electronics, Shenzhen, China) equipped with a 5 MHz micro-convex traducer (Mindray 65C15EAV®, Mindray Bio-Medical Electronics, Shenzhen, China) connected to a follicular aspiration probe (Watanabe Tecnologia Aplicada®, São Paulo, Brazil) and a stainless-steel needle. Follicular puncture was carried out using an 18 G disposable hypodermic needle (Jelco®, Fibra Cirúgica, Santa Catarina, Brazil) connected to a 50-mL conical tube (Corning®, Acton, MA, USA) with a silicone tube (Watanabe Tecnología Aplicada®, São Paulo, Brazil). A vacuum pump (WTA model BV-003®, Watanabe Tecnología Aplicada, São Paulo, Brazil) with negative pressure adjusted to 60-80 mmHg was used to keep the pressure level during the aspiration. After the OPU was carried out in both ovaries, the aspiration system was washed with DPBS medium (Nutricell®, Nutrientes Celulares, São Paulo, Brazil), 0.05% sodium heparin (5,000 UI/mL, Hemofol®, Crist alia Produtos Químicos Farmacéuticos, São Paulo, Brazil), and 1% fetal bovine serum (Gibco®, Thermo Fisher Scientific, MA, USA). Immediately after the OPU session, the oocytes were examined and classified according to their morphology (number of layers, expansion of the cumulus cells, and cytoplasm appearance regarding color, homogeneity, and integrity) [14].

***In vitro* embryo production**

The IVF procedures were adapted from Pontes *et al.* [15].

***In vitro* maturation**

The oocytes were cultured in 20-μL drops of BO-IVM maturation medium (IVF Bioscience®, Cornwall, UK), previously warmed. Five oocytes were used per drop, under a mineral oil layer. They were matured in an incubator, with 5% CO_2, at 38.5 °C for 24 h.

Sperm preparation and in vitro fertilization

The oocytes were removed from the culture medium; they were washed and placed in 20 μL drops of previously warmed and gasified fertilization medium (five oocytes per drop), which were covered with a mineral oil layer. Frozen semen from the same Gyr bull was used to fertilize all the oocytes. The semen straws were thawed at 37 °C for 30 s; a sample of the semen was taken to verify its viability and motility. The semen was centrifuged in 45% Percoll gradient at 700G during 3 minutes and, subsequently, it was centrifuged in BO-SemenPrep (IVF Bioscience®, Cornwall, UK) medium at 700G during 1 minute. The spermatozoa were introduced in the drops of the fertilization medium that contained the oocytes. Both gametes were incubated at 38.5 °C, using 5% CO_2, for 18 h.

Maturation evaluation

The oocytes were removed from the fertilization medium and washed with HTF medium added with hyaluronidase (HTF/0.5 % BSA®, Sigma Aldrich, Saint Louis, USA) for 3 minutes; subsequently, they were denuded using a Stripper® (Origio; Ballerup, Denmark) device and were observed under a stereoscopic microscope. The presence of a polar body indicated an appropriate maturation.

***In vitro* culture**

Twenty-four hours after fertilization, the potential zygotes were cultured *in vitro*, in synthetic oviductal fluid (SOF) medium, supplemented with 5% fetal bovine serum and 0.5% bovine serum albumin. The cells were cultured for 24 hours in an incubator (Cook Minc™, USA), with a controlled gas atmosphere (5% CO_2 and 5% O_2, balanced with 90% N_2). On Day 4 (Day 0=IVF), the culture medium was renewed. On Day 6 (60 h after fertilization), the blastocyst rate was evaluated. The blastocysts were introduced into 0.25 cc straws and transported at 37 °C in an embryo transporter (TE-100 Compact WTA®, Watanabe Tecnología Aplicada, São Paulo, Brazil) for a maximum period of 12 h, until the moment of the transfer.

Vitrification technique

An equilibrium solution (ES) and a vitrification solution (VS) were used to vitrify the embryos. The ES was made up of phosphate buffered saline (PBS) (Fisher BioReagents®, Thermo Fisher Scientific Inc., MA, USA), 20% synthetic serum substitute (SSS) (Irvine Scientific® Santa Ana, CA, USA), 7.5% ethylene glycol (EG) (Sigma-Aldrich®, Darmstadt, Alemania), and 7.5% dimethyl sulphoxide (DMSO) (Sigma-Aldrich®, Darmstadt, Germany). The VS was composed by PBS + 20% SSS + 15% EG + 15% DMSO + 0.5 M sucrose (Sigma-Aldrich®, Darmstadt, Germany). One drop of ES and four drops of VS, 20-μL each, were placed in a 35×10 mm Petri dish (Nunc®, Thermo Fisher Scientific Inc., MA, USA). Afterwards, the embryos were introduced into the ES drop for 5-15 minutes; subsequently, the embryos were transferred into each of the four VS drops, where they remained for 5, 5, 10, and 10 seconds. Immediately after they were removed from the last drop (<30 seconds in total), they were placed into Cryotop® devices, which were introduced in a cryogenic tank with N_2L where they were stored until the transfer [16].

Warming of the vitrified blastocysts

The vitrified embryos were warmed before their transfer. Solutions for thawing (TS), dilution (DS) and washing (WS) were used in this process. The TS was made up of PBS (Bioniche®, Pharma, Canada), 20% SSS, and 1 M sucrose (Sigma Aldrich®). The DS was composed by PBS + 20% SSS and 0.5 M sucrose). The WS was made up of PBS + 20% SSS. A 300 μL TS drop was placed in a 60×15 mm Petri dish, at 37 °C. Meanwhile, two DS drops and three 20 μL WS drops were placed in another 100×15 mm Petri dish, at room temperature. In order to warm the embryos, the Cryotop® was removed from the cryogenic tank, opened and immediately immersed in the TS drop for 1 minute. Subsequently, the blastocysts were placed in the DS drops for 3 minutes and after that in the WS drops, remaining 3 minutes in each one [16]. Finally, the embryos were placed in PBS and introduced into 0.25 cc straws to be transferred to the recipients.

Embryo transfer and pregnancy diagnosis

The embryo transfer was carried out by the same veterinarian in all the recipients that had a well implanted corpus luteum (CL) ≥1.5 cm in diameter 9 days after the removal of the intravaginal progesterone device [17]. To carry out the transfer, the recipients were given epidural anesthesia (100 mg lidocaine; Lidocaína®, Lab. Intervet, Mexico) 10 minutes before the procedure took place. In addition, iodine and 70% ethanol (Dermodine Solución™, Degasa, Mexico) were used to carry out a peri-vulvar asepsis. The embryo transfer gun was wrapped with a sterile cover sheath. It was introduced in the vagina and passed through the cervical channel (through transrectal manipulation) and was directed towards the uterine horn ipsilateral to the ovary with the corpus luteum, depositing the content of the straw in the middle third of the horn [18]. Each recipient received one embryo. Pregnancy diagnosis was carried out using a transrectal ultrasound (Aloka SSD500, with 3.5 MHz convex transducer; Japan), 60 days after the transfer.

Experimental design

The experimental design was completely random, with a 2×2 factorial arrangement. The sample size was 280 *in vitro*-produced embryos. The factors were CP and RDD, with 70 replicates per treatment.

Statistical analysis

A threshold mixed model was used with the PROC GLIMMIX procedure (SAS, 2014) to determine pregnancy rate (PR). Based on the fixed and random effects, the PR was assumed to follow a Bernoulli distribution. It was analyzed with the χ^2 ($p \leq 0.05$), using the STATISTICA™ version 10 software (TIBCO Software Inc., StatSoft, 2011, USA).

Statistical model

The following statistical model was used:

$$Y_{ijk} = \mu + B_i + O_j + BO_{ij} + \beta_1\left(x1_{ij} - x^{-1}\right) + \beta_2\left(x2_{ij} - x^{-2}\right) + \beta_3\left(x1_{ij} - x^{-3}\right) + \varepsilon_{ijk}$$

Y_{ijk}=response variable (pregnancy rate); μ=general mean of the response variable; B_j=*i*-th effect of the type of embryo, i=1,2; O_j= *j*-th effect of the RDD, j=1,2; BO_{ij}=effect of the interaction between the *i*-th effect of the type of embryo and the *j*-th effect of the RDD.

$\beta_1\left(x1_{ij} - x^{-1}\right)$= effect of the covariate age; $\beta_2\left(x2_{ij} - x^{-2}\right)$= effect of the covariate weight; $\beta_3\left(x1_{ij} - x^{-3}\right)$= effect of the covariate body condition; ε_{ijk}=experimental error.

where μ=general mean; B_i=*i*-th effect of type of embryo; O_j=*j*-th effect of the RDD; BO_{ij}=double of the interaction of the factors; ε_{ij}=experimental error.

RESULTS AND DISCUSSION

The PR of the fresh and vitrified embryos reached 44.3 and 22.8%, respectively, in RDD 01, and 62.8% and 31.4%, respectively, in RDD 02 (Table 1). The results were acceptable for the *Bos taurus*×*Bos indicus* recipients in the dry tropics. Pregnancy rate is a multifactorially-regulated event. The main factors that can have a negative impact on PR include: embryo development [19], embryo quality [20], the selection of the recipients [21], and the CL diameter (>14 mm) [17]. In all the treatments of this study, only embryos in the blastocyst development stage (early, blastocyst, and expanded) were transferred. Therefore, this variable did not likely have an impact on the pregnancy results. A research carried out in the sub-humid tropics showed that there were no differences in the transfer of fresh blastocysts (48%) or expanded blastocysts (52%) in Brown Swiss heifers [22]. Other authors have reported a similar PR when the transfers were carried out with early (30%), expanded (40%), and hatched blastocysts (50%), produced *in vitro* and vitrified using the Cryotop® device. These similarities could be the result of a synchronization between the embryo development and the uterine environment of the recipient (±24 h) [23], influenced

Table 1. Transferred and pregnant cows, and pregnancy rate post embryo transfer in production units (PU) of the Rural Development Districts (RDD) 01 and 02 in the state of Guerrero.

RDD / Type of embryo	Transferred n	Pregnant n	Pregnancy rate (%)
Fresh	70	31	44.3[b]
01			
Vitrified	70	16	22.8[bc]
Fresh	70	44	62.8[a]
02			
Vitrified	70	22	31.4[b]
Total	280	113	40.3

[a,b,c] Different letters indicate statistical difference (P<0.05).

by the progesterone (P4) from the CL. The P4 plays an important role in reproductive events, mainly in the establishment and maintenance of pregnancy [24]. In addition, there is a positive association between the high concentrations of blood P4 after conception and the elongation of embryos, the increase of interferon tau (IFN-t) production, and the high pregnancy rates resulting from *in vitro* produced embryos [25, 26]. In this study, the P4 plasma concentrations were not determined when the ET was carried out. However, with the aim of providing a sufficient P4 contribution during the next embryo stages, only cows with a CL >14-mm in diameter were included. In synchronized *Bos taurus*×*Bos indicus* recipients there was a positive correlation (r=0.41) between CL size (>14 mm) and the P4 plasma levels, resulting in a greater contribution of P4 for pregnancy maintenance [27]. According to Bó *et al.* [17], there are no differences in PR of recipients with CL of diameters of 14-16 mm (56.1%), 16-18 mm (56.4%), and >18 mm (54.3%).

The quality of the embryo is a main factor to consider when performing ET, as it has the greatest effect on the embryo development since the implantation [20]. Bad quality embryos impact PR, given the poor compaction of their blastomeres, low internal cell mass, presence of vesicles inside the cytoplasm, and scarce uniformity of the pellucid zone [28]. All these factors affect the embryo competence during [29]. Some authors mentioned that there is no difference when quality 1 (excellent) and quality 2 (good) embryos are transferred, due to their appropriate morphological and developmental characteristics according to their age, which are ideal for ET [30, 23]. Only quality-1 fresh and warmed vitrified-embryos were used in this study. They were evaluated before ET was carried out. This factor could have contributed to the appropriate embryo development and, therefore, the acceptable general pregnancy rates recorded per treatment.

Regardless of the type of embryo, PR was higher in RDD 02 than in RDD 01 ($p<0.05$) (Table 2). Irrespective of the RDD, PR recorded the highest percentages with fresh transferred embryos than with vitrified transferred embryos ($p<0.05$) (Table 2). The cattle producers of both RDD had similar livestock, management, infrastructure, and technological level, and they were given the same training and instructions, regarding the implementation of ovulation synchronization for FTET. However, RDD 02 recorded higher PR. Severino *et al.* [32] mentioned that the different technological levels in cattle production in Mexico can make a difference regarding the implementation of a biotechnology. This was the case in this study.

Table 2. Pregnancy rate of *Bos taurus*×*Bos indicus* recipients, considering the main effects RDD (01 and 02) and type of embryo (fresh vs vitrified) in the state of Guerrero, Mexico.

Main effects		Pregnancy rate mean±SE % (pregnant/transferred)
RDD	01	33.6±3.9 (47/140)^{a}
	02	47.1±4.2 (66/140)^{b}
Type of embryo	Fresh	53.6±4.2 (75/140)^{a}
	Vitrified	27.1±3.7 (38/140)^{b}

^{a,b} Different letter indicates statistical difference ($p<0.05$).

Meanwhile, the PR obtained with vitrified embryos was acceptable, although it was slightly lower than that obtained with fresh embryos. The latter was higher than the PR obtained by Gutnisky *et al.* [6] with *in vitro* produced embryos transferred fresh and vitrified (46% for both treatments), and by Do *et al.* [7], who reported 41% and 34% PR with fresh and vitrified *in vitro* produced embryos, respectively. Post-warming *in vitro* viability of embryos vitrified in Cryotop® and Microdrop® devices can reach 70-90% [32, 7], resulting in appropriate embryo development that is reflected in acceptable PR for both *in vivo* and *in vitro* produced embryos (46.7 *vs.* 44.4 %) [33].

Transferring vitrified embryos can result in a 10% lower PR than transferring fresh embryos [30, 34]. Nevertheless, the PR obtained in this study in *Bos taurus*×*Bos indicus* recipients with vitrified embryos can be considered acceptable and with practical application in the field. Vitrification can prevent the discarding of excellent quality embryos, and help make better use of the number of available recipients. Therefore, this method is a feasible alternative for the genetic improvement of commercial cattle herds, under tropical conditions. Nevertheless, embryo vitrification is still a technology with a low percentage of use in the Mexican tropics.

CONCLUSIONS

The fixed time-embryo transfer of fresh and vitrified, *in vitro* produced Girolando embryos, to dual-purpose cows owned by small producers in the dry tropics, resulted in acceptable pregnancy rates. Consequently, this is an alternative for the genetic improvement of such cattle herds.

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Colegio de Postgraduados

Forage and vegetal characterization of three native mexican grasses in Tulancingo de Bravo, Hidalgo

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ABSTRACT

Objective: To carry out an agronomical assessment and a quantitative description of the yield components of switchgrass (*Panicum virgatum*), eastern gamagrass (*Tripsacum dactyloides*), and alkali sacaton (*Sporobolus airoides*) under rainfed conditions in Tulancingo, Hidalgo, Mexico.

Design/Methodology/Approach: A completely randomized block design was used in 31-month-old pastures to determine forage production, morphological composition, seed yield, and weight of 1,000 caryopses. The plants were characterized in 7-month-old pastures, recording (per plant) the number of total and floral stems, as well as the basal twigs in alkali sacaton and switchgrass. Meanwhile, in the case of eastern gamagrass, the dome number and androecium sections were recorded.

Results: The highest forage dry matter production was observed in switchgrass: 9,322 kg ha^{-1} ($P<0.05$). Eastern gamagrass had a higher leaf ratio (1:3). The highest number of seeds was recorded in alkali sacaton: 211 kg ha^{-1}, with 43% physical purity. After 7 months of sowing, a total of 250, 355, and 280 stems and 193, 150, and 87 floral stems were recorded in switchgrass, alkali sacaton, and eastern gamagrass, respectively.

Study Limitations/Implications: Eastern gamagrass produces a low number of seeds; therefore, it must be propagated by plant material. In rainfed soils, grasses help to recover pasture areas, since rainfed agriculture poses a risk in many places as a result of poor rainfall distribution or early frosts.

Findings/Conclusions: The three grasses studied are productive due to the amount of forage accumulated. Alkali sacaton produces more seeds.

Keywords: *Panicum virgatum*, *Sporobolus airoides*, *Tripsacum dactyloides*, agricultural to livestock conversion.

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INTRODUCTION

Quero-Carrillo *et al.* (2014) and Hernández-Guzmán *et al.* (2021) mention that the diversity of climates in Mexico means that each microsite has endemic plants that improve the diversity of plants, including grasses (Poaceae). Moreover, overgrazing of the common rangeland areas (rangelands) has eliminated perennial forage species from certain sites in semi-arid Mexico (Quero-Carrillo *et al.*, 2014).

Rodríguez-Ortega *et al.* (2021) reported dry matter yields of 3,584, 720, and 1,159 kg ha^{-1} of switchgrass (*Panicum virgatum*), alkali sacaton (*Sporobolus airoides*), and eastern gamagrass (*Tripsacum dactyloides*), respectively, during the first year that they were established in Tulancingo de Bravo, Hidalgo, Mexico. In addition, Marra *et al.* (2013) reported 9,222 kg ha^{-1} of switchgrass in the third year of establishment in West Virginia, USA. In Winsconsin, USA, Coblentz *et al.* (2014) reported 4,950 kg ha^{-1} of eastern gamagrass three years after it had been established. Cox *et al.* (1990) reported a 23-145 g plant^{-1} forage yield of alkali sacaton in Arizona, USA. Yield components in grasses include number of total and flowering stems, number of branches, number of twigs, and number of spikelets per branch or twig (Sánchez-Arroyo *et al.*, 2020); these elements help to assess forage plant materials —useful in agricultural to livestock conversion— since, in many places of Mexico, staple crops are destroyed by prolonged intraestival drought or early frosts. On the one hand, the objective of the study was to carry out an agronomical assessment of switchgrass (*Panicum virgatum*), eastern gamagrass (*Tripsacum dactyloides*), and alkali sacaton (*Sporobolus airoides*), 31 months after their establishment, and, on the other hand, to describe the yield components of selected plants, seven months after they had been established in Ahuehuetitla, Tulancingo de Bravo, Hidalgo, Mexico.

MATERIALS AND METHODS

The study was conducted in Ahuehuetitla, Tulancingo de Bravo, Hidalgo, at an altitude of 2,120 m. The soil of the site is clayey vertisol (INEGI, 2017). The plots for propagation of switchgrass, eastern gamagrass, and alkali sacaton were established on April 4th, 2020 under irrigated conditions, using a randomized complete block design, at a density of 8,333 plants ha^{-1}.

In the genetic improvement progress context, switchgrass, eastern gamagrass, and alkali sacaton genotypes were established in the "El Frijol 1" plot, Ahuehuetitla, Tulancingo, Hidalgo in 2020 (year 1, site 1), following the guidelines of Rodríguez-Ortega *et al.* (2021). In addition, during the anthesis, plants that did not meet the species and genotype characteristics were eliminated; the main characteristics included: forage height versus plant height, anther color, leaf shape, color distribution in stems, internode shape, anthocyanin color in leaves, color of glumes at anthesis, and color and shape of veins. At the end of November 2020, switchgrass, eastern gamagrass, and alkali sacaton seeds were collected from uniform plants and preserved at 13% humidity with the help of the LDS-1G moisture meter with LCD display (Beijing). The seeds were then placed in paper bags in the shade, inside plastic containers with lids. In year 2 (2021), site 2 was established 500 m away from site 1 in "El Arroyo"; the soil was prepared according to the recommendations of Quero-Carrillo *et al.* (2014). On June 26th, seeds from site 1 were sown, under rainfed conditions, in two 50-m long furrows separated 1.2 m apart from each other. From September to October 2021, plants that, for various reasons, did not show any of the abovementioned forage and genotype characteristics were eliminated. In summary, 300 original plants per species were counted and seeds were preserved at 13% humidity, in paper bags within plastic containers with lids.

In the year 2022, seeds were sown in the "El Frijol 2" plot (site 3), under drip irrigation conditions; the seeds came from plants of site 2 as homogenous genotype with characteristics specifics. These plants were used to assess the yield components on April 6^{th}.

The first stage was the determination, under rainfed conditions, of the forage and morphological distribution of site 1 in the second week of October 2022. Yield component data was collected at site 3, under drip irrigation conditions from the first to the second week of October 2022. The variables at site 1 were:

Forage production. It was determined in two 5-m long furrows (2.4×5 m$=12$ m^2). Forage was cut 10 cm above the base of the soil and weighed in a Truper® 0.5021 scale model (0.0 kg; 5,000 g capacity; Mexico); a 300 g subsample was then separated into leaves, stems, inflorescences, and dead material. Subsequently, each component was placed in paper bags and arranged in a greenhouse with air flow. A Ciderta R© forced-air oven (Huelva, Spain) was used for total drying at 55 °C for 6 h and the result was immediately weighed on a Hanchen BSM220 digital scale 4 (0.0001 g, Beijing).

Morphological composition. This variable was the result of the morphological distribution (%) of leaves, stems, dead material, and inflorescences.

Leaf:stem ratio. This variable was the result of the division of the dry weight of leaves by the weight of stems.

Seed production. Harvesting was carried out when the inflorescence per block (especially in the glumes) of each species clearly turned beige. Seeds (complete dispersal units) of the three species were placed on paper in a greenhouse with air flow for 14 days and the humidity was determined with a LDS-1G humidity determiner. Once 13% humidity was recorded, yield was calculated according to the methodology developed by Rodríguez-Ortega *et al.* (2021).

Physical purity. To determine this variable, 3 g of complete dispersal units per species and block of switchgrass and alkali sacaton were weighed, according to the methodology developed by Alvarez-Vázquez *et al.* (2022). Subsequently, caryopses were extracted over a corrugated rubber using friction with mat covered with the same material and weighed. Data for this variable did not have a normal distribution; therefore, they were transformed to a square root function + 0.5. In addition, due to its low caryopsis content and poor feeling, eastern gamagrass was not considered. Therefore, propagation with plant material is recommended.

Weight of 1,000 caryopses and dimension. Eight replicates of 100 caryopses per block were weighed and the mean was multiplied by 10 (Hernández-Guzmán *et al.*, 2021). Twenty-five caryopses per block were measured to determine caryopsis dimensions (length, width, and thickness) with a Truper® IP64 digital vernier. Data for both variables were transformed to a square root function + 0.5. The GLM procedure of SAS (2004) was used to analyze the data and the means were compared with Tukey's test ($\alpha=0.05$).

Yield components on site 3

The yield components of each grass were recorded on 10 homogeneous plants. The number of total stems, flower stalks, branches, twigs, and spikelets on the basal branch or twig were counted. In eastern gamagrass, the number of cupules in the gynoecium and the number of sections in the androecium were counted.

RESULTS AND DISCUSSION

Temperature was adequate in the 2022 pasture growth period. Since the average rainfall was 550 mm, a 271-mm accumulation (Figure 1) was considered to represent a dry year (Rodríguez-Ortega *et al.*, 2021). The first significant rainfall (20 mm) occurred on August 27th.

Differences were observed in agronomic variables (Table 1; P<0.05): switchgrass (9,322 kg ha^{-1}) was greater than alkali sucaton and eastern gamagrass by 1.8 and 3.3 times, respectively; however, eastern gamagrass had a higher leaf proportion. Forage production in switchgrass was similar to the figures reported by Marra *et al.* (2013), while eastern gamagrass production was 1.76 times lower than that reported by Coblentz *et al.* (2014). Cox *et al.* (1990) just like in this study, considered 8,333 plants ha^{-1} — reported

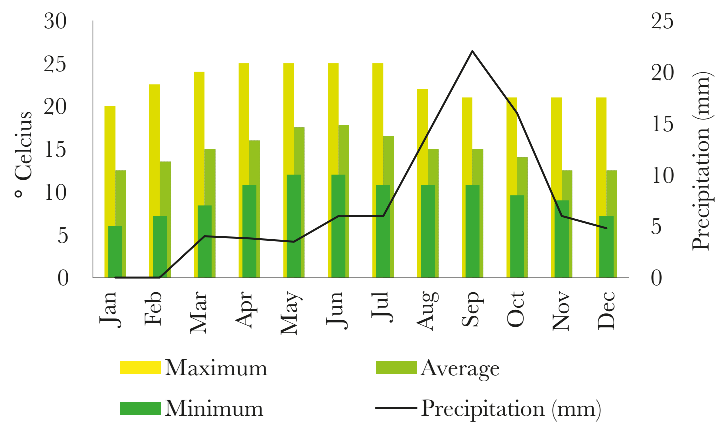

Figure 1. Monthly rainfall (mm) and maximum, average, and minimum temperature in Tulancingo de Bravo, Hidalgo, in 2022.

Table 1. Forage production and its morphological distribution (%), leaf:stem ratio in four pastures under rainfed conditions in Tulancingo, Hidalgo, Mexico.

Especie	Forage yield (kg ha^{-1})	Leaves %	Stems %	Inflorescens	Dead material (%)	Leavs:stems	Seed yield (kg ha^{-1})	Wight of 1000 seeds (mg)	Phisic purity (%)	Pure seed (kg ha^{-1})
Tripsacum dactyloides	2818 b[†α]	52.8 a	40.6 b	4.4 a	2.1 c	1.39 a	No aplica	17.0 a	No aplica	No aplica
Sporobolus airoides	5075 b	31.1 b	30.1 c	0.56 b	38.2 a	1.05 a	211.5 a	0.87 b	43.42 a	91.8
Panicum virgatum	9322 a	20.0 c	53.0 a	1.1 b	25.8 b	0.38 b	159.9 a	0.39 b	2.94 b	4.7
Significancia	***	***	***	***	***	**	NS	***	***	***

[†] Equal lowercase letters per column indicate similar averages (P>0.05). ***P<0.001. **P<0.01. NS=Not significant (P>0.05). No aplica (Not applicable)=seed yield is incipient.

a lower dry matter level in the forage production of alkali zacaton (1,208 kg ha^{-1}). Regarding seed yield, there was no difference (P>0.05) between alkali zacaton (211 kg ha^{-1}) and switchgrass (159.9 kg ha^{-1}). However, this yield was 2.9 times higher than the results reported by Rodríguez-Ortega *et al.* (2021), after 2 years of establishment; in conclusion, plant age is important for the increase in seed quantity (Quero-Carrillo *et al.*, 2014). In the 1,000-seed weight heading, eastern gamagrass caryopses were heavier than in switchgrass and alkali sacaton; these results were typical of these species, but cupule filling was poor, as reported by Rodriguez-Ortega *et al.* (2021). Contrary to eastern gamagrass and switchgrass, alkali sacaton caryopses were 2.3 times heavier than in 2020, which can only be explained by the rainfall (mm). Physical purity in alkali sacaton after 1 year of establishment was higher (43.42%) in the current study than in Rodriguez-Ortega *et al.* (2021; 22.7%); meanwhile, purity decreased in switchgrass (2.94%)), probably due to the low and poorly distributed rainfall (Rodriguez-Ortega *et al.*, 2021; 8.26%).

Caryopsis dimensions were different (P<0.001; Table 2): eastern gamagrass caryopses were longer, wider, and thicker than in alkali sacaton and switchgrass. Likewise, switchgrass caryopses was 1.5, 1.3, and 1.2 times longer, wider, and thicker than in alkali sacaton. Different dimensions are typical of each species; however, measurements should be taken into account for seed sorting or for their consideration for seed drills. Botanical seed size is important, since the vigor of a seedling depends on its intraspecies size (Quero-Carrillo *et al.*, 2017).

Yield components

Panicum virgatum. On average, it recorded 250 total stems, 193 floral stems, 38 inflorescence branches, 5.2 basal branch twigs, and 2.4 spikelets from the first pedicel of the basal branch.

Sporobolus airoides. This forage plant registered 355 total stems, 150 floral stems, 57 branches per inflorescence, 11.2 twigs, 6.5 secondary twigs, 2.7 spikelets on the tertiary twig, and 1.3 spikelets on the tertiary twig.

Tripsacum dactyloides. This grass recorded 280 total stems and 87 floral stems —out of which 75%, 22%, and 3% were single, double, and triple, respectively. In addition, an average of 6.4 cupules per gynoecium and 30.4 androecium sections were counted.

Table 2. Botanical seed dimensions of four grass species grown under rainfed conditions in Tulancingo, Hidalgo, Mexico.

Especie	Length (cm)	Width (cm)	Thickness (cm)
Panicum virgatum	2.20 b[†] [α]	0.89 b	0.69 b
Sporobolus airoides	1.39 c	0.66 c	0.57 c
Tripsacum dactyloides	3.83 a	2.48 a	1.99 a
Significancia	***	***	***

[†] Different lowercase letters per column indicate different averages (P<0.05). ***P<0.001.

CONCLUSIONS

Switchgrass had the highest dry matter yield, followed by alkali sacaton and eastern gamagrass. Seed production was higher and had a higher physical purity in alkali sacaton. The largest caryopses were reported on eastern gamagrass, followed by switchgrass and finally alkali sacaton.

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Colegio de Postgraduados

Chemical treatments in maize seeds to improve germination in acidic soils

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ABSTRACT
Objective: To evaluate the effect of different germination promoters on three maize genotypes grown in Dystric Cambisol soils, since germination problems are linked to latency and restrict agronomic management.
Design/Methodology/Approach: We conducted an experiment at the Instituto Tecnológico Superior de Juan Rodríguez Clara using a split-plot design with a factorial treatment arrangement. The large plot contained genotypes (GEN) G1=MS-405, G2=Arlequin, and G3=MS-404; while the small one comprised promoter (PROMO) HS=humic substance, CI=citrulline, and SA=salicylic acid. We evaluated the following variables: germination speed (GS), emergence percentage (EMERG), stem and leaf volume (S&LV), root volume (RV), chlorophyll (CHL), secondary roots (SECR), stem diameter (DMT), number of leaves (NL), foliar area (FA), root length (RL), and plant height (PH). Then, we conducted a variance analysis and Tukey's tests ($\alpha \leq 0.05$).
Results: For each promoter, we observed main effects in EMERG, CHL, and PH for CI; S&LV, NL, FA, and PH for HS; and RL for SA. In genotypes G2 and G3, variables GS, EMERG, NL, and PH were statistically equivalent, DMT varied only in G2, and there were no statistical differences for S&LV, RV, CHL, SECR, FA, and RL. We observed some simple effects in combinations with CI: GS and PH varied in G3, EMERG in G2 and G3, CHL in G1 and G3, DMT in G1 and G2, and S&LV in G2.
Study limitations/Implications: Soaking corn for one hour in the solution and weighing the correct amount properly are required, since weighing too much may inhibit germination.
Findings/Conclusions: Promoter CI at a dose of 1,000 ppm accelerates the emergence speed of genotypes G2 and G3 in acidic soils.

Keywords: Abscisic acid; Amino acids; Krebs cycle; Emergence.

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INTRODUCTION

Knowing the germination processes is crucial, mainly because of the alterations brought by climate change. When the embryo develops, the endosperm transfers nutrients (Debouza *et al.*, 2021), and once the mitochondria become active, they enter the glycolysis process and then the Krebs cycle (Ali and Elozeiri, 2017). With the testa rupture, cellular respiration increases, and reserves, glycosides, proteins, lipids, hormones,

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enzymes, carbohydrates, and phytins are mobilized in the endosperm (Gómez-Maqueo and Gamboa-de Buen, 2016).

Using pre-germination chemical treatments stimulates germination and decreases abscisic acid (ABA) (Bautista-Rodríguez *et al.*, 2017). Moreover, these treatments remove tissues such as coleorhiza (Anh *et al.*, 2019). At the beginning of the Krebs cycle, using (non-protein) amino acids such as citrulline (CI) (Song *et al.*, 2020) improves nitrogen balance and eliminates hydroxyl radicals. With biostimulants such as humic substances (HS) (Veobides-Amador *et al.*, 2018), the transport of organic and inorganic molecules and the absorption of proteins, amino acids, and ionic nutrients becomes more efficient (Popa *et al.*, 2022). These same processes can be balanced with hormones such as gibberellin (GA) (Tuan *et al.*, 2018) or with salicylic acid (SA). The latter reduces oxidative damage (ROS) due to excess or intoxication (Huang *et al.*, 2021).

Mexico has areas of tropical climate, where soils are acidic. In the state of Veracruz, in municipalities such as Juan Rodríguez Clara and Isla, soils contain scarce Dystric Cambisol organic matter, which limits crop productivity (Tosquy-Valle *et al.*, 2020). With pre-germination chemical treatments, promoters boost maize seed germination in acidic soils. This study assessed the effects of different germination promoters in three maize genotypes.

MATERIALS AND METHODS

We conducted the experiment on June 11, 2021 —during the spring and summer cycle—, on a zero tillage 1,800 m^2 plot at the Instituto Tecnológico Superior (ITS) Juan Rodríguez Clara. The ITS is located in the Municipality of Juan Rodríguez Clara, Veracruz, Mexico (18° 01" 6.1' N, 95° 94" 1.7' W, 133 masl). According to Köppen —modified by García (2004)— the climate is warm subhumid (AW0), with an average temperature of 24.5 °C and an average annual precipitation of 1,100 mm. The soil in the area is classified as Dystric Cambisol by the Norma Oficial Mexicana de la Clasificación Agronómica PROY-NOM-021-RECNAT-2000 (Tosquy-Valle *et al.*, 2020). We soaked the three maize genotypes in the treatments (SA=salicylic acid, CI=citrulline, HS=humic substances) for one hour at a concentration of 1,000 ppm. After sowing, we applied atrazine as herbicide.

Assessed variables

We evaluated emergence percentage (EMERG); germination speed (GS) (Sobarzo-Bernal *et al.*, 2021); stem and leaf volume (S&LV) —using the buoyancy technique by submerging the roots in a cylinder full of water and measuring the displaced liquid in cm^3 (Angulo-Castro *et al.*, 2017)—; root length (RL) in cm; foliar area (FA) in cm^3; chlorophyll (CHL); and number of leaves (NL). We measured EMERG daily (PE) starting the third day after sowing. To determine seedling germination (TE) we followed the formulas suggested by Sharma *et al.*, (2022):

$$PE = \frac{Number\ of\ emerged\ seeds \times 100}{Total\ number\ of\ seeds}$$

$$TE = \frac{N1*T1 + N2*T2 + N3*T3 + \ldots Nn*Tn}{Total\ number\ of\ emerged\ seeds}$$

Where N=number of particles appearing consecutively and T=time elapsed from the beginning of the test to the end of the measurement period.

For S&LV, we used a 50 ml submerged tube. For RL, we used a measuring tape in cm. We counted the number of roots. To obtain the foliar area (FA), we measured leaf length and width (Berdjour *et al.*, 2020). To measure leaf CHL contents, we used a chlorophyll meter (FT Green LLC®, USOS) (Mendoza-Tafolla *et al.*, 2022).

Statistical analysis

We chose a split-plot design in a 3×3 factorial treatment arrangement with three replications. Factor A covered GEN (G1=MS-405, G2=Arlequin, and G3=MS-404), and factor B comprised PROMO (SA=salicylic acid, CI=citrulline, and HS=humic substances). With the data obtained, we conducted a variance analysis and a comparison of means using Tukey's test ($p<0.05$). To process the data, we resorted to the SAS statistical package (SAS, 2009).

RESULTS AND DISCUSSION

Emergence in acidic soils

Due to the hydration of pre-germination treatments, the seeds showed an increase in germination speed (GS) and emergence percentage (EMERG) (Escobar-Álvarez *et al.*, 2021). In the variance analysis (Table 1), we can observe high significance ($p<0.05$) for GEN in germination speed (GS), emergence percentage (EMERG), stem and leaf volume

Table 1. Variance analysis (mean squares and statistical significance) for the following variables: germination speed (GS), emergence percentage (EMERG), stem and leaf volume (S&LV), chlorophyll (CHL), secondary roots (SECR), stem diameter (DMT), number of leaves (NL), leaf area (LA), and root length (RL), plant height (PH).

		Gen	Block	B* Gen	Promo	Gen *Promo	Error	Total	CV (%)
VARIABLES	DF	2	2	4	2	4			
	GS	486.96**	14.44	58.55	7.8	97.02*	6.17	738.4	13
	EMERG	3880.07**	190.29	192.59	492.74*	880.81*	42.7	6148.96	8
	S&LV	25.35**	0.24	0.33	4.16**	2.34*	0.06	33.17	4
	CHL	283.05*	32.39	19.63	530.63*	468.79*	16	1526.62	9
	SECR	1.73	7.16	5.52	8.85	10.21	1.97	56.9	13
	DMT	0.19*	0.039	0.019	0.052*	0.20*	0.0081	0.6	13
	NL	11.87**	0.008	0.016	3.52*	6.36*	0.26	24.9	8
	LA	364.28	791.41*	1090.74*	1007.03*	3885.94*	78.13	8077.04	15
	RL	465.87*	65.34	143.27	363.81*	820.41*	21.9	2121.61	17
	PH	25.80*	0.06	1.71	8.52	4.7	0.66	48.78	10

DF: Degrees of freedom, Gen: genotype, Block, B*Gen: Bloc * Gen: Gen*promo: genotype*promoter, CV: Coefficient of variation. ** Highly significant * Significant (Tukey, 0.05).

(S&LV), and number of leaves (NL), with coefficients of variation (CV) of 13, 8, 4, and 8%, respectively. Regarding PROMO, high significance is only observed for S&LV. Moreover, there is significance ($p<0.0001$) when promoter * genotype interaction (Promoter * Gen) occurs. Dago *et al.* (2021) mention that using biostimulants promotes germination and improves stem length and diameter.

The behavior of means in the double entry table (Table 2) shows main effects of PROMO as follows: CI in variables EMERG, CHL, and PH; HS in S&LV, NL, FA, and PH; and SA in RL. Regarding germination in GEN (G1, G2, and G3), G2 and G3 were statistically equivalent in GS, EMERG, NL, and PH. Only G2 varied in DMT. There was no statistical difference for variables S&LV, RV, CHL, SECR, FA, and RL. We observed some simple effects in combinations with CI: GS and PH varied in G3, EMERG in G2 and G3, CHL in G1 and G3, DMT in G1 and G2, and S&LV in G2. Song *et al.* (2020) mention that citrulline eases nitrogen assimilation, thus increasing the content of chlorophyll and stem and leaf tissue. HS produces variations in EMERG and S&LV in G2 and G3, and in NL, FA, and PH in G3. Bijanzadeh *et al.* (2019) mention that humic substances produce biochemical effects, promote potassium absorption, improve photosynthesis, increase cellular respiration, and improve seedling growth in maize (Figure 1). HS are also related to the amino acid and Krebs cycle metabolism (Popa *et al.*, 2022). As for SA, it produces

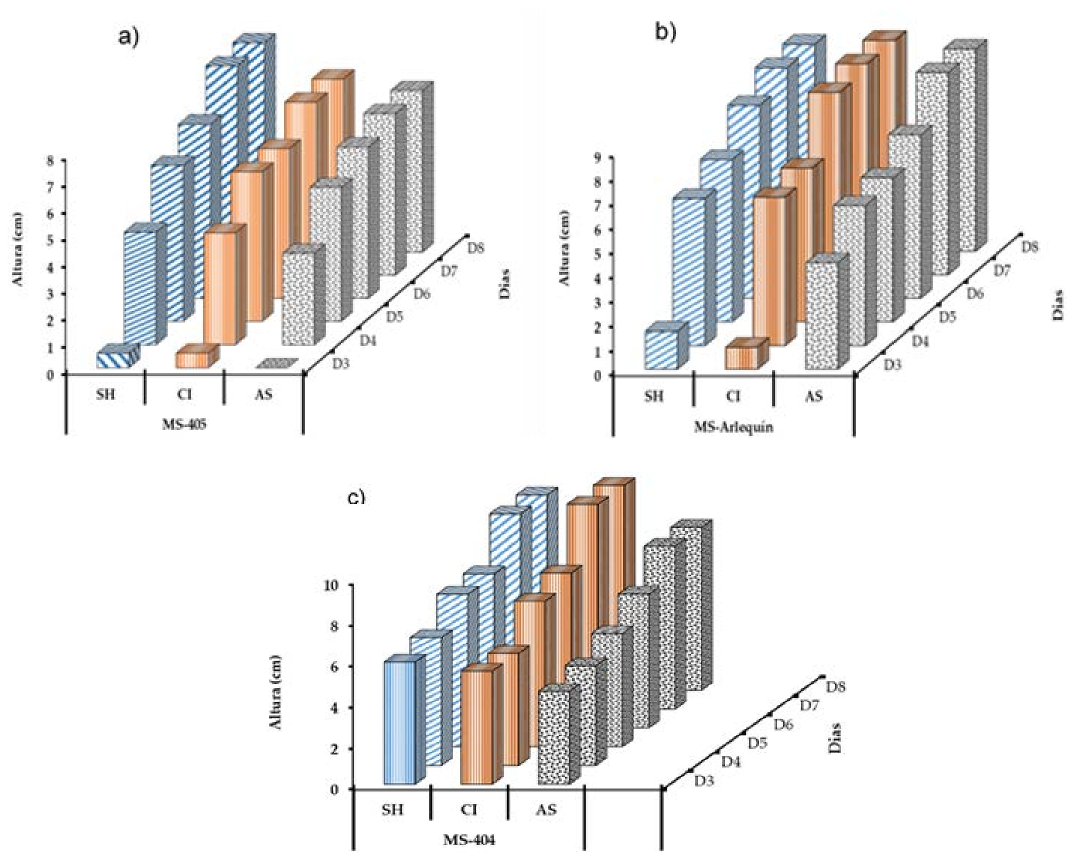

Figure 1. Height of genotypes MS-405, Arlequin, and MS-404, measured for eight days after emergence, considering controllers SH (HS)=humic substance, CI=citrulline, and AS (SA)=salicylic acid.

Table 2. Means for promoter effects (HS=humic substances, CI=citrulline, and SA=salicylic acid) on maize genotypes G1, G2, and G3, and interaction regarding germination speed (GS), emergence percentage (EMERG), stem and leaf volume (S&LV), chlorophyll (CHL), stem diameter (DMT), number of leaves (NL), foliar area (FA), root length (RL), and plant height (PH).

VAR	GEN	Promovedor			
		SH	CI	AS	MEDIA
VG	G1	13.75 cd	15.66 bcd	9.17 d	12.86 b
	G2	18.87 abc	18.21 abc	22.54 ab	19.87 a
	G3	22.63 ab	24.06 a	22.38 ab	23.02 a
	MEDIA	18.41 a	19.31 a	18.03 a	
EMERG	G1	60 b	84 a	60 b	68 b
	G2	90 a	93 a	100 a	94 a
	G3	90 a	94 a	94 a	93 a
	MEDIA	80 b	90 a	85 ab	
VTYH	G1	10.1 ab	12.7 ab	6.2 b	9.6 a
	G2	15.0 a	15.0 a	12.0 ab	14.0 a
	G3	15.0 a	7.0 b	9.0 ab	10.3 a
	MEDIA	13.4 a	11.6 ab	9.1 b	
CLFI	G1	45.6 ab	51.9 a	44.0 ab	47.2 a
	G2	40.1 ac	46.7 ab	53.6 a	44.1 a
	G3	31.9 c	51.5 a	37.0 bc	40.1 a
	MEDIA	39.2 c	50.1 a	44.9 b	
DMT	G1	0.54 bc	0.77 a	0.40 c	0.57 b
	G2	0.70 ab	0.83 a	0.80 a	0.78 a
	G3	0.70 ab	0.60 abc	0.70 ab	0.67 ab
	MEDIA	0.65 a	0.73 a	0.63 a	
NH	G1	6.1 b	6.0 b	4.7 c	5.6 b
	G2	7.0 ab	7.0 ab	7.0 ab	7.3 a
	G3	8.0 a	6.0 b	7.0 ab	7.0 a
	MEDIA	7.0 a	6.3 b	6.2 b	
AF	G1	61.2 abc	63.7 abc	45.5 bc	56.8 a
	G2	51.2 abc	78.2 ab	61.9 abc	74.0 a
	G3	81.9 a	40.5 c	43.5 c	55.3 a
	MEDIA	64.8 a	60.8 ab	50.3 b	
LR	G1	20.2 b	25.7 b	23.0 b	23.0 a
	G2	21.0 b	33.7 ab	25.0 b	28.6 a
	G3	27.0 b	25.0b	47.0 a	33.0 a
	MEDIA	22.7 b	28.1 ab	31.7 a	
ALT	G1	7.81abc	6.46 bc	6.03 c	6.77 b
	G2	8.31 ab	8.67ab	7.88 abc	8.29 a
	G3	9.52 a	9.98 a	7.94 abc	9.15 a
	MEDIA	8.55 a	8.37 a	7.28 b	

[†] The variables VG (GS), EMERG, VTYH (S&LV), CLFI (CHL), DMT, NH (NL), AF (FA), LR (RL), and ALT (PH) presented statistical differences between promoters (SH / HS, CI, AS / SA) and genotypes (G1, G2, and G3) ($p \leq 0.05$). a,b,c: Mean values per column with different letters are statistically different ($p \leq 0.05$). VG (GS)=%, EMERG=%, VTYH (S&LV)=mm, CLFI (CHL)=nm, DMT=mm, AF (FA)=cm^2, LR (RL)=cm, and ALT (PH)=cm.

variations in EMERG for G2 and G3, in CHL and DMT for G2, and in RL for G3. Bijanzadeh *et al.* (2019) and Dzib-Ek *et al.* (2022) mention that SA at concentrations of 1 and 0.01 μM significantly promotes root length and secondary root formation ($p<0.05$), regulates plant growth and development processes, including seed germination, and improves vigor (Chitnis *et al.*, 2014).

CONCLUSIONS

Pre-germination treatment by soaking in citrulline at 1,000 ppm in the MS-404 and Arlequin maize genotypes showed positive effects on germination speed (94%) and percentage (93%), as well as on the development of seedlings in acidic soils during a 15-day monitoring period. Other studies can follow up on the effects of pre-germination treatments and establish whether their application impacts production completion.

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Colegio de Postgraduados

Productive behavior of rabbits fed with *Leucaena leucocephala* (Lam.) de Wit, foliage in the fattening phase

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ABSTRACT

Objective: To assess the productive behavior of rabbits fed with a diet that included 33% fresh *Leucaena leucocephala* foliage during the fattening phase.

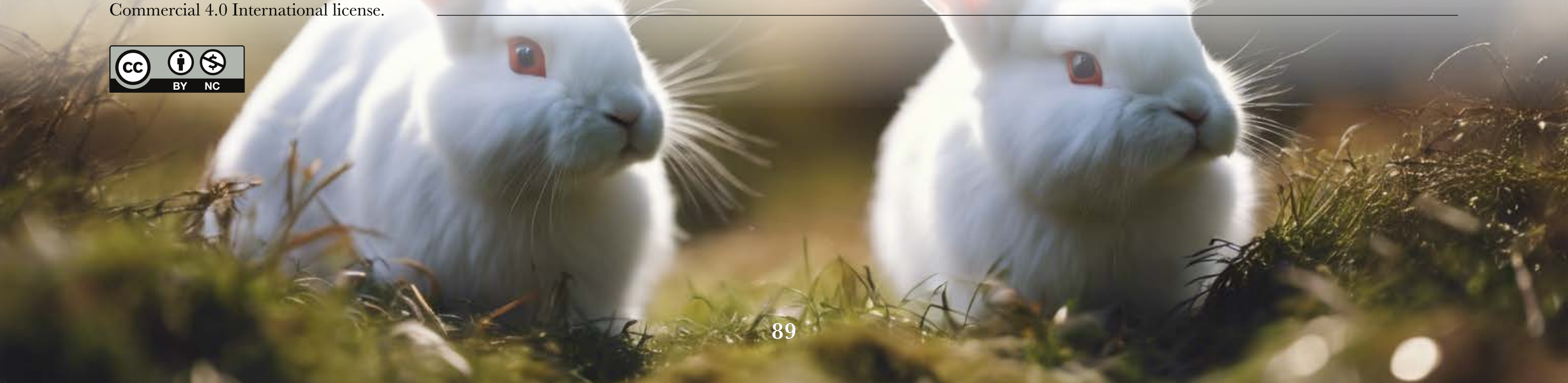
Design/Methodology/Approach: Twenty 60-day-old New Zealand rabbits with an average live weight of 960 g were used. Using a completely randomized, 2×2 factorial design, five male and five female rabbits were distributed among the following treatments: ACCH treatment (300-g commercial feed for female rabbits), ACCM treatment (300-g commercial feed for male rabbits), ACFLCH treatment (200-g commercial feed with 100 g *Leucaena* foliage for female rabbits), and ACFLCM treatment (200-g commercial feed with 100 g *Leucaena* foliage for male rabbits). An analysis of variance and a Tukey's test were applied to determine the effect of the treatments on each of the variables analyzed.

Results: The rabbits that consumed the ACFLCM and ACFLCH diets with the addition of *Leucaena* gained more weight ($P<0.05$): 23.18±6.35 and 21.59±10.23 g/rabbit/day. Feed intake was higher ($P<0.05$) with a *Leucaena* foliage diet: 48.04±0.50 and 47.68±0.84 g/rabbit/day. A significant difference ($P>0.05$) in final weight was observed among treatments (ACCM, 1,971±256.6; ACFLCM, 2,211.8±197.7; ACCH, 2,012.4±275.6; and ACFLCH, 2,184.0±464.9).

Study Limitations/Implications: Proper and correct use of available forage must be made to guarantee that animals have access to food all year round.

Findings/Conclusions: The addition of *Leucaena* foliage in the diet did not generate a decrease in growth or feed intake.

Keywords: Acceptability, consumption, rabbit finishing, weight gain, leguminous plant.



INTRODUCTION

In the tropics of Mexico, rabbit breeding has been of great importance; however, providing them with a balanced diet as a main food source increases production costs (Adedeji *et al.*, 2013). Rabbit meat has several outstanding characteristics, including the quality of its protein and its low-fat content. Meanwhile, rabbit production can be carried out in smaller areas and the flexible diet of rabbits can include food made from plant material, which reduces costs (Martínez *et al.*, 2010; Palma and Hurtado, 2010). Foods such as leguminous shrubs (foliage) are a high-value protein option for rabbit nutrition (García-Trujillo, 1991).

Leucaena leucocephala is found throughout the country. Consequently, it has aroused interest as food for ruminants and non-ruminants —particularly rabbits, to whose diets it can be added up to 20% (Nieves *et al.*, 2005). Its combination with other species of the same family has a 30% acceptance in rabbit feed, improving yields and reducing production costs (Nieves *et al.*, 2002). In this respect, further assessments are needed to determine the maximum percentage of *Leucaena leucocephala* that can be added to the diet of rabbits before it reaches a toxicity level.

Therefore, the objective of this research was to assess the productive behavior of rabbits fed with *Leucaena leucocephala* foliage in the fattening phase.

MATERIAL AND METHODS

Study area location

The study was conducted from June 20 to August 15, 2019, at the Module for Agroecological Rabbit Research and Production (MIPAC) of the National Technological Institute of Mexico - campus Chiná, Campeche, Mexico. The MIPAC is located at 19° 46' 13" N and 90° 30' 13" W, at 33 m.a.s.l. It has a sub-humid climate with summer rains, an average annual temperature of 26 °C, and a rainfall of 1,200 mm/year (García, 2004).

Animals and handling

Twenty 60-day-old New Zealand rabbits (10 males [M] and 10 females [F], all of them weaned) with an average live weight of 960 g were used for the experiment. Rabbits were housed in individual cages with galvanized feeders and drinkers. They were provided with a base diet (300 g/rabbit/day) for 10 days before the test and the corresponding treatments maintained the same amount until the end of the experiment. All animals had water available *ad libitum*. For assessment purposes the cages were also individually identified with each animal.

Experimental procedure

Commercial feed was purchased from a feed distributor. The raw material (forage) was collected in the field. The ration formulation complied with the recommendations of the National Research Council (NRC, 1998). Fresh *Leucaena leucocephala* foliage was cut and included in the feed of the corresponding treatment. The bromatological composition of *Leucaena leucocephala* was also analyzed to determine protein percentages in the diet of the fattening animals (Table 1).

Table 1. Treatment and nutritional composition of *Leucaena leucocephala* for fattening rabbits.

Ingredients	Treatments Food inclusion (g)			
	ACCM	**ACCH**	**ACFLCH**	**ACFLCM**
Commercial food	300	300	200	200
Leucaena leucocephala			100	100
Chemical composition (%)	Commercial food		*Leucaena leucocephala*	
Crude protein	16.5		21.8	
Fat	2.5		13.44	
Fiber	16		18	
Dry matter	88		94	
Ashes	12		6.6	

treatment: 300-g commercial feed for female rabbits; ACCM treatment: 300-g commercial feed for male rabbits; ACFLCH treatment: 200-g commercial feed with 100 g *leucaena* foliage for female rabbits; ACFLCM treatment: 200-g commercial feed with 100 g *leucaena* foliage for male rabbits.

Design and treatments

A 2×2 factorial design completely randomized was used. Five male and five female rabbits were distributed among the following treatments: ACCH (300-g commercial feed for female rabbits), ACCM (300-g commercial feed for male rabbits), ACFLCH (200-g commercial feed with 100 g *leucaena* foliage for female rabbits) and ACFLCM (200-g commercial feed with 100 g *leucaena* foliage for male rabbits).

Productive behavior

Animals were weighed before the morning feeding at the beginning and every 5 days until the end of the experiment. The following productive indicators were quantified: initial live weight (g), final live weight (kg), daily feed intake (g/rabbit), daily weight gain (g/rabbit) and feed conversion (kg).

Statistical analysis

Data were processed using the InfoStat statistical software, using a significance level of $P<0.05$ (Di Rienzo, 2020). The effect of the treatments on each of the variables analyzed was determined with Tukey's test.

RESULTS AND DISCUSSION

Table 2 shows the productive behavior of rabbits during the assessment phase. The final live weight of the rabbits had significant differences ($p \leq 0.05$) between treatments: the animals that consumed ACFLCM and ACFLCH gained more weight than those that consumed ACCH and ACCM.

In relation to sex, Figure 1 shows significant differences in consumption in the treatments ($p \geq 0.05$) that add *Leucaena leucocephala* to the diets for male and female rabbits, compared to animals that only received commercial diet. This result matches the findings of Nieves

Table 2. Productive behavior of male and female rabbits, with the use of commercial feed and *Leucaena leucocephala*.

Productive indicators	Males		Females	
	ACCM	ACFLM	ACCH	ACFLCH
Initial weight (g)	841.6±331.0b	1093.6±546.2a	935.2±325.8b	975.2±285.4a
Final weight (Kg)	1.971±256.6b	2.211.8±197.7a	2.012.4±275.6b	2.184.0±464.9a
Daily weight gain (g/rabbit)	20.18±3.17b	23.18±6.35a	19.24±3.42b	21.59±10.23a
Dry matter intake (g/rabbit)	36.30±0.53b	48.04.2±0.50a	36.14±0.90b	47.68±0.84a
Feed conversion (Kg)	1.80±0.25b	2.04±0.52a	1.92±0.33b	2.76±1.66a
Cost per kg increased (Kg)	10.00	8.00	10.00	8.00

ACCH treatment: 300-g commercial feed for female rabbits; ACCM treatment: 300-g commercial feed for male rabbits; ACFLCH treatment: 200-g commercial feed with 100 g *Leucaena* foliage for female rabbits; ACFLCM treatment: 200-g commercial feed with 100 g *Leucaena* foliage for male rabbits. Different letters in the same row indicate significant differences, according to Tukey's test (p≤0.05).

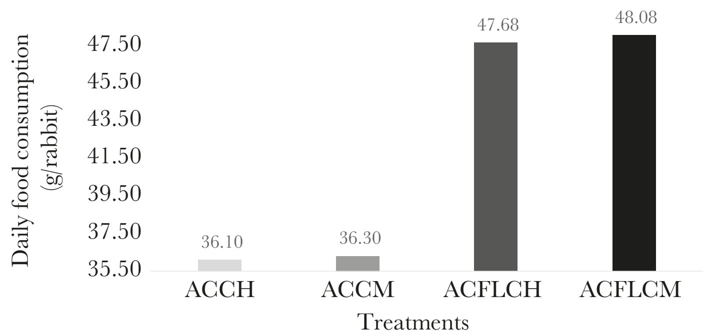


Figure 1. Voluntary consumption by male and female rabbits of commercial feed plus *Leucaena leucocephala*.

et al. (2005), who included 40% *Leucaena* foliage with a higher preference in the diets of fattening rabbits.

Weight gain was significantly higher (p≤0.05) in rabbits subjected to the ACFLCM and ACFLCH treatment than with ACCH and ACCM. These results are similar to the 19.11 g weight gain with a base diet and 40% *leucaena* addition recorded in fattening by Nieves *et al.* (2002) in Guanare, Portuguesa, Venezuela. However, Pilco *et al.* (2018) reported that better productive indexes were observed in the weight gain of the rabbits with a 10% *leucaena* addition to the diet (Figure 2).

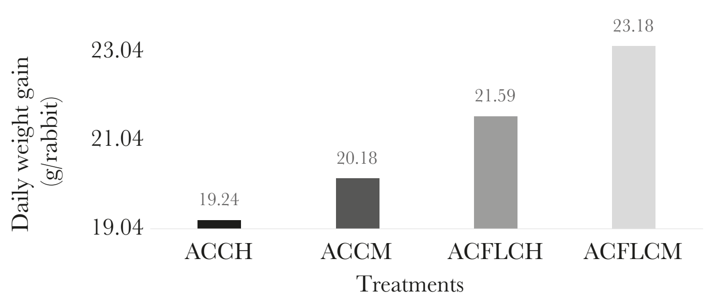


Figure 2. Weight gain in male and female rabbits with commercial feed plus *Leucaena leucocephala*.

CONCLUSIONS

Diets with added *leucaena* are an alternative feed supplement for rabbit production. On the one hand, an increase in daily weight gain of the animal and greater food consumption was found with diets that included *Leucaena*. In conclusion, compared with commercial feed on its own, the addition of *Leucaena leucocephala* foliage in the finishing phases maintained and improved the productive behavior of the rabbits.

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Dry matter distribution of banderita grass [*Bouteloua curtipendula* (Michx.) Torr.] at different plant strata

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ABSTRACT

Objective: To evaluate the dry matter distribution of banderita grass [*Bouteloua curtipendula* (Michx.) Torr.] in different plant strata.

Design/Methodology/Approach: A randomized block experimental design with five repetitions was used for the experiment. Each repetition consisted of three plants which were evaluated at different days after sowing (DAS), in three different plant strata: basal stratum (BS), middle stratum (MS), and upper or apical stratum (AS). The following variables were evaluated: dry matter yield (DMY), morphological composition (MC), leaf area (LA), plant height (PH), leaf:stem ratio (L:SR), and aerial part:root ratio (Ap:rR). An analysis of variance was performed, using the PROC GLM procedure of the SAS software; in addition, a comparison of means was carried out using Tukey's test ($\alpha < 0.05$).

Results: SB made a greater contribution to DMY at 50 DAS, with a 16 g DMY plant^{-1} average, followed by MS, with 9 g DM plant^{-1}, and AS with 3 g plant^{-1}. The MC (g) in the BS registered that the stem made a greater contribution than the rest of the components (average: 12.3 g plant^{-1}), while leaves from the MS and AS made the greatest contribution (2.6 g plant^{-1}) up to 64 DAS. However, they were surpassed by the stem in the MS and by the inflorescence in the AS. In addition, BS registered the highest LA (173.4 cm2). The largest PH was recorded at 120 DAS (96 cm). The highest L:SR ratio reached 1.10 at 50 DAS, while Ap:rR recorded 3.82 at 92 DAS.

Study Limitations/Implications: The experiment was carried out under greenhouse conditions. Therefore, any extrapolation or comparison with field conditions should be done with caution.

Findings/Conclusions: The basal and middle part of a *banderita* grass (*Bouteloua curtipendula*) plant contains the highest forage accumulation (mainly in the leaves and the stem), while the highest biomass content in the apical part is produced by the inflorescence.

Keywords: Native grass, leaf area, morphological composition, plant strata.

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INTRODUCTION

Most of the grasses in the arid and semi-arid areas of Mexico have deteriorated and produce little and bad quality forage (Guerra *et al.*, 2006); consequently, cattle producers face difficulties regarding the availability of forage, because its production depends on the edaphoclimatic and climatic conditions of the area where it is grown (Suttie, 2003) and developed (FIRCO, 2010). Therefore, determining different foraging alternatives —which have a higher tolerance to seasonal extreme temperatures in northern Mexico— is important. Several foraging species have qualities that complement the diet of cattle. Some of these plants belong to genus *Bouteloua*, which includes approximately 60 species, with a wide genetic variability and that are mainly distributed in northern Mexico (Peterson *et al.*, 2015). *Banderita* grass [*Bouteloua curtipendula* (Michx.) Torr] is native to Mexico, grows in plains and rocky low hills, and has an excellent foraging value for extensive grazing (Morales-Nieto *et al.*, 2016). *Banderita* grass produces plenty of forage, adapts to different climatic conditions, and is tolerant to drought; therefore, it is considered the second most agronomically important species within its genus (Morales-Nieto *et al.*, 2008). Its plants can grow to >75 cm tall and have 50-70% digestibility values (Corrales *et al.*, 2016). The forage yield depends on the phenological state of the plant. Additionally, it keeps its foraging value longer than other grasses (Morales-Nieto *et al.*, 2006). The annual average forage yield of *banderita* ranges from 1,850 to 2,000 kg DM ha^{-1}, with annual precipitations of 350-500 mm (Beltrán *et al.*, 2013). Consequently, the use of this high-value foraging species is an alternative for cattle raising, because it can help the economy of producers from the northern region of the country, increasing grazing in arid and semi-arid regions. Therefore, the objective of this study was to evaluate the dry matter distribution of *banderita* grass, in three plant strata (basal, middle, and upper or apical), at different days after sowing, during the spring-summer growing cycle.

MATERIALS AND METHODS

Study area description

The crops were established on April 25, 2022, in the greenhouse of the Departamento de Recursos Naturales Renovables, Universidad Autónoma Agraria Antonio Narro (UAAAN), Unidad Saltillo, located in southeastern Coahuila (25° 35' 35"N and 101° 03' 60" W, at 1,783 m.a.s.l.). The spring-summer growing cycle (April-August 2022) was evaluated. Maximum, minimum, and mean temperature, as well as relative humidity, was recorded using a FCCERoHS WS08 digital hygrometer.

Experiment establishment and experimental design

B. curtipendula var. NdeM-303 was used for a completely random block design, with five repetitions. Forty 8-kg plastic pots were established as experimental units (EU), using a previously sterilized substratum —made up of hill soil (50%), peat moss (25%), and vermiculite (25%)— was used in the experiment. Fifty seeds were directly sown in the pots. Subsequently, the seeds were covered with a light substratum layer and —in order to increase temperature, decrease evaporation, and speed up germination— the pots were covered with transparent plastic. The experimental units were placed on wooden platforms, which increased the ventilation and water leakage in each irrigation. The first irrigations after the sowing were light and carried out with a watering can, in order to prevent exposing the root due to water impact. As the age of the plant increased, the

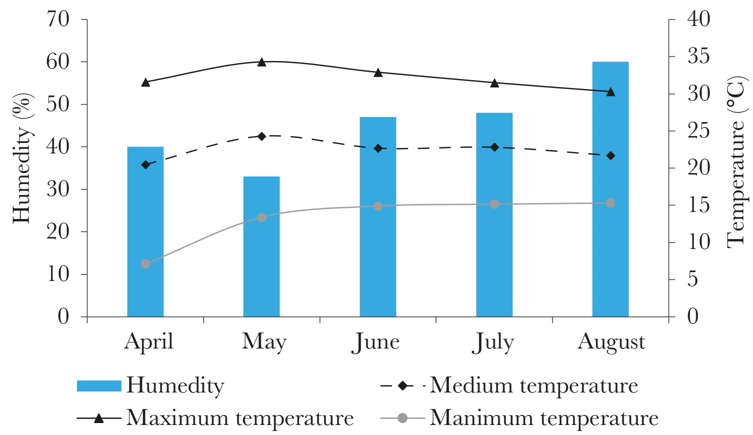

Figure 1. Monthly temperature and humidity recorded from April 25 to August 23, 2022, at the greenhouse of the Departamento de Recursos Naturales, UAAAN-Unidad Saltillo, using a WS08 digital hygrometer.

irrigation consisted of 0.5-1.0 L day^{-1}, until field capacity was reached. A thinning was carried out at 15 DAS, in order to select the three most vigorous individuals of each EU (pot). Successive measurements were carried out every 14 days, from 22 to 120 DAS. These measurements matched eight treatments (plant age), with 5 repetitions each and three individuals.

Evaluated variables

Dry matter yield and morphological components

Destructive sampling consisted of the extraction of whole plants. Afterwards, the roots were washed and placed in previously labelled plastic bags. Subsequently, the aerial parts of the plants were separated from the roots; they were then weighted and the length of each plant was measured. The aerial part was divided into three strata of the same length, according to the age of the plant: basal (BS), middle (MS), and upper or apical (AS) strata. Each stratum was divided into its morphological components: leaves, stem, dead material (DM), and inflorescence. The samples were placed in labelled paper bags and dried in a forced air oven (POM-246F, Serial No. P6-800), at 55 °C for 72 h or until they reached a constant weight. Once they were dehydrated, total and per component dry matter yield was estimated (average of the three plants).

Leaf area

The iMAGEJ software (Rasband, 2007) was used to estimate the leaf area (LA) of fresh leaf blade samples. The leaves were arranged on a previously labelled white sheet, which included the data of the sample and a centimeter scale, based on Rincón-Carruyo *et al.* (2012).

Plant height

Before each sampling, the height of all the individuals of each EU was estimated using a ruler. The ruler had a device that allowed a higher accuracy (Rojas-García *et al.*, 2021).

Leaf:stem ratio

The dry weight of the leaf/stem of each substratum was divided. This operation was based on the morphological composition at 50 DAS, because, from 22 to 36 DAS, the presence of the stem was practically null and the L:S ratio formula could not be applied.

Aerial part:root ratio

This ratio was calculated dividing the dry weight of all the aerial components (leaves, stem, dead material, and inflorescence) per stratum and repetition by the root dry weight.

Statistical analysis

The PROC GLM procedure of the SAS software (Statistical Analysis System, v. 9.4 for Windows; SAS Institute, Cary, NC, USA) and Tukey's means comparison test ($p<0.05$) were carried out for the statistical analysis.

RESULTS AND DISCUSSION

Dry matter yield per stratum

The average dry matter yield per *B. curtipendula* stratum recorded a 16.4, 9.1, and 3.8 g plant^{-1} trend for BS, MS, and AS, respectively ($p\leq0.05$; Figure 2). From 22 to 50 DAS, the dry matter produced per stratum recorded no significant statistical differences ($p\geq0.05$) regarding the DAS averages and the average fluctuated between 0.018 and 0.37 g plant^{-1}. From 50 to 120 DAS (study period), the BS statistically surpassed the MS and AS, accumulating a maximum of 39.1 g plant^{-1} dry matter. AS was the stratum that made the lowest contribution to the total yield per plant, recording a maximum of 11.7 g plant^{-1}, at 120 DAS (Figure 2). In this regard, Sánchez-Arrroyo *et al.* (2020) proved that, based on its DMY results, the NdeM-303 (*B. curtipendula*) genotype have better foraging characteristics than other commercial varieties. For their part, Quero-Carrillo *et al.* (2018) registered a 2.5 g plant^{-1} average yield, in samplings cut with 20-day intervals. Finally, Morales-Nieto *et al.* (2016) studied *banderita* grass populations under similar greenhouse conditions, reporting a 4.0-260 g plant^{-1} dry matter average.

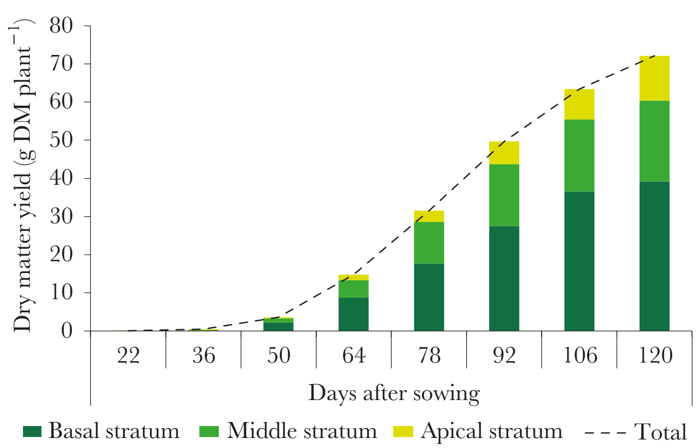

Figure 2. Dry matter yield (g MS plant^{-1}) of *banderita* grass, harvested at different DAS, in three plant strata, during the spring-summer growing cycle.

Morphological composition

At the beginning of the plant's growth (22-36 DAS), the leaves of the BS recorded the highest total yield. Nevertheless, from 50 to 120 DAS, the stem surpassed all the other components ($p \leq 0.05$; average: 12.3 g plant^{-1}), followed by the leaves (2.4 g plant^{-1}), and the dead material (1.7 g plant^{-1}). Since this species shows a trend towards an erect growth, inflorescence was not registered throughout the evaluation in this stratus (Figure 3). In this plant stratum, the stem accounted for 60% of the total yield average, statistically surpassing the leaves (34%), the dead material (5%), and inflorescence (0%) ($p \leq 0.05$) (Figure 3). In addition, from the beginning of the evaluation until 22-64 DAS, the leaves recorded the highest value of all the components in the MS. However, along with the rest of the components, the leaves were surpassed by the stem, at the end of the evaluation period (78-120 DAS). The dead material and the inflorescence always obtained lower values than the leaves and the stems. Nevertheless, unlike what happened in the BS, inflorescence appeared in the MS from 64 DAS (Figure 3). The average dry matter accumulation per component recorded the following sequence: stem>leaf>inflorescence>dead material (4.8>2.0>1.3>0.9 g MS plant^{-1}). The contribution of the morphological components to total plant yield in the MS was more homogenous than in the BS: the leaves recorded the highest average (48%), followed by the stem (39%), inflorescence (7%), and dead material (5%) (Figure 3). Like in the lower strata, leaves registered the highest dry matter yield in the AS of the plant (0.01 and 0.9 g plant^{-1}), at 64 DAS, with 65 to 99% ratios. This situation indicates that plants in the AS are mostly made up of leaves, followed by 1-35% (stem), 0-17% (inflorescence), and 0% (dead material). However, as the plant grows older (78 DAS), the presence of inflorescence surpassed the leaves, stem, and dead material, achieving a 11.6 g plant^{-1} maximum yield, at 120 DAS. This yield accounts for a 99% contribution and a null presence of the stem and leaves, and just 1% dead material (Figure 3). The yield and contribution average of the components showed an inflorescence, leaf, stem, and dead material trends of 86, 10, 3, and 1, respectively. Consequently, as the plants grew older, significant changes were registered in the morphological components of the different strata (Figure 3). In their study, Ramírez-Meléndez *et al.* (2020) recorded differences in the dates and caryopsis of the morphological components, at 56, 67, 81, and 96 DAS, particularly a large caryopsis (0.0005-0.0010 g) for bigger leaves and roots, as well as an increase in total yield. For their part, Morales-Nieto *et al.* (2008) described the morphology of the native *banderita* grass populations and found that the foraging potential of this species is related to its stem production.

Leaf area

There were no differences regarding the leaf area per plant strata between the 22 and 36 DAS ($p \geq 0.05$), because the number of leaves (0.01-0.90 g plant^{-1}) was similar (Figure 3), while the leaf area values fluctuated between 1.9 and 15.9 cm^2 plant^{-1} (Figure 4). From 50 to 120 DAS, the values were higher in the MS, reaching a maximum leaf area of 567.6 cm^2 plant^{-1} at 78 DAS. The greatest total leaf area recorded at 92 DAS was 1,082.2 cm^2 (8.8 g plant^{-1}). There were no statistical differences between BS and MS ($p>0.05$): 191 and 216 cm^2 plant^{-1} were recorded for BS and MS, respectively. Meanwhile, AS registered

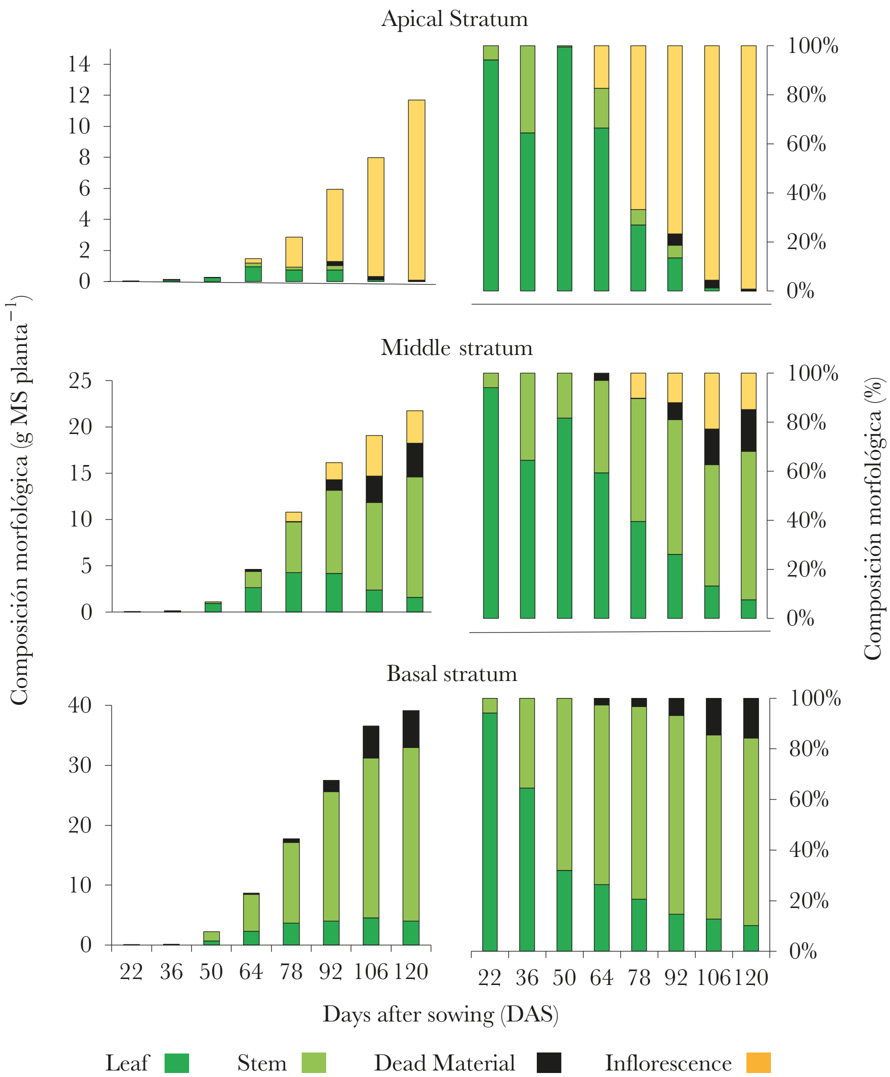

Figure 3. Morphological composition (gr MS plant^{-1}) and percentages of *banderita* grass [*Bouteloua curtipendula* (Michx) Torr] using different plant strata and DAS, during the spring-summer cycle.

a lower value (47 cm^2 plant^{-1}). This difference may explain the low number of leaves on the AS, which is mostly occupied by inflorescence (90%). Pérez-Amaro *et al.* (2004) recorded LA four weeks after the plants were established (28 DAS). For their part, Fagundes *et al.* (2001) recorded leaf area values that fluctuated between 0.21 and 3.7 cm^2, at plant heights between 5 and 20 cm. Meanwhile, Velazco *et al.* (2001) mentioned that a larger leaf area is recorded in summer, when plants experience better growth conditions.

Plant height

The PH of the *banderita* grass (*Bouteluoa curtipendula*) increased as the plant grew older (Figure 5). The maximum PH was 93 (106 DAS) and 96 (120 DAS) ($p>0.05$); therefore, the plant strata under study had a 37.6 cm average length. At 22 and 36 DAS, with a lower height (PH: 13 and 21 cm), the plant strata were established between 4.3 and 7.0

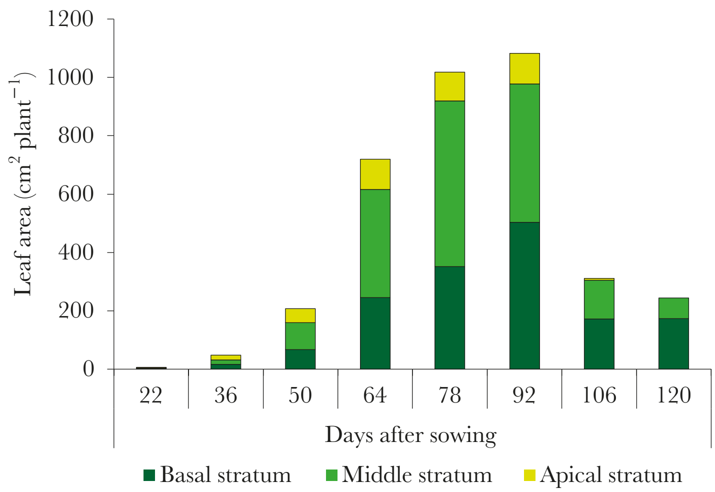

Figure 4. Leaf area (cm^2) of *banderita* grass, harvested at different DAS, in three plant strata, during the spring-summer growing cycle.

cm, showing that the changes in the morphological components are related to plant height at an older age. More leaves can be found in the lower strata of younger plants, while inflorescence prevails in the upper strata of older plants (Figure 3). Morales-Nieto *et al.* (2008) analyzed the main components of the plant and they found that the foraging potential of *banderita* grass (*B. cutipendula*) is one of the main variables that determine plant height. This result accounts for 63.32% of the variation between the studied ecotypes. In addition, heights between 29.9 and 50.0 cm were reported for the Coahuila ecotypes. Meanwhile, Morales-Nieto *et al.* (2016) carried out a morphological and molecular characterization of *banderita* grass populations and reported that plant height varied between 40 and 104 cm.

Leaf:stem ratio

Although the leaft:stem ratio recorded a decreasing trend (L:SR) (Figure 6), no values were recorded in the first cut, as a result of the minimum weight of the stem —resulting

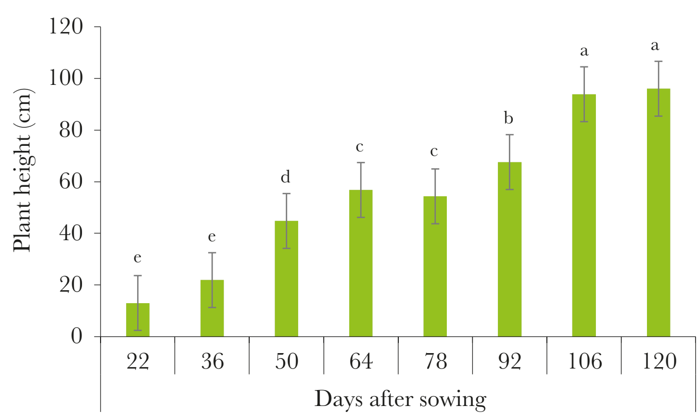

Figure 5. Plant height of *banderita* grass [*Bouteloua curtipendula* (Michx.) Torr.], during the spring-summer season (SSS), at different days after the sowing. Similar letters on top of the bars are not statistically different (Tukey; $\alpha > 0.05$).

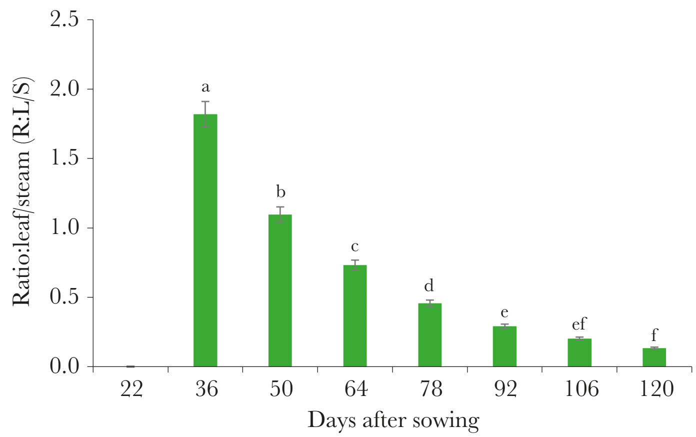

Figure 6. Leaf:stem ratio of *banderita* grass [*Bouteloua curtipendula* (Michx.) Torr.], during the spring-summer season. * L:SR was not estimated at 22 DAS, due to the lack of stems. Similar letters on top of the bars are not statistically different (Tukey; α=0.05).

from the morphological composition of this phenological stage. The ratio recorded its maximum value ($p<0.05$) at 36 DAS (1.82), while the lowest values were obtained at 92, 106, and 120 DAS (0.29, 0.20, and 0.13, respectively), without statistical differences ($p<0.05$) and a 0.59 average value. Fernández *et al.* (2012) mention that, as the plant grows older, the number of stems increases during the days following the sowing or the regrowth, while the number of leaves decreases. Consequently, establishing the optimal harvest moment is fundamental. In this regard, Pérez *et al.* (2002) recorded values between 1.1 and 1.4, which are similar to the findings of this study. Meanwhile, Madera *et al.* (2013) analyzed elephant grass (*Pennisetum purpureum* L.) and recorded values between 1.24 (at 45 DAS) and 0.39 cm^2 (at 120 DAS). These results indicate that the age of the plant has a negative impact on the leaf area and decreases the senescence of the leaf.

Aerial part: root ratio

Regarding the ratio of the biomass produced, the aerial part recorded a higher ($p<0.05$) average value (2.5) than the roots (Figure 7). The lowest value (1.2) was obtained at 22 DAS; this result was statistically different to the values recorded for the other ages of the plant under study. As the plant grew older, the aerial part:root ratio directly increased regarding DAS, reaching a 3.8 maximum value at 92 DAS. These results showed no statistical difference at 106 (3.2) and 120 (3.5) DAS ($p\geq0.05$). Dalrymple and Dwyer (1967) studied the growth of grasses and reported values of 4.92; these results are similar to the values obtained in this study. The lowest SSS values were 1.28, 1.35, and 1.60, at 22, 36, and 50 DAS, respectively. The aerial part registered a greater weight than the root. For their part, Rincón-Carruyo *et al.* (1997) registered 2.44 and 1.86 values for Buffel grass (*Pennisetum ciliaris* (L.) link) grown at an average temperature of 28 °C. Finally, Melgoza *et al.* (2014) recorded a 1.13:1.9 ratio in rose natal grass (*Melinis repens*).

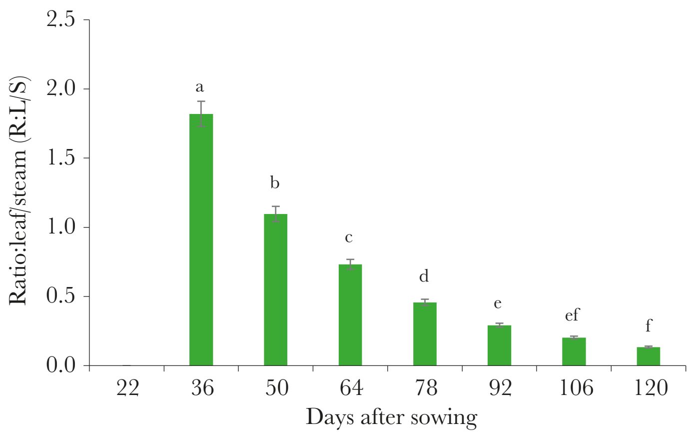

Figure 7. Aerial part:root ratio of the *banderita* grass [*Bouteloua curtipendula* (Michx) Torr], during the spring-summer season, at different DAS. Similar letters on top of the bars are not statistically different (Tukey; α= 0.05).

CONCLUSIONS

During the spring-summer growing cycle, the highest dry matter yield was recorded in the basal stratum of the *banderita* grass (*Bouteloua curtipendula*) plant. Stem made the highest contribution, followed by the leaves, the inflorescence, and the dead material. Nevertheless, the greatest leaf area was registered in the middle stratum of the plant, followed by the basal stratum, and the apical stratum. Height plant and aerial part:root ratio increased as the plant grew older, while the leaf:stem ratio decreased during the days after the sowing.

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Colegio de Postgraduados

Corn kernel and corn fodder yield in four maize varieties in the humid tropics of Mexico

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ABSTRACT
In southeastern Mexico, maize is planted for its corn kernel. Additionally, its bracts are used for *tamales* and handicrafts, its cobs are used as vegetables for human consumption, and its fodder is used to feed cattle.
Objective: To evaluate the growth and yield of maize planted for corn kernel and fodder production in Loma Bonita, Oaxaca, Mexico.
Design/Methodology/Approach: The DK7500, H-520, A7573, and VS-536 maize genotypes were used as treatments for the production of corn kernel and fodder, using a randomized blocks design with three replications. Several variables were measured: plant height, stem diameter, chlorophyll, leaf length, leaf width, and leaf area. Finally, the corn kernel and fodder yields were estimated (kg ha^{-1}) at the time of harvest.
Results: The genotypes under study showed significant differences ($P \leq 0.05$) in plant height, stem diameter, leaf length, leaf width, and leaf area. The A7573 genotype recorded the highest corn kernel yield (20,409 kg ha^{-1}), while fodder yield was statistically the same in the four genotypes.
Study Limitations/Implications: An analysis of different environments in a multi-year period would help to verify the information obtained.
Findings/Conclusions: A7573 maize had the highest corn kernel yield (20,409 kg ha^{-1}): 15.5% higher than that of H-520 and 12.5% higher than VS-536 (control). Fodder yield was statistically similar between genotypes, ranging from 40,529 kg ha^{-1} (H-520) to 42,104 kg ha^{-1} (VS-536).

Keywords: *Zea mays* L., Poaceae, Papaloapan area.

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INTRODUCTION

Maize (*Zea mays* L.) has been planted for thousands of years. Maize remains were found at the Guilá Naquitz caves in Oaxaca, Mexico, dating back 5,400 years (Serratos, 2009). This Poaceae has provided various civilizations with grain, leaves (bracts) for *tamales*, and fodder to feed cattle. Moreover, its kernels can be consumed in soups, roasted, boiled, or used to make bread (Rojas-Polanco *et al.*, 2022).

In 2021, 68,937 ha of maize were sown in Mexico to produce corn kernel, generating 1,059,260 t (average yield: 15.37 t ha^{-1}). The main producing states were Puebla (15,714 ha), Morelos (9,789 ha), San Luís Potosí (8,906 ha), Jalisco (7,999 ha), and the State of

Mexico (4,973 ha) (SIAP, 2023). Corn kernels are used as a fresh vegetable in their milk stage, which occurs 20 to 22 days after pollination (Mehta *et al.*, 2020). The grains contain 18-20% carbohydrates, 5-6% sugars, and 2.1-4.5% proteins; additionally, they have a $<$70% moisture content. The remaining fodder is used to feed livestock (Revilla *et al.*, 2021).

Ortiz-Torres *et al.* (2013) determined the yield and quality of corn kernel production in Tehuacán Puebla, Mexico. Their study determined that the corn kernel yield of the TEH77 maize variety, reached 9,576 kg ha^{-1}, with 11.3 °Brix and 30.7 grains per row. TEH77 also stood out in cob length and width.

Another study conducted in Chiapas, Mexico, found that the plants of native varieties were late grower, reaching a notable height and producing longer and wider corn ears than the control hybrids (Coutiño *et al.*, 2015). Some attributes to consider in corn kernel production include: sweet flavor (up to 13.8 °Brix), associated with the starch content of the endosperm; outstandingly long corn ears and adequate sanitary appearance, with no physical damage; larger, sweeter pieces of corn which consumers are willing to pay a premium for; and, preferably, an organic production system (Fernández-González *et al.*, 2018; Rojas-Polanco *et al.*, 2022).

In Loma Bonita, Oaxaca, 1,384 ha are sown with maize for corn kernel production (SIAP, 2023). These plants are commercial hybrids or native varieties with white or colored (yellow, red, or blue) grains. Producers frequently sell large corn ears by the piece or the ton and use the leftover plants as fertilizer, stubble, or silo material, to help their livestock endure the dry season. The objective of this research was to conduct an analysis of the growth and yield of commercial maize used to produce corn kernel and fodder in Loma Bonita, Oaxaca, Mexico.

MATERIALS AND METHODS

Location of the study site

This research was conducted at the Universidad del Papaloapan, in Loma Bonita, Oaxaca, Mexico (18° 06' N, 95° 52' W, and 25 m.a.s.l.). The climate is warm and humid, with summer rains, an annual precipitation of 1,845 mm, and an average temperature of 24.7 °C (INEGI, 2005).

Land preparation, treatments, and experimental design

Land preparation consisted of fallowing, harrowing (twice), and plowing. Subsequently, the DK7500, A7573, VS-536, and H-520 maize genotypes sown were and used as treatments in a random block design with three replications. The experimental unit (EU=16 m^2) consisted of five 4-m long rows. The separation between rows was 0.8 m and between plants 0.2 m, resulting in a 62,500-plants ha^{-1} density.

General crop management

Weeds were controlled by hand and the crop was fertilized with two applications of the 120-60-20 NPK formula: the first on day 20 and the second on day 45. Foliar fertilizer (Bayfolán forte, 0.75 L ha^{-1}) was sprinkled weekly to correct microelement deficiencies. Chlorpyrifos ethyl (0.75 L ha^{-1}) was used to control fall armyworms.

Variables under study

Plant height (Ph, cm) was measured weekly in five plants per EU, from the base of the plant to the growth point. A digital vernier was used to measure stem diameter (Std, cm). Leaf chlorophyll (Chlo) was measured in SPAD units in the foliar laminae of the intermediate leaves of the plant using the Minolta® SPAD-502 chlorophyll determinant. The following variables were measured: number of total leaves per plant (Tol), leaf length (Ll, cm) and leaf width (Lw, cm). The leaf area per plant (La, cm^2) was calculated with the following formula: $La=Ll \times Lw \times 0.75$. After the morphological components of the plant were separated, an Ohaus Scout SPX2201 digital scale (2,200$\pm$0.1 g) was used to calculate the fodder yield (Foy, kg ha^{-1}) and the corn kernel yield, including bracts (Cly, kg ha^{-1}).

Statistical analysis

A variance analysis was conducted using Proc ANOVA and the means were compared with Tukey's test ($P \leq 0.05$). The SAS statistical package (SAS, 2013) was used in both instances.

RESULTS AND DISCUSSION

Growth characterization

As a result of the accelerated growth, A7573 and DK7500 recorded a much greater plant height (Ph) than VS-536, during the first stages of development and until the third sampling (Figure 1A). However, no Ph differences were recorded from the fourth to the eighth sampling. In the last three stages, A7573 recorded the lowest plant height (180 cm) among all the other genotypes. From November 2022 to February 2023, rainfall levels were low. These conditions may have affected the growth of A7573 plants, since this maize variety blooms early (at 52 d), while VS-536 blooms at 58 days, with a Ph of 201 cm (Estrada, 1998). Sierra-Macías *et al.* (2010) recorded a Ph of 229 cm and 234 cm for A7573 and VS-536, respectively, in the state of Veracruz, Mexico. The disparities between both studies can be explained by time of the year in which each study took place: spring-summer (Sierra-Macías) and autumn-winter (this study). In Campeche, Mexico, A7573 reached a Ph of 183 to 221.5 cm, which Rivera-Hernández *et al.* (2009) attributed to changes in the soil water regime. This information does not contradict prior findings.

Stem diameter (Std) present statistical differences ($P \leq 0.05$) between treatments on sampling dates 1 to 11, except on sampling dates 3, 5, and 10 (Figure 1B). From the beginning until the eighth sampling, stems were thicker in DK7500 (2.6 cm) and A7573 (2.56 cm). These figures are similar to those recorded by Rivera-Hernández *et al.* (2009), who reported a Std of 2.7 cm in A7573 maize.

The number of leaves per plant did not vary between genotypes ($P > 0.05$) (Figure 1C), except at 56 and 73 d. When kernels develop an important demand for photoassimilates, leaves specialize in the capture of photosynthetically active light, which is crucial for the CO_2 fixation process (Taiz *et al.*, 2017). This phenomenon explains increases in leaves per plant. Throughout the samplings, the length and width of the leaves were always different ($P \leq 0.05$) for each of the genotypes (Figure 2A and 2B). Given its precocity, the Ll and Lw of A7573 were statistically lower ($P \leq 0.05$) than the remaining genotypes in the last two

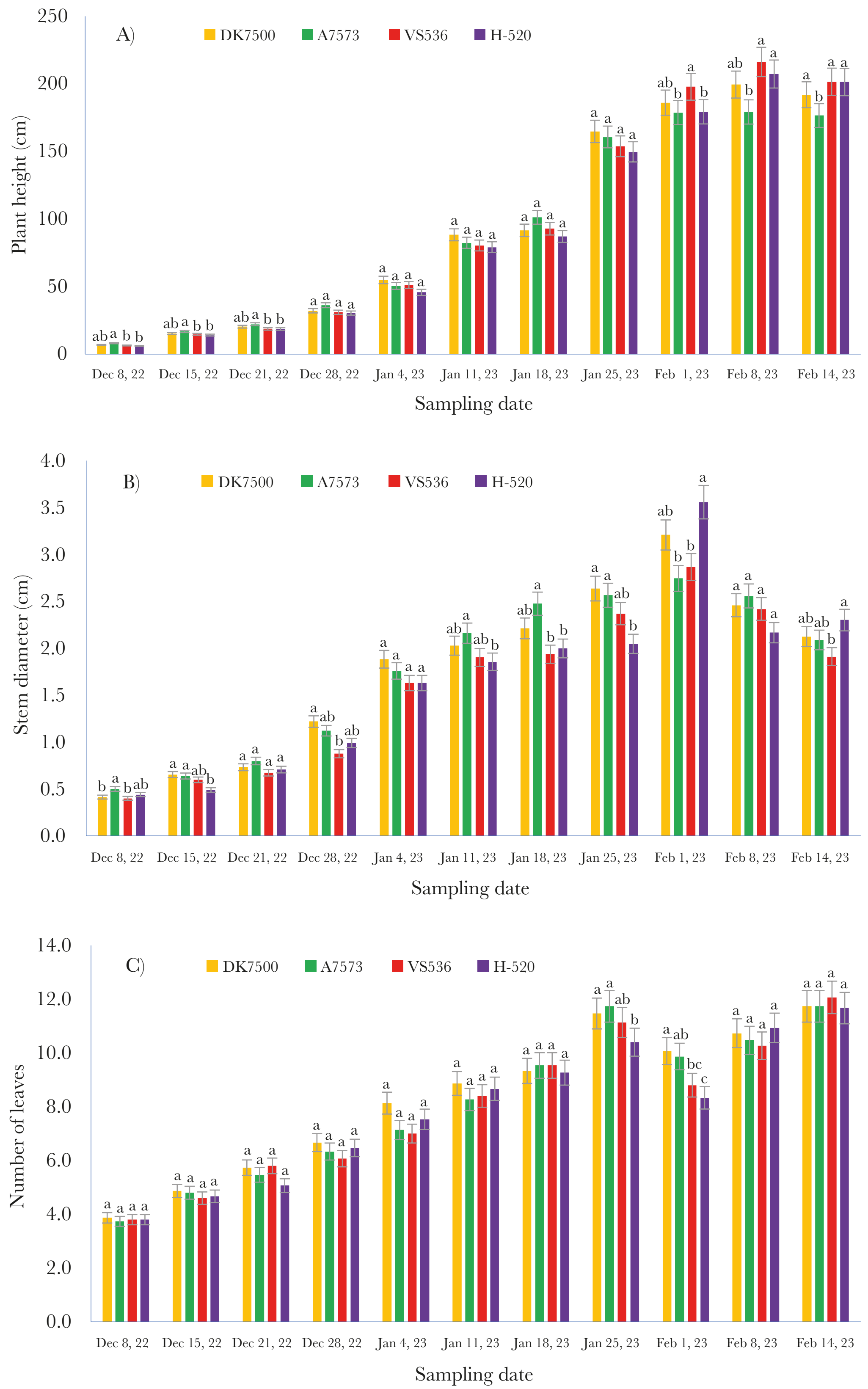

Figure 1. A) Plant height (cm), B) stem diameter (cm), and C) number of leaves in DK7500, A7573, VS-536, and H-520 maize varieties.

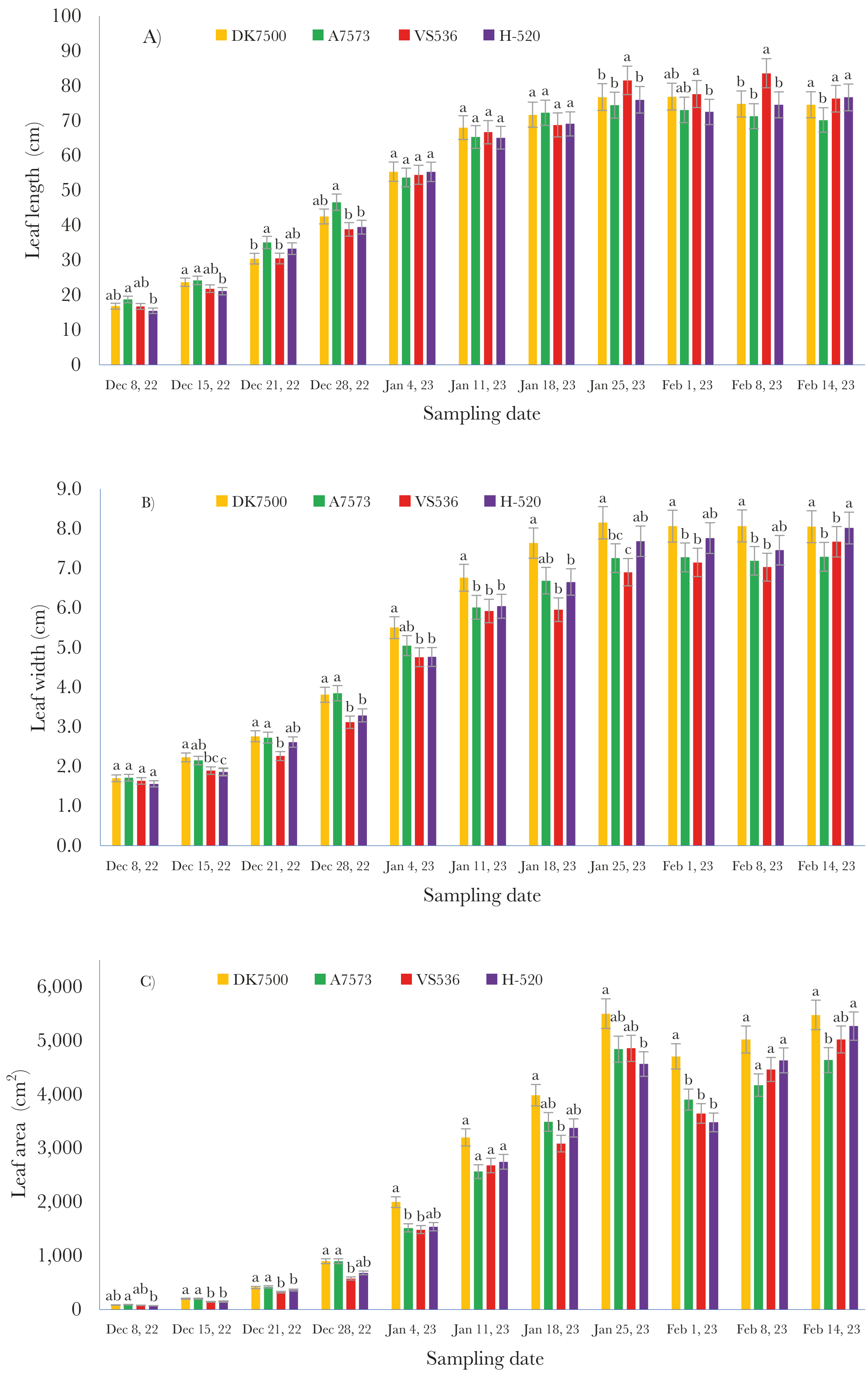

Figure 2. A) Leaf length (cm), B) leaf width (cm), and C) leaf area (cm^2) in DK7500, A7573, VS-536, and H-520 maize varieties.

samplings. Additionally, Ll and Lw were helpful to calculate the leaf area per plant, which is essential to determine important dry matter values. Leaf area per plant was higher in DK7500 (Figure 2C). However, on the last sampling date, the said genotype reached a La of 5,479 cm^2, a statistically similar result to that of H-520 and VS-536.

In the final stages, A7573 had a reduced leaf area (4,638 cm^2); these results matched the minimum leaf width and length values for this genotype. The control variety (VS-536) reached a La of 5,022 cm^2, which is similar to the results reported by Sánchez *et al.* (2021), for VS-536: a La of 4,510 cm^2 at 85 days, in maize apt for fodder production. Chlorophyll in leaves varied significantly ($P \leq 0.05$) in the first four sampling dates and in the ninth and tenth sampling dates (Figure 3). It was lower in H-520 in the first two samplings, since this hybrid maize could require more nitrogen.

In samplings five to eight (35 to 56 days) and the last sampling, there were no variations in chlorophyll between genotypes; during this vegetative stage, values of 52.9 to 54.9 SPAD units were recorded (Figure 3). Rincón and Ligarreto (2010) indicated that chlorophyll in maize ranges from 50 to 54 SPAD units. They also stated that, to obtain adequate yields in corn, chlorophyll in leaves must exceed 50 SPAD units.

The highest corn kernel yield occurred in A7573 (20,409 kg ha^{-1}), while H-520 showed the lowest production (17,233 kg ha^{-1}) (Figure 4). In a study conducted in Campeche, Mexico, Rivera-Hernández *et al.* (2009) registered a corn kernel yield of 16,680 kg ha^{-1} in A7573 maize, which was 18.3% lower than the result obtained in the present study.

Andrés-Meza *et al.* (2017) evaluated corn hybrids with corn kernel production potential in Amatlán de los Reyes, Veracruz, during spring and summer, and reported a corn kernel yield of 14,909 kg ha^{-1} in A7573 maize. These findings corroborate the results obtained in this research.

However, fodder yield was statistically the same in the four genotypes. H-520 recorded 40,529 kg ha^{-1}, while the value of VS-536 (the control variety) rose to 42,104 kg ha^{-1}. On this matter, Sánchez *et al.* (2019) estimated the fodder yield of maize in Loma Bonita,

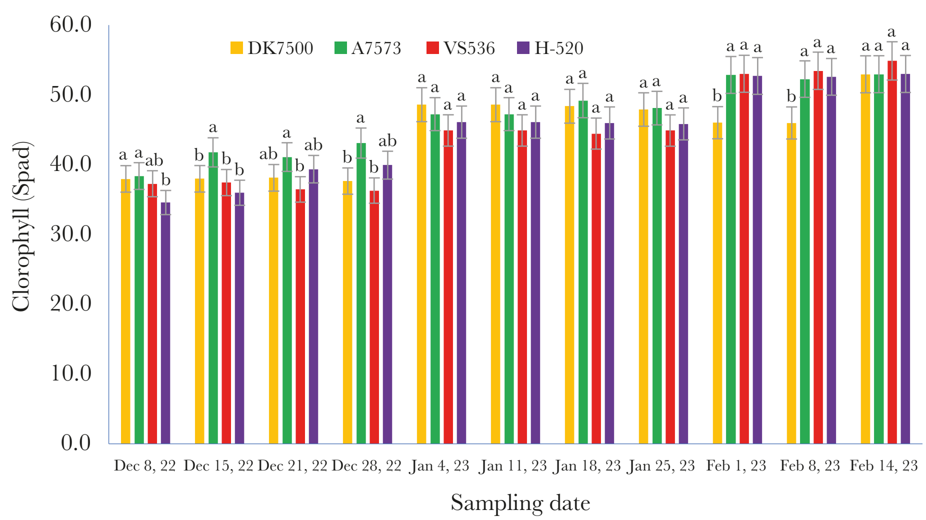

Figure 3. Chlorophyll in leaves (SPAD Units) in the DK7500, A7573, VS-536, and H-520 maize varieties.

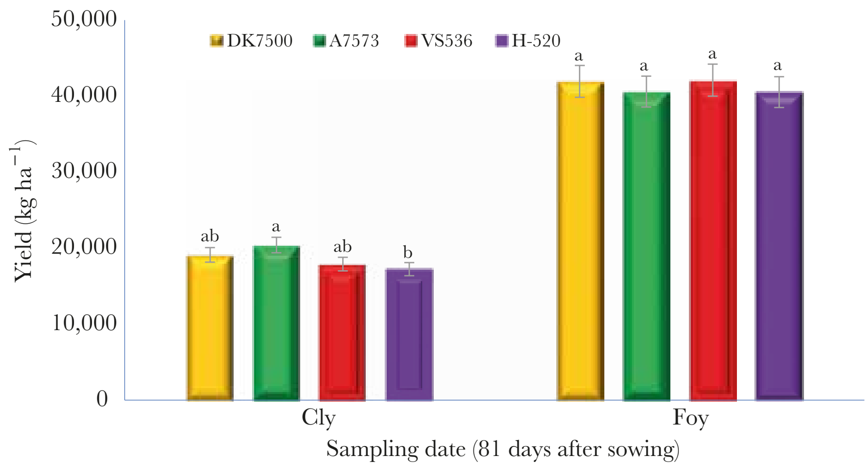

Figure 4. Corn kernel with leaf yield (Cly, kg ha^{-1}) and fodder yield (Foy, kg ha^{-1}) in the DK7500, A7573, VS-536, and H-520 maize varieties.

Oaxaca, and found that VS-536 produced 38,800 kg ha^{-1} of green fodder, while the H-520 hybrid produced only 33,300 kg ha^{-1}. Compared to the results of this study, these figures are 7.8% and 17.8% lower for VS-536 and H-520, respectively. Therefore, the maize varieties under study have the potential to produce corn kernels and fodder.

CONCLUSIONS

The A7573 maize variety produced the highest corn kernel yield (20,409 kg ha^{-1}). However, green fodder yield was similar for all genotypes, ranging from 40,529 kg ha^{-1} (H-520) to 42,104 kg ha^{-1} (VS-536). In its initial growth stages, DK7500 maize stood out regarding plant height, stem diameter, leaves per plant, leaf length, leaf width, and leaf area.

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Colegio de Postgraduados

Carcass yield and primal cuts of lambs fed on different diets in the humid tropics

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ABSTRACT

In the humid tropics of Mexico, sheep production is an important economic activity, mainly focused on the sale of barbacoa and barbacoa tacos; however, no primal cuts of lambs are offered.

Objective: To assess carcass yield and primal cuts of lambs fed on different diets in Loma Bonita, Oaxaca, Mexico.

Design/Methodology/Approach: Five diets were assessed to measure carcass yield and primal lamb cuts: corn stover, corn silage, Chinese hibiscus (locally known as *tulipán*), cracked corn, and grazing. A completely randomized experimental design was used. The following variables were measured: hot and cold carcass weight, hot and cold carcass yield, neck weight, leg weight, shoulder weight, loin weight, rib weight, rib eye area determination, and fat thickness.

Results: Lambs fed on cracked corn achieved a higher carcass yield (44.2%) than the other treatments ($P \leq 0.05$), a leg weight of 3.87 kg, and loins 2.57 kg heavier than the other treatments, in average ($P \leq 0.05$).

Study Limitations/Implications: It was difficult to obtain homogeneous groups of lambs in terms of age and weight.

Findings/Conclusions: Lambs fed on cracked corn recorded higher carcass yields and better leg and loin primal cuts than lambs fed on the other diets.

Keywords: Lamb meat, sheep feed, tropical region.

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INTRODUCTION

In 2008, world sheep population consisted of 1.209 billion individuals and the sheep industry generated 9.78 million tons of meat (FAO, 2020). In Mexico, sheep inventory is made up of 8.7 million heads, with a production of 65,846 t of meat in 2021. The states of Mexico, Hidalgo, Veracruz, Jalisco, Puebla, and Zacatecas account for 54.2% of the sheep meat produced (SIAP, 2020).

Sheep meat is rich in protein (19.1%), fat (22.0%), ash (0.88%) (Du *et al.*, 2019), iron, zinc, selenium, vitamins A, B3, and B12, and folic acid (de Andrade *et al.*, 2016).

Commercial carcass weight in Portugal, Italy, and Spain is 8, 9, and 11 kg, respectively, while in Northern Europe carcass weight ranges from 16 to 23 kg (Vnučec *et al.*, 2014).

In Mexico, approximately 800 g of lamb meat are consumed. In the central zone of the country, 85% of the sheep meat is consumed as barbacoa (90%) and the remaining 10% is prepared *al pastor* and *al ataúd*, served as *mixiotes* and lamb *birria*, and used to substitute roast goat kid (as *cordero lechal* and *cordero*), as well as sold as fine lamb cuts (Partida *et al.*, 2013, Espinoza-Marín *et al.*, 2017).

Given its high quality, consumers consider sheep meat as a luxury product with great nutritional attributes (Sañudo, 2006) and to date there has been an increased interest in lean lamb cuts (Anderson *et al.*, 2015).

The objective of this study was to determine the carcass yield and primal cuts of lambs fed on diets based on corn stover, corn silage, Chinese hibiscus (*Hibiscus rosa sinensis*), cracked corn, and African star grass in Loma Bonita, Oaxaca, Mexico.

MATERIALS AND METHODS

Location of the study site

The work was carried out at the Universidad del Papaloapan, in the municipality of Loma Bonita, Oaxaca, Mexico. Its geographical coordinates are 18° 06' N and 95° 52' W and it is located at 25 m.a.s.l. It has a warm humid climate, with rainfall in summer, and an average temperature of 24.7 °C (INEGI, 2005).

Treatments and experimental design

Forty male lambs with 18±2 kg live weight were used and the following diets were tested: 1) Diet 1 with 38% corn stover, 37% star grass (SG), 19% soybean paste (SP), 5% molasses (MO), and 1% mineral salts (MS), 16.2% crude protein (CP), and 2.3 Mcal kg^{-1} MS^{-1}; 2) Diet 2 with 41% corn silage, 35% SG, 18% SP, 5% MO, 1% MS, 16.7% CP, and 2.5 Mcal kg^{-1} MS^{-1}; 3) Diet 3 with 26% *Hibiscus rosa sinensis*, 59% SG, 9% SP, 5% MO, 1% MS, 16.7% CP, and 2.6 Mcal kg^{-1} DM^{-1}; 4) Diet 4 with 44% cracked corn grain, 32% SG, 18% SP, 5% MO, 1% MS, 16.8% CP, and 2.8 Mcal kg^{-1} MS^{-1}; and 5) Diet 5 with grazing (*Cynodon nlemfuensis*) and commercial concentrate (15% protein). Lambs were fed: 1) reception diet for 7 days, consisting of African star grass forage; 2) adaptation diet for 15 d; and 3) fattening diet. Finished lambs (90 d) were transferred to the laboratory where they were weighed and slaughtered, recording a LW of 30 kg and a ≥3 body condition, according to the Body Condition Scaling developed by Russell (1969), which ranges from 1 (thin) to 5 (overweight).

Variables under study

Hot carcass weight (kg) is the quantity of carcass after slaughter and skinning. Cold carcass weight (kg) is the quantity of carcass preserved at 4 °C for 24 h. Hot carcass yield (%) is the proportion of the animal's carcass, expressed as the ratio of hot carcass weight (HCW) to live weight (LW) at slaughter ($HCW = HCW/LW \times 100$). To determine cold carcass yield (%), the carcass was refrigerated at 4 °C and the weights obtained 24 h post-mortem were recorded, and the yield was calculated as the difference between the hot carcass yield and the cold carcass yield. Neck weight (kg) was determined separating the head at the atlanto-occipital joint to the 7th cervical vertebra. The weight of the rib (kg) was established sectioning from the 1st to the 12th thoracic vertebrae. Loin weight (kg) was determined making a section from the 12th and 13th thoracic vertebrae to the 6th and 7th lumbar vertebrae. The front leg was weighed (kg) separating the scapula from the thorax up to the carpal-metacarpal joint. Leg weight (kg) was determined by a cut from the pelvic joint to the edge of the tarsometatarsal joint. The rib eye area (*Longissimus dorsi* muscle, between the 12th and 13th ribs) was outlined on acetate and the area (cm^2) was calculated with AutoCAD®. Finally, fat thickness was measured in mm at the height of the 13th thoracic vertebra.

Statistical analysis of the information

The experimental design was completely randomized. The information was subjected to an analysis of variance, while the variables that showed statistical significance were subject to a comparison of means (Tukey; $P \leq 0.05$), using the statistical package SAS 9.1 (SAS Institute, 2013).

RESULTS AND DISCUSSION

Hot carcass weight

Hot carcass weight (16.7 kg) was higher with the cracked corn-based diet than with the other treatments ($P \leq 0.05$; Table 1), because corn (3.7 Mcal kg^{-1} DM^{-1}) contains 7.3% protein, 3.3% fat, and 63.8% starch (FEDNA, 2016), which promote muscle formation and fat deposition, generating lambs with greater live weight (kg) and body size.

With corn silage and *Hibiscus*, hot carcass weights were similar to those reported by García *et al*. (1998) for Pelibuey sheep fed on Rye grass and oat straw (hot carcass weight: 10.5 kg). Hernández-Montiel *et al*. (2016) fed lambs on *Canavalia* seeds and quantified

Table 1. Carcass yield of Pelibuey lambs fed on different diets.

Diet	LWS (kg)	HCW (kg)	CCW (kg)	CCY (%)	Diference
Corn stubble	27.0c[†]	10.4c	10.1c	38.6b	6.9c
Corn silage	28.1bc	10.9c	10.5c	38.7b	7.5c
Tulip (*Hibiscus*)	27.9c	10.6c	10.0c	38.0b	7.4c
Cracked corn	37.7a	16.7a	16.5a	44.2a	17.7a
Crazing	30.6b	12.0b	11.8b	40.0b	10.5b
Mean	30.3	12.1	11.8	40.0	10.0
HSD	2.7	0.9	0.8	3.3	2.7
CV (%)	4.1	3.5	3.2	3.8	12.5

LWS: live weight at slaughter (kg); HCW: hot carcass weight (kg); CCW: cold carcass weight (kg); CCY: cold carcass yield (%). "Diferencia" refers to the difference between initial weight and the weight of lambs at slaughter. [†]a, b, c: literals in columns indicate a statistically significant difference ($P \leq 0.05$). HSD: Tukey's honestly significant difference test ($P \leq 0.05$); CV: Coefficient of variation (%).

12.4 kg in hot carcass weight, a similar result to the one obtained in this work with the grazing diet (12.0 kg) (Table 1).

Cold carcass weight

Cold carcass weight (16.5 kg) of lambs fed on cracked corn is similar to the results of the study conducted with Pelibuey lambs (16.6 kg) in a hot dry climate by Macías-Cruz *et al.* (2010). However, the cold carcass weight of lambs provided with corn silage and grazing diet had values of 10.5 kg and 11.8 kg, respectively. This result was in line with the results of Hernández-Montiel *et al.* (2016) who reported a 12.1 kg weight for the cold carcass weight of sheep fed on *Canavalia*.

In this trial, cold carcasses weighted 10.1 kg and 10.0 kg for the corn stover and *Hibiscus* diets, respectively (Table 1). This result is attributed to the varied energy content of the diets; therefore, the animals were smaller and lighter at the time of slaughter.

Cold carcass yield

There were significant differences among treatments. Cracked corn diet provided to sheep showed the highest carcass yield (44.2%), while, in the other treatments, it ranged from 38.0 to 40.0% (Table 1), possibly as a result of the higher live weight at the time of slaughter. Similar results were obtained by Partida and Martinez *et al.* (2010), who reported a carcass yield of 41.1% in Pelibuey sheep fed on sorghum, soybean, and corn silage, and 30 kg at slaughter. Frías *et al.* (2011) recorded a meat yield of 42.0% in Pelibuey sheep. Carcass yield in this study was attributed to differences in the energy content of the diets, which was reflected in the productive behavior and; therefore, affected carcass yields.

Yield of primal cuts of sheep meat

Neck weight

Neck weight was not significant between treatments (Table 2).

Table 2. Yield of primal cuts of sheep fed different diets in Loma Bonita, Oaxaca, Mexico.

Diet	Neck (kg)	Rib (kg)	Loin (kg)	Foreshank (kg)	Leg (kg)	REA cm^2	BFT (mm)
Corn stubble	0.79a[†]	1.47ab	0.71c	1.13c	1.24c	14.7bc	0.3a
Corn silage	1.31a	1.65a	0.84b	1.15c	1.39b	14.2bc	1.2a
Hibiscus	0.77a	1.49ab	0.73c	1.20b	1.24c	12.3c	0.3a
Cracked corn	1.04a	1.72a	2.57a	1.75a	2.18a	17.2a	1.3a
Crazing	0.84a	1.20b	0.78b	1.19b	1.35b	15.3b	0.5a
Mean	0.95	1.51	1.13	1.28	1.48	14.7	0.7
HSD	0.55	0.29	0.10	0.03	0.07	0.9	1.2
CV (%)	26.9	9.0	4.1	1.3	2.3	8.3	26.4

CV=coefficient of variation (%). HSD=Tukey's honestly significant difference ($P \leq 0.05$). [†]a, b, c: literals in columns indicate a statistically significant difference ($P \leq 0.05$). REA=Rib eye area (cm^2); BFT=back fat thickness (mm) in the *Longissimus dorsi* muscle.

Rib weight

There were significant differences in rib weight between diets: control had the lowest value, while corn silage and cracked corn resulted in the highest rib weights (Table 2). Estrada *et al.* (2012) calculated 1.6 kg in rib weight when whole sorghum was offered to sheep. Ríos *et al.* (2012) recorded a rib weight of 2.9 kg when Pelibuey × Katahdin sheep were fed on waste chickpeas. Rib weight differences in this trial are related to the slaughter weight of the lambs (Table 2).

Loin weight

There were differences in loin weight between treatments (Table 2): it reached 2.57 kg with cracked corn, while it ranged from 0.71 to 0.84 kg with diets prepared with corn stover, corn silage, *Hibiscus* and the control. These results are explained by the different weight of the animals. Estrada *et al.* (2012) reported a loin weight of 1.21 kg in Pelibuey × Dorper lambs. The results of the cracked corn diet matched the findings of Magaña-Monforte *et al.* (2015) who reported a loin weight of 2.61 kg in Pelibuey × Katahdin sheep. A corn grain diet increased the size of the sheep, resulting in greater loin weight. Corn provided energy that was converted into muscle; additionally, animals expend less energy under stabling conditions.

Shoulder weight

Significant differences were recorded between treatments regarding shoulder weight. Cracked corn grain diet produced 1.75 kg, a higher value than the other diets, whose shoulder weight values ranged from 1.13 to 1.20 kg. Ríos *et al.* (2012) reported a shoulder weight of 3.18 kg when feeding chickpea grains to sheep. These variations are caused by differences in dietary energy and body weight of sheep at slaughter.

Leg weight

There were significant differences between treatments. Cracked corn diet (2.18 kg) was superior ($P \leq 0.05$) to corn silage, grazing, *Hibiscus*, and corn stover, which ranged from 1.24 to 1.39 kg (Table 2). Estrada *et al.* (2012) reported a leg weight of 2.23 kg in Pelibuey lambs fed on sorghum —an equivalent value to that of the cracked corn diet. This suggests that cereals favor muscle conversion and fat deposition, resulting in lambs of greater size and live weight.

Rib eye area (REA)

REA reached 17.2 cm^2 and 12.3 cm^2 with cracked corn and *Hibiscus*, respectively ($P \leq 0.05$). Hernández-Espinoza *et al.* (2012) reported 14.2 cm^2 in Pelibuey lambs with REA; similar results were obtained with silage (14.2 cm^2) and corn stover (14.7 cm^2) (Table 2). A REA of 15.3 cm^2 was determined in the case of grazing lambs —a result equivalent to that of Ríos *et al.* (2012), who estimated a REA of 15.7 cm^2 in Pelibuey × Katahdin sheep. In this study, carcass size and weight modified the REA dimensions.

Back Fat Thickness (BFT)

No significant differences were recorded between diets. The highest BFT (1.3 mm) was obtained with cracked corn, while grazing recorded 0.5 mm. These results are in line with Hernández-Montiel *et al.* (2016), who obtained 0.7 mm of BFT for Pelibuey sheep.

CONCLUSIONS

A higher carcass yield was achieved with confined lambs fed on a cracked corn grain-based diet. Finally, including an appropriate feeding program in sheep production is a good alternative for obtaining value-added meat cuts, which will result in a higher economic income for the producers of Loma Bonita, Oaxaca, Mexico.

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Colegio de Postgraduados

Yield potential of onion genotypes in the Planicie Huasteca, Mexico

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ABSTRACT

Objective: To identify new generation onion genotypes with high yield per unit that meet the bulb quality characteristics required by the export market.

Design/Methodology/Approach: Eight onion hybrids with yellow bulb, six hybrids with white bulb, and three hybrids with purple bulb were evaluated. The experiments were established with drip irrigation and fertigation. The agronomic characteristics of the plant and the production and quality of the bulb were evaluated, using the USDA specifications for bulb width and shape. The experiments were carried out using a completely randomized block design, with three replications; the statistical analysis was performed using the SAS software, version 9.2.

Results: The best onion genetic materials for the Planicie Huasteca were the yellow bulb hybrids Wayne and Don Víctor, the white bulb hybrids Blanca Grande, Monja Blanca, and Don Alberto, and the purple bulb hybrid Rasta, all of which have high production capacity and bulb quality.

Study Limitations/Implications: Onion (*Allium cepa* L.) is the most extensive horticultural crop in the Planicie Huasteca, Mexico. Although regional producers establish outstanding genotypes, they require information about the new generation genetic materials, if they are to remain competitive in the export market. Therefore, the new genotypes with the best high yield potential and bulb quality must be evaluated to select those that meet the requirements of the market.

Findings/Conclusions: Yellow, white, and purple bulb onion genotypes with high production capacity that meet the requirements of the markets were identified.

Keywords: *Allium cepa* L., bulb quality, production, hybrids.

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INTRODUCTION

Onion (*Allium cepa* L.) is an important source of food, for both urban and rural populations (Shigyo and Kik, 2007). In addition, the production of this crop has economic value (Omoloso and Vagi, 2001; de la Fé and Cárdenas, 2014), since it is the second most widely cultivated vegetable worldwide, only surpassed by tomato (*Lycopersicon esculentum* Mill) (Financiera Nacional de Desarrollo Agropecuario, Rural, Forestal y Pesquero, 2014). The United States is the first global importer and Mexico is its main supplier (Gómez, 2010; Valencia and Zetina, 2017). In the Planicie Huasteca of northeastern

Mexico (northern Veracruz, eastern San Luis Potosí, and southern Tamaulipas), onion is the most extended vegetable; during the autumn-winter cycle, its growing area fluctuates between 4,000 and 9,000 hectares (SIAP, 2022). Onion production is mainly sold to the export market. Yellow onion is the most produced crop (70%), followed by white onion (25%), and purple or red onion (5%) (Mata *et al.*, 2011). Local producers unknowingly grow varieties or hybrids that may not be appropriate for their region and, sometimes, producers report substantial losses, because the crops experience severe disease problems or the bulbs have limited production potential and quality (Velásquez and Reveles, 2011; Velásquez *et al.*, 2013). Although the producers of the Planicie Huasteca establish outstanding genotypes, they require information about the new generation genetic materials, if they are to remain competitive in the export market. Consequently, institutions such as the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP) must support the identification of outstanding onion varieties or hybrids (with higher yields and bulb quality) that will be able to adapt to regional conditions. They should also make this information available to the producers. Therefore, the objective of this study was to identify new generation onion genotypes that can obtain high yield per unit and that meet the requirements of the export market.

MATERIALS AND METHODS

This research was carried out during the 2019-2020, 2020-2021, and 2021-2022 autumn-winter (A-W) cycles, at the INIFAP-Campo Experimental Las Huastecas, located in Villa Cuauhtémoc, Altamira Tamaulipas, Mexico (22° 34' 6" N, 98° 10' 5" W). Eight hybrids of yellow onion, six hybrids of white onion, and three hybrids of purple onion were evaluated, including the commercial hybrids used as control: Don Víctor (yellow onion), Carta Blanca (white onion), and Rasta (purple onion) (Table 1). The experiments were established with the dripping irrigation and fertigation technology developed by the INIFAP (Mata *et al.*, 2011). Eighty-five percent of the regional production in Mexico is sent to the United States; therefore, the agronomic characteristics and the bulb shape and quality were evaluated, based on the width, bulb shape, and quality classification specifications issued by the United States Department of Agriculture (USDA) (NOA, 2006; USDA, 2016). The genetic materials were established under open sky conditions and were divided into three groups, according to the color of the bulb (Table 1).

A completely randomized block design with three replicates was used for the field distribution. The experimental plot was made up of a 1.8 m wide × 5 m long bed and the harvest was carried out in 4 m at the center of the plot, resulting in a 7.2 m^2 useful area. Four rows of plants were established in each bed, with a plant density of 7 plants per lineal meter, resulting in 150,000 plants per hectare. The harvest started when the foliage bent over (an indication of the commercial maturity of the bulb). Based on the export standards, the following elements were recorded: days until harvest, total production, average bulb weight, exportation bulb, and predominant dimensions of the bulb (Asgrow, 2000; USDA, 2016). The combined statistical analysis of the three cycles of each variable was carried out using the SAS version 9.2 software (SAS, 2009).

Table 1. Onion genetic material evaluated under fertigation conditions in southern Tamaulipas at the INIFAP-Campo Experimental Las Huastecas (2019-2022).

Group by bulb color	Number	Genotype
Yellow	1	Hornet
	2	Plethrora
	3	Vulkana
	4	Dulciana
	5	Don Víctor (Control)
	6	Veronica
	7	Tusker
	8	Wayne
White	1	Don Alberto
	2	Florentina
	3	Blanca Montejo
	4	Carta Blanca (Control)
	6	Blanca Grande
Purple	1	Rasta (Control)
	2	Sofire
	3	Red Sensation

RESULTS AND DISCUSSION

Precocity at the time of the harvest. In the Planicie Huasteca, precocity is an important characteristic for onion cultivation, because an early harvest is generally associated with a good sale price, both in the domestic and export markets. There were significant differences ($P<0.05$) in the overall analysis of variance of the genotypes (Table 2). Regarding its commercial maturity, the Vulcana yellow onion was the most precocious cultivar, followed by Tusker, and the Don Victor hybrid (control); from the transplant to the moment when the foliage bend over, the process took 96, 101, and 134 days for each of these cultivars, respectively (Table 2). Meanwhile, the most precocity to harvest was the Blanca Montejo white onion hybrid, whose foliage bend over at 105 days. Meanwhile, there were no significant differences regarding purple onions ($P>0.05$) (Table 2). Amarananjundeswara *et al.* (2020) carried out a study in Karnataka, India, using 28 onion genotypes and determined that onions reached their physiological maturity between 80 and 128 days. The difference between both studies could be the consequence of the evaluated genotypes and the environmental conditions.

Bulb quality. Most of the evaluated materials are of excellent quality. There was a high uniformity in size and bulb color. Most of the genotypes recorded a >95% export quality production. Nevertheless, the Monja Blanca white onion, Plethrora yellow onion, and Sofire purple onion total production recorded an export quality of 94, 93, and 84%, respectively (Table 3). Regarding the shape of the bulb, all the materials mostly recorded flattened globe and globe shapes (Figure 1). The Verónica and Sofire hybrids had a round bulb, while Dulciana had a flat bulb (Table 3). This variable is fundamental, because 85% of the onion production is exported to the USA (TRADE MAP, 2022).

Table 2. Precocity (days at the start of the harvest) in yellow, white, and purple onions, under in southern Tamaulipas. INIFAP-Campo Experimental Las Huastecas (2019-2022).

Group by bulb color	Genotype	Days to harvest after transplanting			
		A-W 2019-2020	A-W 2020-2021	A-W 2021-2022	Average*
Yellow	Don Victor	138	134	129	134 a
	Wayne	137	116	131	128 ab
	Hornet	134	112	123	123 ab
	Verónica	130	116	115	120 ab
	Dulciana	123	118	114	118 abc
	Plethrora	123	110	108	114 bcd
	Tusker	98	100	104	101 cd
	Vulkana	92	100	97	96 d
White	Florentina	139	143	134	139 a
	Blanca Grande	139	147	123	136 a
	Don Alberto	139	139	124	134 a
	Carta Blanca	139	138	111	129 a
	Monja Blanca	126	114	123	121 ab
	Blanca Montejo	119	100	97	105 b
Purple	Rasta	145	147	115	136 a
	Red Sensation	129	146	127	134 a
	Sofire	131	110	110	117 a

*Amounts with the same letters are statistically equal (Tukey, $P<0.05$).

Production was classified by bulb size, according to the exports market quality standards (Asgrow, 2000; Gómez, 2010; USDA, 2016): 1) super-colossal (>10.8 cm width); 2) colossal (9.53-10.79 cm width); 3) jumbo (7.62-9.52 cm width); 4) medium (5.72-7.61 cm width); and 5) small (<2.54-5.71 cm width). Most yellow onions were super-colossal (or extra-large) and colossal, the sizes preferred by the export markets (Mata *et al.*, 2011), where northeastern Mexican yellow onion production is sold. The Wayne and Hornet hybrids recorded the biggest bulbs, with an average weight of 480 and 447 g, respectively (Table 3). Islam *et al.* (2007) reported a positive correlation with the fresh weight of the bulbs. White onions recorded mainly jumbo and colossal produce. The Blanca Grande genotype was the exception: it recorded a 455-g bulb average weight and was classified into the super colossal and colossal categories (Table 3). Purple onions had the smallest bulbs and were classified as medium, jumbo and, to a lesser degree, colossal. In statistical terms, the three evaluated materials were equal regarding average bulb weight ($P>0.05$) (Table 3). The preferred sizes of white and yellow onions are medium, jumbo, and colossal. Consequently, all the genetic materials had a high-quality exportation size, although the domestic Mexican market prefers jumbo- and medium-sized onions (Mata *et al.*, 2011).

Total production of commercial-quality bulbs

Significant differences ($P<0.05$) were recorded between the yield variables of the genotypes. The Wayne yellow onion hybrid recorded the highest yields, with an average

Table 3. Bulb quality and size characteristics of onion genotypes. INIFAP-Campo Experimental Las Huastecas (2019-2022).

Bulb Color	Genotype	Export bulb (%)	Bulb shape	Bulb size	Bulb weight (g)*
Amarillo	Wayne	98	GL	C-SC	480 a
	Hornet	98	FGL	C-SC	447 a
	Dulciana	96	GL	C-SC	426 ab
	Don Victor	98	GL	J-C-SC	418 ab
	Plethrora	93	F	J-C-SC	410 ab
	Verónica	98	R	J-C-SC	386 ab
	Tusker	98	FGL	J-C	319 b
	Vulkana	95	GL	J-C	317 b
Blanco	Blanca Grande	97	FGL	C-SC	455 a
	Carta Blanca	98	GL	J-C-SC	424 a
	Don Alberto	96	FGL	J-C-SC	412 ab
	Monja Blanca	94	FGL	J-C	376 ab
	Blanca Montejo	97	GL	J-C	374 ab
	Florentina	95	FGL	J-C	372 ab
Morado	Rasta	98	GL	J-C	345 a
	Red Sensation	98	FGL	M-J-C	343 a
	Sofire	84	R	M-J-C	329 a

* Amounts with the same letter are statistically equal (Tukey, $P<0.05$); Bulb size: SC=Super-Colossal; C=Colossal, J=Jumbo, M=Medium, S=Small; Bulb shape: R=Round; GL=Globe; FGL=Flattened Globe; F=Flat.

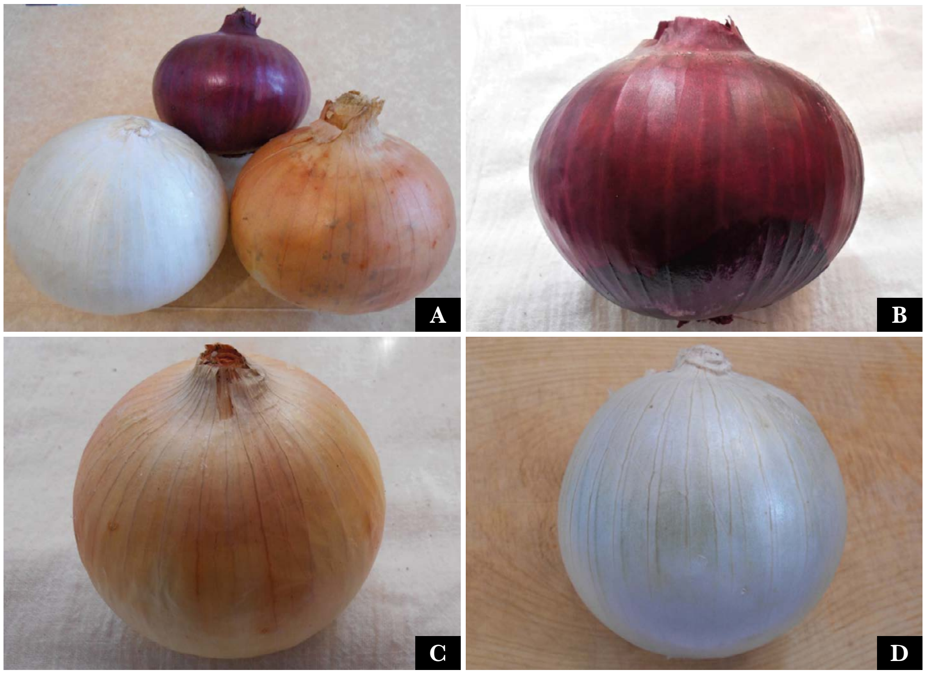

Figure 1. Onion bulbs. A) Group by bulb color. B) purple onion hybrid (Rasta). C) yellow onion hybrid (Wayne). D) white onion bulb (Blanca Grande).

production of 57.9 t ha^{-1}, during the three evaluation cycles. Ninety-eight percent of its production had exportation quality, surpassing the Don Victor hybrid (control), which obtained a 51.4 t ha^{-1} yield, with the same percentage of exportation quality in its production. The Tusker hybrid recorded the lowest yield (39.5 t ha^{-1}), although 98% of its production had exportation quality (Table 3 and 4).

For their part, white onions recorded significant differences ($P<0.05$). The Blanca Grande genotype recorded the highest production (52.5 t ha^{-1}), 97% of which had exportation quality. Blanca Grande was followed by Monja Blanca, Don Alberto, Carta Blanca, and Florentina, whose yields fluctuated from 44.5 to 49.8 t ha^{-1} and had a 94-98% exportation quality. The lowest production was recorded by the Blanca Montejo hybrid (40.3 t ha-1), 97% of whose production had exportation quality (Tables 3 and 5).

Purple onions recorded the lowest average production yield (37.5 t ha^{-1}) regarding the yellow and white onions, which obtained a 48.4 and 47.2 t ha^{-1}, respectively. The three purple onion genetic materials evaluated were statistically equal ($P>0.05$) regarding bulb production (Table 6). Overall, the Sofire purple onion genotype produced the lowest number of bulbs for exportation (86%). This situation could be the result of the high number of splitting bulbs (onions with double or triple bulbs in the same plant) and their characteristic jumbo-medium bulbs (Table 3).

Table 4. Production per cycle and average of yellow bulb onion genotypes. INIFAP-Campo Experimental Las Huastecas (2019-2022).

Variety or Hybrid	Yield per Cycle t ha^{-1}			Average* t ha^{-1}
	AW 2019-2020	AW 2020-2021	AW 2021-2022	
Wayne	51.9	47.1	74.8	57.9 a
Don Víctor	44.7	43.4	66	51.4 ab
Hornet	32.5	44.4	73.4	50.1 ab
Plethrora	42.9	46.5	60.1	49.8 ab
Verónica	37.9	42.3	67.0	49.1 ab
Dulciana	37.7	44.9	62.7	48.4 ab
Vulkana	37.8	34.2	50.9	41.0 b
Tusker	32.7	31.2	54.5	39.5 b

*Amounts with the same letters are statistically equal (Tukey, $P<0.05$).

Table 5. Production per cycle and average of white onion genotypes, in southern Tamaulipas. INIFAP-Campo Experimental Las Huastecas (2019-2022).

Variety or hybrid	Yield per cycle t ha^{-1}			Average* t ha^{-1}
	AW 2019-2020	AW 2020-2021	AW 2021-2022	
Blanca Grande	50.9	47.2	59.5	52.5 a
Monja Blanca	41.4	40.7	67.2	49.8 ab
Don Alberto	43.7	40.9	60.4	48.3 ab
Carta Blanca	41.3	43.1	58.7	47.7 ab
Florentina	39.3	41.8	52.3	44.5 ab
Blanca Montejo	33	35.7	52.1	40.3 b

* Amounts with the same letters are statistically equal (Tukey, $P<0.05$).

Table 6. Production per cycle and average of purple onion genotypes. INIFAP-Campo Experimental Las Huastecas (2019-2022).

Variety or hybrid	Yield per cycle t ha^{-1}			Average* t ha^{-1}
	AW 2019-2020	AW 2020-2021	AW 2021-2022	
Sofire	35.6	28.4	52.3	38.8 a
Rasta	36.9	32	47	38.6 a
Red Sensation	26.2	34.7	44.2	35.0 a

* Amounts with the same letters are statistically equal (Tukey, $P<0.05$).

CONCLUSIONS

The best onion genetic materials in the Planicie Huasteca were Wayne and Don Victor hybrids (yellow onion), Blanca Grande, Monja Blanca, and Don Alberto (white onion), and Rasta (purple onion). These hybrids recorded a high productive capacity and had all the characteristics and qualities required by the market. Consequently, they meet all the requirements of the producers of the Planicie Huasteca region.

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Colegio de Postgraduados

Insecticide resistance level of *Aedes aegypti* L. (Diptera: Culicidae) in northern Mexico

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ABSTRACT

Objective: To determine the response of the larvae of three *Ae. aegypti* populations from the Mexican North Pacific region to insecticides with different mode of action.

Design/Methodology/Approach: Three colonies were obtained placing ovitramps in peridomestic sites in Guadalajara (Jalisco), Culiacan (Sinaloa), and La Paz (Baja California Sur). Based on the methodology proposed by WHO, the bioassays were carried out with F_1 larvae in the early fourth instar.

Results: The larvae from the three field colonies had high resistance to permethrin and low resistance to deltamethrin; however, they were susceptible to Spinosad and *Bacillus thuringiensis* var. *Israelensis*. The Culiacan strain showed a high resistance to the malathion and propoxur insecticides.

Study limitations/Implications: The results provided valuable information about the response of these populations to insecticides, which are useful to establish resistance in the lab. Consequently, further studies should be carried out to complement the information obtained in these field tests.

Findings/Conclusions: The data indicated resistance levels to pyrethroid insecticides (mainly permethrin), as well as to organophosphates and carbamates.

Keywords: resistance, *Aedes aegypti*, biorational, pyrethroids, carbamates.

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INTRODUCTION

The *Aedes aegypti* L. mosquito is one of the most important insects for the public health sector, because it spreads several diseases, including classic dengue, dengue hemorrhagic fever, chikungunya virus, Zika virus, Mayaro virus, and yellow fever. This mosquito lives in urban habitats and it mainly reproduces in artificial containers. Unlike other mosquitoes, it feeds during the day; however, its biting activity intensifies in the early morning and at dusk (Souza *et al*., 2019).

Image by WikiImages at Pixabay

Given its adaptability, *Aedes aegypti* can currently be found in climates and altitudes where it had not been previously reported. Some studies point out that dengue prevails in 128 countries, impacting 3.900 billion people (Brady *et al.*, 2012).

In Mexico, management has mainly been focused on the use of insecticides such as pyrethroids and organophosphates to control adults and larvae, respectively. Nevertheless, this practice has resulted in resistance problems in several states, including Guerrero (Chino-Cantor *et al.*, 2014), Veracruz (Flores *et al.*, 2013), and Quintana Roo (Flores *et al.*, 2006).

As a consequence of the increased range of insecticides authorized for the control of the *Aedes aegypti* larvae, the susceptibility to the said authorized insecticides must be reviewed (Zettel and Kaufman, 2008). Therefore, the objective of this study was to evaluate the current state of the response of three *Ae. aegypti* populations from the Mexican states of Jalisco, Sinaloa, and Baja California Sur to insecticides.

MATERIALS AND METHODS

Individuals from three *Ae. aegypti* populations were collected in Guadalajara, Jalisco (20° 39' 58" N and 103° 21' 07" W), Culiacan, Sinaloa (24° 48' 00" N and 107° 23' 00" W), and La Paz, Baja California Sur (24° 08' 32" N and 110° 18' 39" W). The New Orleans strain —which has been certified as susceptible to insecticides and was provided by the Universidad Autónoma de Nuevo León— was used as a point of comparison. This strain had been kept three years in the lab where the bioassays were carried out.

The field material was gathered during April and May 2017 (La Paz), June 2017 (Culiacan), and May and June 2017 (Guadalajara). The samples were collected with ovitramps. These tramps were made up of 1-L black plastic containers. The insides were covered with a 12 cm wide × 27 cm long white Pellon interlining fabric (model F-1600); the fabric had a hole in the ½ liter measurement capacity. The ovitramps were left in the peridomestic sites for a month. During that period, they were checked on a weekly basis.

The cloth with the eggs was extracted, dried, and sent to the lab. A total of 119 fabric pieces (23,746 parental eggs), 66 fabric pieces (15,353 parental eggs), and 227 fabric pieces (40,779 parental eggs) were obtained from the colonies found in Guadalajara, La Paz, and Culiacán, respectively. However, the egg feasibility percentage was <1% in all the colonies, because most of them had hatched or were dehydrated.

The larvae were fed every third day, with dust from pet food (Rodent Lab Chow®5001). The containers were kept in a TFFU2065FWA bioclimatic chamber (Thermo Fisher Scientific, Waltham, MA, USA), at 27 °C and with a 12:12 (L:O) photoperiod. The pupae were extracted and placed in polystyrene glasses. The said glasses were introduced into 30×30×45 cm entomological cages, wrapped in organza fabric, at 27 °C ± 2, with a 75% ± 5 relative humidity, and the same photoperiod than the larvae, in order to help them to reach adulthood.

Adults were fed with a 10% sugary solution. The females were given *Sus scrofa domesticus* L. pig blood, with 4 mL of heparin sodium 1,000 un/mL per liter of blood as anticoagulant. The blood was warmed in a bath Marie until it reached 37 °C. Afterwards, 5 mL of blood were poured into polystyrene glasses, covered with heat-resistant Parafilm-M® sealing film which had been soaked with human sweat on the outside. The glasses were placed upside-down at the top of the cage (Carvalho *et al.*, 2014).

Eight insecticides from different toxicological groups were used: organophosphates, carbamates, pyrethroids, spinosyns, and Bacillus (Table 1).

In the case of the insecticide formulations, distilled water was used to prepare the required concentrations, while Meyer® reagent grade acetone was used for the active ingredients.

The bioassays followed the standardized procedures of the WHO (2012). Twenty larvae in the early four instar were placed in two 120-mL polystyrene containers filled with 100 mL of distilled water. During the biological response window, nine concentrations of each insecticide were established, starting from 0.01% and logarithmically decreasing until 0 and 100% mortality results were obtained, after a 24 h exposition. Subsequently, nine intermediate concentrations (five repetitions per insecticide and an untreated control) were evaluated. In order to obtain the 0-100% mortality ranges, the dead larvae were counted. In addition, the larvae that were unable to move vertically or that did not perform their characteristic movements when touched with the stimulus-responsive brush were considered to be dead (Flores, 2014). The maximum mortality accepted for the control was 10%; this result was adjusted using Abbott's formula (Abbott, 1925).

The 50% and 95% mortality (RF_{50} and RF_{95}) factors were obtained dividing the LC_{50} or LC_{95} lethal concentration of the field population by the LC_{50} o LC_{95} of the susceptible population. The Mazzarri and Georghiou (1995) criteria were used to determine the resistance degree of the field populations: <5 resistance factor (RF) indicates a low resistance level; 5-10, a moderate resistance level; and >10, a high resistance level.

The data of the bioassays were analyzed using the PROC PROBIT procedure of the SAS software, version 9.0 (SAS Institute, 2002). The LC_{50} and LC_{95} values were calculated, as well as their confidence intervals (95%). The lack of overlap between confidence intervals was taken into account to determine if there was a significatively different response between one and two populations subjected to insecticides of the same toxicological group, regarding the LC_{50} and LC_{95} values.

Table 1. Insecticides used in the bioassays.

Active Ingredient & Trade Name	Formulation	Percentage or purity	Formulator
Spinosad, Natular EC	Concentrated emulsionable	20.6%, 230 g i. per litre	Public Health Supply and Equipment de México S. A. de C. V.
Bacillus thuringiensis var. israelensis VectoBac®WDG	Water-soluble granules	34.7%	Bayer de México S. A. de C. V.
Temefós, Temephos	Concentrated emulsionable	500 de i. a. per litre	Química Lucava S.A. de C.V.
Clorpirifós etílico, Clorpirifós	Liquid in mineral oil	122.8 g de i. a. per litre	Public Health Supply and Equipment de México S. A. de C. V.
Permetrina, Aqua Reslin Super	Aqueous solution	108.7 g de i. a. per litre	Bayer de México S. A. de C. V.
Deltametrina, Aqua K-Othrine®	Aqueous emulsion	20 g de i. a. per litre	Bayer de México S. A. de C. V.
Malatión, Verthion	Concentrated Solution	410 g de i. a. per litre	Agricultura Nacional
Propoxur	Technical Grade	Puresa 99.5%	Chem service, West Chester, PA

RESULTS AND DISCUSSION

The colonies of Guadalajara, Culiacan, and La Paz showed high resistance levels (>10 x) to permethrin, recording 40.81, 42.85, and 69.38x resistance factor (RF_{50}), respectively (Table 2). Meanwhile, the three colonies showed even higher resistance levels (RF_{95}). However, the La Paz colony recorded the highest resistance factor (217x) and RF_{50} among the three colonies.

Meanwhile, the three colonies recorded low resistance levels to the deltamethrin insecticide: 3.73, 1.56, and 2.77x (RF_{50}) for the Guadalajara, Culiacan, and La Paz colonies, respectively. For its part, RF_{95} reported a similar trend towards low resistance levels. In all cases, the slope response of the populations to this insecticide was >2. This phenomenon indicates that the populations have a drastic mortality response to a dose increase. Nevertheless, this was not the case with permethrin, whose slope results were ≤1.61 in all the cases. These results indicate a high resistance trend: when the dose increases, mortality slowly increases.

None of the colonies showed resistance to Spinosad. The LC_{50} and LC_{95} of the field colonies recorded lower results than the New Orleans strain control (Table 3). This insecticide was first applied in Mexico for the management of the *Aedes aegypti* larvae in 2014. However, it was not used in all the states were the health campaign against *Aedes aegypti* has been implemented and this may explain the results obtained.

The slope of all the colonies where Spinosad was applied was ≥2.4, which indicates that these colonies have a similar mortality response to a dose increase.

All the field colonies were susceptible to *Bacillus thuringiensis* var. *Israelensis*: their LC_{50} and LC_{95} fiducial limits overlapped with the susceptible New Orleans strain control. Likewise, the slope values of the bioassays were ≥3.24 in all the cases, which indicates that this insecticide has a low resistance trend.

Although the temephos insecticide is widely used to manage mosquito larvae in Mexico, the three colonies (LC_{50}) showed low resistance levels, reporting 1.42, 1.47, and 3.23x values for the colonies of Guadalajara, Culiacan, and La Paz, respectively (Table 4). LC_{95} showed a similar behavior, recording <5x resistance factors.

Table 2. Pyrethroid toxicity (mg L^{-1}) in *Aedes aegypti* larvae of the Mexican North Pacific.

Insecticide	Strain	[1]N	[2]b±SE	[3]LC_{50} LC%95	[4]Pr>χ^2	[5]RF_{50}	LC_{95} LC95%	RF_{95}
Permetrina	New Orleans	800	1.65±0.19	0.0098 (0.0069-0.013)	0.052		0.096 (0.054-0.25)	
	Guadalajara	480	1.61±0.16	0.4 (0.32-0.49)	0.96	40.8	4.2 (2.98-6.86)	43.7
	Culiacán	540	1.27±0.13	0.42 (0.33-0.52)	0.98	42.8	8.42 (5.05-18.08)	87.7
	La Paz	720	1.1±0.091	0.68 (0.55-0.85)	0.4	69.3	20.89 (12.08-44.09)	217
Deltametrina	New Orleans	900	1.97±0.13	0.0083 (0.007-0.0097)	0.98		0.056 (0.042-0.079)	
	Guadalajara	480	2.29±0.16	0.031 (0.027-0.036)	0.82	3.73	0.16 (0.12-0.22)	2.8
	Culiacán	320	2.42±0.27	0.013 (0.011-0.016)	0.92	1.56	0.066 (0.051-0.097)	1.1
	La Paz	420	2.03±0.17	0.023 (0.02-0.028)	0.9	2.77	0.15 (0.11-0.23)	2.6

[1]N: number of events; [2]b±SE (SE): slope and standard error; [3]LC (LC): lethal concentration; [4]Pr>χ^2: chi-square probability; [5]RF (RF): resistance factor.

Table 3. Toxicity (mg L^{-1}) of microbials in *Aedes aegypti* larvae of the Mexican North Pacific.

Insecticide	Strain	[1]N	[2]b±SE	[3]LC_{50} LC%95	[4]$Pr>\chi^2$	[5]RF_{50}	LC_{95} LC95%	RF_{95}
Spinosad	New Orleans	500	3.78±0.3	0.11 (0.1-0.12)	0.23		0.31 (0.25-41)	
	Guadalajara	420	3±0.2	0.035 (0.031-0.039)	0.82	0.31	0.12 (0.1-.16)	0.38
	Culiacán	420	2.41±0.19	0.037 (0.032-0.043)	0.51	0.33	0.17 (0.14-o.24)	0.54
	La Paz	700	3.39±0.3	0.027 (0.023-0.031)	0.11	0.24	0.082 (0.065-0.11)	0.26
Bacillus thuringiensis var. *israelensis*	New Orleans	540	2.43±0.1	0.021 (0.019-0.024)	0.55		0.1 (0.083-0.13)	
	Guadalajara	360	3.87±0.39	0.011 (0.01-0.012)	0.29	0.52	0.03 (0.024-0.04)	0.3
	Culiacán	540	3.25±0.2	0.027 (0.024-0.029)	0.72	1.28	0.086 (0.073-0.1)	0.86
	La Paz	420	3.24±0.2	0.018 (0.016-0.021)	0.8	0.85	0.06 (0.049-0.077)	0.6

[1]N: number of events; [2]b±SE (SE): slope and standard error; [3]LC (LC): lethal concentration; [4]$Pr>\chi^2$: xhi-square probability; [5]RF (RF): resistance factor.

Table 4. Organophosphate and carbamate toxicity (mg L^{-1}) in *Aedes aegypti* larvae in the Mexican North Pacific.

Insecticide	Strain	[1]N	[2]b±SE	[3]LC_{50} LC%95	[4]$Pr>\chi^2$	[5]RF_{50}	LC_{95} LC95%	RF_{95}
Temefós	New Orleans	540	2.15±0.15	0.021 (0.018-0.024)	0.27		0.12 (0.094-0.16)	
	Guadalajara	480	2.25±0.17	0.03 (0.026-0.035)	0.24	1.42	0.16 (0.12-0.22)	1.33
	Culiacán	540	2±0.15	0.031 (0.027-0.036)	0.14	1.47	0.2 (0.16-0.29)	1.66
	La Paz	700	4.55±0.43	0.068 (0.06-0.079)	0.12	3.23	0.18 (0.14-0.25)	1.5
Malation	New Orleans	600	1.56±0.18	0.017 (0.014-0.022)	0.22		0.19 (0.11-0.45)	
	Guadalajara	420	2.93±0.28	0.1 (0.095-0.11)	0.18	5.88	0.38 (0.29-0.55)	2
	Culiacán	480	2.6±0.3	0.29 (0.23-0.36)	0.022	17.05	1.24 (0.84-2.39)	6.52
	La Paz	420	2.81±0.38	0.1 (0.08-0.13)	0.07	5.8	0.4 (0.26-0.93)	2.1
Clorpirifós	New Orleans	600	3.58±0.33	0.013 (0.012-0.015)	0.18		0.039 (0.031-0.052)	
	Guadalajara	480	2.05±0.17	0.045 (0.038-0.053)	0.94	3.46	0.28 (0.2-0.45)	7.17
	Culiacán	420	2.34±0.58	0.096 (0.066-0.28)	0.0017	7.38	0.48 (0.2-22.12)	12.3
	La Paz	420	2.89±0.22	0.022 (0.02-0.026)	0.51	1.69	0.084 (0.067-0.11)	2.15
Propoxur	New Orleans	900	1.48±0.15	0.096 (0.07-0.13)	0.074		1.23 (0.68-3)	
	Guadalajara	420	4.03±0.57	1.21 (1-1.49)	0.01	12.6	3.11 (2.29-5.7)	2.52
	Culiacán	420	2.93±0.54	1.79 (1.15-2.75)	0.03	18.6	6.52 (3.87-23.18)	5.3
	La Paz	420	3.36±0.45	2.64 (2.04-3.58)	0.006	27.5	8.15 (5.45-17.34)	6.62

[1]N: number of events; [2]b±SE (SE): slope and standard error; [3]LC (LC): lethal concentration; [4]$Pr>\chi^2$: chi-square probability; [5]RF (RF): resistance factor.

The field colonies of Guadalajara and La Paz showed a moderate resistance to Malathion in their LC_{50}, both recording 5.88x resistance factor values. However, the Culiacan strain recorded a high resistance to this insecticide and its RF_{50} reached 17.05x.

The Guadalajara and La Paz strains showed low resistance levels to clorphyrifos in their LC_{50}: 3.46 and 1.69x, respectively. However, the Culiacan strain recorded a moderate resistance (7.38x RF_{50}). Comparing the LC_{95}, the La Paz strain recorded a low resistance (>5x), while the Guadalajara and the Culiacan strains showed moderate (7.17x) and high resistance (>10x), respectively. Meanwhile, the response of the three colonies to the three organophosphate insecticides recorded slopes values between 2 and 3. Meanwhile, only the La Paz strain recorded a 4.55 slope against the temephos insecticide. Among the three colonies this strain recorded the highest RF_{50} against this insecticide.

All the field strains had a high resistance to propoxur (carbamate): their RF_{50} were 12.6 (Guadalajara), 18.64 (Culiacan), and 27.5x (La Paz). Nevertheless, regarding the resistance factors of the LC_{95}, the Guadalajara strain had a low resistance (2.52x), while the Culiacan and La Paz strains recorded a moderate resistance (5.3 and 6.62x, respectively). The highly variable slope values ranged from 2.93 to 4.03, indicating that the response to this insecticide is not even (Table 4).

The three colonies showed low resistance levels (>5x) to deltamethrin, a type II insecticide; however, they recorded high resistance levels to permethrin, a type I pyrethroid. The only metabolic resistance mechanism of the first type of insecticide is made up of mixed function oxidase (MFO). Meanwhile, permethrin has a metabolic resistance mechanism made up of esterase and MFO. In addition, permethrin has been used for a longer period in the campaign against adult mosquitoes and is widely applied in the agricultural sector. For its part, the use of deltamethrin is recent and is mainly recommended for the impregnation of pavilions; consequently, the pressure to select a single insecticide is lower than in the apple cultivation sector, where permethrin is the chosen product.

Although neither deltamethrin pyrethroids, nor permethrin were applied to the larvae, Zettel and Kaufman (2008) pointed out that the use of adulticides can have a marginal selection effect on the larvae, because spraying can spread on water bodies where the larvae develop. Therefore, the larvae frequently become resistant to adulticides.

Meanwhile, although several other species (mainly agricultural pests) have shown resistance to Spinosad, only one resistance case (*Culex quinquefasciatus*) has been recorded among urban pests; this case was induced by selection pressure in the lab (Su and Cheng, 2014). Consequently, field strains frequently keep their susceptibility status. In addition, this insecticide was introduced for urban use in Mexico in 2014.

There is no record about the mosquitoes' resistance to *Bacillus thuringiensis* var. *Israelensis*, although it has been used in some places for more than 10 continuous years, as the only option for the control of larvae (Tetreau *et al.*, 2013).

The abuse in the use of temephos in Mexico —which has been employed since 1980— as a control method against Ae. aegypti larvae has caused numerous resistance cases. Although temephos recorded low resistance levels (>5x), the selection pressure of this insecticide causes a crossed resistance with other insecticides of the same toxicological group (such as chlorpyrifos) and other toxicological groups (such as propoxur) (Rodríguez *et al.*, 2002; Rodríguez *et al.*, 2005).

CONCLUSIONS

The data obtained indicate certain resistance level against pyrethroid insecticides (mainly permethrin). Organophosphates and carbamates also registered resistance levels. Consequently, this work should encourage other researchers to carry out further field tests to confirm that the application of these insecticides is not impacting the conditions. If this situation is confirmed, insecticides should be used with a rotary arrangement. Another proposal is to stop using some insecticides, at least until the resistance levels decrease. In order to achieve this reduction, bioassays should be carried out to monitor mosquito larvae.

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Colegio de Postgraduados

Induction of estrus as a strategy to improve the economic efficiency of the sheep flock

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ABSTRACT
Productivity and profitability in sheep production systems are strongly influenced by the reproductive capacity of the flock.
Objective: To evaluate the use of reproductive biotechnologies (*e.g.*, the induction of estrus) and its impact on the economic efficiency and the productive and reproductive performance of technified sheep production systems, during the seasonal anestrus.
Design/Methodology/Approach: Four-hundred recently weaned (60 days postpartum) ewes of the Katahdin breed were randomly divided into two treatments: T1=natural mating (n=200) and T2=induction of estrus (n=200). Estrus was induced through the application of intravaginal sponges, impregnated with 20 mg of chronolone, plus the injection of 400 IU of Equine Chorionic Gonadotropin. The aim was to evaluate the reproductive and economic efficiency of the flock.
Results: The induction of estrus during the seasonal anestrus recorded increases ($p<0.001$) in prolificacy (32%), fertility (86%), and kilograms of lamb weaned per year per ewe (48%), while decreases ($p<0.05$) were recorded in the number of open days (25%), calving interval (11%), cost per open days (23%), and the kilograms of lamb required per ewe per year (10%).
Study Limitations/Implications: The constant variations in the price of supplies and services caused changes in the economic indicators.
Findings/Conclusions: The use of reproductive biotechnologies (such as estrus induction) has a positive impact on production units, improving their profitability.

Keywords: sheep, reproductive biotechnologies, profitability, fertility, costs.

INTRODUCTION

In 2020, sheep stock in Mexico amounted to 8,708,246 heads (SIAP, 2022). Nevertheless, the market fails to meet the domestic meat demand and, consequently, lamb meat is mainly imported from New Zealand and Australia (FOASTAT, 2020). The low productivity of the sheep production systems is mainly the result of poor nutrition, lack of health management, and low efficiency (Gastelum-Delgado *et al.*, 2015; Martínez-González *et al.*, 2017). Increasing the reproductive capacity of the flock can be an alternative to improve the productivity and profitability of the flocks. The reduction of the unproductive period of the sheep can diminish fixed production costs and variables and increase production, obtaining three births per ewe in two years (Hinojosa-Benavides *et al.*, 2019).

The use of biotechnologies is one of the reproductive management strategies that improves flock performance. The synchronization and induction of estrus are two biotechnologies that use effective and easily applied pharmacological methods. These methods facilitate the manipulation of the reproductive physiology of ewes, consequently homogenizing lamb production, reducing the open days, and directly improving the productive and economic aspects of sheep production (Lozano-González *et al.*, 2012).

In view of this situation, the hypothesis of this study was that the implementation of reproductive biotechnologies (*e.g.*, the induction of estrus) can reduce production costs and improve the productive and reproductive performance of technified sheep production systems. Therefore, the objective of this study was to evaluate the technical and economic feasibility of implementing a protocol for the induction of ewe estrus in a commercial production system.

MATERIALS AND METHODS

Study area location

The study was carried out from March to July, 2020 (seasonal anestrus) in a Commercial Flock, located in the Texcoco municipality, State of Mexico, at 19° 29' N and 98° 53' W, at 2,240 m.a.s.l. The area has a subhumid warm weather, with summer rains. Its climatic formula is Cb(w0)(w)(i') (García, 2004).

Animals and feeding

Four-hundred recently weaned (60 days postpartum; 1.28 prolificity) and multiparous ewes of the Katahdin breed were used; they had a 55.7±4.8 kg average weight and a 2.5 average body mass index in the scale proposed by Russel *et al.* (1969). The specimens were placed in pens with automatic feeders and water dispensers (20 ewes per pen). The ewes consumed a 2.5-kg integral portion (13% crude protein; 2.4 Mcal of metabolizable energy; and 20% crude fiber), made up of 70% corn silage and 30% concentrated feed and prepared in the production unit.

Health management

The ewes were subcutaneously dewormed with 10 mg of ivermectin (Iverfull®, Aranda); additionally, 10.95 mg of sodium selenite and 50 mg of vitamin E were applied through an intramuscular injection (MUSE®, MSD).

Induction of estrus protocol

The induction of estrus was carried out using progestogens. The method consisted of the application of an intravaginal sponge, impregnated with 20 mg of chronolone (Chronogest®, MSD). A speculum was used to introduce the sponges in the fundus of the vagina, where they remained for 12 days. Forty-eight hours before the removal of the sponge, 400 UI of Equine Chorionic Gonadotropin were applied through an intramuscular injection (eCG, Novormon®, VIRBAC).

Treatments and description

The sheep were randomly divided into two treatments: T1=natural mating (n=200), and T2=induction of estrus (n=200).

Natural mating: The ewes were kept in a mating stage for 60 days (three 17-days estrus cycles), with 20 ewes per ram. Suitable rams were subjected to a physical and semen evaluation to determine their reproductive characteristics. During this period, rams used nylon marking harnesses. Ewes with a color mark between their coxal tuberosities were considered to have been mounted by the ram. The ewes mounted by the rams were registered in the daily 8:00 am tour. The color of the males' marking harnesses was changed every 14 days to control the mating. Ewes stained with two different colors were considered as non-pregnant after the first mating and, consequently, they returned to the estrus stage.

Induction of estrus

The detection of estrus was carried out for 120 minutes, every 6 hours, with the help of a ram. This process started 24 hours after the removal of the sponge. Ewes with signs of estrus were separated and inseminated twice by natural mating, at the start and at the end of a twelve-hour period. This procedure was carried out 48 h after the starting of the mating. Ewes that did not respond to the treatment were included in a natural mating group for 43 days, in order to restart their reproductive activity.

Gestation diagnosis

The gestation diagnosis was carried out 47 days after the induction of estrus. In the case of natural mating, the diagnosis was carried out 90 days after it started. The diagnosis consisted of a real-time ultrasound carried out with a MINDRAY® DP 10 Veterinary micro-convex array transducer, calibrated at 5 mHz. The ewes were classified as positive or negative, depending on whether or not they had a well-formed fetus.

Costs

Variable costs. This indicator was the sum of the costs of labor plus feeding, animal health, hormonal treatments, and other costs paid during the period of the analysis.

Fixed costs. These costs included depreciation, which is related to the investment in assets and management expenses.

Total costs. They were calculated as the sum of the variable costs plus the fixed costs of the company, during the analysis period.

Evaluated variables

The study variables included: fertility, prolificity, and open days. The first divides the number of pregnant sheep by the number of inseminated sheep and expresses it as percentages. The second is the number of lambs born divided by the number of sheep that gave birth. Both variables were determined at birth, counting the number of lambs born per ewe. Finally, the third variable takes into account the number of days from the moment of the birth to the moment when the next gestation takes place.

The feeding and production records and the financial data of the production unit were used to determine the costs of open days and the Interval between births for the two strategies in question. Additionally, the components of the fixed, variable, and total costs were determined as a whole. Subsequently, the annual weight (kg) of weaned lambs per sheep (KCDA) and the cost:benefit ratio of the induction of estrus technique were calculated. Finally, the annual break-even point of lamb weight (kg) produced per sheep that the production unit (KNDA) requires to be profitable was also calculated.

Fixed costs were calculated based on the information included in the documents of the ranch. After consulting the bills and receipts of the ranch, the costs paid for each heading were divided by the number of animals in which they were spent, in order to obtain a daily cost per sheep. The fixed costs taken into account for this study were: feed consumption, water consumption, labor, and drug administration; however, the depreciation of the infrastructure was not included, given the lack of information about the facility costs.

A cost of $9.086 Mexican pesos per feeding and per sheep was obtained, based on a 30% concentrate feed and 70% silage corn. Each sheep voluntarily consumed 2.0 kg of feed per day. Daily water consumption was estimated at 4.5 liters per sheep per day, reaching a cost of $0.225 Mexican pesos per day. The ranch had four employees, who were paid a weekly wage of $1,500 Mexican pesos each. The cost of labor amounted to $1.7 Mexican pesos per sheep per day. The monthly cost of the drugs for the basic first-aid kit was divided by 400 animals. The drug cost amounted to $0.1 Mexican pesos. Finally, the monthly fuel bills were divided by 400 animals, resulting in a daily cost per sheep of $0.4 Mexican pesos.

The variable costs were those used in the protocol for the induction: initial gestation diagnosis ($15.0); induction of estrus treatment ($250.0); and a corn grain-based supplementation ($1.5).

Statistical analysis

The fertility and prolificity variables were analyzed using the χ^2 and the Kruskal–Wallis tests. The rest of the variables were subjected to an analysis of variables (ANOVA). Meanwhile, Tukey's test ($p<0.05$) was used to determine the mean differences between the effects of the protocol type (induced or natural mating) on the reproductive and economic parameters. The data was analyzed using the PROC GLM of the SAS statistical package (SAS Institute Inc., Cary, NC, 2008).

RESULTS AND DISCUSSION

Reproductive variables

Table 1 shows that the prolificity and fertility reproductive variables recorded higher results (32 and 86%, respectively) with the induction of estrus treatment ($p<0.001$). Meanwhile, the group of animals subjected to the induction of estrus recorded 25% and 11% less ($p<0.05$) open days (25%) and shorter intervals between births (11%) than the group subjected to a natural mating. These improvements in the reproductive behavior of the group of sheep subjected to the induction of estrus treatment during the seasonal anestrus can be the result of the use of pharmacological methods that enable the manipulation of the luteal and follicle phases of the estrus cycle). These methods include the insertion of intravaginal devices with progesterone and similar drugs, the intramuscular injection of Equine Chorionic Gonadotropin, and the use of prostaglandins and similar drugs. The resulting changes in the functions of the hypothalamus-pituitary-ovary axis helps to intensify animal production, improving fertility and prolificity (Lozano-González *et al.*, 2012). The productivity of sheep production systems mainly depends on the number of births, which is related to fertility and prolificity. Both fundamental indicators influence the profits of the production unit, because the fixed cost per womb is the same, regardless of its productive level. Consequently, the increase of these indicators determines the feasibility of a production system (Macedo and Castellanos, 2004).

The reduction on the number of open days is important, because this is the period when the sheep consume feed, outside their reproductive stage. Even more importantly, its improvement of the energy balance of the diet allows animals to recover their body condition. On this matter, Alvarado *et al.* (2021) pointed out that those sheep that recover their body condition sooner have a shorter first birth-estrus interval. This parameter is determinant for the profitability of a production unit. Under intensive conditions, the reduction of open days is fundamental to reincorporate the sheep into the productive system and to obtain enough lambs to guarantee the profitability of the system. González-Reyna *et al.* (2020) define productivity as the relationship between the outputs and the inputs of a production period or cycle. Therefore, a minimum-cost production involves a

Table 1. Means and standard deviation of the reproductive variables of Katahdin sheep, during the seasonal anestrus, with natural mating and induction of estrus.

Variable	Protocol type (Mean ± S.D.)		P>F
	Induced (n=185)	Natural mating (n=151)	
Fertility (%)	73.1	39.7	***
Prolificacy (lambs)	1.5±0.6	1.1±0.3	***
Days Open (Days)	79.5±9.2b	104.2±18.6a	***
Lambing interval (days)	225.9±9.6a	250.7±18.1b	***
Birth per year	1.6±0.0a	1.4±0.1b	***

a, b: Values with different literals in the row are different ($P<0.05$). S.D. Standard deviation. ***: $P<0.0001$.

maximum efficiency in the use of the inputs to achieve a point of balance, where a positive cost:benefit ratio and other economic variables can be achieved. Reproductive seasonality is a very important factor, because most ewes need an almost 7-month birth-conception interval; the reproductive seasonality of a high percentage of the specimens includes a 5-month sexual repose (De Lucas *et al.*, 1997). This situation increases the feeding and maintenance costs of the sheep.

Economic variables

Regarding the economic variables, Table 2 shows a reduction ($P \leq 0.0001$) in the cost of the open days and the interval between births (23%). In the case of the group subjected to an induction of estrus, the annual lamb weight (kg) required per ewe to guarantee the profitability of a company (10%) likewise recorded a decrease, compared with the group subjected to natural mating. Meanwhile, the annual weaned lamb weight (kg) per ewe increased by 48% ($P \leq 0.0001$).

The economic profitability of the sheep production units directly depends on reproductive efficiency and, consequently, on the productivity of every sheep (Gonzáles-Reyna *et al.*, 2003). Since the use of hormonal treatments during the seasonal anestrus reduces the unproductive period (open days), the group of sheep subjected to the induction of estrus protocol had a better profitability in the production unit. Since food accounts for 60% of the total costs of livestock production systems, the feeding expenses diminished, favoring the profitability of the production (Herd *et al.*, 2003). In addition, 70% of the food required for sheep production is consumed by ewes (Hogue, 1987).

Meanwhile, in the Mexican sheep production systems, 75.7% of the income comes from the sale of lambs, whether as animals with market weight or as weaned lambs (Góngora-Pérez *et al.*, 2010). Consequently, the increase of prolificity and the reduction of the interval between births among sheep subjected to the induction of estrus treatment, during the seasonal anestrus, could increase the efficiency of lamb production (Gonzáles-Reyna *et al.*, 2003) and distribute the maintenance and production cost of the ewes among a larger number of born lambs, increasing the number of sold lambs (Dickerson, 1970).

Table 2. Means and standard deviation of the economic variables used to evaluate the induction of estrus protocol and natural mating of Katahdin sheep, during the seasonal anestrus.

Variable	Protocol type (Mean ± S.D.)		P>F
	Induced (n=185)	Natural mating (n=151)	
Cost Days Open ($)	916±109	1,200±210	***
Cost LI ($)	2,601±115	2,886±211	***
KWLEY (kg)	49±21	33±12	***
KLREY (kg)	52±2.3	58±4.2	NS
Difference	-2.39±22	-24.3±14	***

KWLEY: Kilograms of weaned lamb/ewe/year, KLREY: Kilograms of lamb required/ewe/year. S.D. Standard deviation; T: Synchronization protocol type; ***: $P<0.0001$.

The increase on the productivity of the sheep from the group subjected to the induction of estrus treatment —defined as the weaned lamb weight (kg) per sheep— is a parameter that includes lamb productivity, along with the reproductive characteristics and the maternal ability of the ewe (Snowder and Fogarty, 2009). In this regard, the increase in profitability and the reduction of the interval between the births reported in this study enabled an increase in the productivity per ewe and guaranteed the profitability of the production unit. This situation takes place, always supposing that the number of lambs born per birth does not exceed the capacity of the ewe. The number of lambs born per birth can vary according to the different production systems, animals, weather, and quantity and quality of available food. The increase in profitability can have a negative impact on the growth and survival of the lamb; in addition, it can make the births harder for the ewes, increasing the mortality rate of the lambs (Gootwine *et al.*, 2007; Gootwine *et al.*, 2008).

The annual weaned lamb weight (kg) per sheep is the most important production indicator, as a result of its multifactorial nature. It includes the open days, fertility, prolificity, mortality, and weight gain of the lambs. In addition, the production subsystem must fulfill this parameter to be profitable. In most cases, this subsystem is subsidized by the feedlot subsystem, which is more profitable. However, lamb production takes up most of the costs and risks, because it involves the daily feeding of the ewes and the loss or death of the ewes and lambs. It also includes most of the fixed costs of the production unit. Therefore, determining the annual kilograms of produced and weaned lambs per ewe is fundamental to determine how much they must pay for their maintenance in the production unit.

In this study, this indicator was determined based on the sum of fixed and variable costs. The total was divided between the cost per lamb kilogram at the moment of this study to determine the KCDA. A higher value was observed in the induction of estrus treatment, as a result of the cost of the pharmacological products. Table 2 shows that the use of the induction of estrus protocol increased the annual weaned lamb kilograms per sheep by 48.5%. The use of induction estrus treatments did not allow the ewes to achieve the lamb kilograms required to pay for their stay in the farm. Using this method, 2.39 kilograms more were still required to reach a point of balance. For its part, the natural mating treatment group required 24.3 more kilograms to achieve a balance. This difference will be the result of an efficient intensive fattening of lambs and the increase of the sale prices. Guzmán *et al.* (2022) pointed out that a lower weight per litter at the moment of the weaning has a positive relation with the weight of the lambs at the moment of the sale. Consequently, increasing the kilograms of weaned lambs per sheep can increase the kilograms of lamb sold, improving the profitability of the production unit. Ponce *et al.* (2013) evaluated the technical and economic efficiency of two estrus synchronization protocols in Pelibuey sheep and determined that these pharmacological control techniques —used to manipulate the reproductive activity of sheep— increased the production costs. These results match the findings of this study. However, the protocols also increased lamb production and the income of the production unit.

CONCLUSIONS

The use of a protocol for the induction of estrus in Katahdin sheep increases prolificity and fertility and decreases the number of open days. These changes have a positive impact on the production unit, reducing the expenses generated by the open days and the intervals between births. Additionally, it also decreases the lamb kilograms required per sheep to reach a point of balance between the production costs and the product, improving the profitability of the production unit, through a yearly increase in the weaned lamb kilograms per sheep.

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Analysis of the growth of Chetumal grass established in a tropical climate

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ABSTRACT
Objective: To evaluate the growth of Chetumal grass (*Urochloa humidicola* CIAT 679), in order to determine the optimal moment for the first harvest.
Design/Methodology/Approach: A completely randomized experimental block design, with measurements repeated over time, and three repetitions was used. Morphological composition (MC), growth rate (GR), plant height (PH), intercepted radiation (IR), leaf:stem ratio (L:S), leaf:no-leaf ratio (L:NL), and aerial biomass (AB) —as well as leaf biomass (LB), stem biomass (SB), dead material (DM), net growth (NG), and total biomass (TB)— were evaluated every fifteen days, except for the two first samplings, which were carried out on a monthly basis. Data were analyzed using the GLM procedure of the SAS software and Tukey's mean comparison test ($\alpha \leq 0.05$).
Results: The morphological composition (MC) of the Chetumal grass was statistically different ($p<0.05$), during the different growth ages. The maximum accumulation of total biomass (TB) (13,324 kg DM ha^{-1}), leaf biomass (LB) (2,569 kg DM ha^{-1}), and growth rate (GR) (99 kg DM ha^{-1} d^{-1}) was reached at 135 DAS. On that day, the prairie reached a 68 cm plant height (PH) and 100% intercepted radiation (IR). The L:S ratio decreased from 1.62 to 0.31, while L:NL ratio changed from 1.62 to 0.22.
Study Limitations/Implications: The *Brachiaria humidicola* cv. Chetumal grass reached its highest potential during the rainy season.
Findings/Conclusions: The first cut of the *Urochloa humidicola* cv. Chetumal grass can be carried out at 135 DAS, when the highest accumulation of total biomass (TB), leaf biomass (LB), and growth rate (GR) is recorded.

Keywords: *Brachiaria humidicola*, *Urochloa humidicola* cv. Chetumal, plant height, growth, and intercepted radiation.

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INTRODUCTION

In Mexico, cattle raising must be productive, if it is to achieve profitability and competitiveness. However, there are divisions between regions, 12% of which are located in the humid tropics (Torres *et al.*, 2020). Twenty-nine percent of the pastures are native to those regions and account for a dry matter production of 183 million tons and a low load capacity (Enríquez et al., 2021). However, there are other alternatives regarding grass species, including genus *Urochloa* (Bastidas *et al.*, 2023). Nearly 2.6 million hectares (≈6.4% of the national territory) are covered by these grasses.

Prairie productivity depends on physiological, agronomic, and environmental features (Merchant-Fuentes and Solano-Vergara, 2016). Therefore, germination, introducing new species, or selecting different varieties can help to improve the vigor of the grasses and to preserve their populations or their descendants (Barker *et al.*, 2021). The CIAT 679 material has two propagation methods: seed and stolon (Bastidas *et al.*, 2023). This material can be associated with forage production, if its morpho-physiological characteristics are taken into account, including plant height, leaf:stem ratio, leaf expansion rate, and tiller dynamics (Álvarez *et al.*, 2020; de Dios-León *et al.*, 2022). Cruz-Hernández *et al.* (2017) analyzed two intensities (13-15 and 9-11 cm plant height) of *Brachiaria humidicola* cv Chetumal and recorded an increase in crude protein and a higher forage accumulation after 28 d of grazing. Therefore, the objective of this study was to evaluate the growth of Chetumal grass (*U. humidicola* CIAT 679), during its establishment in a tropical climate, and to determine the optimal moment for its first harvest.

MATERIALS AND METHODS

The research was carried out in the Papaloapan Experimental Unit of the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), located in the municipality of Isla, Veracruz, at 18° 06' N and 95° 31' W, at 65 m.a.s.l. According to the modifications made by García (2004) to the Köppen climate classification, the area has an Aw0 climate, with a 1,000-mm average precipitation and a 25.7 °C mean annual temperature (Figure 1). The region has an orthic acrisol soil, with a sandy loam texture, and a 4.0-4.7 pH (Enríquez and Romero 1999).

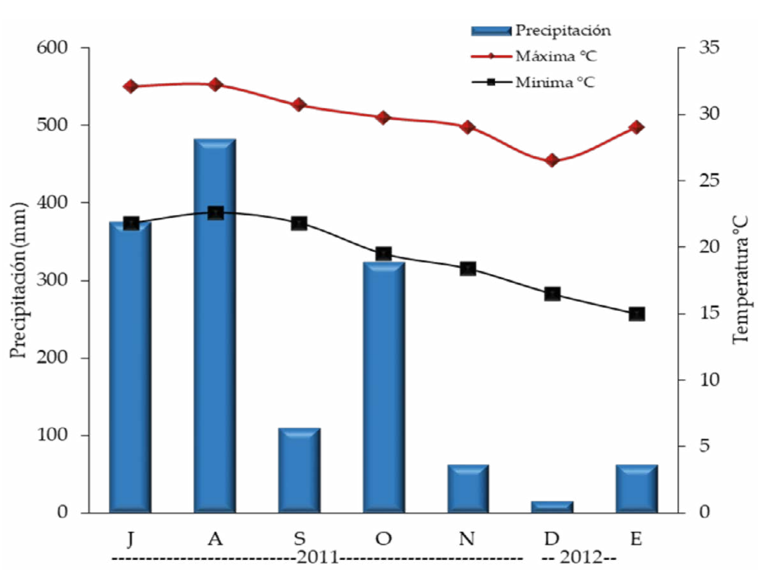

Figure 1. Accumulated monthly precipitation and monthly average maximum and minimum temperatures during the study period in Isla, Veracruz.

The experiment was established on July 22, 2011. The experiment consisted of 3 repetitions and was carried out in 5×16 m wide and long plots. Botanical seeds were sown in furrows, with a 0.50-m separation, resulting in a 14 kg ha^{-1} density. Two 120-80-00 kg ha^{-1} NPK fertilization doses were applied at 43 and 112 days after sowing (DAS).

The variables evaluated were the different growing ages (30, 60, 75, 90, 105, 120, 135, 150, 165, and 180 DAS). A destructive and random sampling was carried out. For each plant age, the forage was harvested at soil level, in 1-m lineal transects per plot. The total fresh weight of the transect of the harvested material was determined; subsequently, the material was dehydrated in a forced air oven (at 55 °C) and, then, it was weighted again, to obtain dry matter (DM) yield. A sub-sample was divided into its morphological components; afterwards, the leaf:stem (L:S) and the leaf:no-leaf (L:NL) ratios were determined.

The cutting yield was used to calculate growth rate (GR), according to the following formula:

$$GR = HF/t$$

Where: HF=harvested forage (kg ha^{-1} DM) and t=days from the sowing date to the cutting (Peters *et al.*, 2022).

In order to determine the intercepted radiation (IR), five random readings were carried out at approximately 12:00 h, using a 1-m rule (divided into cm). The readings were divided by age and plot. The shadow cast on the rule was taken as the radiation intercepted by the canopy (Calzada-Marín *et al.*, 2014; Mendoza-Pedroza and Álvarez-Vázquez, 2022). The quadrant method (1 m^2) was used to determine coverage (Herrera-Haro and García, 2021). Plant height was established with five random measurements, placing the rule at ground level.

A completely randomized experimental block design, with repeated measurements, 10 treatments (the same number as the growth stages), and 3 repetitions was used. In addition, a regression analysis was conducted for each variable. This analysis described the trend, from the moment that the best model is selected, according to the coefficient of determination and the significance degree of the model. Data were analyzed using the SAS GLM procedure and Tukey's mean comparison test ($\alpha \leq 0.05$).

RESULTS AND DISCUSSION

Regrowth age had a significant effect ($p<0.01$) in the total biomass (TB), leaf biomass (LB), stem biomass (SB), dead material (DM), and net growth (NG) accumulation. Consequently, they have a positive relationship with the regrowth age (Figure 2). The biomass accumulation of the different components was adjusted to a third-degree polynomial, with a high determination coefficient ($R^2>0.90$). While Álvarez-Adán (2019) mentioned that temperatures >30 °C favors growth rates, Gichangi *et al.* (2017) reported similar models for the Mulato (*Brachiaria hybrid*) and Fountain (*Pennisetum* sp.) grasses.

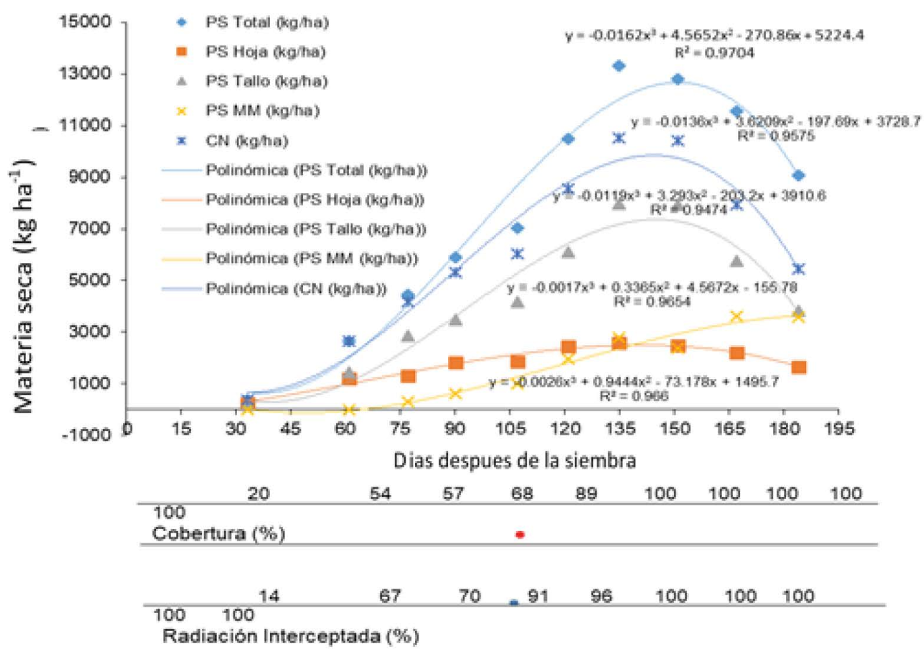

Figure 2. Accumulation dynamics of the total forage of Chetumal grass, by morphological component, plant height, intercepted radiation, and coverage percentage.

The highest TB accumulation (13,324 kg ha^{-1} DM) was recorded 153 days after the sowing (DAS), reaching the highest LB (2,569 kg ha^{-1} DM), SB (7,969 kg ha^{-1} DM), and NG (10,538 kg ha^{-1} DM). In later ages, TB, LB, SB, and NG decreased, as a result of the increase of biomass losses, caused by senescence and decomposition. This phenomenon was reflected in a DM (Gusmão *et al.*, 2020). Solofondranohatra *et al.* (2021) reported that, when plants reach their optimal leaf area index, the shadow on the lower layers of the canopy increases and senescence is higher than the growth of the leaves. Meanwhile, a high forage production and a low accumulation of dead material can be obtained, when 95% of the radiation is intercepted during the vegetative stage (Niinemets, 2018). This phenomenon took place in this study at 135 DAS, recording a 100% IR and a 68 cm plant height. In grasses such as *Digitaria eriantha* Steud, the highest regrowth height recorded higher senescence rates, while optimal defoliation height took place between 35 and 45 days after the regrowth (Gusmão *et al.*, 2020)

Growth rate (GR) was statistically different ($p<0.01$) as a result of the regrowth age. The GR-adjusted model was a third-degree polynomial, with a high determination coefficient ($R^2>0.90$). Emergence increased until it reached a 99 kg DM ha^{-1} day^{-1} maximum growth at 135 DAS. Subsequently, it decreased to 50 kg ha^{-1} day^{-1}, towards the end of the study (Figure 3). The increase of GR is directly related to the vegetal biomass, the canopy increase, and the photosynthetic capacity, as a result of the accumulation of more leaf area, reaching a maximum growth (Martínez-Méndez *et al.*, 2020). The highest GR (Figure 3) was recorded by the accumulation of the leaf biomass (Cruz-Hernández *et al.*, 2020).

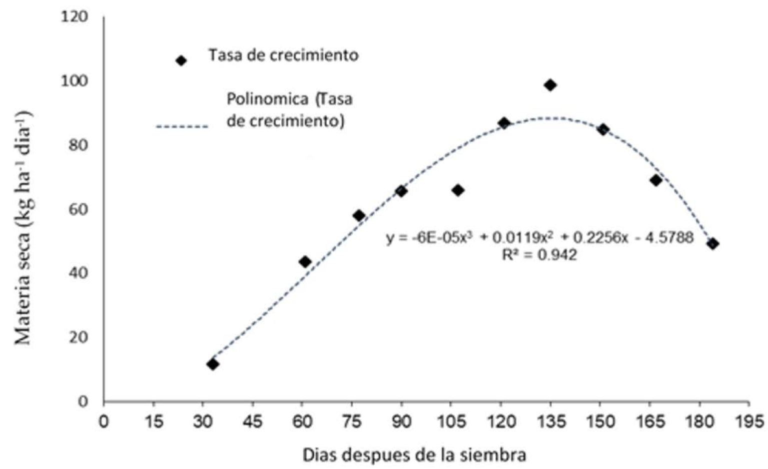

Figure 3. Changes in the growth rate of Chetumal grass over time.

Calzada-Marín *et al.* (2019) pointed out that a higher IR accumulation (95%) results in a higher photosynthetic activity and consequently, a maximum GR. In conclusion, the optimal moment to carry out the first cutting or the grazing is when the GR reaches its maximum point. Figure 4 shows that the morphological components (the leaves) are related to forage quality, while animal productivity is connected to the proportion of leaves included in their diets. Cruz-Hernández *et al.* (2017) studied *Brachiaria humidicola* grass and recorded that the highest leaf accumulation took place during the rainy season, with grazing every 28 days. The morphological composition of the plants in this study consisted of 62% leaves and 38% stems at 33 DAS. At day 77, the number of leaves diminished, while stem proportion increased and DM started to appear. Meanwhile, at 135 DAS, the

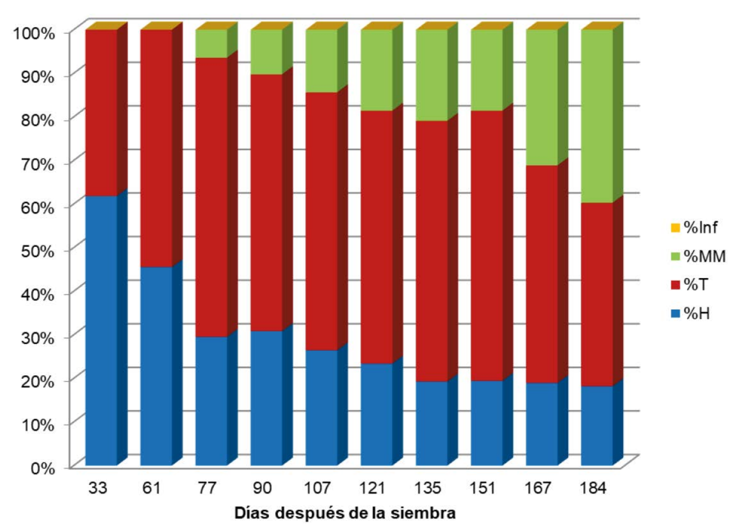

Figure 4. Morphological changes of *Urochloa humidicola* CIAT 679 grass, at different growing stages. Inf%: inflorescence percentage; %MM (DM%): dead material (mm); %T (S%): stem percentage; %H (L%): leaf percentage.

following percentages were recorded: 19% (leaves), 60% (stem), and 21% (dead material) (Rojas García *et al.*, 2020).

The L:S ratio was adjusted to a regression model using a third-degree polynomial (R^2=0.97), with 1.62-0.31 variations, while the L:NL ratio was determined with a potential regression model (R^2=0.95), recording 1.62-0.22 values during the growth stages (Figure 4). Both ratios show a decrease trend as the plant matures, given the increase of the stem and dead material biomass (Figure 2). The difference between both ratios was noticeable at 135 DAS, as a consequence of the DM increase. Calzada-Marín *et al.* (2018) reported this behavior in *Dactylis glomerata* L. and *Pennisetum purpureum* Schum.

CONCLUSIONS

Based on the results obtained in this study, the first defoliation or cutting of the Chetumal grass (*Urochloa humidicola* CIAT 679) should be carried out between 120 and 135 DAS or when the grass of the prairie reaches a plant height of 66-68 cm and 100% of the radiation is intercepted.

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Colegio de Postgraduados

Growth and yield models for black beans under magnetization and pH variation in a greenhouse

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ABSTRACT

Objective: To estimate growth and yield variations in common beans (*Phaseolus vulgaris* L.) treated with a magnetized nutrient solution considering two factors: magnet exposure time and pH level. The significance of this crop lies in its nutritional and economic value.

Design/Methodology/Approach: We used a hydroponic system with magnetized Steiner nutrient solution. The design was completely randomized, with a 4×6 factorial treatment arrangement and three replications. Factor A comprised exposure times (0.333 hours, 2 hours, chronic, and without magnetization), while Factor B covered solutions with different pH levels (3, 4, 5, 6, 7, and 8). We then applied a multiple regression analysis using the SAS software.

Results: Models for vegetative growth variables (plant height, root length, root dry weight, and foliar biomass) and seed yield components (number of pods, number of grains per pod) were statistically significant ($p<0.0001$). Coefficients of determination ranged from 59.7% to 82%, percentages considered appropriate to explain the observed variability.

Study limitations/Implications: While the models showed acceptable coefficients of determination, it is essential to consider other factors that were not assessed in this study: exposure to sunlight, insect influence, and diseases that could impact the responses of the bean crop.

Findings/Conclusions: Appropriate models to describe vegetative growth and seed yield of the common bean, concerning magnetization time and nutrient solution acidity, include variables such as plant height, root length, root dry weight, foliar biomass, total biomass, number of pods, and number of grains per pod.

Keywords: *Phaseolus vulgaris* L.; Greenhouse; Magnets.

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INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is crucial for human nutrition in tropical and subtropical regions. Latin America leads in common bean production and consumption, comprising 45% of global production (Smith and Rao, 2021). This crop has a significant economic and nutritional role —it contains iron, potassium, magnesium, phosphorus,

vitamins B1 and B6, folate, and niacin (Gonzalez-Gonzalez and Guertin, 2021; Ramirez-Cabral, Kumar, and Taylor, 2016). Historical crop data are essential for predicting yields (Gonzalez-Gonzalez and Guertin, 2021). These data are collected throughout the growth cycle, considering management and conditions (Poudel *et al.*, 2022). In Mexico, simulation models have been used to anticipate bean yields (Medina-García *et al.*, 2010; Spoorthi *et al.*, 2022). Applying magnetic fields improves bean growth, nutrient absorption, and productivity (Mroczek-Zdyrska *et al.*, 2016; Hozayn *et al.*, 2017; Vashisth and Joshi, 2017). Magnetic fields affect water's physicochemical and molecular properties (Krishnaraj *et al.*, 2017; El-Sabrout and Hanafy, 2017). On a biological level, they affect membrane permeability, ion flow, enzymatic processes, and metabolic pathways (Pang, 2014; Sheykina, 2016). This study aims to model the growth and yield of common beans in a greenhouse, considering the nutrient solution's pH level and magnetization time.

MATERIALS AND METHODS

We conducted our experiment in February 2019 at the greenhouse of the Universidad Autónoma Agraria Antonio Narro, Unidad Laguna, in Torreón, Coahuila, Mexico (25° 31" 11' N, 103° 25" 57' W, 1123 masl). The area has a hot desert climate (BWh) with maximum temperatures of 40 °C and minimums of 6 °C. The average precipitation is 250 mm (García, 2004). We used a hydroponic system with Steiner (1966) nutrient solution, making pH adjustments with sodium hydroxide (NaOH) and sulfuric acid (H_2SO_4) (Santos-Cavalcante *et al.*, 2016). The solution was magnetized with neodymium magnets 2.5 cm long and 2.0 cm in diameter with a field intensity of 0.380 Tesla. The experimental design was completely randomized with a 4×6 factorial treatment arrangement and three replications. Factor A considered different exposure times (0.333 hours, 2 hours, chronic, and without magnet), while factor B covered pH variations (3 to 8) in the nutrient solution. We employed growing degree days to assess vegetative development, using the formula $GD=\sum_{i=0}^{n}(Xi-Tb)$. For physiological time, we considered the accumulation of heating degree days since the transplant of the bean plants into the greenhouse (Romero Félix *et al.*, 2019). We conducted multiple regression analyses using the Statistical Analysis System program (SAS, 1999).

RESULTS AND DISCUSSION

Heat units in seed growth, development, and yield

The total heat units (HU) accumulated during growth, development (PH), and seed yield (NP and SY) equaled 1410 °C d. This accumulation was distributed between transplant, start of flowering (522 °C d), and harvest (HAR) in approximately 126 days. We observed that the accumulation of heat units during the phenological cycle of black bean cultivation follows a linear equation: (HU=31.67210+331dds) R^2=0.98 (Figure 1).

Vegetative development

The best model for vegetative development based on the exposure time and pH of the magnetized nutrient solution was quadratic. A cubic model offered the highest coefficient

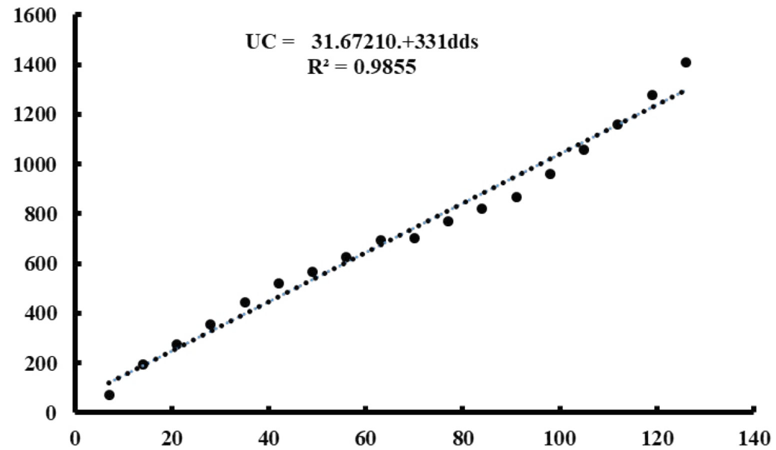

Figure 1. Heat unit accumulation in black beans in an experiment conducted under greenhouse conditions at the Universidad Autónoma Agraria Antonio Narro Unidad Laguna, Torreón, Coahuila, Mexico, 2018-2019.

of determination (R2) in plant height, root length, root dry weight, aboveground biomass, and total biomass.

$$PH=122.07\ \mathrm{pH}-21.54\mathrm{pH}^2+1.19\ \mathrm{pH}^3-192.01$$

$$RL=3.37MET-0.13MET^2+62.07\ \mathrm{pH}-10.05\mathrm{pH}^2+0.48\mathrm{pH}^3-86$$

$$RDW=7.43\ \mathrm{pH}-1.29\mathrm{pH}^2+0.07\mathrm{pH}^3-12.14$$

$$AGB=-7.36MET+4.41MET^2-0.17MET^3+43.18\mathrm{pH}-7.62\mathrm{pH}^2+0.42\mathrm{pH}^3-69.47$$

$$TB=-8.06MET+4.81MET^2-0.19MET^3+50.61\mathrm{pH}-8.91\mathrm{pH}^2+0.49\mathrm{pH}^3-81.61$$

Where *PH*=plant height; *RL*=root length; *RDW*=root dry weight; *AGB*=aboveground biomass; *TB*=total biomass; pH=pH of the magnetized solution; and *MET*=magnet exposure time.

Models for plant height, root length, root dry weight, aboveground biomass, and total biomass were statistically significant ($p<0.0001$) for all variables. Their coefficients of determination (R^2=0.597, 0.700, 0.576, 0.655, and 0.693) are acceptable and explain the variability from 59.7% to 70% (Table 1). These results are similar to those reported by Medina-García *et al.* (2010); Liu *et al.* (2019), and Vashisth and Joshi (2017), who indicate that magnetized water promotes the growth of seedlings, contributing to the development of roots and mineral nutrient contents. In this regard, Çelik *et al.* (2008) show the effects of irrigation with magnetized water compared to untreated water, and show that the former increases plant growth rate, noticeable in the increase in biomass.

Table 1. Multiple regression analysis for the model of vegetative development components in the Veracruz black bean variety, determined in an experiment under greenhouse conditions at the Universidad Autónoma Agraria Antonio Narro Unidad Laguna, Torreón, Coahuila, Mexico, 2018-2019.

Variables	Parameter	Estimator	Standard error	T-value	p>t	p>F	R^2
Plant height	Intercept	−192.01	28.48	−6.74	0.0001	0.0001	0.597
	pH	122.07	17.24	7.08	0.0001		
	pH^2	−21.54	3.28	−6.56	0.0001		
	pH^3	1.19	0.2	5.98	0.0001		
Root length	Intercept	−86.58	29.01	−2.98	0.004	<0.0001	0.7
	Time	3.37	0.96	3.52	0.0008		
	$Time^2$	−0.13	0.04	−3.22	0.002		
	pH	62.07	17.55	3.54	0.0007		
	pH^2	−10.05	3.34	−3.01	0.0037		
	pH^3	0.48	0.2	2.37	0.021		
Root dry weight	Intercept	−12.14	1.79	−6.8	0.0001	0.0001	0.576
	pH	7.43	1.08	6.87	0.0001		
	pH^2	−1.29	0.21	−6.27	0.0001		
	pH^3	0.07	0.01	5.67	0.0001		
Biomass area	Intercept	−69.47	9.71	−7.15	0.0001	0.0001	0.655
	Time	−7.36	2.26	−3.26	0.0018		
	$Time^2$	4.41	1.15	3.82	0.0003		
	$Time^3$	−0.17	0.04	−3.87	0.0003		
	pH	43.18	5.87	7.35	0.0001		
	pH^2	−7.62	1.12	−6.82	0.0001		
	pH^3	0.42	0.07	6.27	0.0001		
Total biomass	Intercept	−81.61	10.32	−7.91	0.0001	0.0001	0.693
	Time	−8.06	2.4	−3.36	0.0013		
	$Time^2$	4.81	1.22	3.93	0.0002		
	$Time^3$	−0.19	0.05	−3.97	0.0002		
	pH	50.61	6.24	8.11	0.0001		
	pH^2	−8.91	1.19	−7.5	0.0001		
	pH^3	0.49	0.07	6.88	0.0001		

Yield components

The best model for yield components based on the exposure time and pH of the magnetized nutrient solution was quadratic. A cubic model offered the highest coefficient of determination regarding number of pods (NP), number of grains per pod (NGP), and seed yield (SY).

$$NP = 16.80\text{pH} - 1.55\text{pH}^2 - 33.00$$

$$NGP = 2.34MET - 1.04MET^2 + 0.04MET^3 + 18.77\text{pH} - 3.12\text{pH}^2 + 0.17\text{pH}^3 - 31.25$$

$$SY = -2.50MET + 1.45MET^2 - 0.06MET^3 + 14.83\ \text{pH} - 2.61\text{pH}^2 + 0.14\text{pH}^3 - 24.27$$

Models for number of pods (NP), number of grains per pod (NGP), and seed yield (SY), were statistically significant ($p<0.0001$) for yield components. The coefficients of determination ($R^2=0.65$, 0.821, and 0.708) were acceptable since they explain the variability from 65.0% to 82.1% (Table 2). While studying bean cultivation, Moussa (2011) Australia, USA, China and Japan indicated the type of irrigation water and its interaction with the plants. According to this study, irrigation water treated with magnetization achieved an increase in photosynthetic activity and better photoassimilates translocation efficiency. Fouad Abobatta (2019) reports that applying magnetic fields to Valencia orange increases yield, number, and quality of fruits, as well as number of flowers. Magnetic fields also increase the yield of strawberry fruits.

El-Ssawy *et al.* (2020) reported that using the NFT (nutrient film technique) hydroponic technique with magnetized water at a high intensity (level 3) led to a significant increase

Table 2. Multiple regression analysis for the model of seed yield components for the Veracruz black bean variety, determined in an experiment under greenhouse conditions at the Universidad Autónoma Agraria Antonio Narro Unidad Laguna, Torreón, Coahuila, Mexico, 2018-2019.

Variables	Parameter	Estimator	Standard error	T-value	P>t	P>F	R^2
Number of pods	Intercept	−33	3.92	−8.41	0.0001	0.0001	0.65
	pH	16.8	1.52	11.04	0.0001		
	pH^2	−1.55	0.14	−11.27	0.0001		
Number of grain per pod	Intercept	−31.25	3.25	−9.62	0.0001	0.0001	0.821
	Time	2.34	0.76	3.1	0.0029		
	$Time^2$	−1.04	0.39	−2.7	0.0087		
	$Time^3$	0.04	0.01	2.67	0.0095		
	pH	18.77	1.96	9.55	0.0001		
	pH^2	−3.12	0.37	−8.35	0.0001		
	pH^3	0.17	0.02	7.31	0.0001		
Performance seed	Intercept	−24.27	2.93	−8.27	0.0001	0.0001	0.708
	Time	−2.5	0.68	−3.66	0.0005		
	$Time^2$	1.45	0.35	4.18	0.0001		
	$Time^3$	−0.06	0.01	−4.21	0.0001		
	pH	14.83	1.77	8.35	0.0001		
	pH^2	−2.61	0.34	−7.73	0.0001		
	pH^3	0.14	0.02	7.09	0.0001		

in nutrient concentrations (N, P, and K) and total soluble solids, although the pH of the nutrient solution had to be reduced for lettuce. They also found an increase in water productivity, fresh yield, and number of lettuce leaves, along with high chlorophyll content.

CONCLUSIONS

Models for vegetative development and seed yield components comprised plant height, root length, root dry weight, aboveground biomass, total biomass, number of pods, and number of grains per pod. These components are the most appropriate to study the Veracruz black bean variety based on the time of exposure to magnetization and the acidity degree of the nutrient solution under greenhouse conditions.

Further studies should be conducted to determine the model's applicability in nutrient solutions based on magnet exposure time and pH variation.

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Colegio de Postgraduados

Harvest age of *Urochloa* hybrids regarding yield, chemical composition, and *in vitro* biogas production

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ABSTRACT

Objective: To evaluate the chemical composition, fermentation, and *in vitro* biogas production of the Cayman and Cobra cultivars, at different cutting ages.

Design/Methodology/Approach: Dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), hemicellulose (HEM), and cellulose (CEL) were determined at 28, 35, 42, and 49 cutting days. *In vitro* dry matter degradation (IVDMD), pH, and concentration of volatile fatty acids (VFA) were determined during fermentation. The biogas volume was estimated at 6, 12, 24, 48, and 72 h; the volume of methane (CH_4) and carbon dioxide (CO_2) in the Cobra and Cayman forages was determined at 72 h. A completely randomized design was used for the experiment.

Results: There were no differences ($P>0.05$) in DM production during the different cutting ages. CP was higher ($P<0.05$) in both cultivars, at 28 and 35 days after the cutting. The NDF, ADF, HEM, and CEL percentages were different in both cultivars. IVDMD was higher ($P<0.05$) between day 28 and day 42. Finally, CH_4 production was lower ($P<0.05$) at 28 and 35 d after the cutting.

Study Limitations/Implications: The chemical composition of pastures is influenced by climate and, therefore, further analysis must be carried out during different periods or seasons of the year.

Findings/Conclusions: The optimal cutting age of the Cobra and Cayman cultivars under drought conditions is between day 28 and day 35 of regrowth. During that period, they have the best chemical and fermentation characteristics.

Keywords: Cobra and Cayman cultivars, cutting time, fermentation, methane production.

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INTRODUCTION

The drought conditions of the tropical areas limit the productive potential of forages (Rojas-García *et al.*, 2018), causing changes in the chemical composition and low biomass production in the prairies (Pratti-Daniel *et al.*, 2019). Different improved genotypes of grasses have been selected in recent years, including hybrids of genus *Urochloa*, such

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as Cayman (BR/1752) and Cobra (BR/1794). These hybrids have recorded excellent biomass production yields and have adapted well to the tropical climate (Pizarro *et al.*, 2013; Vandremini, 2014). Arroyave *et al.* (2013) reported that these cultivars obtain an annual yield of 2 to 4 t DM ha^{-1}, without fertilization, during long drought periods. In addition, they can also grow in alkaline or acid soils (Morales-Bautista *et al.*, 2011). Age impacts the nutritional quality of forages, producing changes in the chemical composition, which affect animal production. The ruminal degradability of grasses is related to the cell wall percentages, because the degradability of the rumen decreases as the fiber content increases (Ledea *et al.*, 2018). Ku-Vera *et al.* (2014) reported that tropical forages had a low CP content and a high NDF percentage, as well as a low digestibility and a lower metabolizable energy content. When tropical grasses are harvested at an appropriate age, they have a lower cell wall content and a higher CP protein and soluble sugars content, which reduces the CH_4 and CO_2 emissions produced by ruminants (Camacho-Escobar *et al.*, 2020).

Eckard *et al.* (2010) reported that between 3% and 10% of the net energy consumed by a bovine becomes CH_4, which is mainly eliminated when the animals belch. Consequently, reducing these emissions has a double impact. On the one hand, the greenhouse gas emissions (GGE) that causes global warming to diminish and, on the other hand, the loss of energy used for animal production also diminishes. The hypothesis of this research was that, during the harvest age, the chemical composition, the fermentation parameters, and the *in vitro* biogas (CH_4 and CO_2) production of the Cobra and Cayman *Urochloa* hybrids were impacted by drought conditions. Therefore, the objective of this study was to evaluate the chemical composition, fermentation, and *in vitro* biogas production of CH_4 and CO_2 of the Cobra and Cayman cultivars, at different harvest ages, during the drought season, in the coastal region (La Costa) of Oaxaca, Mexico.

MATERIALS AND METHODS

The research was carried out at the microbiology and nutrition labs and in the Área de Cultivos Forrajeros of the Campo Experimental of the Universidad del Mar, Campus Puerto Escondido, located in the Bajos de Chila, municipality of San Pedro Mixtepec, Juquila, Oaxaca (15° 55’ 27.54” N and 97° 09’ 04.09” W), at 12 m.a.s.l. The climate is warm sub-humid (A(c)w2 type), with an annual average precipitation of 930.8 mm-1668 mm and an annual average temperature of 26 °C (Serrano-Altamirano *et al.*, 2005).

Sampling collection

The Cayman and Cobra cultivars were sown by hand in a 480 m^2 area, using PAPALOTLA® certified seeds. The seeds were sown in small holes, one next to the other (1 cm apart), with a 6.0 kg ha^{-1} density. The distance between the furrows was 50 cm. The experimental area was divided into eight 60-m^2 plots. During the growing period, a single 50 kg ha^{-1} urea dose was applied. Auxiliary irrigation was carried out once a week. A standardization cutting was made 90 days after the sowing, when the cultivars had grown 10 cm above ground. Cutting intervals took place 4, 5, 6, and 7 weeks after the regrowth.

Chemical composition

The samples consisted of forages harvested at intervals of 4, 5, 6, and 7 weeks after regrowth. The moisture and dry matter content of the samples were determined. Dry matter content was determined following the recommendations of Herrera (2014), using a drying oven (Felisa®, México) at 65 °C for 48 h. The samples were processed in a Werker MF 10 grinder (Ika®, USA), with a 1 mm sieve. Subsequently, CP was determined using the Kjeldahl method (AOAC, 1997); meanwhile, NDF, ADF, CEL, and HEM were determined following the recommendation of Van Soest *et al.* (1991). The samples were analyzed at the Animal Nutrition lab of the Departamento de Ciencias Básicas Para la Salud, Universidad de Guadalajara, Centro Universitario del Sur, Ciudad Guzmán, Jalisco. Mexico.

Culture medium preparation

In order to determine the *in vitro* dry matter degradation (IVDMD), pH, VFA, and biogas, CH_4, and CO_2 production, a culture medium for rumen microorganisms was used. Based on Cobos and Yokoyama (1995), the culture medium was prepared with glucose, cellobiose, starch, and fresh ruminal fluid (GCA-FR). The source of the inoculum was fresh ruminal fluid from a Zebu × Brown Swiss adult, with 500 kg live weight and a rumen fistula. The fluid was extracted 2 h after the specimen was fed with a 100% forage diet. The handling of the bovine complied with the regulations for the use of animals with research purposes of the Universidad del Mar (NOM-024-ZOO-1995).

***In vitro* dry matter degradation (IVDMD)**

In order to determine *in vitro* dry matter degradation (IVDMD), 0.5 g DM from each treatment was weighted in triplicate, adding 120 mL viral serology. Forty-five mL of the sterile culture medium for total bacteria was added to each vial (Cobos and Yokoyama, 1995). The vials were inoculated with 5 mL of fresh ruminal fluid. Each vial was considered both as a bio-fermenter and an experimental unit. IVDMD was determined after 72 h, following the recommendations of Mellenberger *et al.* (1970).

***In vitro* rumen fermentation 72 h after the incubation**

An Orion® portable potentiometer (USA) was used to determine pH. A Perkin Elmer® gas chromatograph (USA) with a flame ionization detector was used to determine VFA molar concentration, based on the recommendations of Erwin *et al.* (1961). The following retention times were recorded: 1.26 m for acetate, 1.6 m for propionate, and 2.09 m for butyrate. The analysis was carried out in the Microbiología Ruminal and Genética Microbiana labs of the Colegio de Postgraduados, Mexico.

Biogas emissions measurement

Biogas production was estimated using the technique reported by Krabill *et al.* (1969) and modified by Cobos *et al.* (2018). The bio-fermenters were placed in a bath Marie (39 °C); they were connected to the biogas capture traps with a Tygon® hose (internal diameter: 3/32”). Two Terumo® 20 g × 1 inch needles were connected to each end of the hose. The bio-fermenter was connected to one end and the biogas capture trap was connected to the other. A needle was connected to the latter as a release valve. The trap was placed upside-down in a plastic test tube. Biogas volume was quantified at 6, 12, 24, 48, and 72 h of incubation.

Estimation of the *in vitro* production of CH_4 and CO_2

The CH_4 and CO_2 ratios were determined using a 500 μL sample of the biogas obtained from the traps. The sample was injected in a Perkin Elmer® gas chromatograph (USA), which had a thermal conductivity indicator and a Poropack packed column. Retention times were 0.71 m and 1.0005 m for CH_4 and CO_2, respectively. The analyses were carried out in the Microbiología Ruminal and Genética Microbiana labs of the Colegio de Postgraduados, Mexico.

Statistical analysis

The chemical and *in vitro* rumen fermentation variables were analyzed using a completely randomized design, with four treatments and three repetitions per treatment for each cultivar. All the evaluated variables were subjected to an analysis of variance, using the GLM procedure of SAS (2011) and Tukey's mean comparison test (α=0.05) (Steel and Torrie, 1988).

RESULTS AND DISCUSSION

The DM yield of the cultivars did not record statistical differences (P>0.05) in the different harvest ages. Meanwhile, there were differences (P<0.05) in the chemical composition regarding the harvest age for the Cobra and Cayman cultivars (Table 1).

Table 1. Effect of the harvest age in the yield, chemical composition, and IVDMD of the Cobra and Cayman cultivars, during the drought season in the tropic.

Cultivar	kg MS ha^{-1}	PC	FDN	FDA	HEM	CEL	DIVMS
Cobra							
28d	1858.24^{a}	10.26^{a}	52.27^{c}	26.74^{b}	25.91^{b}	10.86^{b}	61.27^{a}
35d	2273.02^{a}	9.54^{b}	56.78^{b}	35.42^{a}	22.29^{a}	14.45^{a}	61.09^{a}
42d	1877.28^{a}	7.94^{c}	61.57^{a}	32.79^{a}	27.73^{d}	11.89^{b}	56.87^{a}
49d	2596.44^{a}	7.74^{c}	54.86^{b}	29.25^{b}	24.13^{c}	11.95^{b}	49.39^{b}
MS total	6746.74						
EE	205.84	0.12	0.47	0.58	0.36	0.3	1.01
Cayman							
28d	1662.26^{a}	11.15^{a}	55.83^{b}	27.54^{b}	23.90^{a}	10.86^{b}	62.11^{a}
35d	1848.51^{a}	10.75^{a}	57.47^{b}	28.19^{b}	19.87^{b}	14.45^{a}	58.30^{ab}
42d	2201.96^{a}	9.44^{bc}	57.73^{ab}	30.34^{a}	24.43^{a}	11.89^{b}	58.80^{ab}
49d	2711.99^{a}	8.26^{c}	59.38^{a}	31.68^{a}	23.86^{a}	11.95^{b}	53.52^{b}
MS total	8424.72						
EE	179.91	0.37	0.68	0.38	0.25	0.3	1.66

abc=in the column, indicates statistical differences (P<0.05) in the harvest age.
28, 35, 42, 49=harvest age of the cultivar. EE (SE): standard error.

The lack of differences in DM production in the cultivars is the result of the lower moisture content of the soil, which may have been the cause of the lower nutrient availability for the grasses. Consequently, the reduction in the weight of the stems and leaves caused a lower biomass production. Pizarro *et al.* (2013) reported that, under adverse conditions, the Cayman and Cobra hybrids have a specialized genetic potential for forage production. The accumulated DM production in this study reached 8,424.72 and 6,746.74 kg ha^{-1} for Cayman and Cobra, respectively. These results match the findings of Garay-Martínez *et al.* (2018), who reported a forage production of 6,310 and 8,890 kg ha^{-1}, under drought conditions, for the Cobra and Cayman cultivars, respectively. The lowest DM yield among the cultivars under study was the consequence of the low precipitation levels recorded during the evaluation. The lack of humidity impacted the nutrient availability in the soil, changing the photosynthesis biochemical process of the plants (Cardoso *et al.*, 2015). In addition, the short intervals between harvests limit the capacity of the plants to accumulate reserves, preventing a vigorous regrowth, which results in a lower growth rate and a lower biomass accumulation (Cruz-Hernández *et al.*, 2017). Meanwhile, plant metabolism increases along with harvest age (Castro *et al.*, 2013), which causes a higher leaf production that subsequently increases the biomass of the grass.

The CP content recorded differences depending on the harvest age ($P<0.05$) of both cultivars. The Cobra hybrid recorded its highest CP content at a younger harvest age (28 days), while its percentages decreased at an older harvest age (42 and 49 d). The same trend was recorded for Cayman. The results obtained in this experiment are lower than the findings of Garay-Martínez *et al.* (2018), who reported CP percentage results, at 28 d of age, of 16.2 and 16.1 for Cayman and Cobra, respectively. For their part, Rojas-García *et al.* (2018) reported a 14.4% CP for the Cobra cultivar, at 35 d of regrowth. In this study, CP recorded a decreasing trend as the age of the plant increased. Meanwhile, Garay-Martínez *et al.* (2018) determined that the protein content of *Urochloa* hybrid grasses decreased in older plants. On more mature grasses, DM content and cell walls tend to increase and, consequently, the CP percentage tends to diminish. Therefore, determining the optimal cutting age —in which grasses have the best nutritional characteristics for the animals— is fundamental (Rojas-García *et al.*, 2018). At an early age, grasses have a higher leaf:stem ratio, which increases the plants' CP concentrations —which can be found in higher amounts in its leaves. At an older age, the cellulose, hemicellulose, and lignin content of the cell wall synthesis increases and, consequently, the nutritional quality of the grass decreases (de Dios-León *et al.*, 2022).

Differences in NDF percentages ($P<0.05$) depended on the harvest age; the lowest values were recorded at day 28 in both cultivars. The highest value recorded by Cobra grass was 61.57%, at day 42, while Cayman grass recorded 59.36%, at day 49. Overall, the NDF of both cultivars increased along with the harvest age. The NDF value for Cobra grass was higher at day 35 than the value obtained by Cayman at day 49. The ADF results have a similar trend than the results of NDF. These values do not match the findings of López-Garrido *et al.* (2022), who reported 67.75% NDF and 40.75% ADF values for Cobra grass. Regarding Cayman grass, they reported 71.55% NDF and 44.53% ADF. These percentages were obtained with a 60-day harvest age. Villalobos and Arce (2013) pointed out that a high NDF and ADF content decreases the degradation and nutrient content of the forages. On the one hand, the differences ($P<0.05$) in the hemicellulose percentage depend on the harvesting age: the highest content was recorded by Cobra at day 42 (27.73%), while Cayman recorded lower values at day 35 (19.87%). On the other hand, the highest value (24.43%) was obtained 42 days after the harvest. In this regard, Reyes-Pérez (2022) carried out a study with *Brachiaria decumbens*, during a period with scarce rain, and reported 30.18% and 30.58% values, at 30 and 45 harvest days, respectively. This study found out differences ($P<0.05$) in the cellulose percentage of the cultivars, at 42 and 49 harvest days; the Cobra grass obtained the highest value. A trend towards a cellulose content increase was recorded as the plant grew older. As the age of the plant increases, the cell wall ratio also increases. This ratio is associated with the increase in the DM, NDF, ADF, and hemicellulose content. Older plants are related to the reduction of new leaves, the increase of vascular bundles, the loss of water, a lower cell content, and a higher stem ratio (de Dios-León *et al.*, 2022).

The IVDMD in both cultivars decreased ($P<0.05$) with age; the highest value was recorded at 28, 42, and 45 harvest days. The results of this study match the findings of López-Garrido *et al.* (2022), who reported DM degradation percentages of 64.61% (Cobra) and 63.57% (Cayman), under moderate salinity conditions, at 60 harvest days. In this regard, Barrera *et al.* (2015) related the degradation decrease with the structural carbohydrates increase. Ledea-Rodríguez *et al.* (2018) pointed out that the DS degradation decrease is related to the increase of the age of the plant and the rumen environmental conditions, because DM degradation is related to the microbiota that colonizes food particles. Overall, de Dios-León *et al.* (2022) concluded that the IVDMD decrease is related to the increase of the age of the grasses, because, grasses have more leaves and a lower proportion of stems at an early harvest age (30-40 d) than at a mature harvest age (40-60 d), when high DM, NDF, and ADF percentages are recorded. As the forages mature, the stem proportion increases and the leaf proportion diminishes and, consequently, the structural carbohydrate and lignin content increases, reducing its degradation in the rumen.

No differences in pH were recorded ($P>0.05$) regarding the harvest age (Table 2). This behavior was similar for both grasses. Therefore, this situation suggests that the rumen bacteria had an appropriate development during the *in vitro* fermentation, because they require an almost neutral pH to carry out their enzymatic activity (Barboza *et al.*, 2009).

There were differences ($P<0.05$) in the concentration of acetate during the *in vitro* fermentation and the harvest age (Table 2). The Cobra grass and the Cayman grass recorded their highest concentration at day 49 and 42, respectively. López-Garrido *et al.* (2022) studied the same grass varieties, recording a lower acetate concentration at 60 harvest days, with values of 17.22 mM L^{-1} (Cobra) and 18.88 mM L^{-1} (Cayman). The propionate and butyrate concentrations of both cultivars did not record any differences ($P>0.05$) between harvest ages. A difference ($P<0.05$) in the total VFA concentration depended on the harvest age of both cultivars, recording a higher concentration at day 49 (Table 2).

Table 2. pH values and VFA concentration 72 h after the in vitro incubation of the Cobra and Cayman grasses, at different harvest ages.

Cultivar	pH	Ace	Prop	But	AGV Total	Ac:Pro
		mM L^{-1}				
Cobra						
28	6.35^{a}	25.77^{ab}	13.73^{a}	8.15^{a}	47.66^{ab}	1.87^{a}
35	6.29^{a}	25.48^{b}	12.71^{a}	7.18^{a}	45.37^{ab}	2.00^{a}
42	6.34^{a}	24.08^{c}	13.01^{a}	7.25^{a}	44.34^{b}	1.85^{a}
49	6.44^{a}	27.03^{a}	13.85^{a}	7.58^{a}	48.47^{a}	1.95^{a}
EE	0.07	0.27	0.29	0.37	0.73	0.03
Cayman						
28	6.33^{a}	23.76^{ab}	12.80^{a}	7.33^{a}	43.90^{ab}	1.85^{a}
35	6.37^{a}	23.38^{ab}	12.51^{a}	7.87^{a}	43.77^{ab}	1.87^{a}
42	6.32^{a}	22.07^{b}	12.32^{a}	7.79^{a}	42.18^{b}	1.79^{a}
49	6.41^{a}	23.94^{a}	13.25^{a}	8.09^{a}	45.28^{a}	1.81^{a}
EE	0.03	0.37	0.3	0.27	0.62	0.04

abc=in the column, indicates statistical differences ($P<0.05$) in the harvest age.
28, 35, 42, 49=harvest age of the cultivar. EE (SE): standard error.

The higher NDF percentage in the grasses provides more cellulose and hemicellulose, which can potentially be fermented by cellulolytic bacteria (such as *Ruminococcus flavefaciens*, *Ruminococcus albus*, and *Fibrobacter succinogenes*), degrading glucose and even acetate and butyrate (Ley de Coss *et al.*, 2018).

There were no differences ($P>0.05$) in biomass production, resulting from the harvest ages under study (Table 3). However, there were differences related to harvest age during the estimation of the CH_4 and CO_2 of the grasses. The highest CH_4 production was recorded at day 35 for both cultivars. Meanwhile, Cobra recorded the highest CO_2 production at day 49, while Cayman reported its highest production at day 42.

Biogas production increases or decreases during the *in vitro* fermentation depending on the nutritional value of the forage (Camacho-Escobar *et al.*, 2020). Feeds with a higher structural carbohydrate content and a lower CP content tend to produce a higher biogas volume, while feeds with a higher soluble carbohydrate content and higher CP percentages produce a lower biogas volume.

The results of this study confirm the findings of previous studies. A lower CH_4 production —along with a higher CP content and a lower NDF, ADF, and cellulose percentage— was recorded at day 28 in both cultivars. These elements are components of the cell wall of vegetables and of the acetate:propionate ratio recorded between both evaluated cultivars. Methane production is the result of the fermentation of the structural carbohydrates. Rumen with a higher quantity of acetate results in the creation of two molecules: CO_2 and 8 H^+. Butyrate production also develops two molecules: CO_2 and 4 H^+. As a result of their metabolism, *Archaeas* use CO_2 and hydrogen to produce CH_4 and ATP (Bedoya-Mazo *et al.*, 2016).

Table 3. Biogas, CH_4, and CO_2 (mL g^{-1} DM) in vitro production of the Cobra and Cayman grasses, at different harvest ages.

Cultivar	Producción de biogás total (mL g^{-1} MS)					CH_4	CO_2
	6 h	12 h	24 h	48 h	72 h	72 h	
Cobra							
28d	23.46[a]	25.20[a]	56.13[a]	34.00[a]	10.66[a]	37.06[c]	104.41[c]
35d	29.00[a]	25.40[a]	49.33[a]	27.73[a]	11.73[a]	43.55[b]	117.38[b]
42d	31.86[a]	26.66[a]	54.86[a]	35.80[a]	8.73[a]	47.53[a]	120.25[b]
49d	27.13[a]	30.33[a]	33.20[a]	29.33[a]	6.20[a]	45.01[a]	135.28[a]
EE	2.98	6.16	5.82	3.27	2.25	0.84	2.18
Cayman							
28d	35.46[a]	25.26[a]	58.20[a]	37.33[a]	9.66[a]	41.29[c]	121.06[b]
35d	37.33[a]	29.20[a]	44.20[a]	24.80[a]	12.20[a]	43.66[b]	134.05[a]
42d	36.53[a]	31.13[a]	51.53[a]	23.66[a]	13.86[a]	46.54[a]	135.38[a]
49d	18.66[a]	14.60[a]	27.20[a]	30.33[a]	10.40[a]	42.48[bc]	120.31[b]
EE	8.52	4.55	9.11	8.71	2.77	0.51	0.87

abc=in the column, indicates statistical differences ($P<0.05$) in the harvest age.
28, 35, 42, 49=harvest age of the cultivar. EE (SE): standard error.

CONCLUSIONS

The drought season had an impact on the DM production of the Cobra and Cayman cultivars. The best age to harvest these cultivars ranges from 28 to 35 d, when they have a higher CP content and a lower NDF, ADF, hemicellulose, and cellulose content. This phenomenon resulted in a higher *in vitro* degradation of the DM of both cultivars.

During the *in vitro* fermentation, both cultivars recorded their lowest methane production between 28 and 35 harvest days; consequently, under drought conditions, the best age to harvest the Cobra and Cayman grasses, in the coastal region of Oaxaca, is between 28 and 35 d after the regrowth.

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