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Directorios

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Conclusiones: Son la generalización de los resultados obtenidos; deben ser puntuales, claras y concisas, y no deben llevar discusión, haciendo hincapié en los aspectos nuevos e importantes de los resultados obtenidos y que establezcan los parámetros finales de lo observado en el estudio.

Agradecimientos: Son opcionales y tendrán un máximo de tres renglones para expresar agradecimientos a personas e instituciones que hayan contribuido a la realización del trabajo.

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> Ogata N. (2003b).

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AGRO PRODUCTIVIDAD

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Distribution of the monthly global solar irradiation in the state of Tabasco, Mexico

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ABSTRACT

Objective: To estimate the monthly average global solar irradiance (R_g), using observed cloudiness data (% of cloudy days), as well as its spatial distribution for the state of Tabasco, Mexico.

Design/Methodology/Approximation: The proposed model by Tejeda-Martínez *et al.* (1999) was adjusted to estimate the R_g of 35 meteorological stations in the state of Tabasco. The adjustment was performed with daily observed R_g data from eight automated weather stations and cloudiness data from eight ordinary weather stations.

Results: The proposed model reports a good fit, given that its prediction was optimal according to Willmott's comparison parameter (c=0.89), and excellent based on the Nash-Sutcliffe efficiency index (E=0.99) and had a high corrected determination coefficient of R_c^2=0.87.

Study limitations/implications: It is necessary that in the state of Tabasco the number of automated stations increase, as well as technical maintenance to the existing stations.

Findings/conclusions: The estimated R_g is statistically reliable. The highest R_g values occurred during the dry season, with a maximum of 22.99 MJ m^{-2} d^{-1}, distributed mainly in the northern part of the state. The lowest R_g values occurred during the northeast season (12.52 MJ m^{-2} d^{-1}), distributed in more than 80% of the total state area.

Keywords: Willmott's index, cloud cover, transmissibility, heliophany.

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INTRODUCTION

The information of the spatial and temporal variation of global solar irradiance values (R_g) is of utmost importance to calculate the net primary productivity of plant species (Bouchouicha *et al.*, 2019); as well as for estimating the agricultural crop's potential yield (van Ittersum *et al.*, 2013). In Mexico, as in many areas of the world, R_g is historically

Image by pixolotl at Pixabay

the least recorded meteorological variable (Quej *et al.*, 2016). The R_g has to be estimated from empirical models based on other readily available meteorological variables. Detailed knowledge of the spatial and temporal variation of R_g is still limited for the state of Tabasco, Mexico, and the southeast of the country because most of the existing meteorological stations are conventional and do not record it. The objective of the present research was to estimate the monthly average global solar irradiance, using observed cloudiness data, to elaborate monthly maps, showing its spatial distribution.

MATERIALS AND METHODS

Evaluated climatological variables

Daily data on the percentage of cloudy days (cloudy and medium cloudy days) were obtained from the ERIC III v.2 program from 34 ordinary climatological stations and a meteorological observatory at the state of Tabasco, Mexico (IMTA, 2009). This program allows the extraction of information contained in the CLICOM historical database of the National Meteorological Service (SMN) of Mexico.

Daily global solar irradiance data were obtained from the records of nine automatic meteorological stations (AMS's) of the SMN in Tabasco (SMN, 2016). Daily data were obtained from 2012 to 2015, as these were the most recent and complete. From the daily data of both variables, monthly averages of cloudiness (cloudy and half cloudy days) and global solar irradiance (R_g) in ($W\ m^{-2}$) were obtained. The data from each AMS was related to that of an ordinary climatological station that was less than 5 km away to ensure that the climatic conditions were similar. Table 1 shows the relation of the selected AMS's and their associated ordinary climatological station, from which the R_g data and the percentage of cloudy days (cloudiness) were obtained.

Table 1. List of the automatic meteorological stations (AMS) and their associated ordinary climatological station at Tabasco, Mexico.

Automatic Weather Station	Lat.	Long.	Alt.	Ordinary weather station	Lat.	Long.	Alt.
Boca del Cerro, Tenosique	17.54	91.49	14	Boca de Cerro, Tenosique	17.43	91.32	27
Dos Patrias, Tacotalpa	17.61	92.80	25	Dos Patrias, Tacotalpa	17.61	92.80	25
E. Zapata, Emiliano Zapata	17.75	91.76	20	E. Zapata, Emiliano Zapata	17.74	91.78	26
Huimanguillo, Huimanguillo	17.85	93.40	20	Huimanguillo, Huimanguillo	17.87	93.47	36
Paraíso, Paraíso	18.42	93.02	4	Paraíso, Paraíso	17.97	93.22	6
Pueblo Nuevo, Centro	17.87	92.87	15	Pueblo Nuevo, Centro	17.85	92.88	60
San Pedro, Balancán	17.77	91.03	50	San Pedro, Balancán	17.79	91.16	36
Tres Brazos, Centla	18.38	92.61	11	Tres Brazos, Centla	18.23	92.60	2
Gaviotas, Centro	17.97	92.91	10	Villahermosa, Centro**	17.98	92.92	24

Lat=Latitude; Long=Longitude; Alt=Altitude (m); (**) Meteorological observatory.

Model for estimating relative heliophany (*n*/*N*)

With the cloudiness monthly average values, the relative heliophany (n/N) was estimated using the equation (1) developed by Tejeda & Vargas (1996).

$$\frac{n}{N} = 0.31 + 0.48 * PQ \tag{1}$$

Where: n is monthly average daily heliophany (hours), N is monthly average daily astronomical heliophany (hours) and PQ is the complement of the monthly average point cloudiness, calculated with equation (2):

$$PQ = \frac{Desp + (0.50 * MedNub)}{Nub + MedNub + Desp} \tag{2}$$

Where: *Desp* is the average number of clear days in the month, *MedNub* is the average number of half cloudy days in the month and *Nub* is the average number of cloudy days in the month.

Model for estimating global solar irradiance

The monthly average relative solar irradiance (R_g/R_a) was calculated following the equation proposed by Tejeda-Martínez *et al.* (1999). The adaptation of Tejeda-Martínez *et al.* (1999) for Mexico was to adjust the empirical coefficients "*a*" and "*b*" (0.26 and 0.51) shown in equation (3).

$$R_g = \left[0.26 * Cos(Lat) + 0.51 * \left(\frac{n}{N}\right)\right] * R_a \tag{3}$$

Where: R_g is the monthly mean daily global solar irradiance (MJ m^{-2} d^{-1}), R_a is the monthly mean daily extraterrestrial solar irradiance (MJ m^{-2} d^{-1}), determined following the procedure proposed by Allen *et al.* (2006), *Lat* is the latitude of the meteorological station (degrees), n is the monthly mean daily heliophany (hours) and N is the monthly mean daily astronomical heliophany (hours) according to Allen *et al.* (2006). The model estimating R_g was calibrated using linear regression techniques, similar to the work by Liu *et al.* (2009).

Calibration and validation of the R_g model

The R_g model was calibrated using monthly average data from eight weather stations. The calibrated model was validated using data from the Villahermosa weather station.

Evaluation of model performance

To evaluate the performance degree of the resulting global solar irradiance model, in addition to the corrected coefficient of determination $(R_c)^2$, two additional criteria were used: a) the comparison parameter (c) proposed by Willmott (1981) and b) the efficiency index (*E*) proposed by Nash-Sutcliffe (1970), as criteria to validate the model fit. The comparison parameter (*c*) of Willmott (1981) is calculated using equation (7):

$$c = R * d \tag{7}$$

Where: c is the comparison parameter (dimensionless), R is the correlation coefficient (dimensionless) and d is the Willmott (1981) or concordance index (dimensionless).

The efficiency index (*E*) or Nash-Sutcliffe (1970) criterion is calculated with equation (9):

$$E = 1.0 - \left[\frac{\sum_{i=1}^{N} \left(P_i - O_i \right)^2}{\sum_{i=1}^{N} \left(O_i - O_m \right)^2} \right] \tag{9}$$

Where: E is the Nash-Sutcliffe (1970) efficiency index, "dimensionless", P_i are values estimated or predicted by the model, O_i are the observed values, O_m the average of the observed values.

The corrected coefficient of determination $(R_c)^2$ is calculated with equation (10):

$$\left(R_c\right)^2 = 1 - \left(1 - R^2\right) * \left[\frac{(n-1)}{n - (k+1)} \right] \tag{10}$$

Where: R^2 is the coefficient of determination, n is the sample size and; k is the number of independent variables.

The general regression model used was:

$$\hat{y} = \beta_0 + \beta_1 X_1 + \varepsilon \tag{11}$$

Where: $\hat{y}$ is the estimated value of "*y*" for a specific value of X_1. β_0 and $\beta 1$ are the parameters of the model, ε is the error random variable, which accounts for the variability in "*y*" that cannot be explained by the linear relation between x and y.

Spatial distribution of monthly average R_g

With the validated global solar irradiance model, the latitude data and the historical monthly average cloudiness values of more than 30 years of record for the rest of the 35 meteorological stations, the corresponding monthly average R_g values were estimated for each station. With these values, monthly maps of the spatial distribution of R_g were constructed. The maps were prepared using ArcMap GIS software (ESRI, 2004). The maps of the monthly spatial distribution of R_g were constructed at a scale of 1:250 000 using the Kriging Universal spatial interpolation method, included in the ArcMap 10.2 GIS software.

RESULTS AND DISCUSSION

Table 2 shows the regression equations, their corrected coefficient of determination $(R_c)^2$ and the resulting standard error (SEE), for the eight stations used to fit the model proposed by Tejeda-Martínez *et al.* (1999) with observed monthly average global solar irradiance data. Reviewing the corrected determination coefficients $(R_c)^2$ (Table 2), it can be observed that there is a good fit between the estimated values of R_g by the model and the observed ones. The $(R_c)^2$ values ranged from 0.85 to 0.89, which are alike to the values reported by Liu *et al.* (2009). The standard error values (SEE) shown in Table 2 are small, given the used units, and imply little scattering of the data (Figure 1), which shows the plot resulting from comparing all the observed monthly average R_g data from the eight automatic stations (AMS's), with all the estimated R_g data for the eight ordinary climatological stations.

The resulting equation $y=1.0366*X$ was used to estimate the monthly average R_g for the Villahermosa station to validate and evaluate the goodness and degree of performance of the model. The estimated R_g values obtained with the model were compared with those observed in the AMS of Villahermosa, Tabasco, using regression analysis. The resulting equation was as follows:

$$y = 1.0326 * x; \quad \left(R_c^{\,2}\right) = 0.87; \quad SEE = 0.53 \tag{12}$$

(R_c^2)=corrected coefficient of determination estimated with equation 10.

Table 2. Regression equations of the relation between the estimated monthly average global solar irradiance (equation 3) and that observed at eight meteorological stations at Tabasco, Mexico.

Meteorological station	Regression equation	$(R_c)^2$	SEE
Boca de Cerro, Tenosique	y=0.9691*X	0.87	0.26
Dos Patrias, Tacotalpa	y=1.0660*X	0.89	0.36
Emiliano Zapata, Emiliano Zapata	y=1.0629*X	0.85	0.57
Huimanguillo, Huimanguillo	y=0.0836*X	0.88	0.38
Paraíso, Paraíso	y=1.0172*X	0.85	0.19
Pueblo Nuevo, Centro	y=1.0486*X	0.86	0.53
San Pedro, Balancán	y=1.0644*X	0.85	0.31
Tres Brazos, Centla	y=0.9813*X	0.86	0.72

y=estimated global solar irradiation from cloudiness (MJ m^{-2} d^{-1}); X=observed global solar irradiation (MJ m^{-2} d^{-1}); $(R_c)^2$=corrected coefficient of determination; SEE=standard error of the estimate (MJ m^{-2} d^{-1}).

The validation of the Tejeda-Martínez *et al.* (1999) model resulted in equation (12), which allows a reliable model to estimate the R_g for the entire state of Tabasco from cloudiness data. Therefore, the regression equation shown in Figure 1 was used to estimate the R_g for all the months of the rest of the 35 selected stations (26 stations), from which only cloudiness data were available.

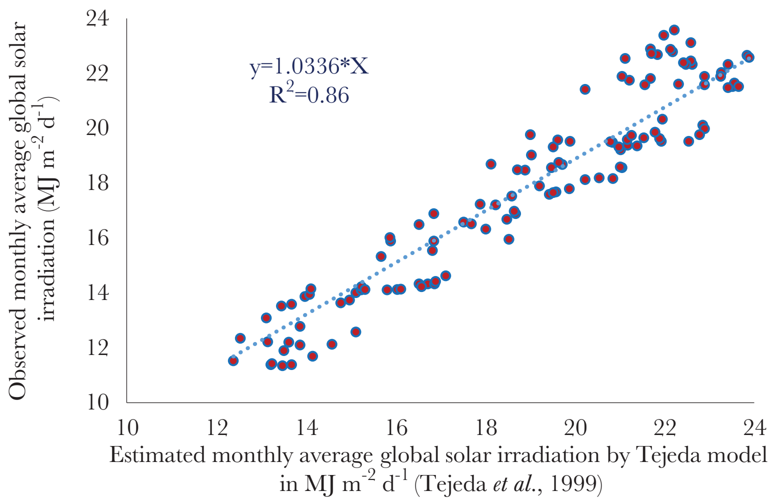

Figure 1. Relationship between the estimated and observed monthly average global solar irradiance values for the complete set of eigh stations.

Evaluation of the degree of model performance

When determining the values of the Willmott comparison parameter (c) (1981) and the Nash-Sutcliffe (1970) efficiency index (E), between the estimated and observed values of the Rg for the Villahermosa station, the values were: c = 0.89 and E = 0.99. With c>0.85, the degree of performance and fit of the model is rated as optimal, and with E>0.81 the rating is excellent.

Spatial distribution of global solar irradiation

Due to the agricultural importance of the seasons (north winds, dry and rainy seasons) according to the precipitation regime in Tabasco, the analysis of the spatial distribution of irradiation was carried out according to these seasons. Figure 2 shows the spatial distribution of R_g for November, December, January and February, the period of north winds. These maps show that the lowest R_g value (12 MJ m^{-2} d^{-1}) occurs during December and is the highest in February (18 MJ m^{-2} d^{-1}). The estimated R_g values at the monthly average level are similar to those reported by the Geophysics Institute of the UNAM (UNAM, 2014); with the difference that the maps made in this research show greater detail, due to its greater number of meteorological stations.

During the northern winds season, the lowest global solar irradiation is received during the year. These low R_g values are mainly due to high cloudiness and prolonged periods of precipitation (IMTA, 2009), as well as daytime heliophony, which decreases global solar irradiance (De Jong & Stewart, 1993).

During November, December and February, a similar distribution pattern of R_g is observed, although with different values. The areas with the highest global solar irradiation incidence during the northern wind season, which occur in February and include parts of the municipalities of Tacotalpa, Teapa and Jalapa in the Sierra subregion; in the area bordering the state of Chiapas in the municipality of Tenosique in the Los Ríos subregion; and in the coastal strip of the municipality of Centla, in the Pantanos subregion. This may be because in February there is a 14-day period (between the 9th and 23rd) with a water deficit (Ruíz-Álvarez *et al.*, 2012) as well as clouds absence.

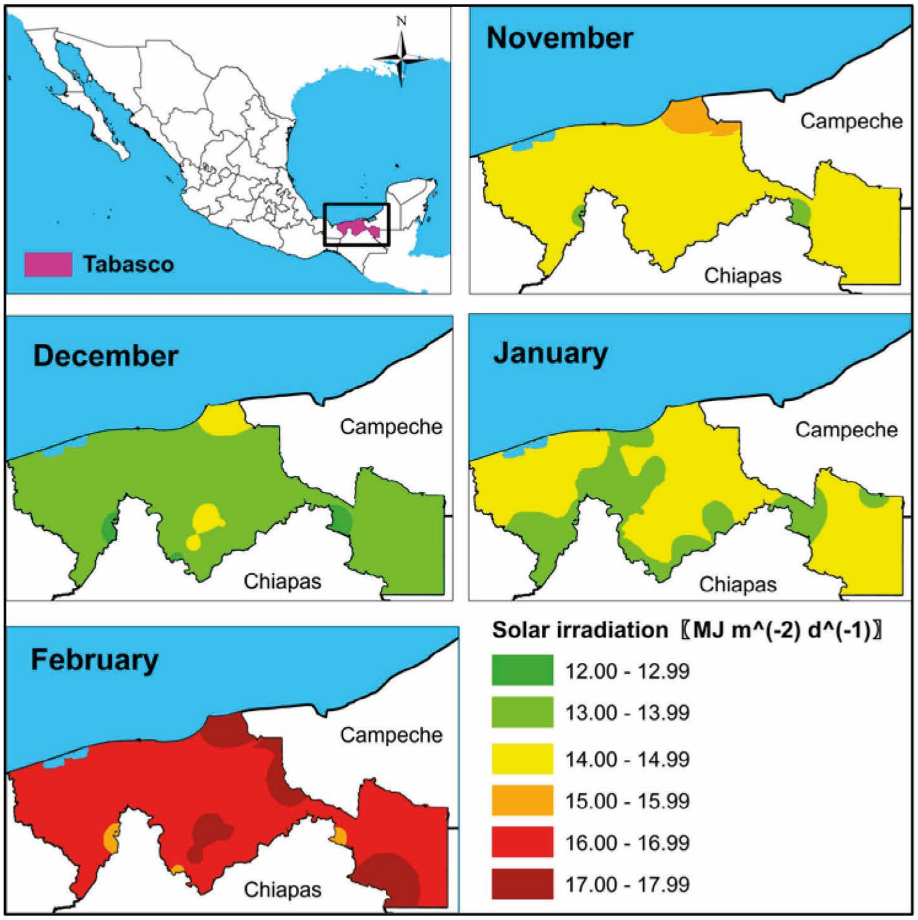

Figure 2. Average global solar irradiance for november to february, corresponding to the period of north winds.

The maps in Figure 3 correspond to the dry season when the highest global solar irradiation of the year occurs. During March, there is a transition from the northeast to the dry season, which is why the value of irradiation increases due to the greater number of clear days, as well as the decrease in precipitation, compared to the northeast season. The largest area of the state is covered by the irradiation interval of 19.00-19.99 MJ m^{-2} d^{-1} (Chontalpa and Centro subregions) and the highest irradiation interval of 20:00-

21:00 MJ m^{-2} d^{-1}, located in the municipalities of Centla, Jonuta, Emiliano Zapata, Balancán and Tenosique, which make up the lower basin of the Usumacinta River.

April and May have the highest global solar irradiation of the year. This is because these are the months with the greatest water deficit (Ruíz-Álvarez *et al.*, 2012), as well as the lowest cloud cover (IMTA, 2009). In April, the largest irradiation interval (22.0-22.99 MJ m^{-2} d^{-1}) covers most of the state, and in May the same interval covers almost the entire state. The highest irradiance in May is in a few small areas in the subregions: Chontalpa (municipalities: Cardenas and Huimanguillo), Pantanos (municipalities: Centla, on the coastal strip and Jonuta, an area bordering the state of Campeche) and Ríos (municipality: Tenosique, the area bordering the state of Chiapas).

Figure 4 shows the R_g spatial distribution during June, July, August, September and October, which are the rainy season. The maximum R_g begins to decrease from June (21.99 MJ m^{-2} d^{-1}) to October (17.99 MJ 17.99 MJ m^{-2} d^{-1}). The observed maximum value in June is because, until the middle of the month, there still are clear days and the longest day length throughout the year. Likewise, for June the Rg interval > 22.00 MJ m^{-2} d^{-1} distributes in the coastal strip of Centla municipality; however, this interval disappears by July to reappear again in August and September. In October, the interval of 16.00-16.99 MJ m^{-2} d^{-1} is predominant in most of the state of Tabasco.

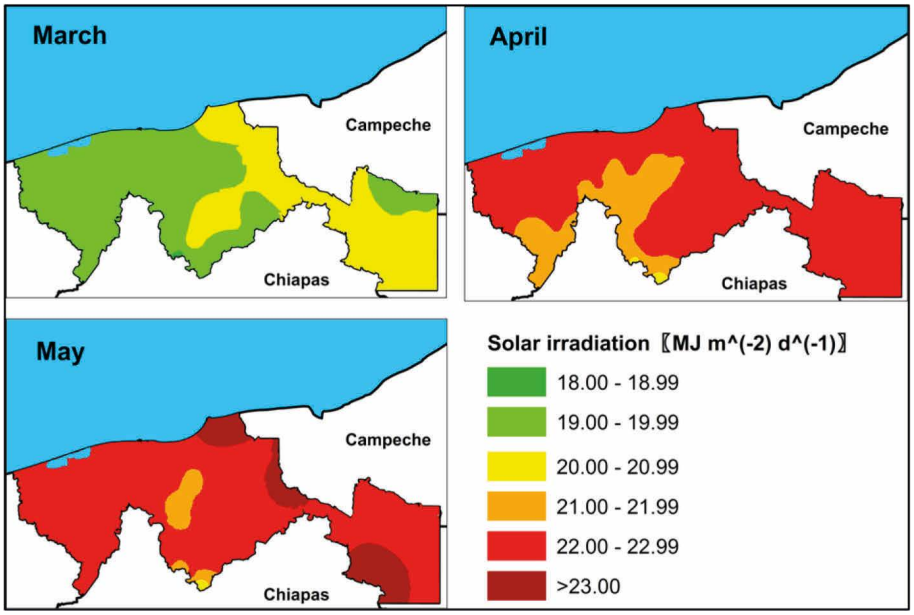

Figure 3. Average global solar irradiance for march, april and may, during the dry season.

An agronomic interpretation of global irradiance variation in Tabasco imposes biophysical limits on crop growth and development rate (van Ittersum *et al.*, 2013), given there is a high correlation between global irradiance and net biomass production (De Wit, 1959). For example, the energy conversion efficiency in maize plants without water stress is 1.2 to 1.6 g MJ^{-1} m^{-2} of intercepted solar radiation (Muchow, 1994), for sorghum is 0.66 to 1.39 g MJ^{-1} m^{-2} (Hernández-Cordova & Soto-Carreño, 2013). Considering the energy conversion data for maize and sorghum, it is expected that the highest net biomass yields are obtained during the dry season for maize and sorghum, because C4 plants require high temperature and light intensity. However, this would only be possible if irrigation was to be available since rainfall during the dry season is scarce and insufficient.

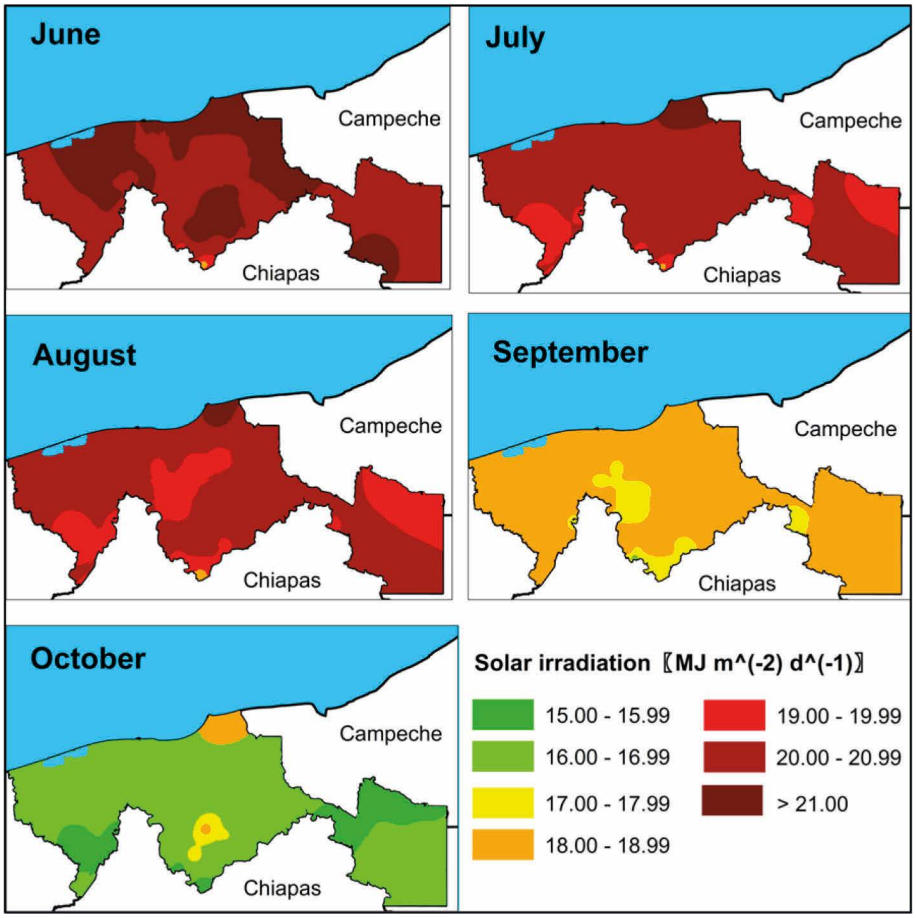

Figure 4. Average global solar irradiance for june, july, august, september and october, corresponding to the rainy season.

CONCLUSIONS

The monthly R_g average values estimated with the proposed model from observed daily R_g data and percentage of cloudy days (cloudiness) reported a good fit. The spatial distribution of the global monthly average R_g irradiation in the three climatic periods spatially varies throughout the year. During the north windy season, the minimum R_g values occur in December are the highest in February, while in the dry season the minimum R_g occurs during March and the maximum in May. In the rainy season, the R_g incidence gradually decreased from June to August.

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AGRO PRODUCTIVIDAD
Colegio de Postgraduados

Morphological divergence of seedlings *Calophyllum brasiliense* cambes collected in São Paulo and Tocantins

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ABSTRACT
Objective: The objective was to quantify the morphological diversity of *Calophyllum brasiliense* Cambes from four municipalities of the State of Tocantins employing morphological characteristics, to strengthen information on conservation and future breeding of the species.
Methodology: Seeds were collected from four municipalities in the State of Tocantins and one in the State of São Paulo. The following were evaluated: plant height, stem diameter, root length, number of leaves, leaf area, root dry mass, shoot dry mass, total dry mass, and Dickson's quality index. The data were subjected to univariate analysis of variance, Tocher grouping method, and UPGMA, obtaining a dendrogram through the generalized Mahalanobis distance.
Results: The results showed a statistical difference of 1 and 5% probability. Dueré stood out in stem diameter (5.52 mm), Sandolandia in height (34.84 cm) and root length (42.13 cm). Formoso stood out in the number of leaves (34 leaves). Lagoa da Confusão in leaf area (856.28 cm^2) and São Paulo in root dry mass (16.20 g), shoot dry mass (12.38 g), total dry mass (16.20 g), and Dickson's quality index (1.57).
Implications: Variations in morphological characteristics can be used as a tool for genetic studies of guanandi progeny according to their similarity and/or differences.
Conclusions: The morphological divergence evidenced that among the five studied areas it is possible to direct the collection of seeds to subsidize conservation strategies and future breeding of the species.

Keywords: *Calophyllum brasiliense*, characteristics, conservation, genetics.

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INTRODUCTION

Calophyllum brasiliense Cambess is a species native to Brazil, known as guanandi, and belongs to the genus *Calophyllum* that comprises approximately 190 species, only eight of them occur in Central and South America (Soerianegara and Lemmens, 1993). It occurs from Mexico to South America, always in plains with temporary floods (Carvalho, 2003). Guanandi is considered climatic because due to abundant regeneration in the shade and is native to the Amazon and Cerrado regions (second largest Brazilian biome). The *Calophyllum* genus has multiple applications since the bark of the tree produces excellent-

Image by Bishnu Sarangi at Pixabay

quality wood, and oil can be extracted from the seeds. This species can be used as an alternative to substitute mahogany and cedar that have problems with the shoot borer (*Hypsipyla grandella*), a pest that makes commercial cropping of both unfeasible (Wrege *et al.*, 2017). Furthermore, it is currently becoming highly relevant due to its high medicinal potential, with various pharmacological uses due to its antidepressant, antioxidant, and antimicrobial effects (Bernabé *et al.*, 2015). *C. brasiliense* is on the list of Amazonian tropical forest species that should be considered within *in situ* and *ex situ* genetic resource conservation programs (Tropical Flora Reflorestadora, 2014).

The floodplains are among the ecosystems that suffer the most disturbances, either due to the presence of relatively fertile and humid soils, ideal attributes for agriculture and pasture formation or due to the high diversity of species that serve as a source of wood and non-wood forest products. (Reis *et al.*, 2009). The State of São Paulo has a large marginal area for the commercial cultivation of guanandi, being a transition region for the Cerrado and total precipitation is low, which can influence the yield of commercial crops (Wrege *et al.*, 2017).

In the State of Tocantins, the floodplains have been widely exploited by agriculture, with cropping of rice, soybeans, watermelons, among others. With all this, many tree populations have already been reduced or eliminated, as a direct consequence, there is a reduction in the size and isolation of the populations, which will result in inbreeding and genetic drift (Botrel *et al.*, 2006). In addition, the state also presents marginal conditions similar to those of São Paulo for the production of guanandi, however, there are no studies that report these facts.

The conservation of native species and, in general, of natural resources as a whole, serves to maintain the representativeness of the species, conserve natural resources to mitigate human and environmental catastrophes, preserve the maximum genetic variability, and form ecological corridors, being its use one of the viable alternatives for the genetic resources conservation.

Quantifying the genetic diversity of guanandi through morphological characteristics can serve as a model for the generation of conservation programs for the species, in addition to guiding material collection programs for germplasm banks and seedling production for reforestation programs.

Currently, the existing knowledge about guanandi is insufficient to indicate it as an alternative to pine and eucalyptus, although its future seems to be promising since more advanced research is still necessary, in which cultural techniques are developed that allow greater production at the lowest cost (Wrege *et al.*, 2017).

The objective was to quantify the morphological divergence of *Calophyllum brasiliense* Cambess. from four municipalities of the State of Tocantins employing morphological characteristics, to strength the information for conservation programs and future breeding of the species.

MATERIAL AND METHODS

The study was carried out at the Experimental Station of the Gurupi University Campus, Federal University of Tocantins (UFT), located at 11° 44’ 42” south latitude, 49° 03' 05" west longitude, and 276 meters above sea level. According to Köppen (1948), the regional climatic classification is of the type B1wA'a 'humid with moderate hydric deficiency. The average annual temperature is 29.5 °C, with an average annual rainfall of 1,804 mm. Tree seeds were collected in four municipalities of the State of Tocantins: Formoso do Araguaia, Dueré, Sandolandia, and Lagoa da Confusão.

The climate of this region is characterized by the Aw type according to Köppen (1948), defined as tropical humid with a rainy season in summer and dry in winter, being the month with the most rain January, and the driest August. As a comparison control, seeds of mother plants from Piracicaba-SP were used, with a Cwa type climate (altitude tropical), at 554 meters above sea level, mean annual rainfall of 1,328 mm and minimum mean annual temperature of 14.8 °C and maximum of 28.2 °C.

The seeds were collected from the fruits of mother plants that naturally detached from healthy, vigorous, and fully mature trees. The areas where the collections were performed in the state of Tocantins were georeferenced and the data was used to represent the geographic locations during 2017.

To obtain the seedlings, the seeds were removed from the pulp and mechanically scarified to break dormancy. The sowing was carried out in beds composed of coarse sand. The seedlings were transplanted when reached 10 cm high to polyethylene bags with a capacity of 2 kg of substrate, where they remained until the end of the experimental period (120 days). Subsoil and tanned manure were used as substrates in a ratio of 2:1 (v/v).

For the fertilization of the substrate, 150 g of N (urea) and 100 g of K_2O (potassium chlorate) were applied for each m^3 (Schorn and Formento, 2003). Four top-dressings were carried out every 30 days, in quantities of 3 g of N and 2 g of K_2O for each plant. During the conduct of the experiment, soil moisture was maintained at field capacity with daily irrigation. The experimental design was completely randomized with five treatments (four treatments from the municipalities of Tocantins and one from São Paulo), with eight replications and 10 plants per plot.

At 120 days, the following morphological characteristics were evaluated: plant height (PH in cm); stem diameter (SD in mm) using a 0.001 mm precision digital caliper; root length (RL in cm); number of leaves (NL); leaf area (FA in cm^2); root dry mass (RDM in g); shoot dry mass (SDM in g), total dry mass (TDM in g), obtained in a forced air circulation oven at 60 °C until obtaining a constant mass, and Dickson's quality index (DQI), estimated with the equation:

$$DQI = \frac{TDM}{\frac{PH(cm)}{D(mm)} + \frac{SDM(g)}{RDM(g)}} \quad [1]$$

The leaf area was determined with the use of known areas of cylindrical perforators, which were used to remove leaf discs. These leaf discs were removed from various parts of the leaves, packed in paper bags, and taken to a forced-air oven until they reached constant mass. The leaves that were not used were placed in another paper bag and were also taken to the oven for drying.

The leaf area was estimated from the relationships between the discs dry mass (DDM), the total disc area, the sum of the areas of all the discs (AD), and the total dry mass of the leaf samples used (DMLS), unused leaves, and discs (Oliveira *et al.*, 2002).

The variance analysis was carried out with the estimation of the mean squares of each morphological characteristic. Their significance was verified by the F test, and the means were grouped by the Scott-Knott test ($p \leq 0.05$) (1974), using the statistical program Sisvar® version 5.5 (Ferreira, 2019).

The genetic divergence between the seedlings collected in the five areas was estimated using morphological data related to the initial seedlings development in the evaluation at 120 days. The variance analysis was performed, and the dissimilarity measures were determined according to the multivariate analysis model, which allowed obtaining the dissimilarity matrix, the residual covariance matrix, and the area means.

The Tocher grouping methods (Rao, 1952) and the UPGMA method (unweighted pair group method with arithmetic mean) were used to obtain a dendrogram using the generalized Mahalanobis distance (D2) as a dissimilarity measure. Singh's (1981) criterion was also used to quantify the relative contribution of these characteristics to the genetic divergence. The analyzes were carried out using the Genes® computer program (Cruz, 2016).

RESULTS AND DISCUSSION

There were significant differences for the characteristics of stem diameter, root length, leaf area, shoot dry mass, total dry mass, and Dickson's quality index, evidencing the significant differences between the origins of the seeds. However, there were no significant differences within the collecting areas for any of the characteristics evaluated. It is fundamental to note that evident when the genetic variability of this species is sought, the seed collections should be performed in different areas (Table 1).

The guanandi seedlings from Formoso do Araguaia and Sandolandia showed similarities in the characteristics of stem diameter, shoot dry mass, total dry mass, and dissimilarity with the seedlings of Lagoa da Confusão and São Paulo (Table 1). As for root length, no differences were observed between Formoso do Araguaia, Dueré, and Lagoa da Confusão, but they differed from those of Sandolandia and São Paulo. The seedlings from Sandolandia stood out among the other locations for root length, with a mean of 42.13 cm.

No significant differences were detected for leaf area between the seedlings from Formoso do Araguaia and Sandolandia, however, they differed from the seedlings from Lagoa da Confusão and Sao Paulo. The Dueré seedlings were different from the four areas. Higher means for leaf area were found in the seedlings of Lagoa da Confusão and São Paulo, with means equal to 856.28 cm^2 and 847.79 cm^2, respectively.

As for the Dickson quality index, only the Formoso do Araguaia seedlings differed from the others, presenting the lowest mean (0.93). The seedlings from Dueré, Sandolandia, Lagoa da Confusão, and São Paulo presented the best DQI, standing out from the other seedlings those of São Paulo presenting higher means (1.57).

Table 1. Summary of the analysis of variance and means of guanandi seedlings from the State of Tocantins and the State of São Paulo. Gurupi - TO. 2018.

	MS			
Characters	**Between Areas**	**Residue**	**General mean**	**CV (%)**
SD (mm)	2.43**	0.13	5.04	7.13
PH (cm)	20.31^{ns}	11.09	32.29	10.31
NL	127.05 ^{ns}	49.78	28.85	24.46
RL (cm)	67.50*	20.71	37.56	12.12
RDM (g)	2.64 ^{ns}	1.03	3.10	32.88
LA (cm^2)	42,1846.40**	38,018.01	621.78	31.36
SDM (g)	62.68**	7.55	9.50	28.92
TDM (g)	79.95**	8.16	12.60	22.67
DQI	0.43**	0.06	1.28	20.26

	Means				
Characters	**Formoso**	**Dueré**	**Sandolandia**	**Lagoa da Confusão**	**São Paulo**
SD (mm)	5.34 a	5.52 a	5.46 a	4.44 b	4.44 b
PH (cm)	31.40 a	32.89 a	34.84 a	31.17 a	31.17 a
NL	34.56 a	24.43 a	29.43 a	25.75 a	30.06 a
RL (cm)	36.46 b	35.71 b	42.13 a	34.89 b	38.63 a
RDM (g)	2.28 a	2.96 a	3.43 a	3.00 a	3.82 a
LA (cm^2)	358.77 c	612.99 b	433.09 c	856.28 a	847.79 a
SDM (g)	6.39 b	9.08 b	7.28 b	12.38 a	12.38 a
TDM (g)	8.67 b	12.05 b	10.71 b	15.37 a	16.20 a
DQI	0.93 b	1.30 a	1.23 a	1.36 a	1.57 a

Where: SD: stem diameter; PH: plant height; NL: number of leaves; RL: root length; RDM: root dry mass; LA: leaf area; SDM: shoot dry mass; TDM: total dry mass; and DQI: Dickson's quality index.
ns - No significant, * and ** significant by the F test at the level of 5 and 1% probability, respectively. Means followed by the same letter in the same line do not differ from each other by the Scott-Knott test ($p \leq 0.05$).

The estimates of the heritability in the broad sense (H^2) of the morphological characteristics evaluated varied from 45.38% (plant height) to 94.66% (stem diameter). The characters with high percentages of heritability were stem diameter (94.66%), followed by leaf area (90.98%), and total dry mass (89.79%) (Table 2).

Table 2. Heritability in the broad sense (H^2), coefficient of genetic variation (CVg), and the relationship between the coefficient of genetic variation and the coefficient of experimental variation (CVg/Cve) in morphological characteristics of guanandi seedlings from four municipalities of the State of Tocantins and one from the State of São Paulo. Gurupi - TO. 2018.

Characteristics	SD (mm)	PH (cm)	NL	RL (cm)	RDM (g)	LA (cm^2)	SDM (g)	TDM (g)	DQI
H^2	94.66	45.38	60.82	69.31	60.73	90.98	87.95	89.79	84.40
CVg (%)	10.62	3.32	10.77	6.44	14.46	35.23	27.62	23.77	16.67
CVg/CVe	1.48	0.32	0.44	0.53	0.44	1.12	0.95	1.05	0.82

Where: SD: stem diameter; PH: plant height; NL: number of leaves; RL: root length; RDM: root dry mass; LA: leaf area; SDM: shoot dry mass; TDM: total dry mass; and DQI: Dickson's quality index.

Most of the analyzed characteristics presented a high coefficient of genetic variation (CVg), with the highest values observed for the leaf area (35.23%), shoot dry mass (27.62%), and total dry mass (23.77%). As well as the heritability in the broad sense (H^2) and the coefficient of genetic variation (CVg), the relationship between the coefficient of genetic variation and the coefficient of experimental variation (CVg/CVe) also presented high values for stem diameter (1.48), leaf area (1.12), and total dry mass (1.05) (Table 2).

The genetic dissimilarity measures estimated from the generalized Mahalanobis distance (Table 3), present a wide range (from 1.52 to 42.26), indicating the presence of genetic variability between the areas. The seedlings from Formoso do Araguaia and São Paulo were more dissimilar (D2=42.26), followed by those from Formoso do Araguaia and Lagoa da Confusão (D2=37.08). The shortest distance was detected between the seedlings of Lagoa da Confusão and São Paulo (D2=1.52) (Table 3).

Table 3. Dissimilarity between municipalities of the State of Tocantins and São Paulo in guanandi seedlings in relation to nine morphological characteristics, based on the generalized distance of Mahalanobis ($D^2_{ii'}$). Gurupi - TO. 2018.

Municipalities	1	2	3	4	5
Formoso do Araguaia (1)	0	14.16	12.15	37.08	42.26
Dueré (2)		0	5.63	23.44	26.29
Sandolândia (3)			0	27.55	28.14
Lagoa da Confusão (4)				0	1.52
São Paulo (5)					0

Three groups of dissimilarities were formed based on morphological characteristics of the four municipalities of the State of Tocantins and one of São Paulo and using the grouping analysis by the Tocher method based on the Mahalanobis distance $\left(D^2_{ii'}\right)$. Lagoa da Confusão and São Paulo were classified in group I, showing that these two areas are very similar genetically. In group II, there are Dueré and Sandolandia. Formoso do Araguaia was placed in group III, being isolated from the others.

Based on the relative contribution of each morphological characteristic to the genetic dissimilarity (Table 4), using the Singh (1981) criterion, the total dry mass, shoot dry mass, and root dry mass were the characteristics that most contributed to the morphological divergence with 49.37%, 44.28%, and 5.41%, respectively, while the other characteristics only contributed with 0.93% (Table 4).

Table 4. Relative contribution (%) of the morphological characteristics to the genetic dissimilarity of guanandi seedlings from five municipalities, one being from the State of São Paulo and four from the State of Tocantins. Gurupi - TO. 2018.

Characteristics	TDM	SDM	RDM	DQI	SD	LA	RL	PH	NL
S.j.	18884.35	16939.82	2069.49	150.25	146.87	35.39	10.09	7.58	7.00
Value (%)	49.37	44.28	5.41	0.39	0.38	0.09	0.03	0.02	0.02

Where: TDM: total dry mass; SDM: shoot dry mass; RDM: root dry mass; DQI: Dickson's quality index; SD: stem diameter; LA: leaf area; RL: root length; PH: plant height, and NL: number of leaves.

From the cluster analysis by the UPGMA method, the dendrogram was obtained (Figure 1). The cut-off point to find the groups was performed visually according to the long-distance jump or level change in the dendrogram (Figure 1).

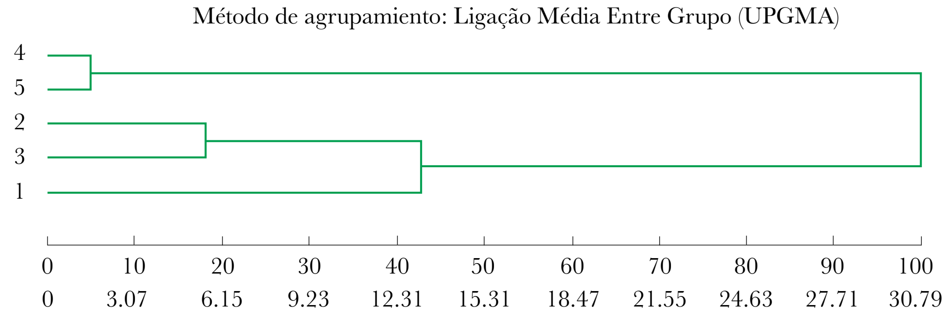

Figure 1. Dendrogram generated with the UPGMA method from the dissimilarities expressed by the Mahalanobis distance between the five guanandi areas obtained from the nine selected morphological characteristics. Gurupi - TO. 2018.

The lack of statistical differences between the areas may be due to the climatic and hydric similarities of the two states (Tocantins and São Paulo), which is confirmed by Wrege *et al.* (2017), presenting the favorable regions for the commercial production of guanandi. Other authors reported significant environmental variations for root length, such as Oliveira *et al.* (2006) studying the genetic variation of Baru (*Dipteryx alata*) progenies and Reis *et al.* (2006) analyzing clones of *Eucalyptus* sp. using root-related characteristics.

Variations in the root-related characteristics may be related to the growth and development capacity of the species. In this sense, a widely developed root system may result in a more vigorous seedling with greater chances of survival in environments with few nutrients and water.

The Dickson quality index considers the solidity and balance of the biomass distribution of the seedling, assessing the results of the important attributes in the quality evaluation. This index is widely employed by researchers in seedlings of forest species (Gomes *et al.* 2003). Thus, the higher the DQI, the higher the seedling quality, *i.e.*, the better its development in the field.

According to Cruz (2005), the broad heritability values can vary from 0 to 100%, considering high when the values obtained are greater than 40%. The characteristics studied present a high heritability in the broad sense. The heritability for root length (69.31%) was lower than that found by Oliveira *et al.* (2006) for Baru (*D. alata*), which obtained heritability values of 84.01% in plant evaluations in the seedling phase.

As stated by Falconer (1987), quantitative characteristics are highly influenced by the environment, and the lower the environmental effect on the genotype, the greater the heritability. Heritability is represented by the fraction of the genetically controlled characteristic when the heritability coefficients are high. Based on these criteria, high heritability coefficients, even in a broad sense, indicate an excellent possibility of success in the selection process.

According to Costa *et al*. (2011), the greater the existing genetic variation, the greater the chances of genetic gains throughout the breeding program. CVg is considered an important parameter to understand the genetic structure of a population since it presents the amount of variation between the progeny in relation to the mean of the trait under study (Baleroni *et al*., 2003). Based on this information, it can be affirmed that the total dry mass, the shoot dry mass, and the leaf area are the characteristics that present possibilities of genetic gains in the selection programs.

According to a survey carried out by Aguiar (2004) on the heritability and the coefficient of genetic variation of the height and diameter of native tree species of Brazil, it can be observed that the estimates of heritability and coefficient of genetic variation of this work are representative and exceed the intervals observed by various authors, especially concerning the coefficient of genetic variation.

On the report of Vencovsky and Barriga (1992), when the CVg/CVe ratio obtains a value equal to or greater than the unity, it indicates that it is possible to obtain representative genetic gain in the breeding process. In this way, the results found confirm the possibility of selection for these characteristics with good expectations of gain. Further experimental investigation should be performed to assess the association of these characteristics with those of importance in terms of the final product of the species.

In this work, variability was only considered between areas, with the need for future studies with a greater number of mother trees per region and characterization of genetic variability within populations. In addition to the incorporation of a greater number of morphological, botanical, and agronomic characteristics allied to molecular techniques. Thus, more complete results will be obtained on the genetic diversity of the guanandi populations of the State of Tocantins.

Guanandi studies are very few and even more those that examine the genetic divergences. The few available studies are with molecular markers (Reis *et al*., 2009). There are a spare number of works that have been reported on the study of the species with morphological data since these are important for the characterization of accessions in germplasm banks.

The formation of groups shows the variability between the areas, verified in the analysis of variance. The different municipalities were in the same dissimilarity group. Siqueira *et al*. (1993) and Carvalho (1994) suggest as an explanation for this minimal diversity among the progeny of the different areas that their origins are from the same ancestral population, or even that these materials may have suffered an anthropogenic action, dispersal of fruits through animals, and cross-pollination.

In the case of seedlings from São Paulo, which is from another state, the similarity with the seedlings from Lagoa da Confusão may be related to environmental factors and adaptability, as the seedlings were planted under the same conditions.

Pasqual *et al*. (2012) observed variability between the morphological characteristics in 16 genotypes of Bacuri (*Platonia insignis* Mart.) that compose the germplasm collection in the Empresa Brasileira de Pesquisa Agropecuaria (Embrapa) Middle-North, and using the same method they reported the formation of five groups.

The characteristics total dry mass (49.37%) and the shoot dry mass (44.28%) were the most efficient to explain the dissimilarity between the progeny and should be prioritized in the selection of parents in the conservation programs and future improvements of the species, when seedling phase studies were considered.

Employing the UPGMA method it was possible to form two different groups, which were almost identical to the groups formed by the Tocher method, except for the municipality of Formoso do Araguaia (1) which was in an isolated group, and in this method, it remained grouped with Dueré (2) and Sandolandia (3) in group II. The most divergent progeny were São Paulo and Formoso do Araguaia, and the most similar were Lagoa da Confusão and São Paulo, reinforcing what was described in the dissimilarity matrix by genetic distance and in Tocher's method.

The optimization methods Tocher and UPGMA are normally used concomitantly to complete the results and help to better distinguish the clusters formed. The use of more grouping methods due to the differences in the hierarchy, optimization, and ordering of the groups allows complementary classification, as each technique employs different criteria, and avoids the adoption of erroneous inferences in the assignment. of elements, within a certain sub-group of genotypes (Arriel *et al.*, 2006).

The relatively low cost of morphological characterization makes it an interesting tool for research in species conservation programs, material collection programs for germplasm banks, production of seedlings for reforestation, in addition to strengthening subsidies for future breeding programs of the species.

CONCLUSIONS

There is morphological variability in the seedlings of *C. brasiliense* Cambess among the five municipalities with 67% of the evaluated characteristics. The characteristics total dry mass, shoot dry mass, and root dry mass contributed the most to the genetic divergence between the municipalities. The results suggest that variations in morphological characteristics can be used as a tool for genetic studies of *C. brasiliense* Cambess progenies according to their similarities and/or differences. The state of Tocantins is suitable to produce *C. brasiliense* Cambess for commercial purposes and for the development of conservation and breeding programs for the species.

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Heat stress mitigation strategies for beef cattle under intensive finishing in the Mexican dry tropics

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ABSTRACT

Objective: Review the heat stress mitigation strategies in intensive cattle feedlots in the tropical region of México.

Approach: Beef cattle production is one of the principal activities of the agricultural sector; therefore, to maintain the inventory in intensive finishing pens, a considerable number of cattle are moved to geographic areas where climatic conditions are not always favorable for most of the year. High environmental temperature combined with relative humidity create heat stress conditions and consequently affecting the productive indicators by compromising the physiological stability of the cattle.

Implications: The improvement of housing conditions to mitigate the effects of heat stress in beef cattle in intensive finishing involves considering living space, available shade area, feeding and watering space that assure the cattle welfare during their stay in livestock production units.

Conclusions: Heat stress mitigation strategies in beef cattle during intensive finishing in practical conditions should contribute to animal welfare and the improvement of the productive indicators at the Mexican dry tropics.

Keywords: cattle, meat production, heat stress.

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INTRODUCTION

In Mexico, beef cattle production represents one of the principal activities of the agricultural sector, due to its contribution to the supply of meat products, as well as its participation in the country's trade balance (Rubio *et al.*, 2013; FIRA, 2019). The evolution of the world beef market and the competitiveness of the countries participating in it influence the dynamics of this activity (Magaña *et al.*, 2020). In this sense, beef production in 2019 reached a historical maximum of two million tons, which is 2.4% higher compared to 2018; 86% of the Mexican beef exports went to the United States. In this activity, the states of Veracruz, Jalisco, San Luis Potosí, Sinaloa and Chiapas stand out, producing 838,930 tons, this implies a contribution of 41.3% of the national total (SADER-SIAP, 2019).

Image by Ulrike Leone at Pixabay

In northwestern Mexico, the Culiacán Valley, Sinaloa is located at 24° 48"00"'N and 107° 23' 00" W and 70 m asl; the average maximum temperature is 36 °C, persistent during the spring-summer-autumn period, and an average minimum of 11 °C during winter; the average annual relative humidity is 68%, its maximum 98% and minimum 14% (CIAD, 2018). It has dry tropical climatic conditions (BS1(h')w(w)(e)) (Garcia, 2004) and an estimated 493,164 head of cattle are annually housed in feedlots, where 106,289 t are produced, it is equivalent to 5.4% of the national beef production. Renaudeau *et al.* (2012) state that the decrease in cattle production indicators in hot regions is affected by several factors. The main one is heat stress, which is generated by high environmental temperatures; this thermal condition occurs once the environmental temperature exceeds the cattle's thermo-neutral zone, which prevents them from dissipating the extra heat (Bernabucci *et al.*, 2010). Therefore, the objective of this research was to review heat stress mitigation strategies in intensive cattle feedlots in the dry tropical region of Mexico.

What is stress?

The biological expression of stress has been used as an indicator of the loss of animal welfare (Broom, 2003) and is defined as the action of sensory and emotional stimuli provoked by the environment on the nervous, endocrine, circulatory and digestive systems of an animal (Broom, 2005). It is referred to as distress when the animal's response to the stressor causes risks to its well-being and life (Mormède *et al.*, 2007).

Physiological response to heat stress

The body temperature of cattle can vary from 37.8 to 40 °C; within this range, their organism efficiently fulfills its cellular and biochemical functions, due to this, they need to generate or dissipate heat towards the environment; when they face diverse environmental conditions to which they are not adapted, they tend to alter their physiological, behavioral and productivity mechanisms, to maintain their body temperature (Arias *et al.*, 2008). For this reason, thermoregulation mechanisms are activated (Sanmiguel and Avila, 2011). Several studies have established that cattle perform better in the thermoneutral zone of 20 °C, which varies between 10 to 26 °C; but when the temperature exceeds 27 °C, especially if relative humidity exceeds 40%. Then, they begin to have difficulties to self-regulate and heat stress is triggered, which manifests as reduced feed intake and consequently lower weight gain (Mader *et al.*, 2007; Lagos *et al.*, 2014). Due to the environmental temperature being above the comfort zone, the heat load causes cattle not to dissipate heat without additional energy expenditure to maintain corporal temperature; this generates physiological and behavioral responses to ease the effect of heat stress (Bernabucci *et al.*, 2010). Both respiratory rate and panting are appropriate indicators to assess the intensity of heat stress experienced by cattle (Brown-Brandl *et al.*, 2005; Gaughan and Mader, 2014).

Heat stress, an approach to beef production

To reduce heat load in high-temperature conditions, cattle tend to reduce heat production through voluntary anorexia, since fermentation of the rumen and digestion generates heat (Cedeño, 2011); which consequently decrease energy consumption, a negative energy balance is generated, which partially explains their weight loss and end up with a poor body condition when subjected to heat stress (Muñoz *et al.*, 2013). Valente *et al.* (2015) mention that cattle of breeds specialized for meat production tend to decrease in 24% their dry matter consumption during the day under heat stress conditions, reducing metabolic heat production; consequently, water consumption increases their physiological response to decrease body temperature. Pereyra *et al.* (2010) observed that the frequency to access water also increases; though, cattle appear to drink, but do not do so, due to a decrease in their body activities, including feed intake and walking. With rumination decreased, increased respiratory rate and panting, the concentration of HCO_3 decreases, generating the risk of ruminal acidosis, affecting weight gain and consequently feed conversion (Malafaia *et al.*, 2011).

To increase the heat loss through evaporation, the respiratory rate increases under heat stress conditions (Morais *et al.*, 2008; Bernabucci *et al.*, 2010). Research has shown that Angus cattle have elevated respiratory rate, even in comfort temperatures, due to the demanding rate of weight gain, which implies an extraordinary metabolic activity and consequently metabolic heat production gets elevated (Valente *et al.*, 2015). Cattle under heat stress tend to lose more saliva and minerals such as sodium and potassium, besides the potential ruminal acidosis due to the excessive saliva loss effect (Hall, 2000). These conditions negatively impact the productive indicators and consequently generates economic losses for the beef industry (O'Brien *et al.*, 2010; Renaudeau *et al.*, 2012).

Temperature and relative humidity index: effect on caloric load

Thom published in 1959 a famous formula to calculate a thermal discomfort index based on the ambient temperature and relative humidity, focused on the human population. Similarly, the effect of climate on animal production has been highly studied, achieving important advances to understand physiological and behavioral aspects of animal behavior under climatic stress conditions, by jointly evaluating factors such as solar radiation, relative humidity, ambient temperature, wind speed and rainfall; together, these variables have a direct effect on animal welfare (Mitloehner *et al.*, 2001; Brown-Brandl *et al.*, 2006). Mader *et al.* (2006) applied the following equation to assess heat stress in feedlot beef cattle:

$$ITH = 0.8 * \textit{ambient temperature} + (\%\ \textit{relative humidity}/100) * (\textit{air temperature} - 14.4) + 46.4$$

and used the Livestock Weather Safety Index (LWSI), published by LCI in 1970, as a reference to assign heat stress levels to the following categories: Comfort, ≤ 74; Alert, $74 < ITH < 79$; Danger, $79 \leq ITH < 84$; and Emergency, $ITH \geq 84$. In this way, the temperature and humidity index are indicators used to measure the heat stress degree to which cattle are subjected (Gaughan *et al.*, 2008; Olivares *et al.*, 2013).

Climatic conditions at Culiacán valley

Table 1 shows a summary of the climatic variables frequently recorded throughout the months in the valley of Culiacán, Sinaloa, México.

Table 1. Annual summary of climatic variables in the Culiacán valley, Sinaloa.

Month	T Min (°C)	T Max (°C)	RH, (%)	THI Min	THI Max	UV	Light (h)	Sun (h)
January	10.9	27.8	72	52.6	78.3	5	10.8	6.1
February	11.3	28.9	70	53.3	79.7	7	11.4	6.7
March	12.1	30.5	67	54.5	81.6	10	12.0	7.4
April	14.5	32.8	65	58.1	84.6	11	12.8	7.1
May	18.0	34.9	64	63.1	87.4	12	13.4	8.0
June	23.2	35.9	67	70.9	89.5	12	13.7	7.4
July	24.1	35.5	72	72.7	90.0	12	13.5	6.2
August	23.8	34.8	75	72.5	89.5	12	13.0	6.4
September	23.6	34.4	75	72.2	88.9	11	12.3	6.5
October	20.7	34.2	72	67.5	88.0	9	11.6	7.4
November	15.6	31.5	71	59.7	83.7	6	10.9	7.1
December	12.2	28.2	72	54.6	78.9	5	10.6	5.9

T: ambient temperature in degrees Celsius; RH: relative humidity in percent; THI: temperature and humidity index; UV: ultraviolet radiation.

The maximum average value of THI indicates that, during winter, cattle are in distress, and from spring to fall in emergency conditions (Table 2). Heat stress is associated with reduced productivity and animal welfare, especially during the summer months (Lees *et al.*, 2019).

Table 2. Heat stress categories for animals in production established by the World Meteorological Organization (1989).

THI	Categories	Interpretation
< 70	Confort	Suitable conditions, the animal is not under any heat stress.
71 - 79	Alert	Approaching the critical limit of production; prepare to take precautions, do not leave animals exposed to the sun.
80 - 83	Danger	Above the critical limit of production; do not subject the animals to too many movements.
> 84	Emergency	Extreme heat stress conditions in production; minimize any activity, activities should be done during the morning.

Therefore, it is considered necessary to establish mitigation measures to ease the heat stress consequences.

Heat stress mitigation measures

To maintain body temperature, cattle need to gain or lose heat from their surrounding environment; this process, called heat balance, is achieved through a constant thermoregulation process that involves the flow of heat through four basic pathways: conduction, convection, radiation and evaporation. When the physiological mechanisms to maintain thermoneutrality are not sufficient, the animal enters what is known as the heat stress zone (Beatty *et al.*, 2006). Cattle can subsist in adverse climatic conditions, for which, various individual characteristics are involved; however, there are geographical areas, such as tropical regions, where mitigation measures need to be implemented. One of the main mitigation measures is shading feedlots, which reduce the impact of solar radiation and heat load by up to 30% (Brown-Brandl *et al.*, 2013). To avoid the excess heat effects, cattle also modify their usual behavior; under heat stress conditions they decrease the time spent consuming feed and lying down, increase the time spent drinking water and standing near water troughs; or places with better ventilation (Arias *et al.*, 2008). If their body temperature reaches a critical level, the animals may die due to the lack of control over the regulation of this physiological indicator (Renaudeau *et al.*, 2012). In this matter, Gaughan and Mader (2014) observed that panting is a heat stress indicator in cattle. In this regard, several authors indicate that shading helps to mitigate heat stress; Mitlöhner *et al.* (2001), Mitlöhner *et al.* (2002), Gaughan *et al.* (2010), Blaine and Nsahlai (2011) and Sullivan *et al.* (2011) agree that shade availability helps cattle to mitigate heat load.

Availability of shade in feedlots

Providing shade in intensive beef cattle finishing pens influences the reduction of direct or indirect losses in livestock (Brown-Brandl *et al.*, 2005). Renaudeau *et al.* (2012) indicated that shade usage helps to mitigate heat stress; similarly, Mitlöhner *et al.* (2001) noted that cattle housed under the shade provided by 80% solar filtering (FS) polypropylene fabric at a 3 m height, had lower respiration rate, as well as higher feed intake and higher weight gain, reaching finishing weight 20 days earlier than cattle without access to shade. Blaine and Nsahlai (2011), in South Africa during the winter season, provided 2.87 m^2 of shade per head, from corrugated iron sheeting, placed at a 5 m height. They observed that cattle housed in shade obtained higher final weight than those housed without shade, as well as higher weight gain and improved feed conversion; also, the carcass weight difference was higher; they also indicated a panting decrease and increased resting time. In Australia, Angus steers were provided with a 3.3 m^2 shade per bovine, with a black polypropylene fabric at 80% FS at 4 m height. Gaughan *et al.* (2010) observed lower body temperature and panting in animals housed with shade, as well as higher CMS, GDP, finishing weight and hot carcass weight. Sullivan *et al.* (2011) assessed the shade availability (0, 2.0. 3.3 and 4.7 m^2/animal) provided by 70% black FS solar fabric at 4 m height; it shows that shade-providing improved animal welfare and performance, while different areas of shade/bovine did not affect productive variables, but shades greater than 2.0 m^2 improved bovine welfare. In a tropical climate, Castro *et al.* (2020) determined that increasing shade space in feedlots tends to linearly increase the average of daily gain and dry matter intake; this effect was more evident between 1.2 and 2.4 m^2 of shade/head.

The recommended shade space is 3.7 m^2 in adult animals (Gasque, 2008); the shade height should be at least 4 m so that it does not interfere with air movement and thus achieve greater projection inside the pen; a strategy to keep the floor of the pen dry is to leave unshaded spaces of 15 cm in the structure (Lagos *et al.*, 2014).

Feeding space

While feeding cattle tend to show hierarchical behavior, because those of higher rank fed first (Méndez *et al.*, 2013); the required space may vary between young animals and large animals; for young animals, a linear space of 0.45 m per head is required, and larger than 300 kg animals require 0.70 to 0.90 linear m per animal (Gasque, 2008; Lagos *et al.*, 2014).

Drinking trough space requirements

Water troughs are an important part of feedlots, since they provide fresh, clean water in necessary quantities, so the required size of water troughs is 30 cm^2 per 10 bovines; these should not be deep, to avoid water stagnation and consequently its contamination, offering less freshwater for cattle (Lagos *et al.*, 2014).

Feedlot density

It is important to take into account for the construction of the pens for intensive feedlots, cattle in production require a living space where they can express their natural behavior while remaining within the finishing cycle (Gasque, 2008); the required living space for fattening animals is of 18. 5 m^2 per animal, but this can be modified according to the animal's weight, requiring up to 15 m^2 per animal when they weigh 300 kg or less, and 20 m^2 per animal over 400 kg; this is why, the number of cattle per pen should be established according to the m^2 of available surface area in the feedlot (Lagos *et al.*, 2014). The scientific information regards the effect of feedlot density and its relationship with the productive performance of cattle is limited; in this sense, Ha *et al.* (2018) researched the density and the productive response of cattle; in that research, the authors state that increasing the space per animal in feedlots can improve the cattle's welfare, since they can express their natural behavior, tend to increase their social behavior and decrease agonistic behaviors, which usually occur in pens with less living space per animal. In the aforementioned study, an improvement in carcass characteristics, such as rib-eye area (REA) and marbling percentage, was also observed; in another study, conducted by Lee *et al.* (2012), they reported that a low density per pen helps cattle grow faster, obtain larger REA, improve feed efficiency, GDP and improve carcass weight.

CONCLUSIONS

Heat stress mitigation strategies in beef cattle in intensive finishing under practical conditions should contribute to animal welfare and improve productivity indicators in the Mexican dry tropics. Heat stress mitigation strategies in beef cattle under intensive finishing directly contribute to productive indicators and animal welfare in the Mexican dry tropics.

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AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

In vitro production of gases with mixtures of *Hyparrhenia rufa* (Nees) and *Leucaena leucocephala* (Lam) de Wit

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ABSTRACT

Objective: To evaluate total *in vitro* gas and methane (CH_4) production in different mixtures of *Hyparrhenia ruffa* (Hr) and *Leucaena leucocephala* (Ll).

Design/methodology/approach: In airtight biodigesters with 200 mL of culture medium, 20 g of the following treatments were incubated by triplicate: T1: 100% Hr, T2: 80% Hr + 20% Ll, T3: 60% Hr + 40% Ll, T4: 40% Hr + 60% Ll; these were inoculated with 20 mL of fresh rumen fluid and incubated at 38±0.5 °C for 24, 48, 72 and 96 h. The total gas and CH_4 production were assessed; the data were analyzed in a completely randomized design.

Results: The addition of 20%, 40% and 60% Ll in mixture with Hr decreased the neutral detergent fiber (NDF), acid detergent fiber (ADF), total gas and CH_4 production, while the crude protein content increased.

Study limitations/implications: *In vivo* studies are required / by including amounts higher than 20% Ll may improve energy utilization efficiency.

Findings/conclusions: Adding more than 20% *L. leucocephala* in a mixture with *H. ruffa* decreases total gas and CH_4 production.

Keywords: methane, ruminants, tropical grasses, forage shrubs.

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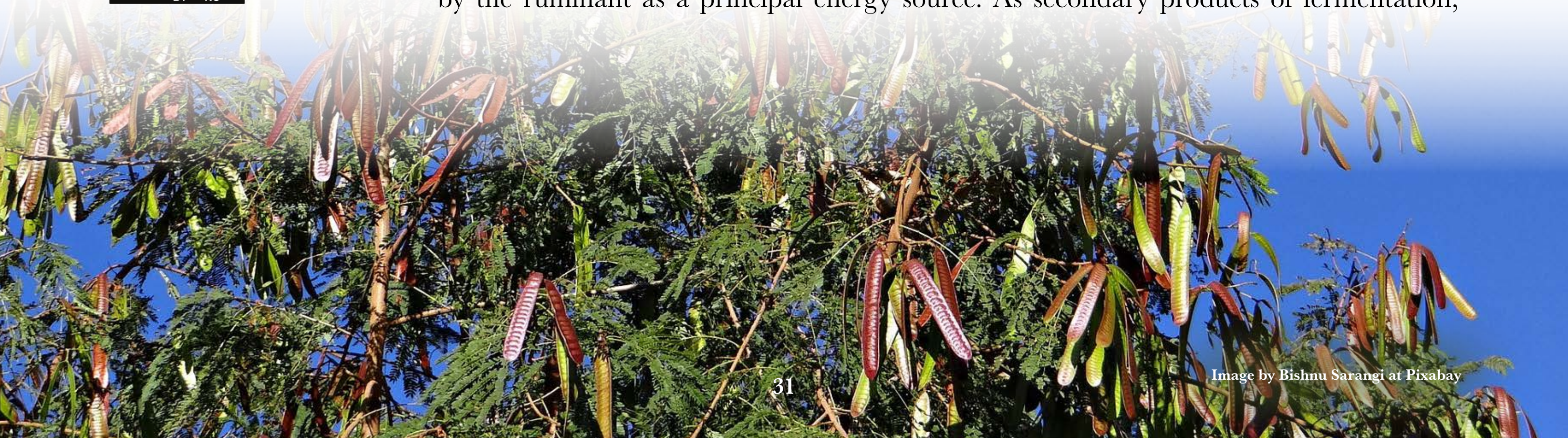
INTRODUCTION

The digestive process of the food consumed by ruminants involves physical, microbiological and chemical processes. The fermentation of food in the rumen is carried out by microorganisms, including bacteria, protozoa and fungi; the products of fermentation are: energy in the form of adenine triphosphate (ATP), which is used by microorganisms to grow and reproduce, the process involves the synthesis of microbial mass that will be later digested in the abomasum and used by the ruminant as a true protein source; volatile fatty acids such as acetic, propionic and butyric are also produced, which will be used by the ruminant as a principal energy source. As secondary products of fermentation,



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carbon dioxide (CO_2), hydrogen (H_2) and CH_4 are produced, these are synthesized from the fermentation of structural carbohydrates products by rumen methanogenic archaea such as *Methanobrevibacter ruminantium*, *Methanobacterium formicicum*, *Methanomicrobium mobile* (Cobos *et al.*, 2018).

The CH4 production is necessary for the oxidation of nicotin adenine dinucleotide (NAD), it is a step in the process for obtaining the energy contained in the neutral detergent fiber (NDF) and other nutrients in the diet, due that this process reduces the accumulation of H_2, it regulates pH and reduces ruminal pressure (Sharp *et al.*, 1998; Ley de Coss *et al.*, 2018) to maintain ruminal stability (Galindo *et al.*, 2010). In a second process, with the manure fermentation, nitrous oxide (N_2O) is produced, a gas with 310 times the potential of heat retaining than CO_2 (Ellis *et al.*, 2012).

Methane emissions from bovine enteric fermentation account for about 39% of the greenhouse gases (GHG) produced by the livestock sector (Arbre *et al.*, 2016), it is necessary to reverse the negative trends of extensive livestock farming, where the increase of CO_2 was of 70% and 40% for CH_4 during the 1970 to 2004 period (IPCC, 2016). To achieve this, it is required to design and evaluate methodologies to accurately calculate the energy flow and its relationship with GHG emissions in livestock activity (De-Vries and de-Boer, 2010).

In the southern and southeastern regions of Mexico, the ruminants' diet is based on forage grasses and shrubs; the former has a higher NDF, acid detergent fiber (ADF) and lower protein content, which favors higher CO_2, H_2 and CH_4 production. Methane production in tropical regions is of 20 L kg^{-1} of fermented dry matter (Piñeiro *et al.*, 2017), with energy losses in the form of CH_4 of up to 18%, so that, by using feeding strategies that allow greater efficiency in energy utilization, emissions of this gas could be reduced and cattle productivity improved (Vanlierde *et al.*, 2016).

The rate of CH_4 emissions from ruminal fermentation is directly related to the intake, nutrient content, physical and chemical characteristics and diet digestibility. Therefore, a strategy to reduce enteric methane production is through feeding management that allows changes in the ruminal fermentation pattern (Piñeiro *et al.*, 2017). The inclusion of forage trees and shrubs in the feeding of ruminants has improved NDF degradation, due to the higher crude protein contribution. When 20% of *L. leucocephala* was included in the diet, CH_4 production was reduced by 26% (Piñeiro *et al.*, 2015). Reduction in CH_4 emission was also observed with the shrubs as *L. leucocephala*, *Sapindus saponaria*, *Calliandra calothyrsus*, *Pithecellobium dulce*, *Heliocarpus velutinus* and *Guazuma ulmifolia*, when supplied with low quality pasture or with *Megathyrsus maximus* (Gaviria *et al.*, 2015; Lopez *et al.*, 2016). Based on the above, the objective was to assess the total gases and CH_4 production, when combined with *Hyparrhenia ruffa* grass and *Leucaena leucocephala* in *in vitro* incubation.

MATERIALS AND METHODS

Location of the study. The research took place from May 1 to June 30, 2019, in the Jaulas Metabólicas area and the Laboratorio de Sanidad Agropecuaria at the Facultad de Ciencias Agronómicas, Campus V of the Universidad Autónoma de Chiapas located at the Centro Universitario de Transferencia de Tecnología (University Center for Technology Transfer, CUTT) "San Ramón", Carretera al ejido 16 de septiembre km 2.5 in Villaflores, Chiapas, Mexico (16° 27' 59" N and 93° 28' 43" W). From September 2 to 16, 2019, the analyses were performed at the Laboratorio de Nutrición Animal of the Programa en Ganadería from the Colegio de Postgraduados, Campus Montecillos, México (19° 27' 39.24" N and 98° 54' 29.19" W).

Chemical analysis of samples. The mixtures of *H. ruffa* and *L. leucocephala* were analyzed for total dry matter (TDM), crude protein (CP) following the Kjeldahl method, ethereal extract (EE) via the Soxhlet method, crude fiber (CF) by the Weende method, ash (C) and organic matter (OM) by difference, all using the techniques described by the AOAC (2012). The fiber fractions (NDF and FDA) determination was performed with alpha-amylase without ash correction as specified by Van Soest *et al*. (1991).

Growth medium and treatments. Rumen liquid (RL) from a 525 kg live weight male Brown Swiss bovine was used, the RL was extracted with an esophageal tube with a vacuum pump. The bovine received a diet with 85% *Hyparrhenia rufa* and 15% of a concentrated feed, containing 2.7 Mcal of ME and 14% crude protein (CP), the culture medium used to determine total gas (TG) production, CO_2 and CH_4 ratios and DM degradation (Table 1), was prepared in sterile conditions and low CO_2 flow.

The evaluated treatments were: T1: 100% *Hyparrhenia rufa* (Hr), T2: 80% Hr + 20% *L. leucocephala* (Ll), T3: 60% Hr + 40% Ll, T4: 40% Hr + 60% Ll.

Table 1. Culture medium for *in vitro* fermentation of dry matter of *Hyparrhenia rufa* and *Leucaena leucocephala* mixtures.

Compound	Quantity (mL) for 100 mL of medium
Destilled water	52.9
Clarified rumen fluid (1)	30.0
Mineral solution I (2)	5.0
Mineral solution II (3)	5.0
Sodium carbonate (Na_2CO_3), 8% (4)	5.0
Sulfide-cysteine solution (5)	2.0
Resazurin solution, 0.1 % (6)	0.1

(1) Clarified rumen fluid filtrated and centrifugated at 17,664 g for 15 min and sterilized 20 min at 21 °C a 15 psi. (2) Contains (in 1000 mL) 6 g K_2HPO_4. (3) Contains (in 1000 mL H_2O), 6 g KH_2PO_4, 6 g $(NH_4)_2SO_4$, 12 g NaCl, 2.45 g $MgSO_4$ y 1.6 g $CaCl_2 \cdot H_2O$. (4) 8 g Na_2CO_3 in 100 mL H_2O destilled. (5) 2.5 g L-cysteine (in 15 mL 2N NaOH) + 2.5 g Na_2S-$9H_2O$ (in 100 mL H_2O). (6) 0.1 mL resazurin in 100 mL.

Production of total gases and CH_4. The *in vitro* production of TG and CH_4 was determined by triplicate with repetition throughout time using 2.0 L capacity biodigesters with a hermetic look, to which the following mixture was added in aseptic conditions and CO_2 flow: 20 g of DM of each treatment plus 200 mL of culture medium (Table 1), each treatment was inoculated with 20 mL of fresh RL, which was filtered in cotton gauze and kept in a water bath at 38±0.5 °C and CO_2 flow. The initial total bacteria concentration was counted with the most probable number (MPN) technique with 1.30×10^8 CFU mL^{-1}

at pH 6.72. The inoculated treatments were incubated for 24, 48, 72 and 96 h in a water bath at 38±0.5 °C. At the end of the incubation period, the TG pressure was measured with a manometer adapted to the equipment. Afterward, the amount of produced TG in the system was assessed using the liquid displacement technique through a trap with Mariotte flasks.

The displaced water was collected in a graduated cylinder and the amount of gas produced per gram of fermented DM (mL of gas g^{-1} of MSf) was determined. In a second run and under the same culture conditions and temperature, a 2N NaOH solution (20 g L^{-1}) pH 13.57 was added to the Mariotte flask traps; the NaOH solution upon reacting with CO_2 formed Na_2CO_3 and the released gases were a mixture of CH_4, H_2, N_2 and H_2S according to the technique described by Ley de Coss *et al.* (2018); the CO_2 trap was coupled to the biodigester using a Taygon hose (5 mm internal 35 cm length) to which a 31.8 mm 20 G hypodermic needle was attached. In all TG production evaluations, the result was corrected by difference to the gas production of the blank samples (200 mL of culture medium plus 20 mL of RL), as well as with the CH_4 concentration measurements using the gas trap technique with saline solution.

Statistical analysis. The experimental design was in a completely randomized design, total gas and CH_4 production data were analyzed with the PROC GLM procedure of the Statistical Analysis System (SAS) (SAS, 2011) and the means of the treatments were compared with Tukey's test ($P \leq 0.05$).

RESULTS AND DISCUSSION

Chemical composition of the diets

Table 2 shows the chemical composition of the *H. ruffa* and *L. leucocephala* evaluated mixtures. By increasing the *L. leucocephala* proportion from 0.0% to 60% of the mixture, the NDF content decreased from 55.7% to 38.2%; FDA from 28.7% to 18.4%; while the CP content increased from 8.3% to 18.1% and the ethereal extract from 0.8% to 2.4%. The increase in CP content and reduction of NDF and FDA could have an impact on the production of TG and CH_4, during *in vitro* fermentation (Piñeiro *et al.*, 2017). One of the factors that modify the fermentation pattern and products is the amount of fermentable NDF; in this study, the 100% *H. ruffa* fermentation, showed a higher production of total gases (Table 3). Grasses from tropical regions such as *H. ruffa* have a higher NDF amount than shrubs, which generates fermentation patterns with higher CH_4 production (Archimède *et al.*, 2011).

Total gas production

Table 3 shows that increasing the *L. leucocephala* content in the mixture from 0.0% to 20% decreased the TG production at 24 and 96 h of incubation ($P<0.05$), at 48 and 72 h there was no difference between both treatments ($P>0.05$). When the *L. leucocephala* proportion increased to 40%, TG production was lower at all incubation hours ($P<0.05$) compared to the treatment without *L. leucocephala*, and there was also a difference ($P<0.05$) compared to the treatment where 20% *L. leucocephala* was included at all incubation hours.

Table 2. Chemical composition of *Hyparrhenia ruffa* and *Leucaena leucocephala* mixtures (g per 100 g of dry matter).

	Proportion of *H. ruffa: L. leucocephala*			
Nutrient (%)	100	80:20	60:40	40:60
Dry material	93.4	92.8	92.1	91.5
Ashes	9.0	8.9	8.8	8.7
Raw Fiber	28.8	26.7	24.7	22.6
NDF	55.7	49.9	44.0	38.2
ADF	28.7	25.3	21.8	18.4
Raw protein (N×6.25)	8.3	11.5	14.8	18.1
Ethereal extract	0.8	1.3	1.9	2.4
NFE	53.3	52.0	50.7	49.4

NDF, Neutral detergent fiber; ADF, Acid detergent fiber; N, Nitrógen; NFE, Nitrogen free extract.

Table 3. Cumulative *in vitro* total gas production (mL of gas per 20 g of fermented DM).

Treatments *Hyparrhenia ruffa*: *Leucaena leucocephala*	**Time (h)**			
	24	**48**	**72**	**96**
100	150.03[a]	248.82[a]	357.75[a]	605.47[a]
80:20	99.60[b]	245.69[a]	353.37[a]	480.89[b]
60:40	91.77[c]	143.91[b]	263.26[b]	348.04[c]
40:60	89.93[c]	123.78[b]	234.72[b]	300.75[c]
SEM	2.84	9.17	26.48	22.85
DMS	7.43	23.99	69.24	42.08

SEM: Standart error of the mean; MSD: Minimum significant difference. [a, b, c] Means with different letters within the same column differ from each otther, according to Tukey test (P<0.05).

When the *L. leucocephala* proportion of the mixture increased to 60%, there was no difference in total gas production (P>0.05) compared to the treatment with 40% of *L. leucocephala*, indicating that with higher than 40% levels in the mixture, TG production did not increase. In all assessed treatments, the highest TG production was recorded between the 48th and 72nd hours of incubation.

When analyzing the proportion increase in the TG at 48 h, when the proportion of *L. leucocephala* increased from 0 to 20%, the production decreased by 1.25%, while when the proportion increased from 20% to 40%, the decrease was 41.42%, and when the proportion of *L. leucocephala* increased from 40% to 60%, the total gas production decreased by 13.98%.

In this research, the result indicates that the greatest effect in the TG decrease production is obtained when 40% of *L. leucocephala* is included. This effect could be related to the NDF and CP content of the different mixtures (Table 2), since, as the proportion of *L. leucocephala* in the mixture increases, the NDF content decreases and the CP content increases, due to a lower proportion of cell walls. With that, the fermentation pattern changes, the amount of potentially fermentable cell walls decreases, and consequently the amount and proportion of volatile fatty acids decreases. This decreases the proportion of acetate, H_2, CO_2 and increases the proportion of propionate. It is reported that in diets with *L. leucocephala* containing 14% of CP, methane production is reduced (Lovett *et al.*, 2004), and when NDF and FDA levels are lower, there is lower total gases production (Waghorn and Hegarty, 2011).

CH_4 production

A similar trend was observed in the CH_4 production to that obtained in TG production. The highest amount of CH_4 produced was in the treatment with 100% *H. ruffa* at 24 and 96 h of incubation ($P<0.05$). At 24 h of incubation, when the proportion of *L. leucocephala* increased from 0.0% to 20%, CH_4 production decreased ($P<0.05$), and the same response was observed when the *L. leucocephala* content was of 40 and 60%. At 48 and 72 h, there was a difference when 40 and 60% of *L. leucocephala* were included. The results obtained in this research show that increasing *L. leucocephala* content in the mixture decreases CH_4 production. Higher NDF and FDA content has been related to higher CH_4 production (Lopez *et al.*, 2016; Vélez *et al.*, 2018). When there is lower availability of digestible nutrients and higher cell wall content (NDF and FDA), CH_4 production is favored, while high levels of fast-fermenting carbohydrates and CP reduce CH_4 production (Owens *et al.*, 1998).

Table 4. Cumulative CH_4 production in *in vitro* incubations (mL of gas per 20 g of fermented DM).

Treatments *Hyparrhenia ruffa*: *Leucaena leucocephala*	Time (h)			
	24	48	72	96
100	111.97^{a}	176.19^{a}	272.45^{a}	451.81^{a}
80:20	66.43^{b}	173.09^{a}	253.46^{a}	354.99^{b}
60:40	47.89^{b}	74.68^{b}	125.21^{b}	167.47^{c}
40:60	40.59^{b}	57.58^{b}	114.22^{b}	148.15^{c}
SEM	10.41	16.25	14.34	21.43
MSD	27.22	42.47	37.49	40.32

SEM: Standart error of the mean; MSD: Minimum significant difference. [a, b, c] Means with different letters within the same column differ from each otther, according to Tukey test ($P<0.05$).

CONCLUSIONS

By including higher than 20% levels of *L. leucocephala* in mixtures with *H. ruffa* grass in *in vitro* incubations, total gas production and CH_4 production decrease, due to the change in the fermentation pattern caused by decreasing the NDF and FDA content of the mixture, and at the same time increasing the crude protein content.

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AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Scientific mapping of the knowledge on the El Cielo Biosphere Reserve

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ABSTRACT

The current knowledge of the El Cielo Biosphere Reserve (CBR), Tamaulipas, Mexico was identified from scientific publications between 1993 and 2019.

Objective: Analyze, synthesize and categorize the published research on the El Cielo Biosphere Reserve (CBR), Tamaulipas.

Design/methodology/approach: The methodology focused on searching, classifying and reviewing existing sources in the SCOPUS database from a knowledge base perspective. The assessment recovered 37 publications that were analyzed and mapped in four dimensions: size, time, space, and composition. The analyzes were performed using the Bibliometrix and Biblioshiny software tools.

Results: The reviewed publications addressed biodiversity studies from a disciplinary approach and from an ecological perspective, which produces a fragmented knowledge of this territory and its problems.

Limitations on study/implications: There may be publications not appearing in the Scopus database.

Findings/conclusions: The knowledge of the El Cielo Biosphere Reserve is fragmented, with a disciplinary approach and from an ecological perspective. The lack of a critical mass of researchers to generate useful knowledge is observed. A weak intellectual structure is also found, which generates a reduced contribution to the conservation of biodiversity. The original contribution of this paper is the synthesis of the current state of the knowledge regard biodiversity and conservation of the El Cielo Biosphere Reserve, which had not been performed previously.

Keywords: Science Mapping, Knowledge basis, Knowledge management, El Cielo Biosphere Reserve.

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INTRODUCTION

The effective conservation of biodiversity is based on knowledge and understanding of ecological patterns and processes (Fischer & Lindenmayer, 2007; Fahrig *et al.*, 2011; Tscharntke *et al.*, 2012). However, research such as that from Giehl *et al.* (2017) documents there is no reliable information on the available scientific knowledge, or if this is used to design conservation processes. Hallinger & Suriyankietkaew (2018) point out that the analysis of the knowledge basis can be done in four dimensions: size, time, space and composition. Size is the volume of accumulated knowledge. The productive knowledge

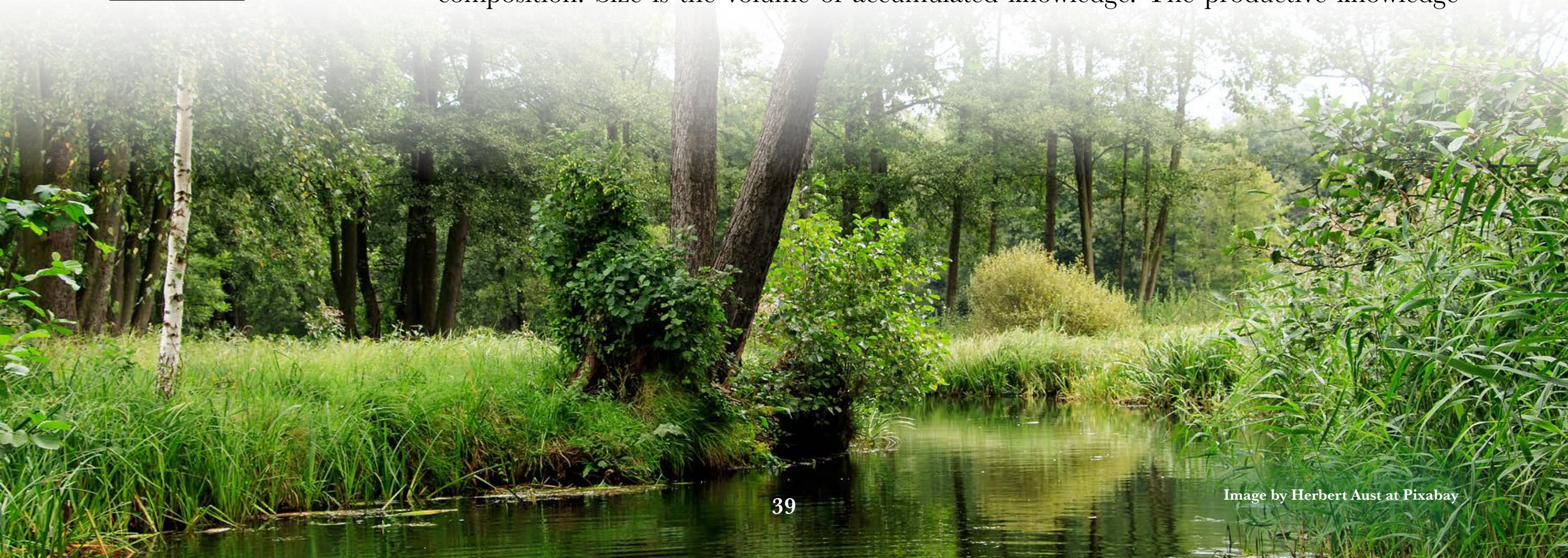

Image by Herbert Aust at Pixabay

accumulation requires a critical mass of research and a long time must pass before a discipline or field accumulates a sufficient body of knowledge. Therefore, knowledge size is necessary but an insufficient precondition for a useful knowledge base (Hallinger & Suriyankietkaew, 2018). In this context, according to Richardson (2013), the accumulation of knowledge is a dynamic process that changes over time. Time refers to the paths that a publication follows within a discipline or research line. Meanwhile, space refers to the geographical distribution of documents within a knowledge base. The spatial distribution offers information on academic ability and reveals concentrations and gaps in the production and knowledge accumulation in different societies (Hallinger & Suriyankietkaew, 2018).

The composition of the intellectual structure of the knowledge basis is the fourth dimension. It allows identifying gaps, orientations, structures and patterns of the research, thus exposing how the study of a territory and the development of knowledge are approached (Topp & Loos, 2019). In this regard, Gazni *et al.* (2012), Khasseh *et al.* (2017), Wagner *et al.* (2017), and López & Sassone (2019) point out that the studies are carried out at a country level and it is necessary to be complemented with what happens in each protected natural areas (PNA), given that these represent specific situations that must be addressed to ensure their conservation. In this sense, it should be noted that no previous studies were found in a PNA in Tamaulipas, Mexico.

The central axis of this research was to analyze, synthesize and categorize the published research from the El Cielo Biosphere Reserve (CBR), Tamaulipas, Mexico. Two characteristics distinguish this review: it is a first on the produced knowledge, and second, its "scientific mapping" used (Zupic & Čater, 2015), a research review method designed to synthesize patterns on the knowledge production within a discipline, rather than synthesizing substantive findings.

For this, four research questions were posed: 1) What is the size, growth trajectory and distribution of the literature on CBR?, 2) What are the topics studied by academics in the CBR area?, 3) What journals have been used by the authors to disseminate their findings?, and 4) What is the intellectual structure of the CBR knowledge base?

In this research structure, in the first place are the antecedents that include aspects of relevant knowledge generation for this research and the previously reported work in the literature. Subsequently, the methodology used for this study is described. Then the obtained results and their discussion are presented. Finally, the main conclusions of the research and future works established that follow up on the research topic are presented.

MATERIALS AND METHODS

To analyze the scientific production on the CBR, the research results described in scientific papers, book chapters and reviews were assessed. A retrospective quantitative analysis was performed based on the articles published in the Scopus database (https://www.scopus.com). For its identification, the combination of the words "Reserva Biosfera El Cielo Tamaulipas México - Biosphere Reserve El Cielo Tamaulipas Mexico" was used. The search took place on March 18, 2019. The search date range of the publications was open. The initial documents selection eliminated ineligible source types (research that cited work done in the CBR or only mentioned it as a context) and duplicate elements.

First, the searches were done for all fields, later, it was carried out by article title, abstract and keywords, to ensure that the articles belonged to the CBR, leaving 37 articles. The first was published in 1993, so the investigation period comprises from 1993 to March 2019, that is, 26 years. For the analysis of these 37 papers, the associated bibliometric data was downloaded from Scopus in BIBX format, the extracted metadata included the authors' names, affiliation information, year of publication, keywords, abstracts, and various citations information. Later, analyzed with the Bibliometrix package through the Biblioshiny application, both implemented in the R statistical software environment (R CoreTeam, 2016).

Descriptive techniques were used to determine the size, growth trajectory and geographic distribution of the publications and to document the main characteristics of the knowledge base on the CBR. Regarding the bibliometric analysis, a citation analysis was applied to evaluate the impact and influence of the authors and documents. The citation analysis examines the direct impact of the documents included in the review database by calculating the number of times each document or author has been cited by other documents located in the Scopus index. Lotka's law (Lotka, 1926; Urbizagastegui, 1999) was used to group authors according to their productivity. While identifying the core publications, Bradford's law (East, 1983; Alvarado, 2016) was used to identify both cases, the most productive researchers and the most relevant journals in this field of study.

RESULTS AND DISCUSSION

The results were organized around the four research questions laid at the beginning of the research.

Size, growth path, and distribution of the literature on the CBR

The knowledge database is made up of 37 publications, published between 1993 and 2019 and represents a reduced set of knowledge, not generated in a systematic manner.

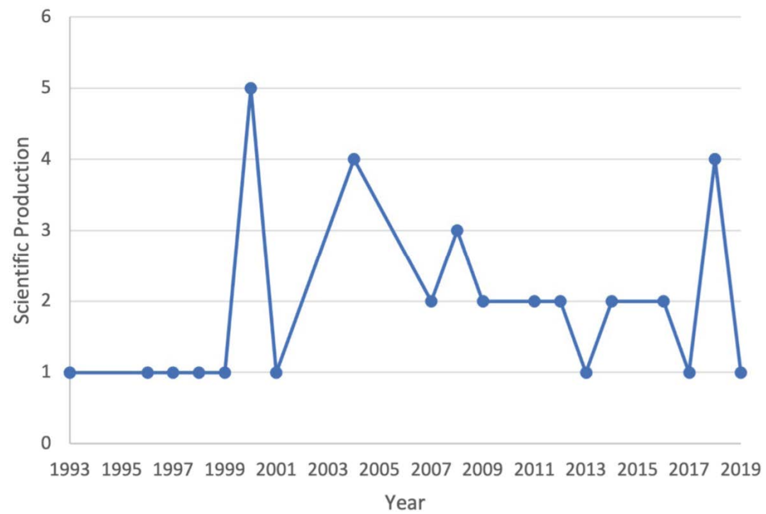

Figure 1. Distribution of the existing literature on the El Cielo Biosphere Reserve between 1993 and 2019.

The main type of published document was as scientific articles and constitutes 94.59% of the production. The remaining 5.41% was made up of a book chapter and a review.

Topics studied by academics in the area of CBR

The most researched topic was its plant community. Twenty publications were identified, followed by 12 on their animals. There is one on mushrooms and four addressing cross-cutting issues. Figure 2 shows the topics addressed overtime in the analyzed publications.

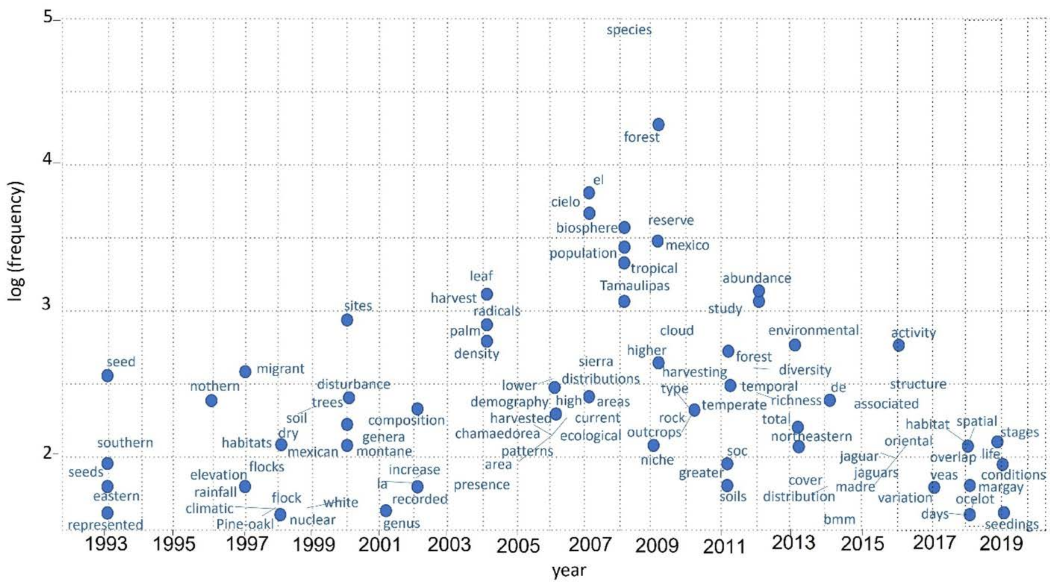

Figure 2. Evolution of the investigated topics on the CBR between 1993 and 2019.

Analysis of influential journals, authors and documents

This research question inquired about the composition of the knowledge database from the perspective of their sources, academics, and documents. The assessed papers were found to be published in 28 journals. The impact of the chosen journals on which the research results on the CBR were published was determined based on the *h*-index, which defines the quality based on the number of citations a scientific article receives (Hirsch, 2005), finding that the most important is Journal of Tropical Ecology of the USA (Figure 3).

The most cited source in the list of reference publications in Ecology with 79 citations, followed by Conservation Biology with 48. Both journals are published in the USA. With bibliometric analysis, key scholars and documents within a research field can be identified. In this sense, based on Bradford's law, five core journals were identified, the most relevant being: the *Journal of Tropical Ecology* from England, *Southwestern Naturalist and Condor* from USA, the *Revista de Biología Tropical* from Costa Rica, and the *Revista de Geofísica* from Mexico.

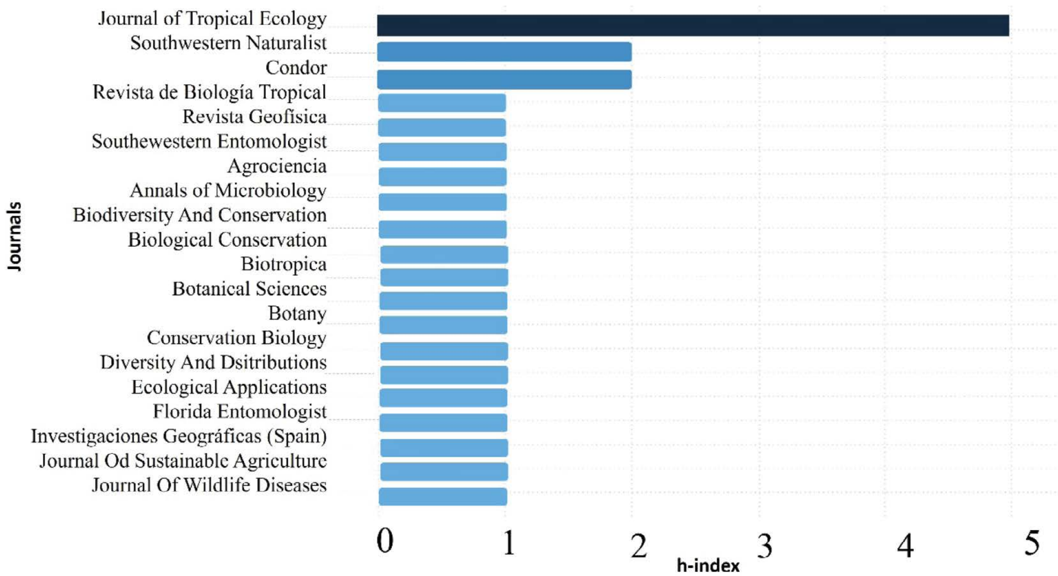

Figure 3. Impact of the journals (*h*-index) chosen to publish research on the CBR between 1993 and 2019.

In the 37 publications on the CBR from 1993 to 2019, 90 authors participated (Figure 4), the most important authors with their publications and citations, where the size of the circles is proportional to the number of documents published in that year, while the color intensity relates to the total of citations per year.

As can be seen, Gorchov DL is the researcher with the highest production (eight articles) and the highest number of citations, followed by Edress B. (five), both from the USA. The Miami University of Ohio State in the USA is the institution from which the largest number of publications were produced. The total number of citations in the analyzed documents was 599. Their highest number was 248 and reported during 2004.

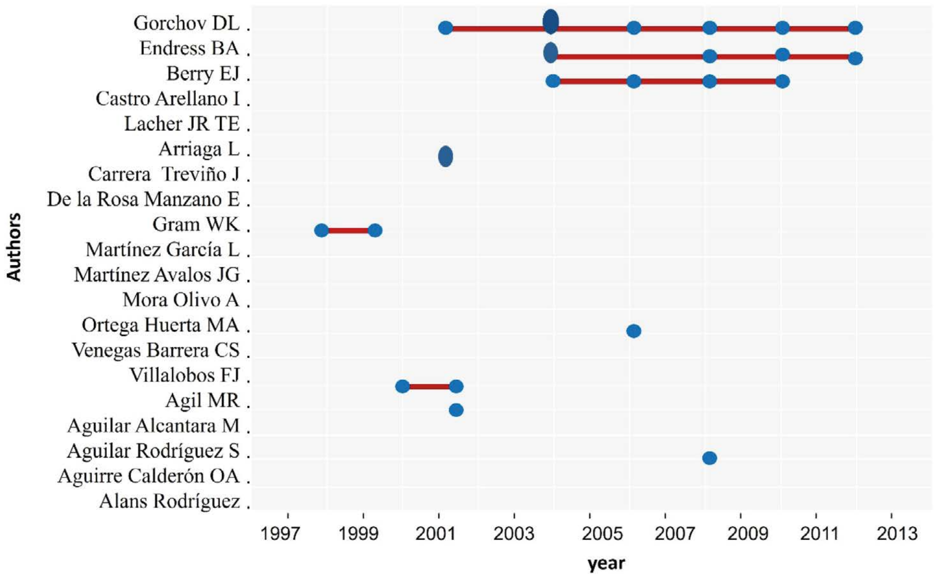

Figure 4. Most relevant authors with their publications and citations.

The analysis of the most relevant documents, presented in Table 1, referring to the most globally cited documents. The data presented should be interpreted in light of two considerations: most of the most cited researchers publishing on the CBR have a low actual level of Scopus citations, and none is identified as leading worldwide. These findings, therefore, reaffirm that the knowledge base is incipient.

Table 1. Documents most cited worldwide.

Source	Total cited	Total cited per year
Ortega-Huerta MA, 2004, Diversity Distrib	103	6.0588
Rojas-Soto OR, 2012, Biodiversity Conserv	62	6.8889
Endress BA, 2004, Ecol Appl	62	3.6471
Endress BA, 2004, Conserv Biol	58	3.4118
Gram WK, 1997, Condor	32	1.3333
Gram WK, 1998, Condor	32	1.3913
Berry EJ, 2004, J Trop Ecol	25	1.4706
Arriaga L, 2000, J Trop Ecol-a	25	1.1905
Berry EJ, 2008, Popul Ecol	21	1.6154
Castro-Arellano I, 2009, J Trop Ecol	20	1.6667
Castro-Arellano I, 2008, J Wildl Manage	19	1.4615
Arriaga L, 2000, J Trop Ecol	18	0.8571
Williams-Linera G, 1993, J Trop Ecol	17	0.6071
Castro-Arellano I, 2009, J Wildl Dis	13	1.0833
Berry EJ, 2007, Biotropica	11	0.7857
Ortega Huerta MA, 2007, Biol Conserv	11	0.7857
Jones FA, 2000, Southwest Nat	11	0.5238
Jones RW, 2012, Rev Mex Biodiversidad	7	0.7778
Cruz-Flores G, 2011, Agrociencia	6	0.6
Ramos-Garza J, 2016, Ann Microbiol	5	1

Intellectual structure of the CBR knowledge base

An approach that has been used in scientific mapping was applied on the author and co-citation analysis (ACA). Table 2, on the summary of countries with several publications with national and foreign authors, includes the number of publications with at least one foreign author (*MCP=Multiple Country Publication*) as well as the number of publications that have national authors (*SCP=Single Country Publication*). The value of the MCP rate is a measure of the intensity of the international collaboration of each country. It can be seen that researchers mainly collaborate with institutions in their own countries.

The geographical distribution of the scientific production by country is presented in Figure 5, where it is observed that Mexico and the USA are the countries that publish the most on the CBR. In the map colors, a strong intensity indicates a lot of academic production while a light intensity represents little. In this sense, Mexico registered 48 coauthors, the USA 35, and Germany and Spain one. The citations contained in the articles are concentrated in three countries: USA with 308 citations, Mexico with 208 and Antigua with 65.

Table 2. Summary of countries with the number of publications with national and foreign authors.

Country	Number of publications	Publications with national authors (*SCP=Single Country Publications*)	Publications with at least one foreign author (*MCP=Multiple Country Publications*)	Value MCP
México	17	14	3	0.176
EUA	13	10	3	0.231
Antigua	2	2	0	0.000
Total	32	26	6	

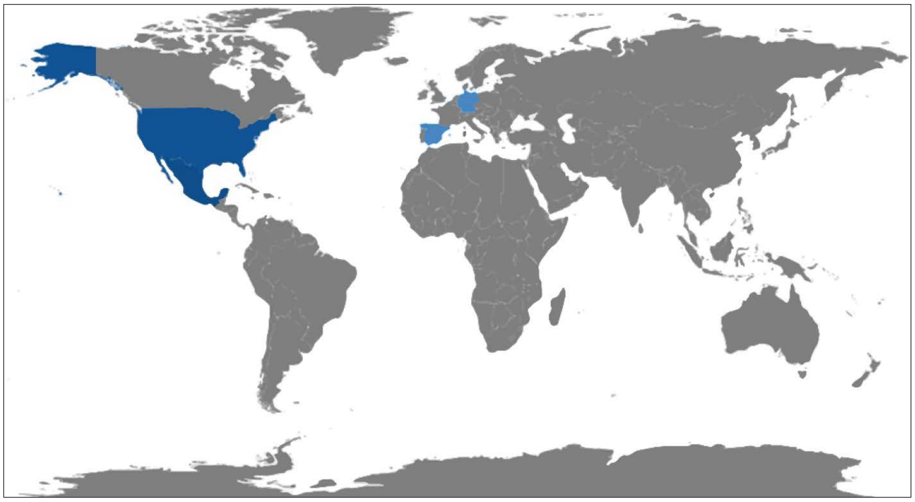

Figure 5. Scientific production by country.

In the Sankey diagram (http://sankey-diagram-generator.acquireprocure.com) shown in Figure 6, the association between authors is emphasized, citations of their works, and keywords. This allows to visually relate the intellectual roots represented by the authors, the citations that they use in their works and the content of the research, represented by the keywords.

CONCLUSIONS

This review revealed that the generated literature is mainly descriptive and qualitative. Advancing the knowledge base on the CBR will require a broader set of research methodologies, capable of documenting and proposing different strategies for conservation, development and social inclusion of its inhabitants. Successful shift towards sustainability in organizations and societies requires leadership to provide a vision, as well as establish direction and motivate people to move towards new goals. Therefore, sustainable leadership is emerging as a new domain of study within the field of management (Hallinger, 2020). Finally, the various findings in this research suggest that the research on the CBR should be encouraged, in addition to carrying out transdisciplinary research based on the expert knowledge of researchers who address the study of natural resources. Identifying major themes, trends, leaders, and core publications form the basis for identifying research gaps.

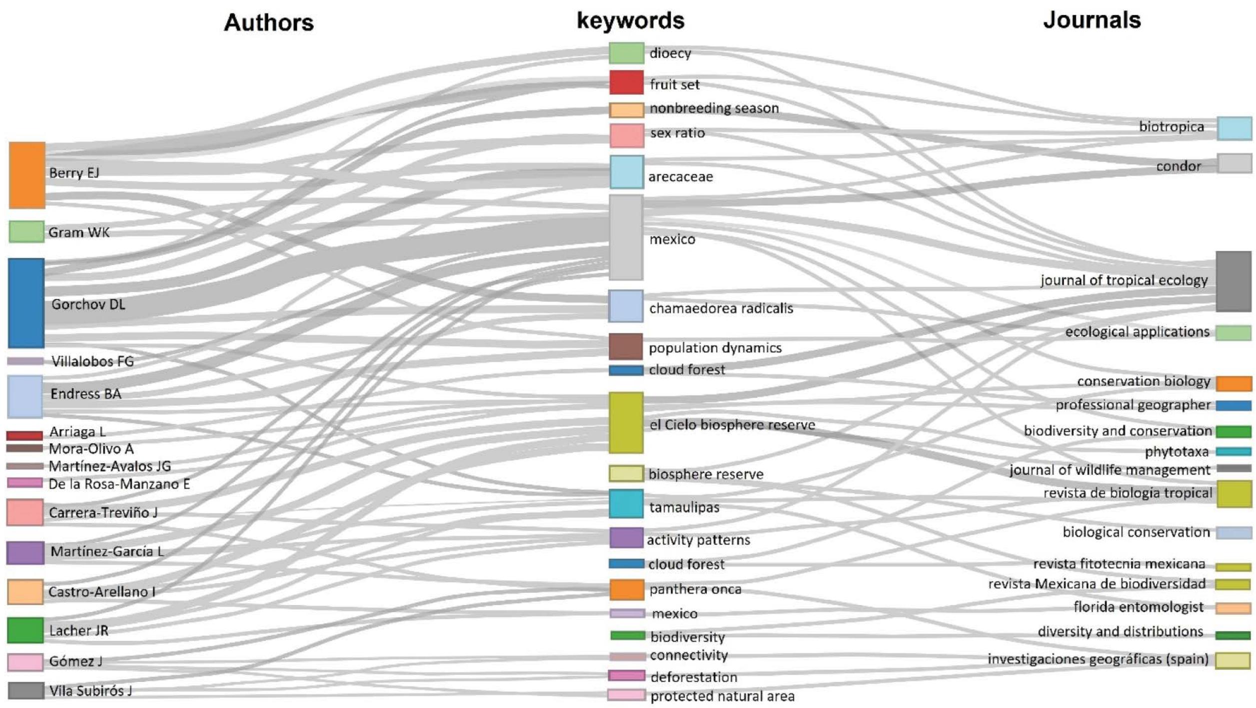

Figure 6. Intellectual roots and content of the research on the CBR.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Degraded forest lands and pine plantations in homogeneous ecological areas in Mexico

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ABSTRACT

Objectives: i) to determine the total degraded areas of EZ in the country, ii) estimate the priority degraded areas for restoration planting, and iii) assess the species and planted areas of the *Pinus* genus and whether these were within their natural distribution range.

Design/methodology/approach: total EZ degradation surfaces and priority degraded areas for restoration plantings were determined with the Germplasm Movement Zones and Restoration Zones of the Comisión Nacional Forestal (National Forestry Commission, CONAFOR), while planted surfaces were estimated from the CONAFOR records from 2016 to 2018.

Results: on degradation, it was shown that three EZ had large areas, six EZ intermediate areas and 32 EZ small areas; two degradation types (III.C and III.D) were prioritized and viable for restoration plantings; four species (23%) were established outside their natural distribution range while ten (59%) were within it, three species were undefined.

Study limitations/implications: for restoration of areas, it is necessary to avoid high initial plant mortality and poor growth.

Findings/conclusions: the north of the country has larger areas with degradation, while the center, north and south have areas with medium and low degradation; planting species outside their distribution range leads to plant adaptation problems.

Keywords: temperate forests, soil conservation, forest restoration.

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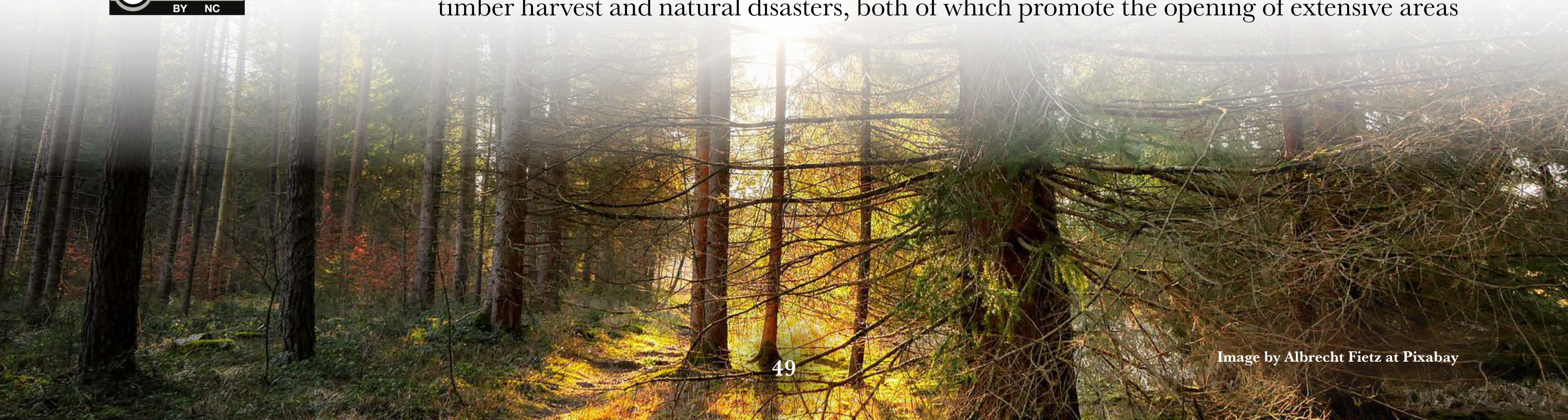
INTRODUCTION

Globally, temperate forests occupy about a quarter of the world's forests and are present to a great extent in the United States and southern Canada, Europe, China, and Australia (Gorte & Sheikh, 2010); however, the areas of this ecosystem type are still decreasing due to deforestation. The common cause of disturbance in developing countries is the land conversion to non-agricultural uses, commercial, residential developments and infrastructure construction such as roads (Flores, Velasco-García *et al.*, 2018; Stein *et al.*, 2005). Other agents reducing the area of this type of ecosystem are the uncontrolled timber harvest and natural disasters, both of which promote the opening of extensive areas



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where reforestation has little success, due to survivorship problems related to drought and competing species invasion (native shrubs, exotic species) (Gorte & Sheikh, 2010).

In Mexico, temperate forests occupy an area of approximately 17% of the country (323 305 km^2) and provide timber and non-timber resources (Galicia *et al.*, 2015). However, these ecosystems have been reduced due to increased land degradation, which, according to studies and official reports, the national projections of deforestation rate vary from 260 000 to 1 600 000 ha $year^{-1}$ the last three decades (Couturier *et al.*, 2012). The government has responded to this situation through nationwide reforestation and soil conservation programs managed by the Comisión Nacional Forestal (National Forestry Commission). However, despite this efforts, the current low percentage of seedling survival (36%) is a problem (Wallace *et al.*, 2015), associated with inadequate plant quality and erroneous species location in areas outside their natural distribution range.

Land degradation is a problem increasing the number of people in poverty (Boer *et al.*, 2017) and is linked to food insecurity and vulnerability to climate change (Barbier & Hochard, 2016). Although the assessment of forest land degradation at the Homogeneous Ecological Zones (EZ) level has not been conducted to assess the effect it has had on forests in Mexico.

A EZ is an area with extensive formations of natural vegetation, but relatively homogeneous, with similar physiognomy although not necessarily identical (Food and Agriculture Organization [FAO], 2001). A viable example of these homogeneous ecological areas are the Germplasm Movement Zones, which have similar conditions for the forest species development and have superior to the conventional operational management (local, state or Umafor). Evaluating the EZ degree of degradation is a key element for planning the resources restoration, based on the adequate establishment of trees from the regions they come from.

Forest plantations in Mexico have a high potential to increase their production (Flores, Muñoz-Gutiérrez *et al.*, 2018) and the recover deteriorated areas. A large part of the established areas has been made with fast-growing trees for timber, and in few states: Tabasco, Veracruz, Campeche, Chiapas and Puebla (Conafor, 2014). In this regard, it is necessary to increase the number of species for the restoration of degraded areas, particularly in regions with temperate climates where conifers prevail (Flores *et al.*, in press). This will contribute to land recovery, carbon sequestration, and ecosystems and biodiversity preservation. Based on the above and with the purpose of assessing the degraded areas of forest land and the areas planted with pine in EZ, the objective here was to determine the total degraded areas of EZs in Mexico, estimating the priority degraded areas for restoration plantations in EZs, and to identify areas planted with species of the Pinus genus within their natural distribution range.

MATERIALS AND METHODS

To determine the total degradation surfaces of the EZs, Germplasm Movement Zones (GMZ) and Restoration Zones (RZ), both made by the National Forestry Commission (CONAFOR, 2016, 2017b), were used (Figure 1 and 2). The GMZ are areas with similar ecological and climatic characteristics that host populations with relatively uniform genotypes or phenotypes (Flores *et al.*, 2014), that reduce the movement of germplasm out of its natural distribution; while the RZ correspond to forest areas with evidence of degradation, in different degrees, and that constitute a risk due the loss of the forest resource (SEMARNAT, 2015). It was proposed to use GMZ as EZs because they are homogeneous areas in terms of climate and latitudinal or longitudinal distribution (Conafor, 2016). The RZs were used because they consider five types of forest land degradation for the country: forest land with high soil degradation (III.A); forest land with severe erosion (III.B); forest land with medium degradation (III.C); forest land with low degradation (III.D); and degraded forest land with management for restoration (III.E). The processing of the GMZ and RZ, and the information representation was carried out using the QGIS software (http://qgis.osgeo.org) (QGIS Development Team, 2015).

The definition of priority degraded areas for restoration plantations was made considering only two types of forest land degradation: III.C and III.D, due to their moderate degree of deterioration (medium and low) (Flores *et al.*, 2019). Degraded areas (ha) were estimated, and the percentages that these represent in the EZs and in the country following the formulas (1 and 2) proposed:

$$\%D_{EZ} = \frac{ADZ}{ATD} * 100 \qquad (1)$$

Where: $\%D_{EZ}$=Percentage type III.C or III.D degradation in Homogeneous Ecological Zones. ADZ=Degraded area (ha) type III.C or III.D in the Homogeneous Ecological Zones. ATD=Total degraded area (ha) of all types (III.A to III.E) in the Homogeneous Ecological Zones.

$$\%D_{P} = \frac{ADZ}{ATDP} * 100 \qquad (2)$$

Where: $\%D_P$=Percentage type III.C or III.D degradation in the country. ADZ=Degraded area (ha) type III.C or III.D in the Homogeneous Ecological Zones. $ATDP$=Total degraded area (ha) of all types (III.A to III.E) in the country.

To estimate the planted areas with species of the *Pinus* genus within its natural distribution range, CONAFOR records for the last three years (2016 to 2018) were used, which are an indicator of the areas in which this work has been done in Mexico. Due to the annual variation of the areas, the average for the analyzed period was calculated. Likewise, it was determined whether these conifers were established in the states that comprise the natural distribution range of the species by EZ. This distribution was determined based on the geographic data (latitude and longitude) of the plots of the National Forest and Soil Inventory 2004-2007 (CONAFOR, 2017a), its representation was done with the QGIS program (http://qgis.osgeo.org) (QGIS Development Team, 2015).

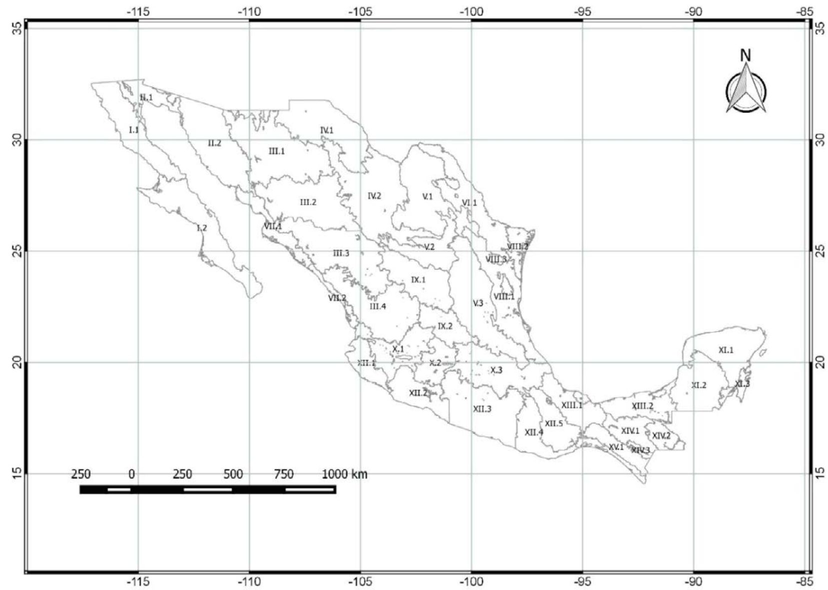

Figure 1. Germplasm Movement Zones of Mexico.

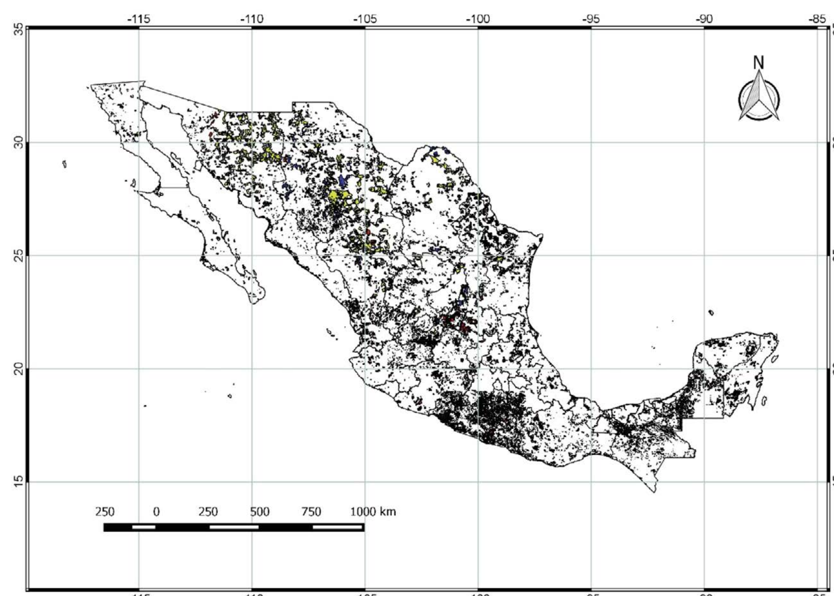

Figure 2. Forest Zoning for Restoration in Mexico.

RESULTS AND DISCUSSION

Degraded areas and priority areas for restoration plantations

With respect to the degradation of EZs, three zones presented the largest area (>18 000 ha), 32 smaller areas (<8000 ha) and six intermediate areas (8 to 18 000 ha) (Figure 3). The results show that the north of the country has the largest areas of forest land degradation, which implies that the production of plants of the analyzed species or others, such as, for example, *P. oocarpa* Schiede ex Schltdl. (VII.1), or *P. durangensis* Martinez (III.2) (Flores *et al.*, 2019) for tree planting in large areas for restoration purposes.

With respect to the intermediate surface areas of degradation distributed in the north, center and south of the country (Figure 3), it is necessary to continue with the use of pinaceae that have already been established in plantations or consider other species, using *P. maximinoi* H. E. Moore (XII.3), *P. lawsonii* Roezl ex Gordon (XII.2) or *P. leiophylla* Schiede ex Schltdl. et Cham. (X.3), which is more effective if the number of forest nurseries in each EZ is considered, since it determines the amount of seedlings to be used (Flores *et al*., in press). For areas with less degraded surface, located in the rest of the country (Figure 3), it should also be considered to expand the species to be used in nurseries that are commonly used for restoration, as well as other species, such as *P. leiophylla*, *P. maximartinezii* Rzed. or *P. teocote* Schiede ex Schltdl. *et* Cham. in IX.1).

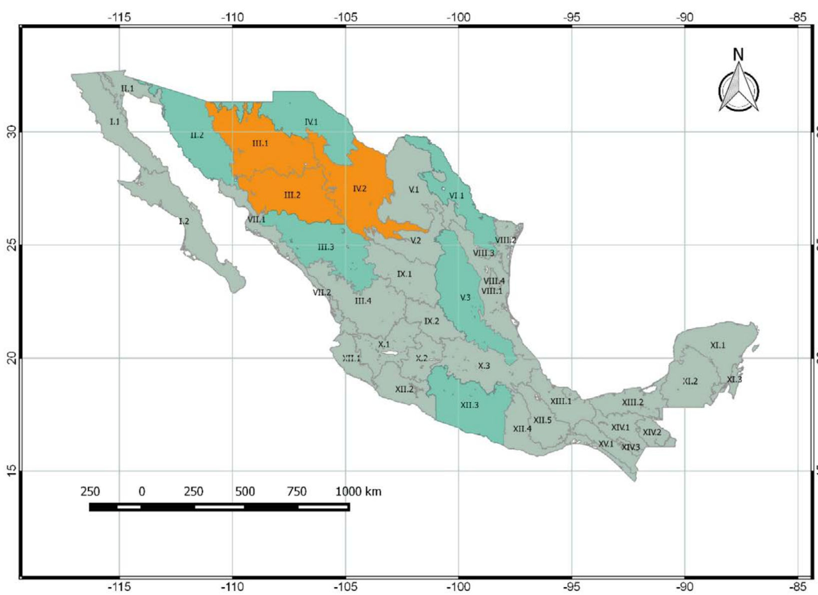

Figure 3. Areas of forest land degradation in the EZs: >18 000 ha (orange color), 8 to 18 000 ha (green color) and <8000 ha (gray color).

The estimation of priority and viable degraded areas to carry out plantations for restoration purposes in degraded land type III.C presented less surface (19 618.53 ha) than III.D (126 706.95 ha), so an effective strategy would be aimed at recovering first the areas with less degree of erosion (III.D). On the other hand, seven EZs with degradation type III.C had 75.69% (14 849.01 ha) of the surface and 26 zones only 24.31% (4 769.52 ha), while six EZs with degradation type III.D covered 71.43% (90 511.85 ha) and 32 zones 28.57% (36 195.10 ha) (Table 1).

In relation to the degraded areas of forest land in the EZs, areas with degradation type III.C presented lower percentages (33.33 to 0.00 %) compared to areas with III.D (96.28 to 0.10 %) (Table 1). Consequently, the same characteristic was presented when the degradation of each EZ was analyzed with respect to the total degraded area of all types (III.A to III.E) of the country; that is, III.C covered lower percentages (11.76 to 0.00 %) than III.D (11.76 to 0.00 %) (Table 1). When considering together areas with degradation

Table 1. Areas with medium (III.C type) and low degradation (III.D type) for forest land in EZ.

EZ	Type of degradation	Area (ha)	Percentage of degradation in EZ	Percentage of degradation in the country
I.1	III D	861.11	97.04	0.44
I.2	III D	435.88	84.50	0.22
II.1	III D	125.59	99.80	0.06
II.2	III D	12,596.90	94.25	6.46
III.1	III C	2,991.40	12.71	1.53
III.1	III D	20,287.40	86.21	10.40
III.2	III C	4,554.13	24.60	2.33
III.2	III D	12,097.31	65.35	6.20
III.3	III C	1,089.64	10.01	0.56
III.3	III D	9,132.12	83.89	4.68
III.4	III C	458.33	9.17	0.23
III.4	III D	2,555.43	51.13	1.31
IV.1	III C	508.67	4.03	0.26
IV.1	III D	12,014.46	95.15	6.16
IV.2	III C	1,717.23	6.70	0.88
IV.2	III D	22,948.89	89.58	11.76
IX.1	III C	739.69	16.29	0.38
IX.1	III D	1,238.91	27.29	0.63
IX.2	III C	412.49	8.51	0.21
IX.2	III D	305.51	6.31	0.16
V.1	III D	7,374.29	94.87	3.78
V.2	III C	771.59	18.34	0.40
V.2	III D	3,136.42	74.56	1.61
V.3	III C	2,676.32	29.89	1.37
V.3	III D	1,873.98	20.93	0.96
VI.1	III C	943.26	8.04	0.48
VI.1	III D	10,566.89	90.10	5.42
VII.1	III D	944.81	96.28	0.48
VII.2	III C	20.99	8.53	0.01
VII.2	III D	24.98	10.15	0.01
VIII.1	III C	114.44	3.00	0.06
VIII.1	III D	2,768.62	72.48	1.42
VIII.2	III C	66.77	11.91	0.03
VIII.2	III D	399.88	71.33	0.20
VIII.3	III C	59.44	5.32	0.03
VIII.3	III D	1,058.10	94.63	0.54
VIII.4	III C	11.16	12.27	0.01
VIII.4	III D	76.20	83.77	0.04

Table 1. Continued.

EZ	Type of degradation	Area (ha)	Percentage of degradation in EZ	Percentage of degradation in the country
X.1	III C	59.21	1.78	0.03
X.1	III D	1,980.02	59.49	1.01
X.2	III C	102.60	8.31	0.05
X.2	III D	36.22	2.93	0.02
X.3	III C	638.90	33.33	0.33
X.3	III D	509.78	26.60	0.26
XI.1	III C	4.06	0.12	0.00
XI.1	III D	18.20	0.53	0.01
XI.2	III C	0.83	0.02	0.00
XI.2	III D	4.12	0.10	0.00
XII.1	III C	13.10	1.73	0.01
XII.1	III D	34.68	4.59	0.02
XII.2	III C	60.82	1.70	0.03
XII.2	III D	72.21	2.02	0.04
XII.3	III C	877.03	8.31	0.45
XII.3	III D	471.20	4.46	0.24
XII.4	III C	206.59	9.87	0.11
XII.4	III D	57.30	2.74	0.03
XII.5	III C	107.90	18.16	0.06
XII.5	III D	94.03	15.83	0.05
XIII.1	III C	11.32	3.47	0.01
XIII.1	III D	199.05	60.92	0.10
XIII.2	III C	15.84	1.89	0.01
XIII.2	III D	6.25	0.75	0.00
XIII.3	III D	117.00	99.60	0.06
XIV.1	III C	273.67	26.16	0.14
XIV.1	III D	141.18	13.50	0.07
XIV.2	III C	9.05	6.18	0.00
XIV.2	III D	95.64	65.27	0.05
XIV.3	III C	1.38	4.25	0.00
XV.1	III C	84.89	32.94	0.04
XV.1	III D	46.39	18.00	0.02
XV.2	III C	15.79	28.91	0.01

type III.C and III.D in the EZs, it is possible to group three groups for restoration work, *i.e.*, 23 areas <1,000 ha, 10 areas between 1,000 and 10,000 ha, and seven areas >10,000 ha (Figure 4).

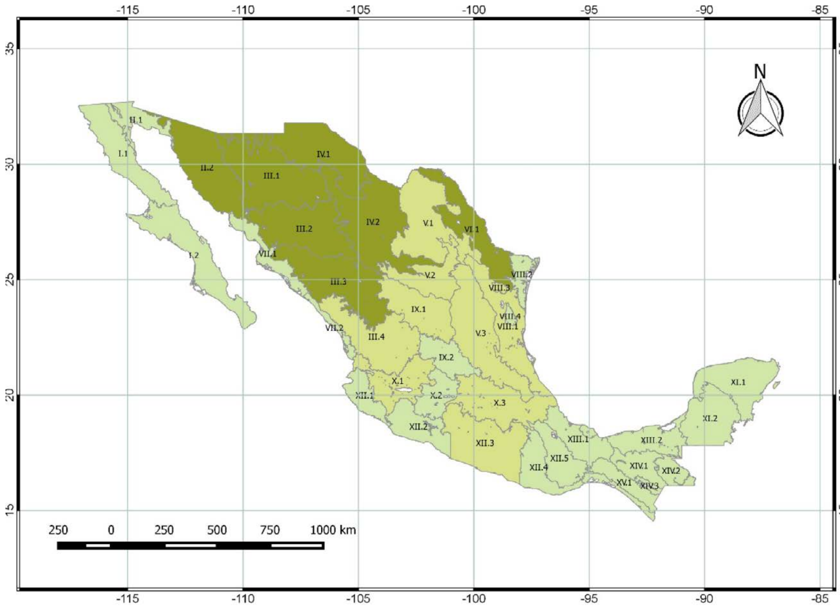

Figure 4. Type III.C and III.D degradation surfaces of EZ for restoration: >10 000 ha (dark green color), 1000 to 10 000 ha (yellow color), <1000 ha (light green color).

There are species with good restoration capacity and a high percentage of survival that can be employed in sites with degradation type III.C and III.D in some local regions of Mexico, for example, in the Monarch Butterfly Biosphere Reserve (Honey-Rosés *et al.*, 2018). *Juniperus flaccida* Schltdl is a conifer that grows in degraded soils and is drought resistant, with good wood quality and rot resistance (Newton & Tejedor, 2011; Willson *et al.*, 2008). Some pine species have good potential for restoration, *e.g. P. pseudostrobus* Lindl.; *P. engelmannii* Carrière, *P. montezumae* Lamb., *P. greggii* Engelm., *P. arizonica* (Engelm.) Shaw, and *P. durangensis* (Flores *et al.*, 2019), which should be taken into account during the implementation of strategies aimed at reducing forest soil loss. There are examples of good survival in pines during restoration works, such as: *P. cembroides* Zucc. 90% (Gómez-Romero *et al.*, 2012), *P. pseudostrobus* 86% (Gómez-Romero *et al.*, 2013), *P. devoniana* Lindl. 80% (Gómez-Romero *et al.*, 2012) and *P. montezumae* 60% (Blanco-García *et al.*, 2008), which could be used in the EZs. In this regard, it is necessary to promote the use of these pines, but above all to carry out restoration evaluations on species for which there is still no information reported.

***Pinus* plantations within their natural range**

In Mexico, an annual average of 2382.09 ha of pine has been established during 2016 to 2018 using 17 species: *P. ayacahuite* Ehrenb. ex Schltdl., *P. arizonica*, *P. caribaea* Morelet, *P. cembroides*, *P. chiapensis* (Martinez) Andresen, *P. cooperi* Blanco, *P. devoniana*, *P. douglasiana* Martinez, *P. eldarica* Medw, *P. engelmannii*, *P. greggii*, *P. hartwegii* Lindl., *P. leiophylla*, *P. montezumae*, *P. oocarpa*, *P. patula* Schiede ex Schltdl. *et* Cham. and *P. pseudostrobus* (Table 2). Most of the conifers used for plantation establishment have been within their natural range, with the exception of four species: *P. greggii*, *P. ayacahuite*, *P. caribea* and *P. cembroides* (Table 2).

Table 2. Areas planted with pine and its location within the natural distribution range of the species by state.

State	Species	Planted Area (ha)¶				Location*
		2016	2017	2018	Mean	
Ags	*P. greggii*	0.00	0.00	67.03	22.34	No
	Subtotal	0.00	0.00	67.03	22.34	
Chih	*P. arizonica*	0.00	70.00	0.00	23.33	Yes
	P. eldarica	8.00	0.00	0.00	2.67	-
	P. engelmannii	155.00	14.00	0.00	56.33	Yes
	Subtotal	163.00	84.00	0.00	82.33	
Dgo	*P. cooperi*	3.10	25.00	0.00	9.37	-
	P. engelmannii	148.00	75.50	158.00	127.17	Yes
	P. greggii	161.90	293.90	79.00	178.27	Yes
	Subtotal	313.00	394.40	237.00	314.80	
Edo Mex	*P. ayacahuite*	78.90	19.75	12.62	37.09	No
	P. greggii	77.70	127.21	71.89	92.27	Yes
	P. hartweggii	170.40	0.00	0.00	56.80	Yes
	P. montezumae	173.10	80.16	0.00	84.42	Yes
	P. patula	158.67	42.75	28.51	76.64	Yes
	P. pseudostrobus	86.75	42.40	2.48	43.88	Yes
	Subtotal	745.52	312.27	115.50	391.10	
Mich	*P. devoniana*	138.21	6.40	0.00	48.20	Yes
	P. douglasiana	0.00	0.00	44.00	14.67	Yes
	P. greggii	124.61	71.00	2.19	65.93	Yes
	P. leiophylla	244.50	82.23	0.00	108.91	Yes
	P. oocarpa	38.20	38.70	18.00	31.63	Yes
	P. patula	40.95	0.00	0.00	13.65	Yes
	P. pseudostrobus	29.00	66.90	23.86	39.92	Yes
	Subtotal	615.47	265.23	88.05	322.92	
Nay	*P. douglasiana*	0.00	42.00	0.00	14.00	Yes
	Subtotal	0.00	42.00	0.00	14.00	
Oax	*P. pseudostrobus*	75.00	75.00	177.00	109.00	Yes
	P. patula	40.86	73.91	0.00	38.26	Yes
	Subtotal	115.86	148.91	177.00	147.26	
Pue	*P. ayacahuite*	0.00	5.53	15.00	6.84	Yes
	P. cembroides	87.91	0.00	0.00	29.30	-
	P. greggii	143.54	31.12	20.62	65.09	Yes
	P. montezumae	230.34	6.00	8.69	81.68	Yes
	P. patula	286.84	137.90	32.77	152.50	Yes
	P. pseudostrobus	63.62	20.46	82.13	55.40	Yes
	Subtotal	812.25	201.01	159.21	390.82	
Tab	*P. caribaea*	303.10	331.82	262.78	299.23	No
	Subtotal	303.10	331.82	262.78	299.23	
Ver	*P. ayacahuite*	0.00	5.00	0.00	1.67	Yes
	P. caribaea	790.46	0.00	265.58	352.01	No
	P. chiapensis	20.00	26.30	0.00	15.43	-
	P. montezumae	5.00	0.00	0.00	1.67	Yes
	P. patula	0.00	13.00	0.00	4.33	Yes
	Subtotal	815.46	44.30	265.58	375.11	
Zac	*P. cembroides*	41.53	0.00	0.00	13.84	No
	P. devoniana	0.00	25.00	0.00	8.33	Yes
	Subtotal	41.53	25.00	0.00	22.18	
	Total	3 925.19	1 848.94	1 372.15	2 382.09	

Base on Conafor's information. * Within the natural range of the species.

The use of species in places that are not within their natural distribution range is considered a serious problem because trees are being planted that will not be able to withstand adverse conditions (low precipitation, frost, drought, high temperature) in regions where they do not come from, for example, *P. hartwegii* presents more than 70% mortality due to water stress (Salas-González & Franco, 2001). Under this condition, plants will present adaptation difficulties and will decrease the restoration potential that is intended to take advantage of them, e.g. plantations of *P. greggii* that have been established in the state of Durango present health and mortality problems, significantly reducing their potential (J. Á. Prieto-Ruíz, personal communication, October 23, 2019).

CONCLUSIONS

For Mexico, the north of the country has the largest areas with forest land degradation, while the center, north and south have areas with medium and low degradation. Restoration of priority degraded areas can be initiated in lands with medium (III.C) and low (III.D) degradation, because they could be restored in a short time: 23 EZs (<1000 ha), ten EZs (1000 to 8000 ha) and seven EZs (>10 000 ha). An effective strategy for the restoration of degraded areas should be aimed at restoring the less eroded areas first (III.D). The average area planted from 2016 to 2018 was 2382.09 ha for pine forest plantations, although this depended on the species and year. In general, the species have been established in places that are within their natural distribution range; however, in some of them such as *P. greggii*, *P. ayacahuite*, *P. caribea* and *P. cembroides* were planted outside this, which could lead to plant adaptation problems.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Revaluation of agri-food waste to obtain bioethanol

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ABSTRACT

Objective: Produce bioethanol from the alcoholic fermentation of agri-food waste.

Design/methodology/approach: food waste was collected for one month and separated into fruit and legumes waste; its size was reduced and then washed with hot acetone. A batch of 100 g of residue underwent acid hydrolysis with 5% H_2SO_4 at 125 °C, the hydrolysate was fermented with *Saccharomyces cerevisiae* at 30 °C for 48 h; the ferment was then distilled at 78 °C. The sugar content was determined following the phenol-sulfuric method. Brix degrees, density and percentage w/w ethanol were measured with a densimeter.

Results: from the fruit residues, an organic fraction was obtained with a total sugar content of 53.3 g/100 g of residue and 9.6 °Brix, generating 45 mL of distillate with 3.8% w/w of bioethanol. From the legumes residues, an organic fraction was obtained with a total sugar content of 19.4 g and 4.140 °Brix, generating 30 mL of distillate with 2.54% w/w of bioethanol.

Study limitations/implications: Rapid decomposition of waste due to bacterial and fungal decomposition complicates long-term storage.

Findings/conclusions: fermentable sugars can be obtained from the evaluated agri-food waste to obtain bioethanol. In this way, they can be integrated into the value chain as raw materials, reducing their accumulation and the environmental impact generated by their final disposal.

Keywords: Agri-food waste, bioethanol, fermentation, waste recovery.

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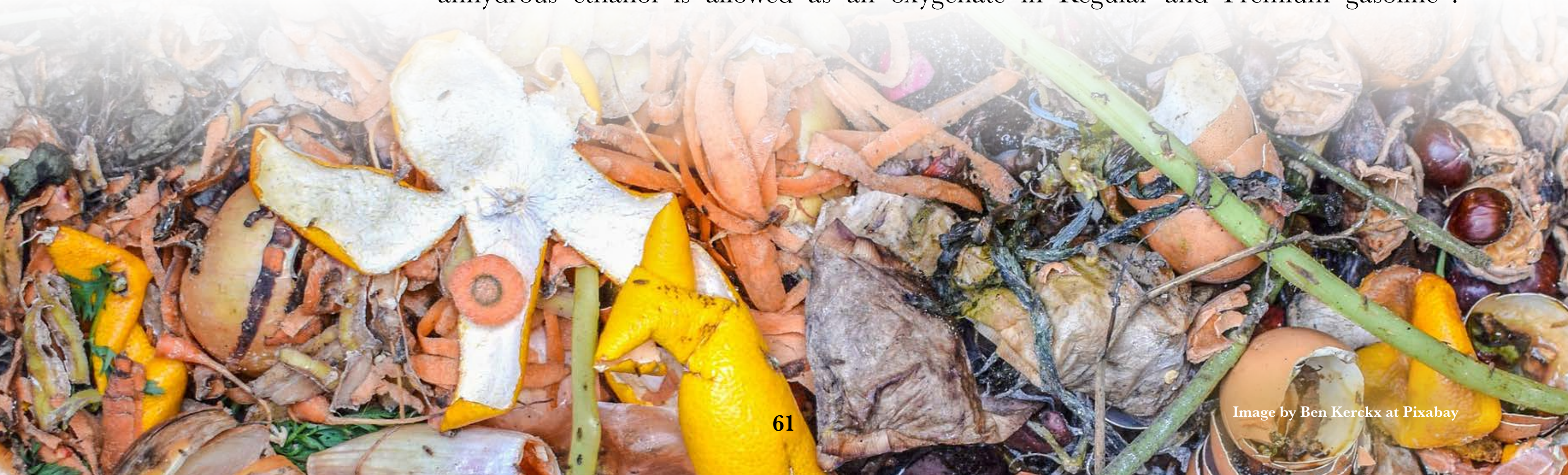
INTRODUCTION

Currently, in several countries of the European Union, as well as in the United States, Argentina, Brazil and Colombia, the use of gasoline with a 5 to 10% bioethanol percentage is authorized, to reduce greenhouse gases emission, a commitment established by the international community in the Kyoto Protocol on climate change. Mexico joined this initiative (Carrillo-Nieves *et al.*, 2019), in the Diario Oficial de la Federación (Official Journal of the Federation) issued the Norma Oficial Mexicana (Mexican Official Standard) NOM-016-CRE-2016, which states that "a maximum content of 5.8% by volume of anhydrous ethanol is allowed as an oxygenate in Regular and Premium gasoline".



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Bioethanol is used both as an antiknock additive and to obtain ETBE (ethyl tertiary butyl ester), a substitute compound for MTBE (methyl tertiary butyl ester), which is a powerful carcinogen (Sanchez & Cardona, 2008). In the immediate future, the bioethanol demand will increase, so raw materials availability will play an important role. The most used crops for bioethanol production are sugar cane, corn, wheat and rice (Zaldivar *et al.*, 2001). Other proposed options are lignocellulosic wastes (Malagón *et al.*, 2017; Rastogi & Shrivastava, 2017) within which are wood, agricultural, agro-industrial and agri-food wastes. These residues are composed of cellulose (35-50%), hemicellulose (15-25%) and lignin (20-25%) with the latter component being the only one that does not contain carbohydrates. Lignin, while providing structural strength to plants, protects them against pathogen and insect invasion (Zhao *et al.*, 2012). For bioethanol production, it is necessary to remove lignin, as it acts as a barrier preventing the penetration of the acid responsible for converting cellulose and hemicellulose into reduced sugars (Alvarez-Castillo, 2012), which, through the fermentation process are converted into ethanol, CO_2 and ATP molecules. Therefore, in the case of lignocellulosic waste, prior treatment is required to hydrolyze the lignin before obtaining bioethanol.

Thus, the process for obtaining bioethanol from lignocellulosic waste consists of four steps: pretreatment, hydrolysis, fermentation and distillation (Aditiya *et al.*, 2016). Although technological improvements are needed to develop from these residues, their use would have the advantage of being accessible, renewable and low-cost. In addition, it contributes to the reduction of waste accumulation in the environment, since there is no management for its final disposal these end up in open dumps. In this sense, Martínez and Montoya (2013) report obtaining 0.5 g of bioethanol per gram of glucose obtained from a mixture of urban solid waste. Other authors such as Malagón *et al.* (2017) evaluated the bioethanol production from waste generated from fruit pulp from lemon, lulo, passion fruit, blackberry and mango production industry, and reported an average yield of 0.8 g bioethanol per g of glucose obtained from each sample and a mixture of them. Considering the accumulation of untreated agro-food waste at the Universidad Popular de la Chontalpa, this research developed a procedure to minimize the environmental impact they generate, using them as raw materials for bioethanol production.

MATERIALS AND METHODS

Agro-food wastes were collected at the Universidad Popular de la Chontalpa. The samples were defined among the collected residues and two materials were established from them: fruit residues, formed by a mixture of fruit peels (banana, papaya, melon, watermelon and pineapple) and legumes residues, formed by a mixture of vegetables (onions, cabbages, potatoes, carrots and lettuce).

Physical and chemical pre-treatment

A 100 g of the biomass to be assessed was weighed and cut into pieces until reduced to a ± 3 cm size. Subsequently, they were immersed in acetone and heated to a boiling point for 10 min in constant agitation. They were then filtered and dried at room temperature (24 °C ± 2).

Bioethanol obtention

Acid hydrolysis: the dry mixtures were placed in glass reagent bottles and a 5% solution of H_2SO_4 was added to them. The bottles were placed in a pressure cooker and heated at 115 °C for 20 min. The resulting juice was then pressed to obtain an organic fraction containing fermentable sugars.

Fermentation: 50 mL of the hydrolysate was fermented with 3 g of the yeast (*Saccharomyces cerevisiae*) at 30 °C for 48 h.

Distillation: the ferment was distilled at 78 °C, separating the head and tail of the distillate.

Quantification of reducing sugars by the Dubois method

A calibration curve was defined using glucose standard solutions (Table 1). For the analysis of the samples, 1 mL of 5% phenol and 3 mL of H_2SO_4 were added to 1 mL of the hydrolysate. The absorbance assessment was performed at 504 nm with a dilution factor of 2 in an Evolution 300 UV-Vis Thermo Scientific spectrophotometer (Dubois *et al.*, 1956).

Calculation to determine total sugars

The following equation was used to calculate the grams of total sugars (Hernández-Galindo, 2017):

$$\frac{g\ sugar}{100g\ dry\ residue} \equiv \frac{Abs * R * F}{1000} * \frac{1\ L}{1000\ mL} * \frac{Vf}{Po} * \frac{100\ g\ \ fresh\ residue}{X\ \ g\ dry\ residue} * 100$$

Where:
Abs: measured absorbance
R: value of the calibration line
F: dilution factor
Vf: final volume of hydrolysate
Po: initial weight of the substrate
X: percentage of real dry residue

Table 1. Standard glucose concentrations.

Tube	H_2O (mL)	glucose (mL)	phenol (mL)	H_2SO_4 (mL)
1	1	0	1	3
2	0.9	0.1	1	3
3	0.8	0.2	1	3
4	0.7	0.3	1	3
5	0.5	0.5	1	3
6	0.4	0.6	1	3
7	0.2	0.8	1	3
8	0.1	0.9	1	3
9	0	1	1	3

Brix determination

4 mL of hydrolyzed juice were injected into a densitymeter model Density Meter Anton Paar DMA 4100 M.

w/w of bioethanol (%)

4 mL of bioethanol were injected into a densitymeter model Anton Paar DMA 4100 M under ISO 5725 OIML-ITS-90.

RESULTS AND DISCUSSION

The obtention of bioethanol began with the recollection of agro-food waste produced at the university cafeterias. Plastic containers with lids were used for this purpose, which indicated not to mix organic waste with other types of waste, such as paper, plastic, aluminum or meat waste. The waste was collected daily and transported to the Sustainable Chemistry Laboratory for treatment.

Table 2 shows the quantification of the waste collected for one month (80.2 kg). This estimate indicates that, at the Universidad Popular de la Chontalpa, an average of 20 kg per week are generated. During a year it is possible that approximately 1 t of agro-food waste could be generated, which, if not treated, is taken to the sanitary landfill in the municipality of Cárdenas, Tabasco, Mexico.

Once the samples were collected, they were grouped into fruit or legumes residues and immediately underwent mechanical and chemical pretreatment with acetone (Figure 2). This pretreatment has two main purposes. First, to hydrolyze the lignins which, as already mentioned, hinders the present reducing sugars released of the biomass. Second, to dehydrate the samples, to avoid their decomposition by bacterial or fungal growth. It is important to note that the solvent used in this step is recovered by filtration and can be reused (after distillation) with another batch of biomass. This minimizes the generation of polluting chemical residues.

The next step consists of hydrolysis of the sample to generate the reducing sugars from cellulose and hemicellulose. Figure 3 shows the assessment of the organic fractions obtained from the waste mixtures by acid hydrolysis. The obtained value was used to calculate the number of total sugars per 100 g of dry residue. A value of 53.3 g from the fruit mixture was obtained, a high value, because in addition to glucose it contains sucrose and fructose; a value of 19.4 g was obtained from the legumes mixture.

Table 2. Total quantification of agri-food waste generated in the university restaurants.

Week	day 1 (kg)	day 2 (kg)	day 3 (kg)	day 4 (kg)	day 5 (kg)	Total quantification of residues (kg)
1	3.3	5.1	4.3	3.5	3.0	19.2
2	5.5	3.0	4.1	3.7	3.8	20.1
3	5.0	4.5	3.4	4.0	3.1	20
4	5.0	4.3	4.5	3.3	3.8	20.9
Total						80.2

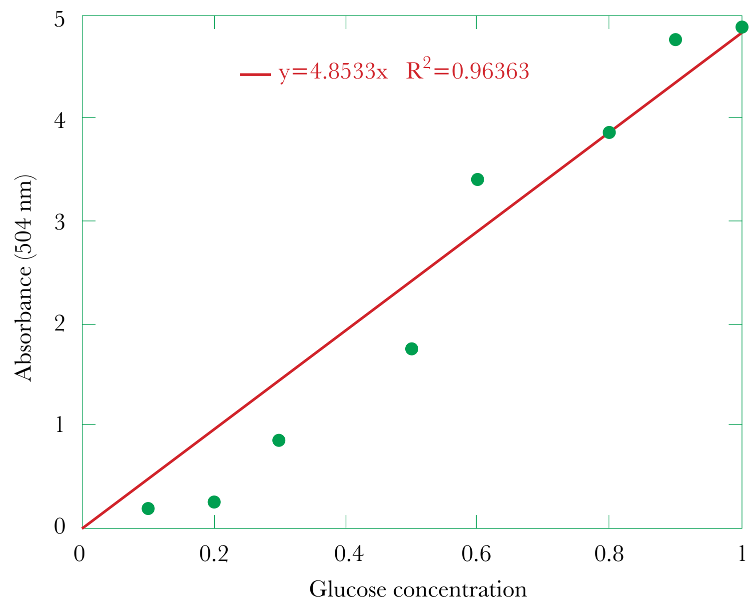

Figure 1. Calibration curve for the glucose concentration determination.

Figure 2. Mechanical and chemical treatment of agri-food waste.

Table 3 shows the characteristics of the obtained organic fractions from each of the mixtures after treatment with acid hydrolysis. The density value for the fruit mixture was 1.03 g/mL and 9.86 °Brix, values close to those reported for mandarin peel hydrolysates 1.15 g/mL and 14 °Brix (Llenque-Díaz *et al.*, 2020). A hydrolysate with a 1.0144 g/mL density and 4.140 °Brix was obtained from the legumes mixture. Brix degrees are the percentage of soluble solids present in some substances. For fruits, the value indicates the amount of present sugar (sucrose), which explains the higher value obtained from the fruit peel mixture compared to the hydrolyzate of the legumes mixture.

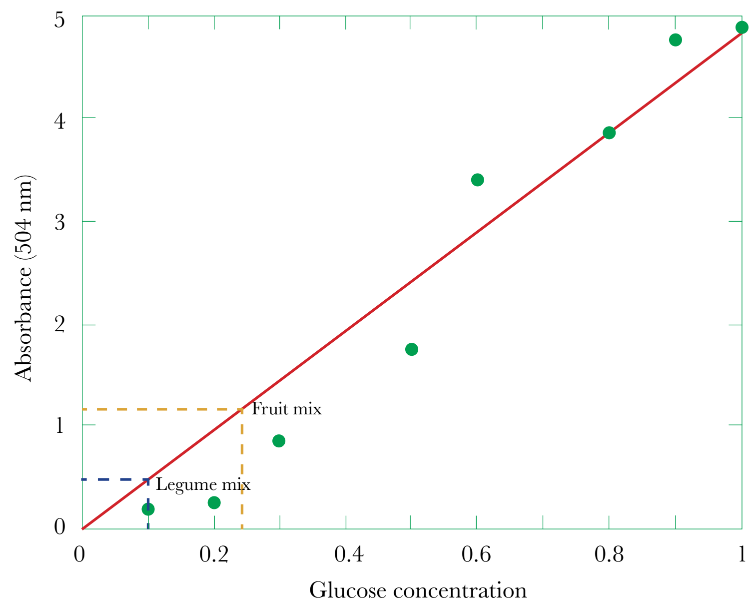

Figure 3. Determination of the amount of glucose in hydrolyzed samples.

Table 3. Characteristics of hydrolyzed organic fractions.

Characteristics	Fruit mix	Legume mix
Density (g/ml)	1.03	1.01
°Brix	9.86	4.14

Table 4 shows the bioethanol yields obtained from the evaluated blends. From the fruit peel blend, a yield of 3.8% w/w of bioethanol was obtained per 45 mL of distillate; while, for the legumes blend, a yield of 2.5% w/w of bioethanol was obtained per 30 mL of distillate under the same test conditions (Figure 4). Values close to those reported by Llenque-Díaz *et al.* (2020), where they obtained 3.8% (v/v) for mandarin peel and 4.2% (v/v) for passion fruit peel per 100 mL of distilled ferment.

Table 4. Characteristics of distillates.

Characteristics	Fruit mix	Legume mix
Ethanol volumen (mL)	45	30
% w/w	3.8	2.5

CONCLUSIONS

Agro-food waste is accessible, low-cost and renewable, and represents a potential source for bioethanol production since it is made up of polysaccharides that can be converted into fuel through a standard chemical process. This study recorded moderate ethanol yield from discarded lignocellulosic material, integrating it into the value chain and contributing to a circular economy of the food industry. In this way, the amount of waste generated at the University, which is generally destined for inadequate final disposal, was reduced.

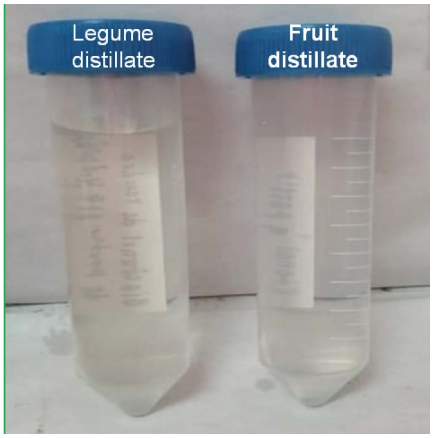

Figure 4. Distillates obtained from the agri-food waste.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Flavonoids quantification in *Acer negundo* L., extracts by HPLC analysis

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ABSTRACT

Objective: The identify and quantify, by high performance liquid chromatography, flavonoids from leaf and stem extracts of *Acer negundo*.

Design/methodology/approach: Ethanolic extracts of *Acer negundo* were analysed with high performance liquid chromatography to quantify and identify their major antioxidant flavonoids.

Results: Leaf extracts had high concentrations of rutin (34.19 μg/mL) and catechin (33.97 μg/mL), intermediate concentrations of apigenin (19.05 μg/mL), gallic acid (19.04 μg/mL), ferulic acid (17.2 μg/mL) and 2.5 dihydroxybenzoic acid (12.72 μg/mL), and low concentrations of caffeic acid (6.15 μg/mL), quercetin-3-β-glucoside (4.97 μg/mL) and isorhamnetin (4.68 μg/mL). In the stem's extracts, the highest concentrations were of ferulic acid (7.96 μg/mL), rutin (5.61 μg/mL) and catechin (4.37 μg/mL); medium concentration were identified for isorhamnetin (3.31 μg/mL) and quercetin-3-β-glucoside (2.01 μg/mL) and apigenin (0.79 μg/mL) was identified at the low concentrations. Gallic acid, caffeic acid or 2,5-dihydroxybenzoic acid were not detected.

Limitations/implications: Some flavonoids have been identified in other *Acer* species but have not been identified and quantified in *Acer negundo*, a Mexican species.

Findings/conclusions: For the first time we report gentisic acid in *Acer negundo* leaf extracts. This analytical method can be standardized to serve as a quality analysis of maple tree products.

Key words: ferulic acid, gentisic acid, flavonoids, HPLC.

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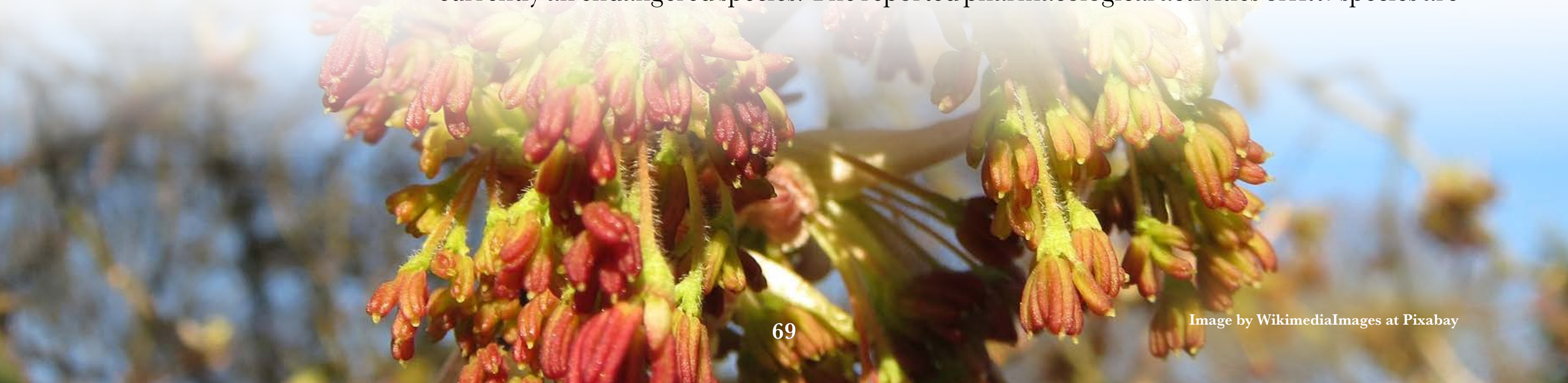
INTRODUCTION

The genus *Acer* belongs to the family Aceraceae and the order Sapindaleae, comprising 180 species (Li *et al.*, 2010). Maples (*Acer* spp.) are important in the northern hemisphere, in regions of East Asia, North America and Europe (Glensk *et al.*, 2009). *Acer negundo* is used in reforestation programs. The sap is a component that acts as a sugar source. It is currently an endangered species. The reported pharmacological activities of *Acer* species are



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antioxidant (Zhang *et al.*, 2014), antitumor (Kim *et al.*, 2015), anti-inflammatory (Ko and Choi, 2015), antibacterial (Maisuria *et al.*, 2015), antifungal, antiviral (Song *et al.*, 2015), antihyperglycemic (Zhang *et al.*, 2015), hepatoprotective (Yoo *et al.*, 2011), antiobesity (Gao *et al.*, 2012) and osteoblastic differentiation stimulator (Ha *et al.*, 2014). There are studies of compounds isolated from *Acer* showing antidepressant, skin-protective, neuroprotective, vasorelaxant, antihypertensive and antimutagenic properties. From the maple trees maple syrup is produced, an inexpensive, highly commercial product containing carbohydrates (glucose, fructose, sucrose and high molecular weight polysaccharides), minerals (Al, Ca, Fe, K, Mg, Mn, Na, and Zn), vitamins (niacin, riboflavin, and thiamine), amino acids (arginine, proline, and threonine) and organic acids (fumaric acid, and malic acid) (Zhang *et al.*, 2014). Many highly bioactive compounds have been extracted from *Acer* species, such as flavonoids, tannins, alkaloids, terpenoids, and phenolic compounds such as lignans, phenolic acids, stilbenes, and coumarins.

The chemical components with the highest bioactivity extracted from the *Acer* genus are benzoic acid derivatives (5.4%), diarylheptanoids (8.8%), simple phenolic compounds (9.7%), terpenoids and phytosterols (9.7%), tannins (12.4%), flavonoids (18.7%), phenylpropanoids (22.4%), among other compounds such as alkaloids and phenylethyl glycosides (Bi *et al.*, 2016). The most characteristic compounds existing in *Acer* species are flavonoids and tannins (Liu *et al.*, 2013). The *Acer* genus also contains important phytochemical compounds such as triterpene saponins (Glensk *et al.*, 2009). Some 331 chemical compounds have been identified from 34 species of the *Acer* genus (Bi *et al.*, 2016). However, most of the compounds from *A. negundo* have not yet been reported.

High Performance Liquid Chromatography (HPLC) is well suited for both qualitative and quantitative monitoring of various trees and has been widely used to evaluate tree and medicinal resources. Therefore, in the present research, HPLC analysis was used to analyze flavonoids from *A. negundo* extracts. There are research reports on the identification of flavonoids in *Acer* species, but do not record their quantification. Therefore, the present work reports the quantification of flavonoids obtained from renewable sources of *A. negundo* trees (leaf and stem extracts) by high performance liquid chromatography analysis, so that they are used as marker compounds for chemical evaluation or standardization of *A. negundo* and its products.

MATERIALS AND METHODS

Standards and reagents. The solvents used for the extraction and high performance liquid chromatography procedures were HPLC and analytical grade, respectively, and obtained from Sigma-Aldrich (St Louis, MO, USA). All stock solutions, standards, samples, solvents, and reagents were filtered through 0.20 μm PTFE membrane filters (Phenomenex, USA) prior to separation or injection into the instrument.

Sample collection. Stem and whole leaves of *A. negundo* were collected during autumn (October-November) 2019 in the vicinity of the city of Morelia, State of Michoacán, Mexico (19° 46''06" N 101° 11''22"' W, 1920 masl).

Obtaining the extracts. Hundred mg samples of dried leaves and 100 mg of dried stems of *A. negundo* were taken by triplicate, these organs are renewable sources so as not to damage the trees. The leaves and stems of *A. negundo* were macerated in a mortar with a pistil. In a 250 mL flask, 100 mL of 80% ethanol were added to each sample, the mouth of the flask was covered with aluminum foil, and each sample was allowed to rest for 24 h. The samples were then filtered on filter paper. Finally, a rotary evaporator (Buchi brand) was used to evaporate the solvent from each extract. The crude extracts were placed in amber bottles for further analysis.

HPLC analysis. The profile of phenolic compounds was determined from the methods modified by Espinosa-Alonso *et al.* (2006) and Valdez-Morales *et al.* (2014), with some modifications. Flavonoids and phenolic compounds were identified and quantified on an automatic injection chromatograph model Ultimate 3000, Dionex brand, equipped with a quaternary pump and a diode array detector. An Acclaim 120, C18 column (4.6 mm × 250 mm, 5 microns, Thermo Sci brand) was used. The mobile phase used consisted of A = acidified water to pH 2.8 with acetic acid and B = acetonitrile, a gradient was used starting with 90% A and 10% B up to 2.5 min, gradually increasing the percentage of B: 12% at 6 min, 23% at 18 min, 35% at 24 min, 95% at 30 min and returning to the initial conditions of 90% A in a final time of 40 min.

The rest of the chromatographic conditions are summarized: flow rate of 0.3 mL/min, injection volume of 10 μL, and the recorded wavelengths were 260, 280, 300, 320, 350, 375 nm. The compounds were identified by comparing their retention times and absorption spectra with the previously run standards and with which the calibration curves were made. The Chromeleon 7.0 software was used for the chromatographic analysis. The concentration values of each phenolic compound were calculated from the area under the signal curve observed in the chromatogram.

Compounds were identified as a function of their retention time and absorption spectra. Identity was only assigned to signals with a purity greater than 980 (1000 being the maximum value). The areas at the wavelength of maximum absorption of each compound were captured, as was the corresponding standard curve used. Flavonoid content was analyzed with a mean comparison test ANOVA ($P<0.05$), in the SAS statistical software (v. 2018).

RESULTS AND DISCUSSION

HPLC analysis. The isolated flavonoids from *A. negundo* extracts and identified by chromatographic analysis are shown in the chromatograms in Figure 1 and 2.

The *Acer* genus is characterized by the biosynthesis of phenolic compounds such as lignans, phenolic acids, stilbenes, coumarins and various flavonoid subclasses. Thirty-one phenolic compounds have been reported for the genus *Acer* (Bi *et al.*, 2016). The phenolic group in flavonoids directly acts by capturing missing electrons from Reactive Oxygen Species (ROS), generating less reactive species. Flavonoids act as buffers, capturing free radicals that generate the less reactive flavin radical, as the missing electrons are delocalized in it. Flavonoids can prevent the cancer occurrence by acting as natural antioxidants that prevent damage to cellular DNA caused by ROS or carcinogens. Flavonoids, both glucosides and glycosides, are also important antioxidant compounds (Wijeratne *et al.*, 2006). Secondary plant compounds, such as flavonoids and other phenylpropanoid derivatives, act as attractants and deterrents to potential insect herbivores (Harborne and Williams, 2000). Phytochemical studies have also been conducted to investigate the phytochemical content in *Acer* species wood. Japanese maple bark has been investigated for its anticancer, anti-inflammatory, antifungal, and antibacterial effects. The bark is used in traditional Japanese medicine to treat liver disorders. Compounds isolated from *A. nikoenses* bark were catechin, rhododendrol, centrolobol, acerogenin A, B, D, K, and M, and acerides I, II, and IV (Li and Seeram *et al.*, 2011).

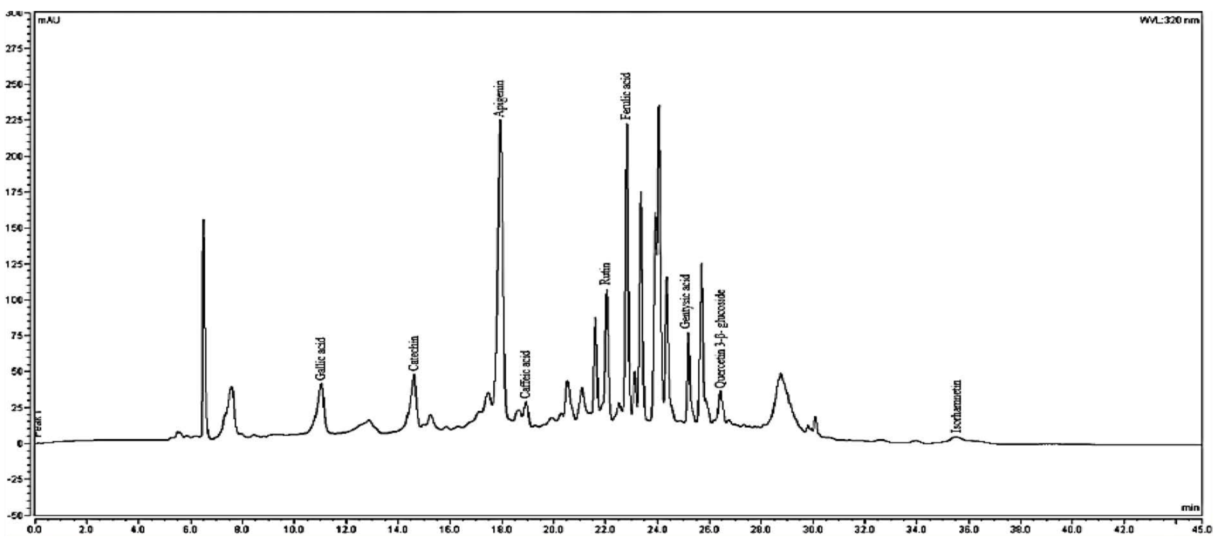

Figure 1. Compound identification by HPLC from leaves of *A. negundo*. Chromatograms of flavonoids, which were obtained from ethanol extract of *A. negundo* by column chromatography. Gallic acid ($C_7H_6O_5$), catechin ($C_{15}H_{14}O_6$), apigenin ($C_{15}H_{10}O_5$), caffeic acid ($C_9H_8O_4$), rutin ($C_{27}H_{30}O_{16}$), ferulic acid ($C_{10}H_{10}O_4$), gentisic acid (2,5-dihydroxybenzoic acid; $C_7H_6O_4$), quercetin 3-β-glucoside ($C_{21}H_{20}O_{12}$), and isorhamnetin ($C_{16}H_{12}O_7$).

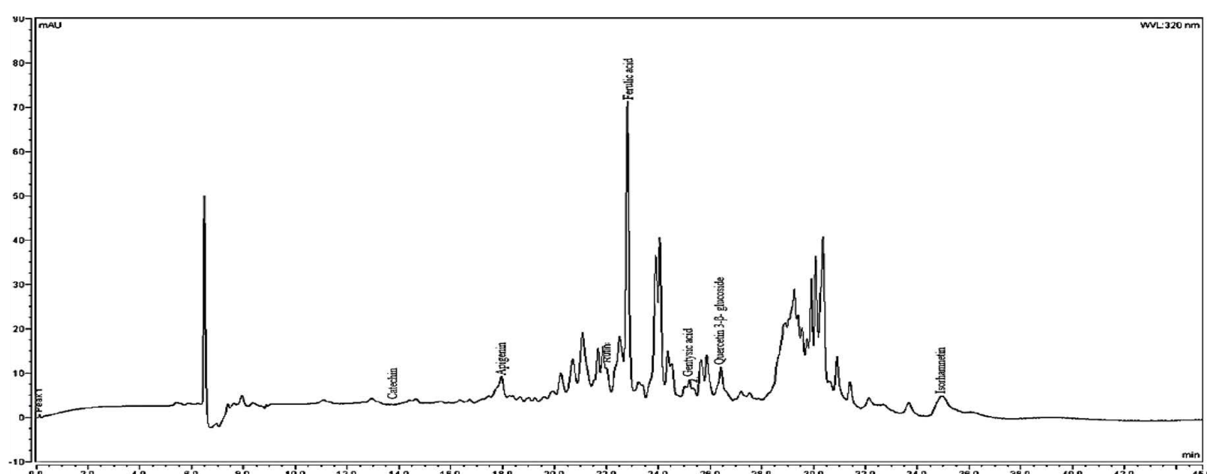

Figure 2. Compound identification by HPLC in *A. negundo* stems. Chromatograms of flavonoids, which were obtained from the ethanol extract of *A. negundo* by column chromatography. Catechin ($C_{15}H_{14}O_6$), apigenin ($C_{15}H_{10}O_5$), rutin ($C_{27}H_{30}O_{16}$), ferulic acid ($C_{10}H_{10}O_4$), quercetin 3-β-glucoside ($C_{21}H_{20}O_{12}$), and isorhamnetin ($C_{16}H_{12}O_7$).

Understanding the differences between the compounds of different species of the *Acer* genus brings us closer to the chemotaxonomic distribution of the compounds. In *A. campestre* wood, cellulose, pentosan/xylan and lignin contents were determined with HPLC (Antczak *et al*. 2013).

1) Gallic acid. Zhang *et al*. (2015) identified gallic acid in leaf extracts of *A. pseudoplatanus* by nuclear magnetic resonance. Gallic acid (tannin) is also present in the stem, leaf and bark of *A. barbinerve*, *A. tataricum*, *A. negundo*, *A. platanoides*, *A. rubrum*, *A. truncatum*, *A. pentapomicum* (Dong *et al*., 2006). In addition, many galloyl-containing flavonoid glycosides have been isolated from the leaves of *A. tataricum* subsp. ginnala (Maxim.) Wesm., *Acer okamotoanum*, *A. rubrum* L. and *A. platanoides* L., and two glycosides exhibited strong inhibitory activity against HIV-1 integrase (Kim *et al*., 1998). Among these flavonoids, anthocyanins have been of great interest because they are responsible for the leaf color change in spring and autumn. Cyanidin-3-(2",3"-digalloyl-β-glucopyranoside) was the first example of a di-acetylated anthocyanin with gallic acid (Fossen and Andersen 1999). In our research we found gallic acid in leaf extracts (19.04 μg/mL) but not in stem extracts.

2) Catechin. Nugroho *et al*. (2015) analyzed three phenolic substances (salidroside, catechin and scopoletin) by HPLC analysis from three methanolic extracts of stem bark, heartwood and leaves of *A. tegmentosum*. The major metabolite produced from the methanolic extract of stem bark was: salidroside: 80.22 mg/g, and in lower concentration catechin: 23.31 mg/g, and scopoletin 9.45 mg/g. Catechin was identified in extracts of stems, stem bark, wood and bark of *A. barbinerve*, *A. mandshuricum*, *A. maximowiczianum*, *A. rubrum*, *A. tegmentosum* (Lee *et al*., 2014). In our research, we found catechin in leaves (33.97 μg/mL) and stems (4.37 μg/mL) of *A. negundo* extracts.

3) Apigenin. In leaf extracts of *A. palmatum* (Aritomi, 1963) and *A. oblongum* (Parveen *et al*., 1988) the flavonoid apigenin was found. In our research, we found apigenin in the extracts of leaves (19.05 μg/mL) and stems (0.79 μg/mL) of *A. negundo*.

4) Caffeic acid. Caffeic acid was identified in dormant shoots of *A. saccharum* (Thakur, 1977). Here, caffeic acid was identified in *A. negundo* leaf extracts (6.15 μg/mL) but not in stem extracts.

5) Rutin. The flavonoid rutin was found in both, leaves and stem bark of *A. tataricum* subsp. ginnala, *A. glabrum*, *A. rubrum* and *A. negundo* (Backheet, 2003). In our study we found rutin in the leaf (34.19 μg/mL) and stem (5.61 μg/mL) extracts of *A. negundo*.

6) Ferulic acid. In dormant shoots of *A. saccharum* they ferulic acid has been identified (Thakur, 1977). In this research we found ferulic acid, a phenylpropanoid, in extracts from the leaves (17.2 μg/mL) and stems (7.96 μg/mL) of *A. negundo*.

7) Gentisic acid (2,5-dihydroxybenzoic acid). As hydroquinone, gentisic acid easily oxidizes and is used as an antioxidant excipient in some pharmaceutical preparations (Kostiuk *et al*., 1988). In this research, ferulic acid was identified in *A. negundo* leaf extracts (12.72 μg/mL) but not present in stem extracts.

8) Quercetin 3-β-glucoside. Quercetin has anti-ulcer properties by protecting the gastric mucosa (de Lira *et al*., 2009). Ma *et al*. (2005) isolated quercetin-3-O-L-rhamnoside from *A. truncatum* by HSCCC (High-Speed Counter-Current Chromatography) type chromatography, the analysis was based on studying an ethyl acetate extract of the leaves.

We obtained 41.9 mg of quercetin-3-O-L-rhamnoside from 366 mg of the crude extract. In our research, we found quercetin-3-β-glucoside in leaf extracts (4.97 μg/mL) and stem extracts (2.01 μg/mL) of *A. negundo*.

9) Isorhamnetin. Isorhamnetin was found in the leaves of *A. glabrum* (Justice *et al.*, 1995). Isorhamnetin-3-O-ruthinoside has also been identified (Backheet, 2003). In our study we identified isorhamnetin in leaf extracts (4.68 μg/mL) and stem extracts (3.31 μg/mL) of *A. negundo*. Glensk *et al.* (2009) identified a triterpene saponin from *A. velutinum* leaf extracts by NMR spectroscopy, 21β-saponin, 22α-O-diangeloylprotoaescigenin, and it exhibited *in vitro* cytotoxic activity against HL-60, B16-F0 and BALB/3T3 cell lines. Also, several authors have analyzed the flavonoid content in maple syrup. Ann (2013) analyzed four grades of maple syrup (extra light, light, medium, and dark) from the 2007 harvest. Twenty-four phenolic compounds were isolated from medium grade syrup and identified by spectral and chemical tests. They were found to have: (a) benzoic acid and several hydroxylated and methoxylated derivatives (gallic acid, 1-O-galloyl-β-d-glucose, and γ-resorcylic acid); (b) cinnamic acid derivatives, coumaric acid, 4-methoxycinnamic acid, caffeic acid, ferulic acid, sinaptic acid, and chlorogenic acid ester); (c) flavonoids, flavanols, catechin and epicatechin, and flavonols of kaempferol and its 3-O-β-d-glucoside, 3-O-β-d-galactoside, quercetin and its 3-O-β-d-glucoside, 3-O-β-L-rhamnoside and 3-O-rhamnoglucoside (rutin). Traces obtained at 280 and 350 nm in the HPLC series of the ethyl acetate soluble fractions of eight samples indicated the presence of several phenolic substances, mostly at very low concentration with some variability in peak heights, but not in retention times, among the syrups.

Authors such as Geoffroy *et al.* (2019) studied hot water extracts of *A. saccharum* bark and shoots, proving that they contain large amounts of phenolic structures that can be used as antioxidant food additives. By performing a replication based on high Performance Liquid Chromatography-High Performance Liquid Chromatography-High Performance Mass Spectrometry (HPLC-DAD-HRMS), it has been showed that hot water extracts of *A. saccharum* bark are rich in simple phenolic compounds and phenylpropanoid derivatives, while the extract of shoots predominantly contains flavonoids, benzoic acids and their complex derivatives, such as condensed and hydrolyzable tannins (Geoffroy *et al.*, 2019).

CONCLUSIONS

Our research revealed that leaf extracts contained a large number of flavonoids compared to stem extracts. *A. negundo* leaf extracts had higher rutin and catechin concentrations; in intermediate concentration were apigenin, gallic acid, ferulic acid, and 2,5-dihydroxybenzoic acid; and in the lowest concentration caffeic acid, quercetin-3-β-glucoside, and isorhamnetin. In *A. negundo* stem extracts there was a higher concentration of ferulic acid, rutin and catechin; in medium concentration were isorhamnetin and quercetin-3-β-glucoside and in the lowest concentration apigenin, but no gallic acid presence, caffeic acid or 2,5-dihydroxybenzoic acid. We report for the first time the presence of gentisic acid in *A. negundo* leaf extracts but not in stem extracts. The method developed to characterize *A. negundo* leaves and stems is rapid and highly sensitive using HPLC. This analytical method can be standardized to serve as a quality analysis for maple products. With the increasing commercial demand for natural products, phenolic profiles of leaf and stem extracts will help promote these Acer negundo derivatives as new sources of bioactive compounds for the food, nutraceutical, and cosmetic industries.

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Potential distribution models of *Sechium tacaco* (Pittier) C. Jeffrey in Costa Rica

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ABSTRACT

Objective: Determine current and potential distribution of *S. tacaco* in Costa Rica with seven Species Distribution Models (SDM), in order to optimize the management of *S. tacaco* genetic resources, aimed at identifying patterns of geographic distribution and possible climatic adaptations allowing to have perspectives on their conservation and genetic breeding.

Design/Methodology/Approach: 21 points of occurrence together with 19 bioclimatic variables and altitude were used to evaluate seven machine learning models and an assembly of these. Open-source libraries running in Rstudio were used.

Results: Distribution models were inferred by the variables bio1, bio2, bio3, bio4, bio12, bio13, bio14, bio18 y bio19. The generalized additive model obtained the highest values of area under the curve (0.96) and True skill statistic (0.90), however, the seven models tested and the assembly showed adequate performance (AUC>0.5 and TSS>0.4). Bioclimatic variables related to temperature were the ones with the greatest contribution to the models and the main limitations in the distribution of *S. tacaco*.

Study limitations/implications: Possibly a greater number of occurrence points are required to evaluate distribution models.

Findings/Conclusions: Areas with high potential distribution suitability for *S. tacaco* are found in central valleys of Costa Rica, covering regions of the provinces of Alajuela, Cartago, San José and Puntarenas. These areas can be sources of germplasm for future conservation and breeding studies.

Key words: SDM, germplasm, conservation, breeding.

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INTRODUCTION

Sechium tacaco (Pittier) C. Jeffrey is an endemic species to the mountainous regions of Costa Rica, where it is locally known as "tacaco" (Wunderlin, 1976). Its possible wild ancestor is also distributed in this country, *Sechium talamancensis* (Wunderlin) C. Jeffrey. Fruits of the tacaco are representative of Costa Rican culture and gastronomy. Through selection, phenotypic variation of the fruits have been achieved based on their weight, equatorial

width, thickness, number of spines and longitudinal sutures. (Monge and Loría, 2017). Generally, tacaco plantations are found in altitudinal ranges of 500-1700 masl. (Wunderlin, 1976; Monge and Loría, 2017) and can reach up to 2000 masl (Lira, 1995). *S. tacaco* is a species underrepresented in germplasm banks and with problems of genetic erosion, due to ignorance of the crop, disturbance of its habitat and introduction of crops (Lira, 1995).

Species distribution models (SDM) are tools that rely on Geographic Information Systems (GIS) and data of real presences to predict areas of suitability for species, this based on their environmental characteristics (Mateo *et al.*, 2011). In general, the methodology used by the SDMs consists of compiling geographic locations of the species of interest; later, spatial data of edaphoclimatic variables are obtained according to the points of occurrence of the species. Spatial data obtained are processed using statistical techniques that can predict suitable areas for the distribution of species. (Hijmans and Elith, 2013). SDMs can be classified into descriptive methods, such as Bioclim, Domain, Mahalanobis distance and Anuclim, which only need presence data to be modeled (Mateo *et al.*, 2011). There are also discriminant techniques such as generalized linear models with their respective variants, for example, generalized additive model and generalized enhanced model; within this group are also automated learning methods such as random trees, maximum entropy and support vector machines, which are used to compute numerical regressions for prediction tasks.

Discriminant techniques based on machine learning are very flexible in terms of computational calculations, they can process a large amount of information and their results are usually more consistent compared to descriptive techniques (Mateo *et al.*, 2011; Hijmans and Elith, 2013; Schmitt *et al.*, 2017). Maximum entropy model (Maxent) is the most applied in species distribution models due to a simple interface in its programming and because it provides adequate results (Phillips *et al.*, 2006). However, it is advisable to optimize the Maxent settings to obtain the best model (Muscarella *et al.*, 2014), and above all to compare it with other algorithms to have multiple perspectives on the species in question.

SDMs can have various applications in agriculture. For example, they are useful for determining the effects of climate change on the distribution of species (Beck, 2012); monitor the presence of pests, invasive species (Lantschner *et al.*, 2018) and pollinating agents (Polce *et al.*, 2013), as well as to detect plant endemisms, threatened habitats, patterns of diversity and conservation studies, among other uses (Mateo *et al.*, 2011). All these applications are vital for the formulation of strategies that seek to maximize plant genetic resources, either to mitigate the damage caused by other species and to prioritize vulnerable areas rich in germplasm (Flores-Tolentino *et al.*, 2019). Regarding genus *Sechium* P. Br., there are few studies on species distribution models, and only *S. edule* y *S. tacaco* are cultivated.

Authors such as González-Santos *et al.* (2017) predicted with Maxent that by year 2050 some varietal complexes of *S. edule* could lose more than half of their current distribution, which is an alarming panorama. In addition, in high-risk areas there are some wild populations that are important to understand the phylogenetic processes of Mexican species of the genus. On the other hand, studies of *S. tacaco* are limited to its morphology (Monge and Loría, 2017) and its phylogeny (Sebastian *et al.*, 2012).

The objective of this study was to determine the current and potential distribution of *S. tacaco* in Costa Rica with seven SDM, in order to guide the management aimed at optimizing geographic distribution patterns and possible climatic adaptations that allow have perspectives on their conservation and genetic breeding.

MATERIALS AND METHODS

Occurrence data and environmental information

For this article, 21 points of occurrence (latitude and longitude) of *S. tacaco* (Figure 1) were obtained from the Global Biodiversity Information Facility database (GBIF, https://www.gbif.org/) and Monge and Loría (2017). It was verified that occurrence points were not atypical and repeated. The 19 bioclimatic variables of WorldClim version 2.1 from period 1970-2000 with spatial resolution ~ 1 km^2 were used (Table 1) (Fick and Hijmans, 2017). Likewise, the altitude raster model with spatial resolution ~ 1 km^2 was used to obtain the elevation data in meters (Fick and Hijmans, 2017); Köppen-Geiger climate classification (Beck *et al.*, 2018) and soil types from Harmonized World Soil Database version 1.2 (Fischer *et al.*, 2008) were also used. The raster values of the 22 environmental layers were obtained with the Point Sampling Tool of QGIS version 3.16.2 (QGIS Development Team, 2020).

Figure 1. Fruits of *Sechium tacaco*, accession 1038-18 of the Germplasm Bank of *S. edule* (BANGESe). Collector: Arévalo-Galarza, M.L. image by Jorge Cadena Iñiguez.

Species Distribution Models (SDMs)

All variables and statistical programs were executed in Rstudio (R Core Team, 2020). For raster data of the 20 environmental variables (19 WorldClim variables and altitude), Pearson correlation was calculated and those variables with correlations ≥0.8 were eliminated, to prevent collinearity and avoid affecting the models (Feng *et al.*, 2019). Climate and soil types variables were used as descriptive information and were omitted in the predictions of the distribution models; in the case of climate type, it is related to some variables such as annual precipitation (bio12) and average annual temperature (bio1); regarding soil types, there is a lack of information regarding its quality; however, by excluding these variables, optimal results can be achieved in SDM (Evans *et al.*, 2010).

Table 1. Bioclimatic variables used for SDMs of *S. tacaco* in Costa Rica.

Variable	Description	Unit
Bio1*	Average annual temperature	ºC
Bio2*	Median diurnal Temperature range	ºC
Bio3*	Isothermality	-
Bio4*	Temperature seasonality	-
Bio5	Maximum temperature of warmest month	ºC
Bio6	Minimum temperature of coldest month	ºC
Bio7	Temperature annual range	ºC
Bio8	Mean temperature of wettest quarter	ºC
Bio9	Mean temperature of driest quarter	ºC
Bio10	Mean temperature of warmest quarter	ºC
Bio11	Mean temperature of coldest quarter	ºC
Bio12*	Annual precipitation	mm
Bio13*	Precipitation of wettest month	mm
Bio14*	Precipitation of driest month	mm
Bio15	Precipitation seasonality	-
Bio16	Precipitation of wettest quarter	mm
Bio17	Precipitation of driest quarter	mm
Bio18*	Precipitation of warmest quarter	mm
Bio19*	Precipitation of coldest quarter	mm
Altitude	Digital elevation model	m

* Variables selected in SDM of *S. tacaco* in Costa Rica.

Machine learning techniques or SDM widely used according to literature were used due to their high performance and optimal results (Mateo *et al.*, 2011), including the following algorithms: Generalized Linear Model (GLM), Generalized Additive Model (GAM), Generalized Power Regression Model (GBM), Classification Tree Analysis (CTA), Maxent, Random Forest (RF) and Vector Machines of Support (SVM). For the execution of these seven models the package SSDM was used (Schmitt *et al.*, 2017) with its default settings and gam dependencies (Wood, 2017), stats (R Core Team, 2020), maxent (Hijmans *et al.*, 2017), rpart (Therneau and Atkinson, 2019), gbm (Greenwell *et al.*, 2020), randomForest (Liaw and Wiener, 2002) and e1071 (Meyer *et al.*, 2019). However, to optimize the Maxent model, the ENMeval package was used (Muscarella *et al.*, 2014) with the following configuration: threshold and hinge functions were deactivated to avoid overfitting the response curves, the quadratic and product functions were also deactivated and the regularization multiplier was 1.25 with a linear function. Evaluation of the models was quantified with area under the curve (AUC), where values >0.5 indicate adequate models; however, to eliminate the spatial classification bias studied by Lobo *et al.* (2007), a point distance sampling was carried out, that is, the difference in distances for presences and absences was calculated in training (75%) and test (25%) data (Hijmans and Elith, 2013). To verify the performance of the models, the kappa and TSS statistics were calculated.

Kappa quantifies the proportion of correctly predicted points after eliminating the probability of random coincidence, its value ranges from −1 to 1; values close to 1 indicate excellent model performance and values close to −1 indicate poor performance. To correct dependence on the prevalence of kappa, the TSS ((Specificity + Sensitivity) −1) was calculated, which optimizes and corrects kappa problems. TSS performance criteria are the same as kappa (Allouche *et al.*, 2006).

Additionally, Jacknife test was carried out to observe the contribution of each environmental variable to SDM, which were calculated together with the statistics AUC, kappa, TSS, sensitivity, specificity, proportion of correct predictions (PCP) and an assembly of the seven algorithms with the same SSDM package (Schmitt *et al.*, 2017). All statistics were averaged from the test results and training data. The raster of the seven models and the assembly were exported with the raster package (Hijmans, 2020) to QGIS Development Team versión 3.16.2 (2020).

RESULTS AND DISCUSSION

Five climate types were found in the environments associated with the points of occurrence of the species: Am (tropical, monsoon, 38.1%), Af (tropical, rainforest, 23.8%), Aw (tropical, savanna, 19%), Cwb (temperate, dry winter, warm summer, 14.3%) and Cfb (temperate, no dry season, warm summer, 4.76%). Soil types found were andosol (38.1%), cambisol (33.3%) and alisol (28.6%). Bioclimatic variables to evaluate SDMs were bio1, bio2, bio3, bio4, bio12, bio13, bio14, bio18 and bio19, which resulted from the correlation analysis. Variables related to temperature showed little variation, for example, bio1 obtained a range of 17.65-21.3 °C, with an average of ~20 °C. For the variables related to precipitation, broader ranges were obtained, for example, for bio12 there were records with a range of 2200-4000 mm of precipitation.

GAM model obtained the highest AUC (0.96) and TSS (0.90). In general, AUC for the seven algorithms was >0.8, thus indicating suitable models. TSS was within optimal performance model range of 0.4>TSS<0.7 (Allouche *et al.*, 2006). The assembly of the seven models was adequate in terms of AUC, kappa and TSS. PPC got values >0.8 and was considered acceptable (Table 2).

Table 2. Statistical parameters for SDMs of *S. tacaco* in Costa Rica.

Model	AUC	PPC	Sensibility	Specificity	kappa	TSS
GLM	0.89	0.88	0.91	0.88	0.22	0.79
GAM	0.96	0.93	0.98	0.92	0.37	0.90
GBM	0.83	0.81	0.83	0.83	0.66	0.66
CTA	0.83	0.83	0.91	0.75	0.66	0.57
RF	0.85	0.83	0.83	0.83	0.66	0.66
Maxent	0.92	0.88	0.83	0.88	0.023	0.71
SVM	0.91	0.92	0.91	0.91	0.83	0.82
Assemble	0.83	0.83	0.83	0.84	0.42	0.67

Generalized Linear Model (GLM), Generalized Additive Model (GAM), Generalized Potentiated Regression Model (GBM), Classification Tree Analysis (CTA), Random Forest (RF), Maximum entropy (Maxent), Support Vector Machines (SVM).

Regarding Jacknife test, the variables with the greatest contribution in the SDMs were bio1, bio2, bio3 and bio14. In the assembly of the seven models, the variable with the greatest contribution was bio1. AAC model assigned the same contribution for all predictor variables (Table 3).

Table 3. Contribution (%) of environmental variables to SDMs of *S. tacaco* in Costa Rica.

Model	Bio1	Bio2	Bio3	Bio4	Bio12	Bio13	Bio14	Bio18	Bio19
GLM	0.22	4.01	8.01	0.1	6.54	12.36	4.76	37.99	26.07
GAM	23.92	0.29	40.19	14.8	1.68	4.47	1.24	1.65	11.73
GBM	26.63	30.42	2.11	18.04	1.66	0.64	0.21	1.05	18.85
CTA	11.11	11.11	11.11	11.11	11.11	11.11	11.11	11.11	11.11
RF	53.74	7.85	6.61	7.9	3.25	5.03	6.85	1.99	6.74
Maxent	16.8	8.4	43.7	3.5	1.6	1.5	0.3	16	8.3
SVM	24.52	3.78	2.79	11.83	2.17	6.87	30.68	13.06	4.24
Assemble	27.48	10.01	10.54	10.10	6.07	8.57	4.72	10.41	12.04

Generalized Linear Model (GLM), Generalized Additive Model (GAM), Generalized Potentiated Regression Model (GBM), Classification Tree Analysis (CTA), Random Forest (RF), Maximum entropy (Maxent), Support Vector Machines (SVM).

GLM and GAM models show large territorial extensions for a suitability of 0.25 (Figure 2A-B). GBM model indicated suitability with probability of 1 for the Central Valleys of Costa Rica (Figure 2C), peculiarly ACC model showed the maximum suitability in a longitudinal axis of Costa Rican territory that covers part of Alajuela, San José, Cartago, and Puntarenas provinces (Figure 2D).

RF, Maxent and SVM models showed suitability of 1 in very similar regions (Figure 3 A-C), however, RF model had a greater territorial extension in Central Valleys and in the South Pacific where Talamanca Mountain is located. The assembly of the seven models (Figure 3D) presented a potential distribution area very similar to that predicted by Maxent model.

Because 80% of the Costa Rican territory has A climate type, it would be expected that the climate type is not a conditional to determine the distribution of *S. tacaco*, considering also that climate is strongly related to the bioclimatic variables bio1 and bio12.

AUC values for training and testing (0.97 and 0.93) determined a good modeling for potential distribution of *S. tacaco*. Values very close to 1 are usually indicative of restricted distribution of species (Phillips *et al.*, 2006), just as it happens for *S. tacaco*. Lira *et al.* (2018) found AUC values higher than 0.95 in wild populations of *S. edule*, which are only distributed in the states of Oaxaca and Veracruz, Mexico. Generally, endemic species tend to have low levels of genetic diversity due to the small size of their populations; however, it is necessary to evaluate them with molecular markers, since in some cases it has been found that these species may have moderate or high levels of genetic diversity (Forrest *et al.*, 2017).

Average annual temperature (bio1) plays an important role in the distribution of *S. tacaco*, although it is a semi-cultivated species, it is difficult to determine the optimal temperature and irrigation requirements as a crop due to the scarce agronomic research.

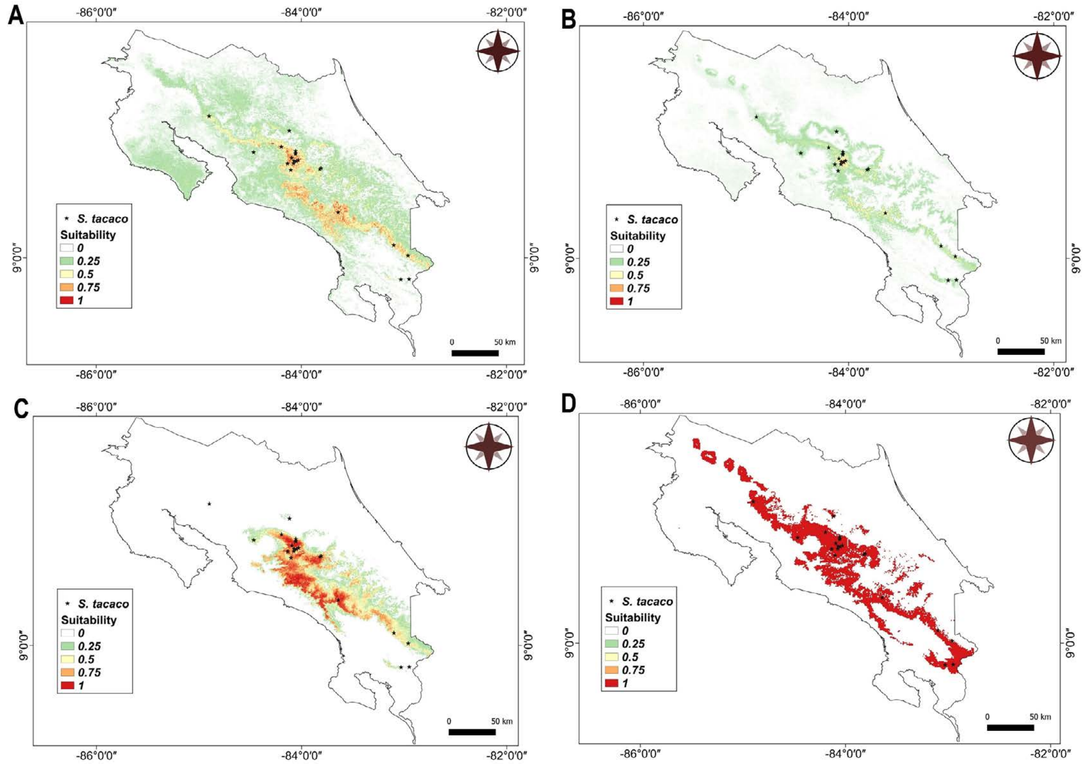

Figure 2. SDMs for *S. tacaco* in Costa Rica. A. Generalized Linear Model (GLM) B. Generalized Additive Model (GAM) C. Generalized Potentiated Regression Model (GBM) D. Classification Tree Analysis (CTA).

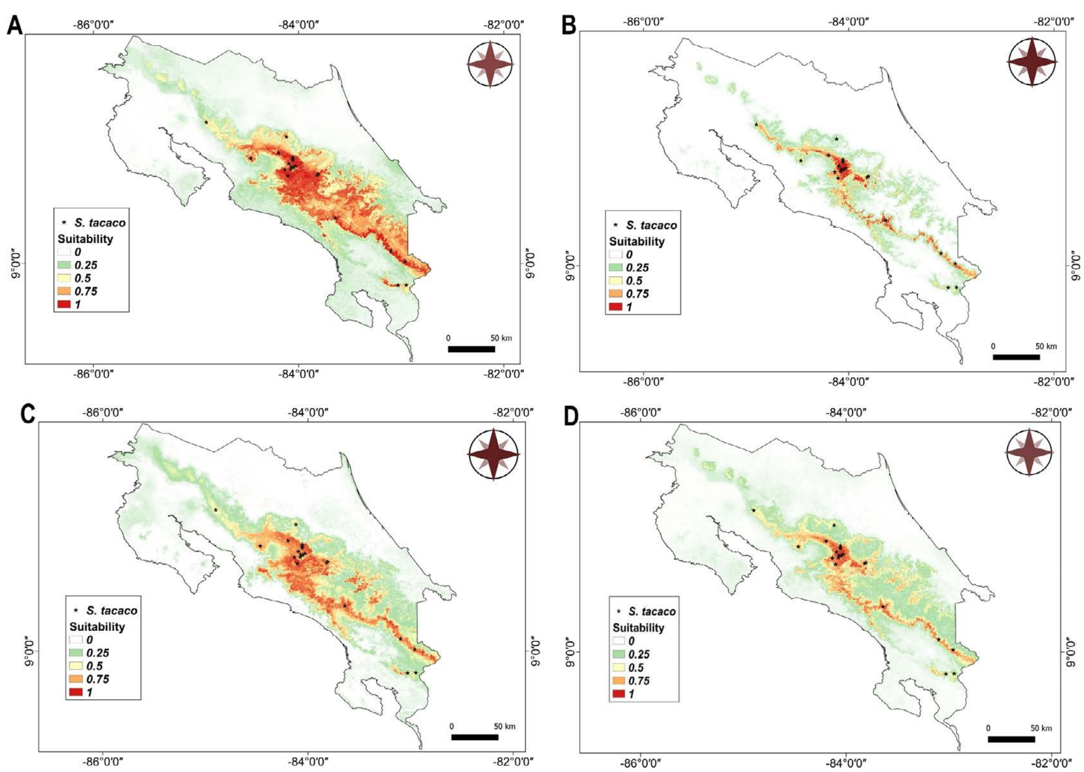

Figure 3. SDMs of *S. tacaco* in Costa Rica. Techniques based on machine learning, A. RF, B. Maxent, C. SVM and D. Assembling of seven models.

Figures 1 and 2, in agreement with Monge and Loría (2017), show a possible trend that *S. tacaco* may be introduced or distributed in Panama, especially with ACC, RF, Maxent y SVM models. This constitutes an important aspect to investigate, agronomic response of S. tacaco in other edaphoclimatic conditions. Andosol and Cambisol soil that predominate in two thirds of the occurrence points, are characterized by being of volcanic origin and having a high cation exchange capacity, which is positively correlated with the amount of organic matter (Dai *et al*., 2018).

Distribution models are predictions of the suitability for species, they provide relevant information on geographic regions that contain germplasm of interest, which is undoubtedly important for conservation and breeding studies. The previous approach is relevant if it is considered that seeds of *Sechium* species are recalcitrant and cannot be conserved in seed banks. Due to its endocarpic nature, the seed germinates even within the fruit, and it does not show signs of senescence, since it is not carotenogenic, that is, it does not change color. In the case of *S. tacaco*, it is relevant to determine real and potential distribution areas for in situ conservation, and for the collection of specimens that contribute to maintaining its diversity.

CONCLUSIONS

Generalized Additive Model (GAM) turned out to have the best area under the curve (AUC) and TSS ((Specificity + Sensitivity) − 1); however, all seven models and the assembly showed adequate performance. Bioclimatic variables related to temperature are the ones with the greatest contribution to models and the main limitations in the distribution of *S. tacaco*, which, being a species with restricted distribution, requires very specific climatic conditions. Areas with high suitability are found in the central valleys of Costa Rica, covering regions of the provinces of Alajuela, Cartago, San José and Puntarenas, which can be sources of germplasm for future conservation and breeding studies.

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AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Obtaining and characterizing bioplastic films obtained from passion fruit (*Passiflora edulis* Sims) waste

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ABSTRACT

Objective: Obtaining films from a vegetable biopolymer from the peel (a by-product) of passion fruit (*Passiflora edulis*) (BPM) and contribute to reduce the environmental pollution generated by the consumption of petroleum-derived plastics.

Design/methodology/approach: By acid hydrolysis at four concentrations of citric acid (0, 1, 2, and 3%), pectin was extracted of passion fruit peels, making a paste mixture with glycerol. The obtained biofilms with an approximate 1 mm thickness were characterized by transformed Fourier infrared spectroscopy (FTIR), X-ray diffraction (XRD) and scanning electron microscopy (SEM) with coupled elemental analyzer (EDS).

Results: XRD diffractograms revealed that passion fruit bioplastic had a semi-crystalline structure and a calculated crystallinity index of 74.6%. Its value reduced by the half as the citric acid increased concentration, the samples with lower concentration with greater flexibility (1%). FTIR analysis suggested alterations in the BMP structures and a decrease of methoxyl groups in the polymeric chains with the increasing in citric acid content.

Limitations/implications: SEM micrographs showed homogeneity in the films, although with some granular irregularities and folding.

Findings/conclusions: The increase in citric acid concentration decreased the degree of gelation in the writing of the obtained biofilms, suggested by EDS and FTIR results, with a consequent reduced flexibility of the GMP films.

Keywords: Passion fruit, bioplastics, agro-industrial waste, physicochemical characterization.

INTRODUCTION

Plastics are among the most widely used commodities in the world. According to recent data, their global plastic production is of about 335 million t yr^{-1}, from which more than 95% are petroleum-based plastic (Xin-Chan *et al.*, 2021; Rivero *et al.*, 2017).

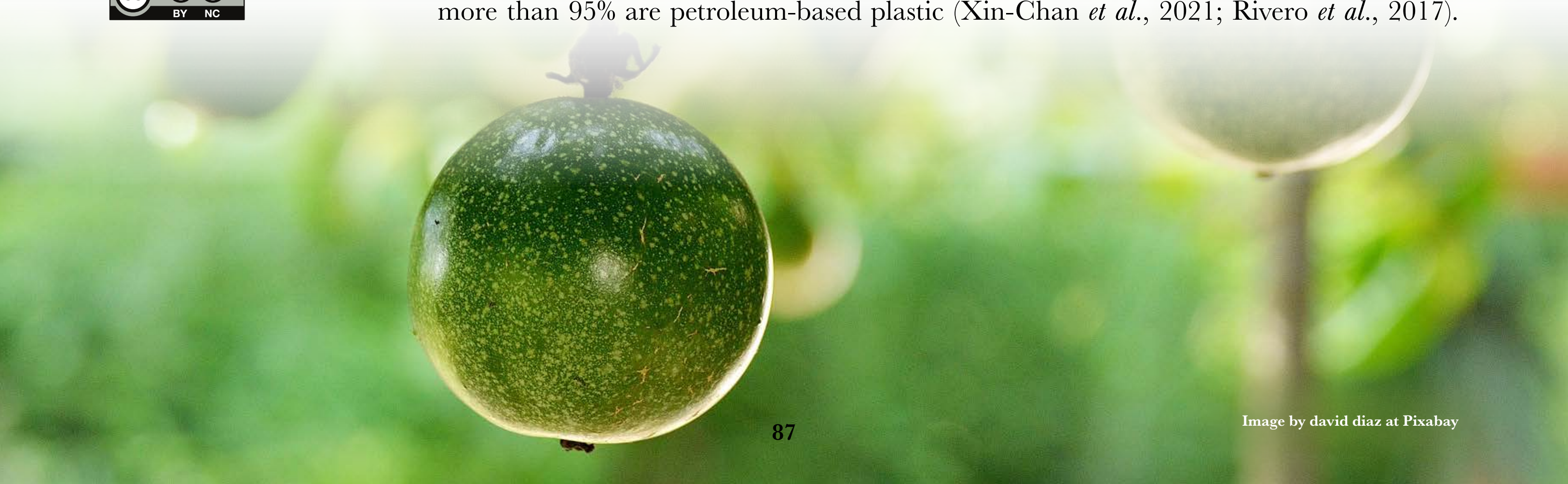

Image by david diaz at Pixabay

For many years, these synthetic materials have been used in a variety of applications in everyday life, such as in food and beverage packaging, automotive, healthcare, electronics, and communication industries (Goncalves *et al.*, 2017). Plastics became indispensable due to their versatility, aesthetic qualities and low cost. However, it is estimated that these constitute 10% of household waste and most of it is dumped in landfills. Plastic waste has several effects on human health and ecosystems. Mismanagement of landfills leads to the release of waste containing chemicals. Its impact on humans and the ecosystem is due to toxic microfragments that easily leach into the surrounding ecosystems (Devasahayam *et al.*, 2019).

The fossil fuels depletion, the energy crisis, the deteriorating environmental conditions and climate change have spurred the development of sustainable technologies derived from renewable sources. In this context, the abundant available biomass represents a potential alternative to manufacture essential products for everyday life. The utilization of biomass such as fiber, cellulose and starch to produce bioplastics and the replacement of fossil materials is a widely accepted strategy to establish a more sustainable society (Karan *et al.*, 2019).

About 3.7×10^{9} t of agricultural residues are annually generated worldwide, which contributes to the problem of waste disposal in landfills, as these are burned, generating greenhouse gases (Grewal *et al.*, 2020). Therefore, valorising these agro-industrial wastes to manufacture new products is essential in the development of a circular economy and combating dependence and reduction of fossil fuels.

The maracuya (*Passiflora edulis* f. flavicarpa) (*Passifloraceae*) is a native species of the American tropics. There are more than 400 species of *Passiflora*, 50 of which are edible and two are commercially cultivated. Maracuya or passion fruit as *P. edulis* is known, is one of the most appreciated exotic fruits in the international markets, mainly for its organoleptic and nutritional properties and the large amount of pectin it contains. Pectin is an additive with no limitations or reserves of use and is a main hydrocolloid in food processing, which modifies the texture of compotes, jams and jellies. Due to its characteristics, passion fruit is a very versatile fruit, used in the cosmetic industry, confectionery, non-alcoholic beverages and particularly in the gastronomic sector (Arias-Domínguez *et al.*, 2019).

For Colombia, passion fruit cultivation is important for its economy, because it is a source of income and employment in the region. In 2020, 19,853 ha were cultivated, with a production of 200,920 t, and a yield of 13.30 t ha^{-1} (DCAF, 2020). In the department of Meta, where the Agroindustrial Center (SENA-Hachón) is located, the producer municipalities Vista Hermosa, Mesetas, Granada, Fuente de Oro and Puerto Lleras are founded, which cultivate 1,320 ha, with an average yield of 15 t ha^{-1}, harvesting approximately 19,800 t, of which only 35% is used (pulp and seeds); the remaining 65% are harvest residues, so in 2020 there were 12,870 t of residues, available for use. Therefore, this research focuses in obtaining films of bioplastic from passion fruit residues (peel), manufactured with citric acid at different concentrations, by the analysis of results acquired with XRD (X-ray diffraction), FTIR (Fourier infrared spectroscopy) and SEM-EDS (scanning electron microscopy - coupled elemental analyser) methods to characterize their crystallinity, functional groups and intermolecular interactions in their structure and morphology.

MATERIALS AND METHODS

The passion fruit residues (peels) were supplied by the Department of Meta, Villavicencio, Colombia and processed at the Meta Agroindustrial Center (SENA-Hachón). In the pectin extraction stage, the following procedure was carried out (Figure 1): (1) the peels were washed with tap water, with the purpose of eliminating contaminant remains present in the raw material, (2) extraction of the pectin by the method of acid hydrolysis, with a citric acid solution ($C_6H_8O_7$) at concentrations of 0 (without additives), 1, 2, and 3% (BPM0, BPM1, BPM2, BPM3, respectively), (3) the peels were immersed in the citric acid solutions for 90 min, at 90 °C and removed from the water, (4) once cooled, the mesocarp was separated with the aid of a spatula. The mesocarp obtained was ground in a stainless steel industrial blender to form a homogeneous paste, which was passed through a sieve with a mesh size of 250 μm to ensure its homogeneity.

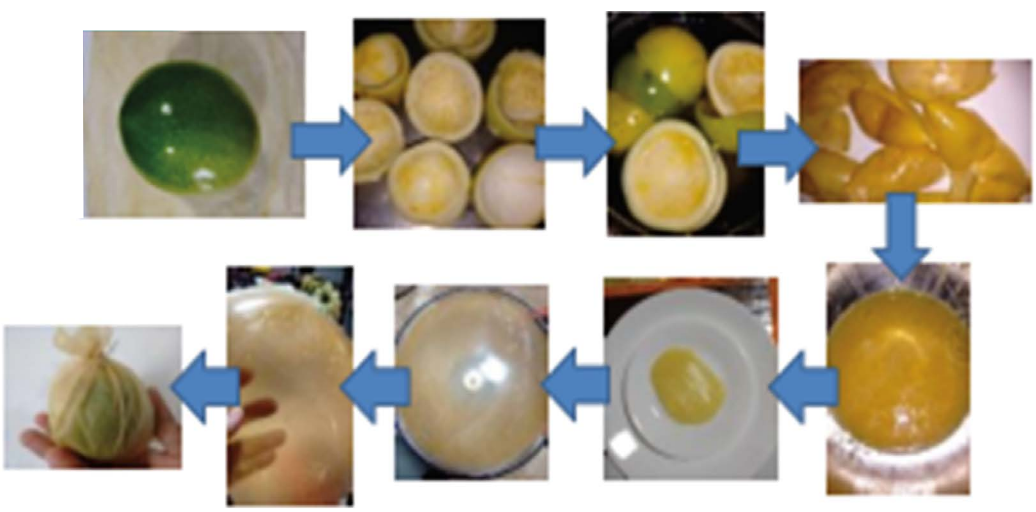

Figure 1. Process of the extraction of the mesocarp from passion fruit residues and its hydrolysis (Henao, 2016).

The elaboration of the passion fruit bioplastic films (BPM) was carried out by the following procedure: the paste obtained from the mesocarp was mixed with glycerol at a concentration of 5%, used as a plasticizer; a portion of the mixture was poured onto smooth expanded polystyrene plates covered with a non-stick plastic film (film), spreading the mixture with the help of spatulas to form thin films. The bottom of the plates were then gently tapped to break air bubbles formed during the pouring or the spreading of the biofilms and were placed in a convection drying oven (ECOSHELL brand) at a temperature of 60 °C for 24 h. Once the drying process was completed, the films were carefully peeled off, avoiding breakage, and were stored in hermetically sealed bags to avoid environmental humidity until their subsequent use.

Characterization methods

Infrared spectroscopy (FTIR)

The determination of the functional groups of the passion fruit bioplastic samples was carried out using the Fourier Transform infrared spectroscopy technique with a Nicolet Magna Protegé 460 FTIR infrared spectrophotometer in the absorbance mode, with a resolution of 4 cm^{-1} and 100 scans. The 1 mg samples were mixed in 100 mg KBr.

Scanning Electron Microscopy (SEM-EDS)

The morphology and elemental composition of the bioplastic were determined by scanning electron microscopy (SEM) with an integrated elemental analyzer (EDS), Bruker D8 Advance. The samples were coated with a gold deposit (thickness less than one micron).

X-ray Diffraction (XRD)

The crystallinity of passion fruit mesocarp was determined by X-ray diffraction spectra, powder method (PXRD), using a Siemens D 5000 Diffractometer equipment, CuKα'spectrum (α=1.5418 Å and energy 8.047 keV).

The percent crystallinity was calculated by the method of Segal *et al.* (1959) Eq. (1):

$$X_c\% = 100\left[1-\left(\frac{I_1}{I_2}\right)\right] \quad \text{Eq. (1)}$$

where: I_1 is the intensity of the minimum peak and I_2 is the maximum intensity of the crystalline peak, respectively.

RESULTS AND DISCUSSION

Fourier Transform Infrared Spectroscopy (FTIR)

Fourier infrared spectroscopy was performed to characterize the molecular interactions in the passion fruit bioplastic (PMB) film. The interferograms of the PMB films, manufactured with different concentrations of citric acid (0, 1, 2 and 3%) are shown in Figure 2 a, b, c and d. The broad absorption area between 3600 and 3000 cm^{-1} was assigned to O—H stretching vibrations, due to inter- and intramolecular hydrogen bonds, characteristic of the pectin structure (Nizar *et al.*, 2019). The absorption band around 2925 cm^{-1} corresponds to the stretching of C—H bonds, involved in CH, CH_2 and CH_3 groups of stretching and bending vibrations (Lorevice *et al.*, 2016).

The bands around 1616 cm^{-1} and 1734 cm^{-1}, corresponding of carbonyl groups (COOH) and acetyl groups ($COOCH_3$) of pectin, confirm the high degree of esterification and the presence of high methoxyl pectin in the passion fruit mesocarp. The free and esterified carboxyl groups are useful for the identification of high and low methoxyl pectins (Manrique, 2002). The pronounced elongation at 1616 cm^{-1} corresponds to the COO— carboxylate ion symmetric strain vibration band. The peaks at 1318′1517 cm^{-1} are attributed to the C—O—H bond stress vibration band. On the other hand, the peaks near 1260 cm^{-1} belong to the asymmetric strain vibration band of the C—O—C bond, and indicate the abundance of methoxyl groups (—O—CH_3).

The strong peak at 1061 cm^{-1} suggests the strain vibration band of the symmetric C—O—C group, which also confirms the high degree of esterification and the presence of high methoxyl pectin (Chasquibol *et al.*, 2008). The interferogram corresponding to the BPM1 sample (bioplastic with 1% citric acid, Figure 2b) shows a similar behavior to that of the film in the absence of citric acid (Fig. 2a).

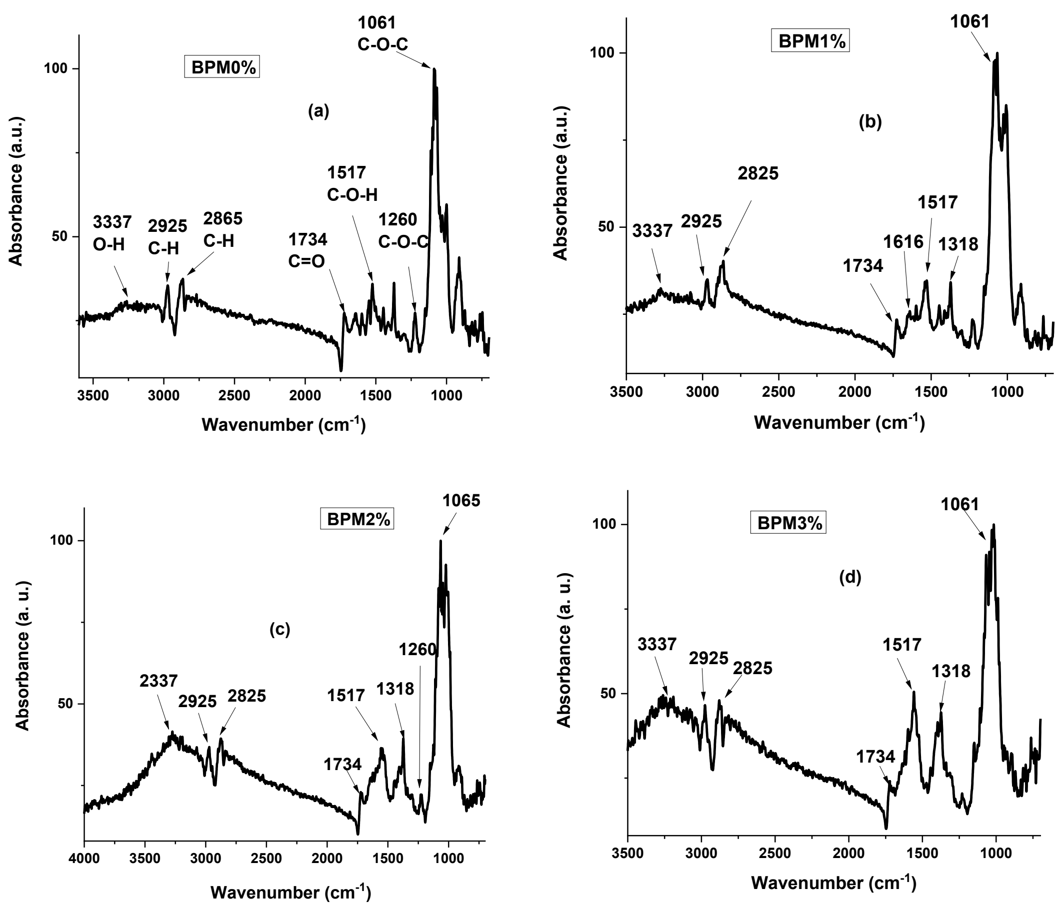

Figure 2. FTIR spectra of passion fruit bioplastic (BMP) films prepared with different concentrations (%) of citric acid: a) BPM0, b) BPM1, c) BPM2 and d) BMP3, e) BPM3, f) BPM3, g) BPM3, h) BPM3 and i) BPM3.

The interferograms corresponding to 2 and 3% citric acid (Figure 2c and 2d) were very similar to each other. However, it should be noted that increased acidity (lower pH, 2 and 3% citric acid) affected the methoxyl content of the extracted pectin, the corresponding band at 1734 cm^{-1} (Figure 2c and 2d). The absence of a pronounced elongation in this band, corresponding to the esterified carboxylic groups, places this pectin as having a low methoxyl content (Vázquez *et al.*, 2008). Pectin forms colloids par excellence (Willats *et al.*, 2006), so it has the property of absorbing large amounts of water. These colloids belong to the family of oligosaccharides and polysaccharides of high molecular mass and contain long chains of 1,4-α-D-galacturonic acid (GalpA) units. According to one report (Willats *et al.*, 2006), it has been possible to separate and characterize three pectic polysaccharides (homogalacturonan, rhamno galacturonan-I and substituted galacturonans) and all contain GalpA acid in a greater or lesser amount.

Scanning electron microscopy with integrated elemental analyzer (SEM-EDS)

The microstructure of the passion fruit bioplastic film is influenced by the inter-spatial organization of its components, as well as the way they have interacted during the drying process. Figure 3a shows that the films of BPM0 bioplastic (prepared without citric acid) showed a smooth microstructure in general, compact and continuous without pores, although with some irregularities such as folding and granules, compared to the surface of BPM2 (Figure 3b). Elemental analysis (EDS) (Table 1) suggests that the main elements are C and O, in the presence of traces of other elements (N, Mg, P, Cl, K and Ca), part of the chemical composition of passion fruit peel (León and Riveros, 2014).

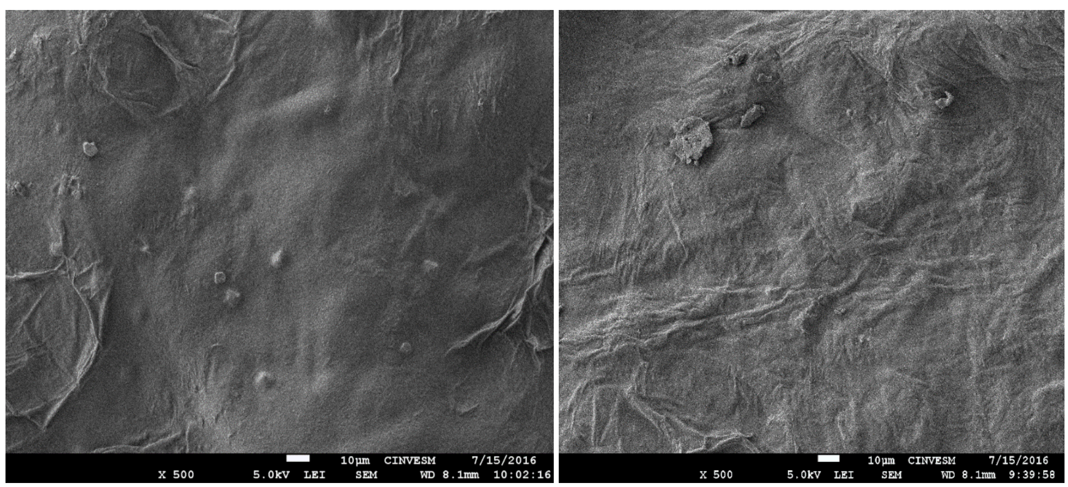

Figure 3. SEM micrographs of passion fruit bioplastic BMP films: a) BPM0 (0% citric acid) and b) BPM2 (2% citric acid).

The high percentage of calcium compared to the other BPM0% minerals (Table 1) is due to the clearly structural role of its metal ion for maintaining the integrity of the membranes of the middle lamella and cell walls, binding to the free carboxyl groups of the uronic acid of pectin in the form of pectates (Simmonds, 1980). The calcium ion induces crosslinks involved in the cell adhesion and tissue texture (Fisher & Bennet, 1991).

Table 1. Elemental analysis (EDS) of passion fruit bioplastic films BMP0 and elaborated with 2 % citric acid (BPM2), SEM.

Samples	Element	C	O	N	Mg	P	Cl	K	Ca	Total
BPM0%	Mass (%)	52.3	39.34	3.63	0.35	0.95	0.43	2.54	0.38	100.00
BPM2%	Mass (%)	44.73	51.91		0.19	0.29	0.43	2.26	0.20	100.00

Note* The EDS analysis of BPM3 is not presented because it shows similar results to BMP2.

When a higher concentration of citric acid was used in the elaboration of the BPM films, the microstructure was slightly altered (Figure 3b) and caused an irregular configuration.

This fact is possibly due to the decrease of esterified carboxylic groups, transforming into a low methoxyl pectin, as can be confirmed by FTIR of BPM2% and BPM3% (Figure 2c and 2d). It has been reported that the gelation power depends also on the concentration of calcium ions that influence the texture of the formed gelatin (Devia, 2003). The ability of calcium to form complexes with pectin is associated with the free carboxyls in the pectin chains. It has been considered that there is an increase in gel formation as the degree of esterification decreases (Anyas and Deuel, 1950). This is corroborated by FTIR analysis (Fig. 2c and 2d), by the absence of a pronounced elongation in the esterified carboxyl groups (band 1750 cm^{-1}). EDS elemental analysis of BMP2% citric acid (Table 1) reveals that the calcium concentration decreased by the half compared to BMP (prepared in the absence of citric acid, Table 1).

Powder X-ray diffraction (XRD)

XRD diffractograms (Figure 4) were analyzed to characterize the crystal structure of the BMP bioplastic. Peaks at 2=12.7, 16.3, 18.40, 25.3 and 40.1° are related to pure pectin (Nizar *et al.*, 2019). A broad peak at 16.3°, as well as a peak of higher intensity at 25.3°, confirmed the presence of some crystalline structure (Figure 4a). In Figure 4b, it is observed that the higher the amount of citric acid (2%), the intensities of the peaks decrease, indicating lower crystallinity index of the film as prepared, which collaborates with the calculated values of crystallinity (Table 2). The results indicate that when 1% of citric acid was introduced, the crystallinity index decreased significantly from 74.6% to 41.8%. For this reason, as the concentration of citric acid increases, the biofilms become more flexible.

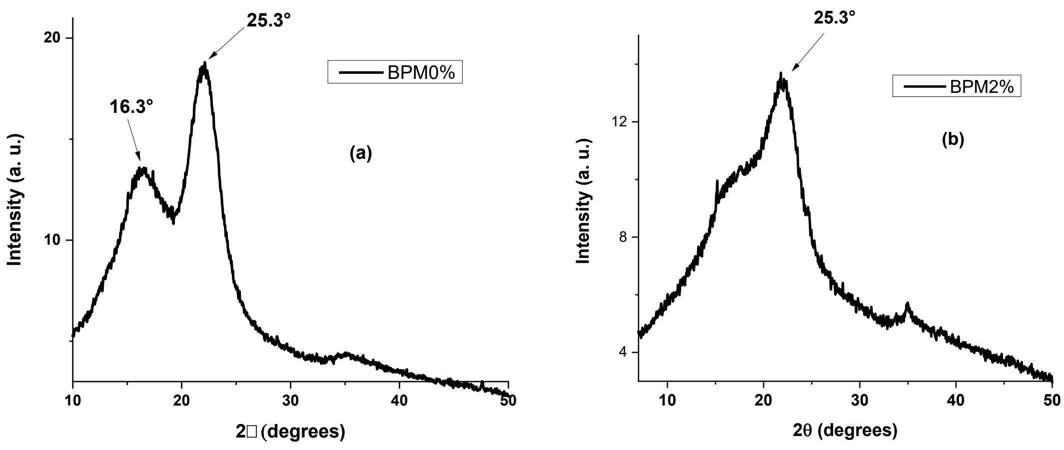

Figure 4. Diffractograms of passion fruit based bioplastic (BMP): a) BPM0% and b) BPM2% citric acid.

In gel formation with high methoxyl pectins, it is reported that at pH 3.0 ≈ 90% of the peptidic acid groups are in undissociated form and are therefore able to form hydrogen bonds with acid or hydroxyl groups of adjacent chains. These binding sites can be considered as "crystalline", whereas the parts of the molecule that do not exhibit cross-linked bonds are in solution (Ferreira, 2007).

Table 2. Crystallinity index of passion fruit BMP bioplastic at different citric acid concentrations.

Citric acid (%)	Cristalinity (%)
0	74.6
1	41.8
2	38.4
3	36.5

CONCLUSIONS

Films of a bioplastic (BPM), obtained from the passion fruit mesocarp, have been elaborated with different concentrations of citric acid (up to 3%). SEM analysis revealed that, as the citric acid content increases, the surface becomes more irregular, granular and folded. The crystallinity index of MPA (74.6%) decreased ≈ 2 times with increasing citric acid and BPM3 had 36.5% crystallinity. This change led to a decrease in gelation and the films resulted in more flexibility. Thus, the BPM3 bioplastic resulted with a better consistency, greater flexibility, due to its less crystalline structure. The results obtained are well corroborated by FTIR analysis, where the absence of a pronounced elongation in the esterified carboxylic groups was observed (band 1750 cm^{-1}).

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Pregnancy Rate in Ewes Injected with Zinc Oxide during an Estrus Synchronization Protocol

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ABSTRACT

Objective: To measure the effect of zinc injection, during an estrus synchronization protocol, on pregnancy rate in sheep from Valle de Mexicali.

Design/Methodology/Approach: The experimental units were 157 ewes, which were allocated in five farms (F): F1 (n=19), F2 (n=27), F3 (n=20), F4 (n=71), and F5 (n=21). In each farm, the ewes were randomly assigned to one of three treatments: control, z-100 and z-200. The ewes from control groups were subcutaneously injected with 4 mL of olive oil as placebo. The ewes from groups z-100 and z-200 were subcutaneously injected with 100 and 200 mg of zinc oxide. The response variables were the preovulatory diameter of the largest follicle and pregnancy rate.

Results: The differences between experimental groups in diameter of the largest preovulatory follicle and pregnancy rates were not significant ($p>0.05$).

Study Limitations/Implications: The ewe's reproductive response to zinc injection might be affected by the animal mineral status, it is recommended to carry on supplementation based on mineral blood concentrations.

Findings/Conclusions: The subcutaneous injection with 100 or 200 mg of zinc oxide did not affect the size of the largest preovulatory follicle and pregnancy rate in ewes.

Keywords: Minerals, reproduction, sheep.

INTRODUCTION

The increasing world population and demand for foods of animal origin (Turk, 2016) urbanization and rising incomes are driving greater demand for animal source food in most parts of the world but especially in developing countries. The United Nations Food and Agriculture Organization (UN-FAO makes it necessary to apply strategies in

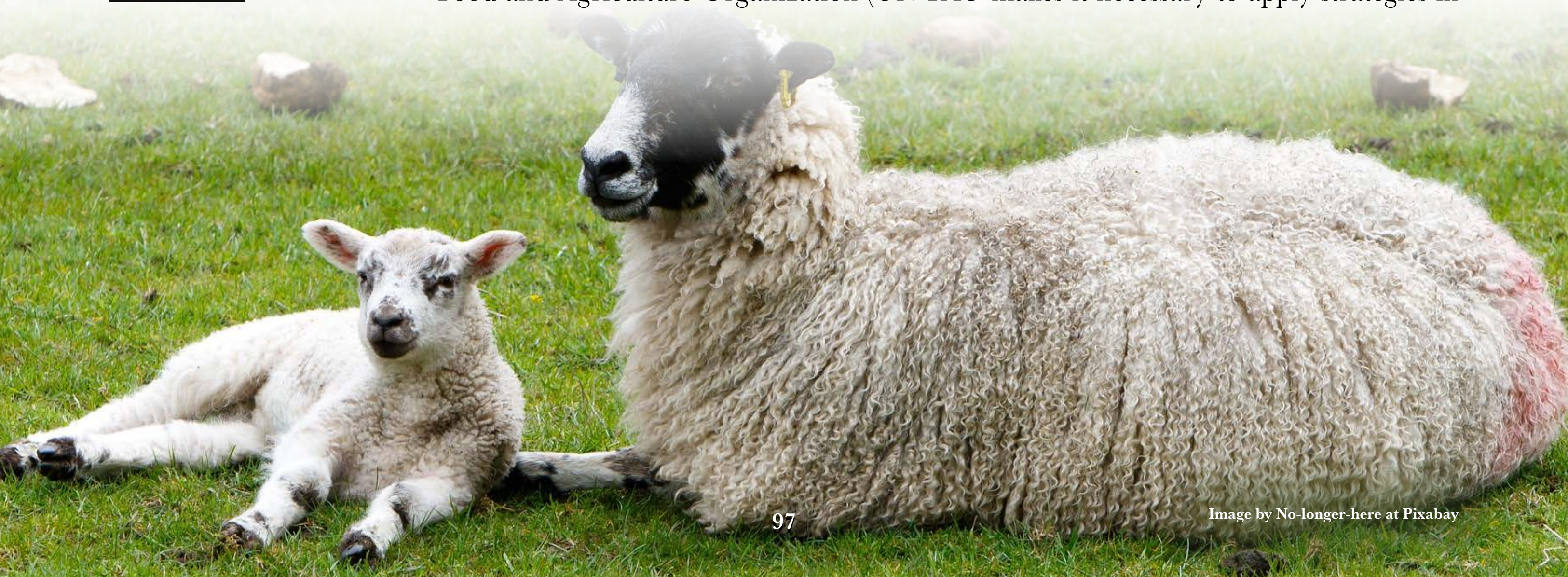

Image by No-longer-here at Pixabay

livestock production systems to improve their productivity. In Mexico, sheep production systems are diversified, regarding their technological advance and the breeds used to produce mutton (Partida de la Peña *et al.*, 2017). There are regions such as Valle de Mexicali where sheep production is considered as a subsistence activity, with a low degree of technification (Martínez-Partida *et al.*, 2011). Therefore, there is a need to manage and promote the development of this activity through research studies.

The profitability of sheep production systems can be improved by applying reproductive biotechnologies and nutritional strategies (Gifford & Gifford, 2013)such as supplying specific nutrients during the occurrence of relevant reproductive events (Delgadillo & Martin, 2015). The list of these nutrients includes zinc and other trace minerals, which are essential for a variety of physiological functions in the animal (López-Alonso, 2012).

Zinc is an essential mineral to sustain life and one of the most abundant trace minerals inside the animal's organism, but its endogenous storage is low and it must be provided to animals by feed or mineral supplementation (Mir *et al.*, 2020; Swain *et al.*, 2016). In the organism, zinc participates as enzymatic cofactor, regulates cell growth, immunity and reproductive function in mammals (Hill & Shannon, 2019; Nasiadek *et al.*, 2020) whereas zinc body deficiency causes low productive (Masters *et al.*, 1985) and reproductive performance (Tian & Diaz, 2012; Tian *et al.*, 2014). Zinc can be administrated to animals orally or by injection. The oral administration of zinc has been effective in improving the reproductive performance in ewes (Monem & El-Shahat, 2011) and goats (Kundu *et al.*, 2014), while a similar effect of zinc injection has been observed in cows (Anchordoquy *et al.*, 2019). The latter is considered as more effective in increasing endogenous zinc concentrations in sheep (Lamand *et al.*, 1983). However, we are unaware of the effects of zinc injection on reproductive variables in sheep.

Therefore, the objective of this study was to measure reproductive variables in ewes injected with zinc, during an estrus synchronization protocol, in Valle de Mexicali.

MATERIALS AND METHODS

Location

The study was undertaken in five sheep farms located in Valle de Mexicali during the increasing photoperiod season (March-April). The climate in the region is dry arid (BWh), the average rainfall is 85 mm and the temperature range throughout the year is 0 to 52 °C (Garcia, 1988). The animals were managed following the Canadian Council on Animal Care guidelines (CCAC, 2009).

Animals and experimental design

The experimental units were 157 crossbred ewes (Dorper×Pelibuer×Katahdin) with at least one lambing and non-lactating. The ewes were distributed in five different sheep farms (F): F1 (n=19), F2 (n=27), F3 (n=20), F4 (n=71), and F5 (n=21). In each farm, the ewes were randomly assigned to one of three treatments: Control (0 mg of zinc oxide), z-100 (100 mg) and z-200 (200 mg). The zinc oxide (Zinc Óxido, Jalmek) was dissolved in 4 mL of olive oil and subcutaneously injected to ewes in z-100 and z-200 treatments on day nine of the estrus synchronization protocol (day 0 is the day of intravaginal device (CIDR®

ovis, Zoetis) insertion). The number of ewes assigned to each treatment within each farm was as follows: Control: 6, 9, 7, 24 and 7; z-100: 6, 9, 6, 24 and 7; z-200: 7, 9, 6, 23 and 7; for F1 to F5, respectively.

Animal nutrition

The ewes in F1 were fed with 2 kg day^{-1} of a total mixed ratio (48% alfalfa, 28.84% Sudan silage and 23.07% oat straw as feed). The ewes in F2 to F4 were fed *ad libitum* with mixed forage hay (*Cynodon dactylon*, *Echinochloa colona*, *Echinochloa crus-galli*, *Sorghum halepense* and *Medicago sativa*). The ewes in F5 had free access to *Lolium multiflorum* Lam pastures during the entire experimental period. The ewes in the five farms did not receive any mineral supplementation before, during or after the experimental period.

Reproductive management

The estrus cycle of the ewes was synchronized by intravaginal CIDR insertion for 12 days. The ewes were injected with 350 IU of equine chorionic gonadotropin (eCG, Novormon® 5000, Virbac) at day 10 of the estrus synchronization protocol. The intravaginal device was removed at day 12 and the ewes were injected with 12.5 mg of dinoprost (Lutayse®, Zoetis). The ewes were inseminated 28-30 h after CIDR removal by cervical insemination method. The semen was provided by the same ram at all times. The semen was collected by artificial vagina just before the artificial insemination; its concentration was determined by Neubauer chamber and diluted with a commerncial medium (Triladyl®) to the desired concentration (300×10^6 sperm cells mL^{-1}). The diluted semen was placed in a plastic tube surrounded by ice during artificial insemination.

Response variables

The response variables to the zinc oxide injection were the diameter of the largest preovulatory follicle before insemination and the pregnancy rate. The diameter of the largest preovulatory follicle was measured in five ewes from each experimental group in every farm by transrectal ultrasonography (Handscan V8, Sunway Medical). The two ovaries were observed and the position of the largest preovulatory follicle was recorded. The follicle diameter was calculated by the average of the horizontal and vertical measurements of the largest follicle. The pregnancy diagnose was carried out by transrectal ultrasonography between 30-35 days after artificial insemination.

Statistical analysis

The residual normality for the diameter of the largest preovulatory follicle variable was tested by the Saphiro-Wilk test. The data from this variable was analyzed using ANOVA and considering the farms as blocks. The means comparison was carried out by Tukey's test. The pregnancy rate was analyzed by Fisher's exact test. However, the data for pregnancy rate from F5 were not considered in the statistical analysis because there was an accidental loss of the semen sample and a lower dose of sperm (100×10^6 sperm cells) was used to inseminate the ewes. A value of $p \leq 0.05$ was considered as significant. The SAS University Edition statistical package was used at all times.

RESULTS AND DISCUSSION

Mineral supplementation is essential to sustain adequate animal reproductive activity (López-Alonso, 2012). The minerals can be supplied to animals in feed or by injection. There is evidence that pregnancy rate in ewes is increased by multiple injections of selenium and vitamin E during a synchronized estrus protocol (Awawdeh *et al.*, 2019). Similarly, oral supplementation of zinc is effective to improve pregnancy rate (Ali *et al.*, 1998) and prolificacy in ewes, possibly from reduced embryo mortality (Masters & Fels, 1980). These results show that mineral supplementation both by oral administration and by injection is effective to improve the ewe's reproductive performance. However, zinc supplementation by injection has been reported to be a more effective way to restore zinc blood concentrations in deficient animals than by oral administration (Lamand *et al.*, 1983). A high zinc blood concentration is reached after three days of mineral injection (Lamand, 1978) and it remains elevated for up to 28 days (Lamand *et al.*, 1980). Therefore, it is suggested that supplementation by injection ensures a good supply of trace minerals to achieve optimum reproductive performance (Stokes *et al.*, 2017).

The effect of subcutaneous injection of zinc oxide on the means of the largest preovulatory follicle diameter, measured in the different experimental groups, is shown in Table 1. In general, the effect of the treatment, block and their interaction were not significant ($p>0.05$). The latter is contradictory to published results in cows, where it was observed that the diameter of the preovulatory follicle increased with the injection of 400 mg of zinc oxide (Anchordoquy *et al.*, 2019). This contradiction can be due to the day when the zinc oxide injection was applied, since in the case of the study in cows by Anchordoquy *et al.*, supplementation was carried out at the beginning of the estrus synchronization protocol, which exposed the animal organism to the effects of zinc for a longer time. It is suggested that the zinc function at the follicular level is directed more to the oocyte (Ménézo *et al.*, 2011), which is particular sensitive to zinc deficiency during the last stages of maturation (Tian & Diaz, 2012). Delayed development of embryos and pregnancy loss have been observed when zinc dietary deficiencies are created four to five days before ovulation (Tian *et al.*, 2014). This agrees with the fact that intrafollicular zinc concentrations increase as the follicle's size increases (Kor *et al.*, 2013).

Mineral supplementation was not part of the feeding program for ewes in any of the farms in the present study. Therefore, there was speculation that sheep had zinc deficiency and that a positive effect from zinc supplementation could impact follicular size, oocyte quality, and pregnancy rate. This study is limited regarding the unknown mineral balance of the ewes before and after zinc injection. In addition, oocyte quality was not evaluated, but pregnancy rate results and the available scientific evidence allow speculating about the results from this study.

The effect of zinc injection on the pregnancy rate in ewes is depicted in Figure 1. In general, no dependency was observed between zinc injection and pregnancy rate ($p>0.05$), which is similar to the results found in ewes supplemented orally with 50-150 ppm of zinc oxide (Monem & El-Shahat, 2011). This is contrary to the results reported in cows (Anchordoquy *et al.*, 2019) and goats (Kundu *et al.*, 2014), where an increase in pregnancy rate was observed by injecting 400 mg of zinc sulfate or by oral administration of 100 ppm of zinc oxide to goats.

Table 1. Diameter of the largest preovulatory follicle (mm, mean ± EE) in ewes injected with 0 (Control), 100 (z-100) and 200 (z-200) mg of zinc oxide in five farms (F1-5).

Group	Preovulatory follicle diameter
Control	6.66±0.23
z-100	6.24±0.23
z-200	6.14±0.23
Block	
F1	6.56±0.30
F2	6.00±0.30
F3	6.46±0.30
F4	6.26±0.30
F5	6.43±0.30

Means with different superscript letter are significantly different ($p \leq 0.05$)

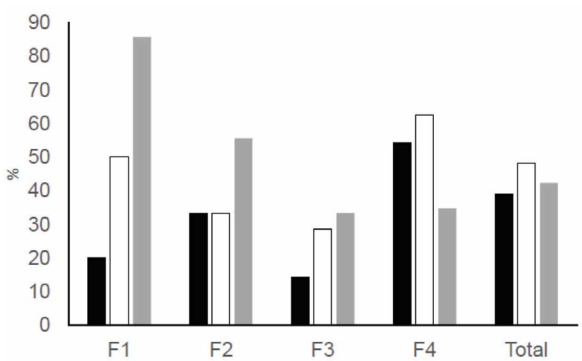

Figure 1. Pregnancy rate of ewes injected with 0 (black bars), 100 (white bars) and 200 (gray bars) mg of zinc oxide on four farms (F1-4).

The beneficial effect of zinc supplementation on pregnancy rate is sustained, at least partly, on the blood concentrations of this mineral being higher in pregnant females than in empty ones (Nazari *et al.*, 2019). In addition, zinc supplementation in laboratory conditions reduces the fragmentation of genetic material in the oocyte (Abdel-Halim *et al.*, 2018), increasing the number of oocytes that reach the blastocyst stage and their quality (Choi *et al.*, 2016; Jeon *et al.*, 2014). In this regard, zinc supplementation to the culture medium increases the size of the embryo inner cell mass, which might be associated to a higher pregnancy rate in cows (Wooldridge *et al.*, 2019). On the other hand, supplementing bulls (Kumar *et al.*, 2006) and adding this mineral to the fertilization medium improves sperm activity and quality (Galarza *et al*., 2020), which suggests that the fertilization process could be improved under *in vivo* conditions.

The lack of statical significant differences between experimental groups in pregnancy rates overrides the beneficial effect of zinc on female reproductive performance. However, it can be suggested that the lack of significant difference between treatments could be due to the small sample size in some sheep farms, such as F1 and F2. In addition, it has been suggested that the intrinsic characteristics of each farm, such as the initial mineral balance of the animals, affects differently the pregnancy rate in response to the zing levels supplemented, since the low levels of pregnancy are only observed in females with a zinc status considered as deficient, but not in those with a marginal or adequate zinc status (Galarza *et al*., 2020).

CONCLUSIONS

The subcutaneous injection of 100 or 200 mg of zinc oxide during an estrus synchronization protocol did not affect the diameter of the largest preovulatory follicle or the pregnancy rate in ewes. It is advisable to perform mineral supplementation according to the female's mineral status.

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AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Analysis of the environmental impact generated by backyard swine production in Tepetlán, Veracruz, Mexico

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ABSTRACT

Objective: To identify and estimate waste production, water consumption and production practices of backyard swine farms at the municipality of Tepetlán; Veracruz, Mexico, to foresee the possible influences on the environment and generate information that promotes new public environmental policies adapted to small producers.

Methodology: 36 backyard livestock production units were identified and studied in Vicente Guerrero and Alto Tío Diego by a census in which structured surveys were applied to obtain data; the results were analyzed by descriptive statistics. The maximum pollutant potential of the load with the installed infrastructure was estimated.

Results: We registered 503 swine heads of diverse zootechnical functions, mainly from the Landrace breed, in 36 studied farms, 54.7% of idle infrastructure was identified. We found that about three tons of excreta are produced per day between the towns of Vicente Guerrero and Alto Tío Diego, from which 78% are dumped into the municipal drainage. The average water consumption per unit of livestock production was 132.2 L and for each unit of animal population 28.5 L, with a standard deviation of 32.2 L per animal population unit. This research also provides information on the feeding and production practices of the animals. It was possible to estimate the volume of swine excreta generated in the assessed localities. However, it is necessary to quantify organic matter, nitrogenous products, phosphorus, and total and fecal coliforms.

Conclusions: It is important to quantify and regulate the generated waste by this livestock activity, to take corrective and regulatory decisions, to establish solutions that protect natural resources without harming the economy of the small producer.

Keywords: swine production, animal population unit, water and soil pollution.

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INTRODUCTION

Mexico produces almost 18 million head per year, from which, 1,751,183 heads were produced at the state of Veracruz during 2019, ranking fourth nation-wise, after Jalisco, Sonora and Puebla (SIAP, 2020b). It is a profitable activity, generating significant profits

Image by Thomas B. at Pixabay

for farmers. As a result, the number of people in the Mexican countryside who engage in swine farming increases each year.

In Mexico, three swine production systems are recognized: technified, semi-technified and backyard systems (Mariscal, 2007). The first two have a defined geographical distribution and are registered and regulated by the authorities; on other hand, the backyard system is present in all the country, more common in Veracruz, which many small producers with land plots of less than one hectare.

The economic activity of breeding and fattening swine, in addition to contributing to the country's gross domestic product, produce large volumes of wastewater with urine, excreta, the runoffs of washing of production units, uneaten feed and various polluting liquids, which are usually not properly handled, and their final disposal are soils, urban wastewater, and/or surface bodies of water near the production units (Mateo *et al.*, 2019; Sandoval-Herazo *et al.*, 2020).

Swines' wastewater enters a high concentration of nutrients in surface waters. This has been increasing notably in recent decades. The degradation of water quality can cause a potential impact on health risks, in addition to the typical negative effects caused to the environment (De la Mora *et al.*, 2014). The NH_3 emitted by swine farms is deposited close to where it is produced, thus contributes to the eutrophication and acidification of ecosystems (Liu *et al.*, 2013), as well as to the reduction of biodiversity (Clark and Tilman, 2008). Swine production accounts for approximately 15% of the worldwide NH_3 emissions associated with livestock (Olivier *et al.*, 1998).

The official Mexican Norm (NOM-001-ECOL-1996) is an instrument to regulate the pollution of national waters by various activities, including livestock; compliance with it is carried out by the competent authority for identified livestock units, mainly those with a large number of animal units (AU). At the same time, the Ley de Organizaciones Ganaderas (law for livestock organization) in Mexico considers as producers those which have a minimum inventory of five animal units, for swines the equivalence corresponds to 15 swine's Regulations of the Livestock Organizations Law (Reglamento de la Ley de Organizaciones Ganaderas, 1999). This leaves out backyard producers, who are not identified and do not register their inventories with the authority and are not regulated.

Tepetlán municipality is in the center of the state of Veracruz, Mexico. It is a small municipality of just over 83 km^2, its population is 9429 people; it has 29 localities, of which 28 are rural and one urban (Subsecretaria de Planeación, 2018). Tepetlán is dedicated to agricultural activities such as sugarcane and lemon crops, and livestock such as dairy and pork production; however, there are no industrialized or technified farms and is therefore not listed as a municipality with high animal production. Yet, it has a large number of backyard producers, with which it reached a production of 157.37 tons of live swine during 2019, with a recorded local production value of $4,482,039.00 Mexican pesos (SIAP, 2020a).

Given the above, it is important to identify backyard swine production units, in addition to estimating their waste production, water consumption and practices production to foresee possible impacts on the environment and generate information that promotes new environmental public policies adapted to small producers.

MATERIALS AND METHODS

The present study took place in Tepetlán municipality, at Vicente Guerrero and Alto Tío Diego localities, where there is a high production of backyard farm swine (FAOCC, 2020).

For this research, support was requested from the General Directorate of Rural Development (Dirección General de Desarrollo Rural) from the Secretary of Agricultural, Rural and Fishing Development (Secretaria de Desarrollo Agropecuario, Rural y Pesca), and from the Autonomous Foundation of Organizations of Farmers and Settlers A. C. (Fundación Autónoma de Organizaciones de Campesinos y Colonos A. C. FAOCC) to identify the backyard swine production units in the included localities in this study. It should be noted that since these are not registered by the authorities, there is not an exact number of backyard swine farms within these localities.

The livestock production units (LPU) considered for this study are backyard farms, with less than five animals, with basic infrastructures, such as block or brick swine pens, and, in some cases, basic production equipment, mainly maternity cages.

Once the LPU's were identified through the census, surveys were applied to identify the number of animals at the time of the assessment, maximum productive capacity, infrastructure and equipment, water supply and final disposal, waste disposal, in addition to identifying the environmental knowledge of the producers.

Subsequently, the number of animal population units (APU), the generation of excreta and the amount of wastewater were estimated.

The number of APU's helps to compare between LPU's dedicated to breeding and those dedicated to fattening; it is determined by their equivalence, one APU is equivalent to 100 kg of live swine (Méndez *et al.*, 2009).

To calculate the biomass in kilograms, the average weights of the animals were taken, considering their zootechnical function. The total biomass of the swine on the assessed farms was obtained and finally divided by 100, to obtain the number of total UPA's (Méndez *et al.*, 2009). A total of 214 kg was recorded for bellies and sires, 16 kg for weaning animals and 75 kg for fattening animals.

Taking values from Drucker *et al.* (2004) and Méndez *et al.* (2009), swine excreta production per APU was determined to be 9 kg d^{-1} for sow and hog, 8.6 kg d^{-1} for weaning and 7 kg d^{-1} during fattening.

To estimate the water consumption of the APU's, each producer was surveyed during a visit; in some cases, water use records were presented; finally, the sum of the water presumably consumed in backyard swine production was added up.

To complement the data, unstructured interviews were conducted using the selective snowball sampling technique used by Di Lorio *et al.* (2020), where the different points of view of backyard production could be analyzed.

RESULTS AND DISCUSSION

We identified and analyzed n=36 backyard swine farms, 23 located in the town of Vicente Guerrero and 13 in Alto Tío Diego. The livestock herd present at the time of the census is described in Tables 1 and 2; which is mainly of the Landrace breed, chosen by the producers for the good performance of the sows, with some Pietrain sires. All farms had idle infrastructure, so it was necessary to determine the maximum APU's for each facility, finding an idle capacity of 54.7%.

A record of the daily and average water consumption per APU was obtained. The sum of the daily water consumption in the assessed farms was 6.09 m^3, with an average of 169.2 L used by each LPU daily, and 28.5 L of water consumed per day by each PAU, with a population average of 14.38 L / PAU; the standard deviation of water consumption per UPA with respect to the mean reached 33.2 L / PAU on average. The calculation of water consumption, if the facilities were at 100% capacity, was estimated to be 24.1 m^3.

Table 1. Summary of information collected in Vicente Guerrero, Veracruz, Mexico.

No.	Breeding sows	Stallions	Fatteners pig	Piglet	APU	APU maximum	Dropping d^{-1}	Dropping d^{-1} maximum capacity	Water L/d/ LPU	Water L/APU
1	8	0	25	14	38.1	88.5	302.1	303.1	200	5.2
2	0	0	5	0	4	9.9	28	30	200	50
3	3	0	13	0	16.4	34.5	126.8	129.8	200	12.2
4	0	0	0	8	1.2	12	10.3	14.3	200	166.7
5	1	0	3	12	6.2	6.2	50.3	55.3	200	32.3
6	0	0	4	0	3.2	12	22.4	28.4	200	62.5
7	1	0	25	0	22	36	158	165	100	4.5
8	2	0	0	11	5.65	19.2	50.2	58.2	100	17.7
9	0	0	2	0	1.6	5.5	11.2	20.2	20	12.5
10	3	0	18	10	21.9	38.5	167.7	177.7	200	9.1
11	2	0	0	6	4.9	28.5	43.7	54.7	100	20.4
12	0	0	10	0	8	23.7	56	68	200	25
13	1	0	25	0	22	34.5	158	171	90	4.1
14	6	2	9	11	24.8	53.7	208.6	222.6	200	8
15	1	0	7	0	7.6	23.5	57.2	72.2	100	13.2
16	0	0	3	0	2.4	25	16.8	32.8	100	41.7
17	1	0	30	0	26	59.5	186	203	400	15.4
18	3	0	15	30	22.5	22.5	176.7	194.7	200	8.9
19	2	1	0	0	6	21	54	73	200	33.3
20	4	0	0	12	9.8	16	87.5	107.5	400	40.8
21	0	0	0	10	1.5	23.7	12.9	33.9	200	133.3
22	3	0	8	0	12.4	25	98.8	120.8	200	16.1
23	2	1	10	0	14	31	110	133	200	14.3
	43	4	212	124	282.2	650.1	2193.2	2469.2	4210	

Seventy-two-point two percent of the farmers discharge their wastewater into the municipal sewage system; 22.2% into crops, such as sugarcane, corn, or livestock pastures; while only 5.6% have septic tanks. Only 5.6% have a septic tank. Twenty-two percent of the farmers report treating the generated solid waste, mainly as fertilizer for sugarcane crops and pastures. Regard antibiotics usage, 92% continuously use them in the production process, especially tetracyclines, and 36% use hormones, mainly oxytocin, to support the females during calving; only 67% use these drugs by prescription.

Only 5.5% of the producers stated that they provide swine with a diet based on agricultural by-products, mainly corn and chayote. Eleven point one percent use balanced feed as a base and supplement with agricultural products; while 83.3% provide a commercial diet based on products containing ground cereals, oilseed pastes, vitamins, molasses, amino acids and minerals.

Table 2. Summary of information collected in Alto Tío Diego.

No.	Breeding sows	Stallions	Fatteners pig	Piglet	APU	APU maximum	Dropping d^{-1}	Dropping d^{-1} maximum capacity	Water L/d/LPU	Water L/APU
1	2	0	6	0	8.8	22.9	69.6	182.7	200	22.7
2	0	0	8	0	6.4	9.6	44.8	67.2	200	31.3
3	0	0	7	0	5.6	8	39.2	56	200	35.7
4	0	0	2	0	1.6	2.4	11.2	16.8	40	25
5	0	0	6	0	4.8	8	33.6	56	50	10.4
6	0	0	9	0	7.2	8.8	50.4	61.6	150	20.8
7	0	0	4	0	3.2	8	22.4	56	150	46.9
8	0	0	9	0	7.2	12	50.4	84	150	20.8
9	0	0	9	0	7.2	28.8	50.4	201.6	100	13.9
10	1	0	22	0	19.6	32	141.2	224	300	15.3
11	3	0	4	0	9.2	28	76.4	196	140	15.2
12	2	0	10	9	13.3	20	103.6	148	100	7.5
13	1	0	6	0	6.8	8	51.6	56	100	14.7
	9	0	102	9	100.9	196.5	744.8	1405.9	1880	484.8

All producers consider that water is a limiting factor for production in this municipality, but 88.8% do not see backyard swine farming as a problem for water resources and are unaware of the swine excrement might have on the environment. No producer has sanitary infrastructure for their waste treatment.

At the time of the census, low pork production was recorded for the infrastructure installed by backyard producers; the instruments applied indicate that this is a consequence of the rise in the costs of balanced feed since it represents 80% of the costs in backyard swine (Méndez-López *et al.*, 2016). However, backyard producers do not usually plan their production, and state they usually need a greater amount of infrastructure, such as pens for certain production periods.

It was possible to estimate a high current production of swine manure in very small localities, which if not correctly treated, can cause negative environmental impacts, such as soil and water contamination and generation of gases that potentiate the greenhouse effect; in addition, when waste is dispersed on land, it causes unhealthy situations due to bad odors, among others; which decreases the quality of life for people; as some local inhabitants mention (Osejos *et al.*, 2018).

In the municipality, water is a scarce resource, it has 40 water supply sources, and a mean daily volume of 3.4 thousand of m^3 of water (Subsecretaria de Planeación, 2018). The recorded water usage for backyard swine was 28.5 L per day per APU on average, very close to that reported by Drucker *et al.* (2004) estimate of 35 L per day per APU for small farms; however, considering the high standard deviation, there were backyard units with consumption exceeding 130 L per day per APU.

Regard the management of backyard production waste, the only recorded treatment for solid waste and wastewater was as fertilizer, which is not healthy for the environment, as ammonium production can cause eutrophication and acidification of ecosystems (Liu et al., 2013).

The common process to dispose of excreta and urine in the sampled farms was through drainage, which is not adequate management, as the municipality only has one wastewater treatment plant, with a basic capacity of 0.5 L min^{-1} (Subsecretaria de Planeación, 2018).

Therefore, we agree with the analysis made by Pérez (2000) that there is an inefficient use of water, with low or null pollutant removal for activity in constant growth; we also agree that an environmental policy according to the backyard swine producer is required and that NOM-001-ECOL-1996 is not sufficient to regulate this production, mainly due to the lack of budget and personnel for monitoring unregistered units.

CONCLUSIONS

In order to care for the environment and water resources, it is important to identify and evaluate the effects of backyard production in rural areas. The main reason for the problem is the lack of knowledge of proper swine management practices and environmental awareness by small producers, as well as the lack of capacity and skills to treat the waste generated by the activity. Therefore, environmental policies must be created to support training and technical assistance for small producers in environmental issues, not just production issues, which should be implemented through social agricultural extension programs and regulated with new control instruments adapted to the size of the small producer.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Reproductive Evaluation of Charolais and Charbray Bulls on the Reproductive Efficiency of Herds in Warm Sub-Humid Climate of Veracruz

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ABSTRACT

Objective: To correlate the Breeding Soundness Evaluation (BSE) of Charolais and Charbray bulls in the warm sub-humid climate and its effect on the pregnancy rate and calving interval of herds to estimate differences between breeds.

Design/Methodology/Approach: BSE was carried out on N=90 Bulls, Charolais (n=33) and Charbray (n=57), evaluated ***in situ*** during the period of 2017 to 2019. Variables for the bull (age, body condition, libido, and scrotal circumference), semen (concentration, motility, and volume), herd (pregnancy rate and calving interval), and environment (rainy and dry season) were analyzed with the one-way ANOVA, Factorial, Multiple regression and Multivariate analysis.

Results: Charolais and Charbray bulls show similar results in the BSE ($p>0.05$) in a warm sub-humid climate.

Study Limitations/Implications: Yearly seasons (rainy or dry) do not affect ($p<0.05$) the BSE rating. Libido is associated ($p<0.05$) with age ($R=-0.42$), sperm motility ($R=0.67$), sperm concentration ($R=0.66$) and pregnancy rate ($R=0.63$).

Findings/Conclusions: Variables with high association ($p<0.05$) with pregnancy rate were libido ($R=0.63$), motility ($R=0.60$), sperm concentration ($R=0.51$) and age ($R=-0.50$); variables with high association ($p<0.05$) with calving interval were: age ($R=0.74$) and libido ($R=-0.33$). Charolais and Charbray bulls show similar reproductive efficiency in herds under a warm sub-humid climate in Veracruz.

Key words: Charolais bulls, Charbray bulls, herd, efficiency.

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INTRODUCTION

An increase in demand for animal protein has resulted in producers on livestock farms including in their reproductive programs purebred bulls and their crosses with genetic traits specialized for meat production (Sitienei *et al.*, 2018). The Charolais breed is a viable alternative to obtain a greater amount of kilograms per animal, due to its large corpulence, additional weight gain at weaning, and peripheral fat (Gagaoua *et al.*, 2018). However, purebred bulls introduced to hot humid climate present difficulties in adapting

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to environmental conditions in the area, which has a high temperature-humidity index (THI), low availability of forage and water, and this condition causes the animals to be subjected to prolonged periods of heat stress during puberty and sexual maturity (Rahman *et al*., 2018). In order to reduce the negative effects related to climatic variables, it has been decided to carry out genetic crosses with *Bos indicus*, specifically with Brahman, resulting in the synthetic Charbray breed (5/8 Charolais and 3/8 Zebu), which shows resistance to high temperatures and humidity, in addition to conserving the characteristics of a meat breed. Under tropical conditions, where there is high environmental temperature and/or humidity, cattle reproduction can be impacted by heat stress (Morrell, 2020). With the above and as consequence of the climatic variability present in tropical zones in recent decades (Dominguez-Mancera, 2017), it was proposed to conduct evaluations of the reproductive capacity of Charolais and Charbray bulls to determine the differences in reproductive performance between breeds on the reproductive efficiency of the herd.

METHODS AND MATERIALS

Experimental animals and semen collection

All handling, immobilization and semen collection procedures performed on bulls within the Livestock Production Units (LPU) by the veterinary services and they were evaluated and approved by the Bioethics Committee of the FMVZ-UV (COBIBA010/2017). Information from the breeding soundness evaluation (BSE) of bulls (N=90; Charolais n=33, Charbray n=57), carried out by the Animal Reproductive Biology Laboratory and the Cell Biology Laboratory of FMVZ-UV, was used. All the bulls were under an extensive grazing system with *Cynodon nlemfuensis* and *Brachiaria humidicola* grasses, ranging in age from 1 to 11 years, with no apparent health deterioration at the time of the BSE. The cows used (N=2274) to measure the reproductive performance of bulls in herds were clinically healthy and fertile at the time the BSE was performed; cows with reproductive abnormalities were excluded from the analysis. Transrectal palpation and ultrasonography of the reproductive tract were performed for gestation detection using a 6.5 MHz linear Minitube probe (Minitube, Verona, WI, USA). In addition, reproductive records from the herd (N=42) were used.

Breeding Soundness Evaluation; Semen evaluation

Semen samples were collected from January 2017 through December 2019, each of these were evaluated in situ immediately after collection (~5 minutes). Semen was collected in a test tube with a graduation of 1 to 15 ml and volume, color and density were measured (Chenoweth, 1983). Electroejaculation was performed on the bulls with a three-electrode probe (Minitube, Verona, WI, USA; Ø: 2"/5.08 cm; length: 33 cm). Individual motility was assessed in a sample diluted with warm saline. A drop of diluted semen was placed on a slide on a thermoplate at 37 °C, covered with a coverslip and examined at 40X. The proportion of sperm moving progressively through the field of view was estimated by finding multiple groups of ~10 sperm and counting how many sperm are progressive versus how many are not (Ibanescu *et al*., 2020). The spectrophotometric method was used to measure sperm concentration (x10^6/mL). Once the sample was obtained, a drop of undiluted semen was taken and placed in the Microcube for SDM-1 (Minitube, Verona, WI, USA) with a capacity of 2 μL, then inserted into the spectrophotometer model SDM-1 (Minitube) calibrated for cattle (Bompart *et al.*, 2019). To evaluate bull libido, the test was conducted in a small pen where the bull and a cow showing signs of estrus could be easily observed; the bull was allowed to be in contact with the cow exhibiting behavioral estrus for a period of 5 minutes; libido was scored (1-10 scale) with the system proposed by Chenoweth *et al.* (2010).

Analysis and collection of climatological data

Climatological data (2017-2019) from the meteorological station located in the municipality of Juan Rodriguez Clara, Veracruz (30143), were used, provided by the National Meteorological Service's Gulf of Mexico forecast center, zone where the herds and bulls analyzed are located. With climatological data, the climatic safety index for livestock, known as the temperature-humidity index (THI), was obtained with the following equation: $THI = 1.8 + T + 32 - (0.55 - 0.55 * HR) * (1.8 * T - 26)$ (Eq. 1). Where: "T" is the average daily temperature in °C and RH is the percentage (%) of relative humidity. Nienaber and Hahn (2007) have considered four categories of THI to evaluate environmental and thermal conditions and their associated impact on breathing per minute. THI values ≤ 74 were considered as Comfort; 75-78 as Alert; 79-83 as Danger and ≥ 84 as Emergency. In addition to THI, the climatic variables analyzed were: accumulated monthly precipitation (mm), dominant wind speed (m/s), daily temperature (°C) and relative humidity (%). With the climatological data, two seasons of the year were recorded (rainy and dry).

Statistical Analysis

The statistical package STATISTICA V10 (StatSoft, 2011) was used for all statistical analyses and the figures were edited with the help of Sigma Plot V11 software (2008). The Shapiro-Wilk test was used to test for normality and Bartlett's test for homoscedasticity. One-way and factorial ANDEVA (GLM) was performed to evaluate main and pooled effects; multiple comparisons by Tukey ($p < 0.05$). Simple linear regression was performed with the model: $Y_i = \alpha + \beta * X + \varepsilon_i$ (Eq. 2) where "Y" is the dependent variable and "X" is the independent variable. 1) Dependent variables: sperm concentration, individual motility, libido, gestation rate, inter-calving interval. 2) Independent variables: bull and semen characteristics. Multiple linear regression analysis was used to obtain statistical models that best explained herd efficiency. Lastly, exploratory-multivariate analyses (Cluster, Correspondence and Principal Component Analyses) were performed to obtain the variables and total variation of the model that best describes the BSE of the bull on the reproductive efficiency of the herd.

RESULTS

Descriptive analysis of BSE of Charolais and Charbray bulls

Table 1 shows the values of the minimum-quadratic means and standard errors for each variable of the BSE of Charolais and Charbray bulls where it can be seen that there is no difference ($p > 0.05$) between breeds; in addition, the reproductive efficiency of the herds with the two breeds is not different ($p > 0.05$).

Table 1. Bull Breeding Soundness Evaluation descriptive análisis of the Charolais and Charbray bulls in warm subhumid climate of Veracruz.

Bull Breeding Soundness Evaluation		Bull breed	
Experimental Unit	**Parameter**	**Charolais (n=33)**	**Charbray (n=57)**
Bull	Body Condition Score (scale 1-5)	2.80±0.06	2.85±0.04
	Age (years)	5.09±0.37	5.00±0.37
	libido (scale 1-10)	7.81±0.21	7.56±0.17
	Scrotal circumference (cm)	36.60±0.30	36.87±0.23
	Semen volume (mL)	3.63±0.16	3.53±0.18
	Sperm concentration ($X10^6$)	649.45±52.76	621.42±50.64
	Sperm motility (%)	72.78±3.15	68.84±2.89
Herd	Pregnancy rate (%)	50.69±2.10	47.82±1.44
	Calving interval (days)	697.75±13.37	681.73±16.42
	Cow / Bull ratio (n)	33.25±0.49	28.80±0.71

Note: Bulls with 1 and 2 years old do not report data on pregnacy rate and calves interval.

Climatological analysis

With the meteorological data, it was decided to perform a climatological analysis of the area, the results of which are shown in Figure 1.

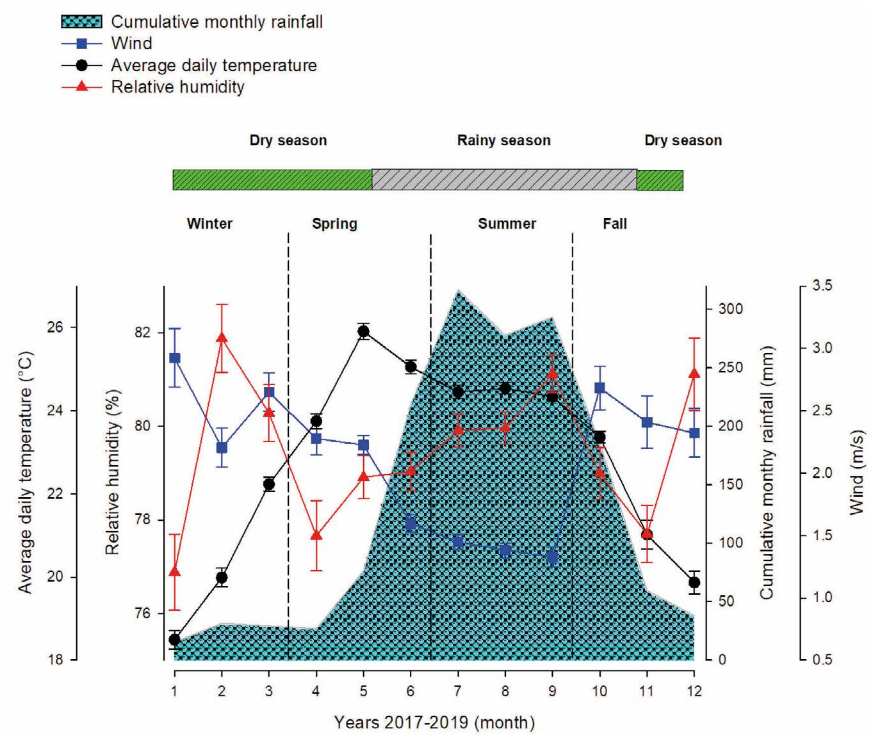

Figure 1. Monthly analysis (mean ± ee) of the climatic variables in warm subhumid climate of Veracruz. Cumulative monthly rainfall (mm, shaded area). Dominant wind speed (m/s, blue box), average daily temperature (°C, black circle), relative humidity (%, red triangle). The vertical dotted lines indicate the change of season in the year. The horizontal bars show the two main seasons of the year in the region: Rainy season (gray) and Dry season (green).

With the combined values of temperature (°C) and humidity (%), the THI was obtained and with this, the periods and seasons of the year when there could be heat stress could occur and affect the reproductive capacity of Charolais and Charbray bulls were determined (Figure 2).

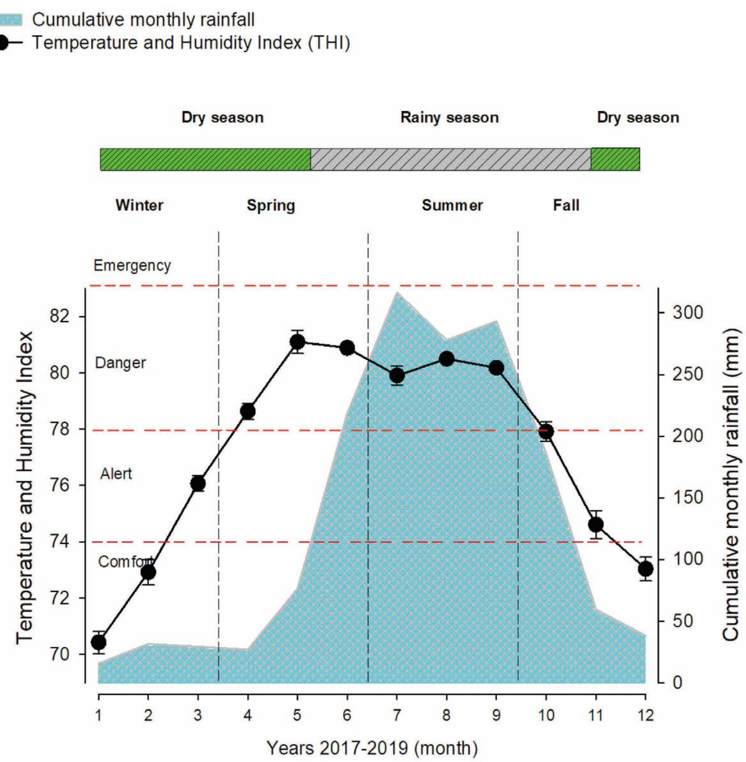

Figure 2. Monthly analysis (mean ± ee) of the Temperature and Humidity Index (THI) in warm subhumid climate of Veracruz. Cumulative monthly rainfall (mm, shaded area). Temperature and humidity index (THI, black circle), The vertical dotted lines indicate the change of season in the year. The horizontal bars show the two main seasons of the year in the region: Rainy season (gray) and Dry season (green).

Effect of time of year on BSE of Charolais and Charbray bulls

Table 2 shows the effect of the main seasons of the year (rainy and dry) on semen parameters and bull behavior (libido); it can be observed that there are no differences ($p>0.05$), both breeds behave similarly in the parameters of semen and libido.

Correlation of the BSE of bulls with herd efficiency

Table 3 shows the results from the correlation analysis on herd efficiency (pregnancy rate and calving interval).

Evaluation of BSE on herd parameters

The main effects and their interactions of pregnancy rate and calving internal with the variables that best explain BSE (age) and bull behavior (libido) were analyzed (Figure 3). The libido of Charolais and Charbray bulls decreases with age ($p<0.05$) (Figure 3a).

Thin bulls (CC 3) and obese bulls (CC 5) show low libido ($p<0.05$) Figure 3b. Sperm motility and concentration decrease with the bull's age ($p<0.05$) (Figure 3c). High libido values are associated with high sperm motility and concentration ($p<0.05$) (Figure 3d). The herd's reproductive parameters are affected by libido (Figure 3e) and age (Figure 3f) of the bull.

Table 2. Analysis of the main seasons of the year on the parameters of the semen of Charolais and Charbray bulls in warm subhumid climate of Veracruz.

Semen evaluation	Season			
	Rainy		Dry	
	Charolais (n = 11)	Charbray (n = 19)	Charolais (n = 22)	Charbray (n = 38)
Volume (mL)	3.50±0.21	3.71±0.30	3.70±0.22	3.48±0.22
Concentration ($X10^6$)	658.54±96.77	536.68±91.74	644.90±64.27	663.78±60.25
Sperm motility (%)	71.72±5.85	63.47±5.50	73.27±3.81	71.52±3.32
libido (1-10)	7.90±0.31	7.57±0.36	7.77±0.29	7.55±0.18

Note: no significant differences ($p>0.05$) are reported between bulls and between seasons.

Table 3. Bull Breeding Soundness Evaluation correlation analysis on herd parameters: Pregnancy rate and Calving interval.

Herd efficiency	Breeding Soundness Evaluation	Simple linear correlation Y= a+b*X							
		R	R^2	Intercept (a)	ee (a)	Value of "p"	slope (b)	ee (b)	Value of "p"
Pregnancy rate	Age (years)	−0.498	0.248	62.509	2.959	0.001	−2.329	0.478	0.001
	Body condition score (1-5)	0.069	0.005	43.521	9.522	0.001	1.960	3.335	0.558
	Scrotal circumference (cm)	0.089	0.008	29.292	26.143	0.266	0.537	0.709	0.451
	Volume (mL)	0.010	0.057	48.776	3.790	0.001	0.081	0.985	0.934
	Sperm motility (%)	0.642	0.412	23.829	3.678	0.001	0.350	0.049	0.001
	Concentration ($X10^6$)	0.538	0.279	37.928	2.308	0.001	0.017	0.003	0.001
	Libido (scale 1-10)	0.640	0.409	10.051	5.605	0.077	5.120	0.725	0.001
Calving interval	Age (years)	0.745	0.554	509.810	20.280	0.001	30.996	3.275	0.001
	Body condition score (1-5)	−0.144	0.007	791.624	84.091	0.001	−36.369	29.452	0.220
	Scrotal circumference (cm)	0.009	0.025	670.159	233.669	0.005	0.503	6.341	0.938
	Volume (mL)	−0.195	0.038	741.394	33.098	0.001	−14.506	8.606	0.968
	Sperm motility (%)	−0.305	0.081	795.586	40.664	0.001	−1.483	0.545	0.008
	Concentration ($X10^6$)	0.264	0.070	737.325	23.512	0.001	−0.075	0.032	0.023
	Libido (scale 1-10)	−0.349	0.110	878.385	60.838	0.001	−24.893	7.867	0.002

Note: R=correlation coefficient; R^2=coefficient of determination; a: Intercept; b: slope; ee=standard error; "p" value: probability significance value.

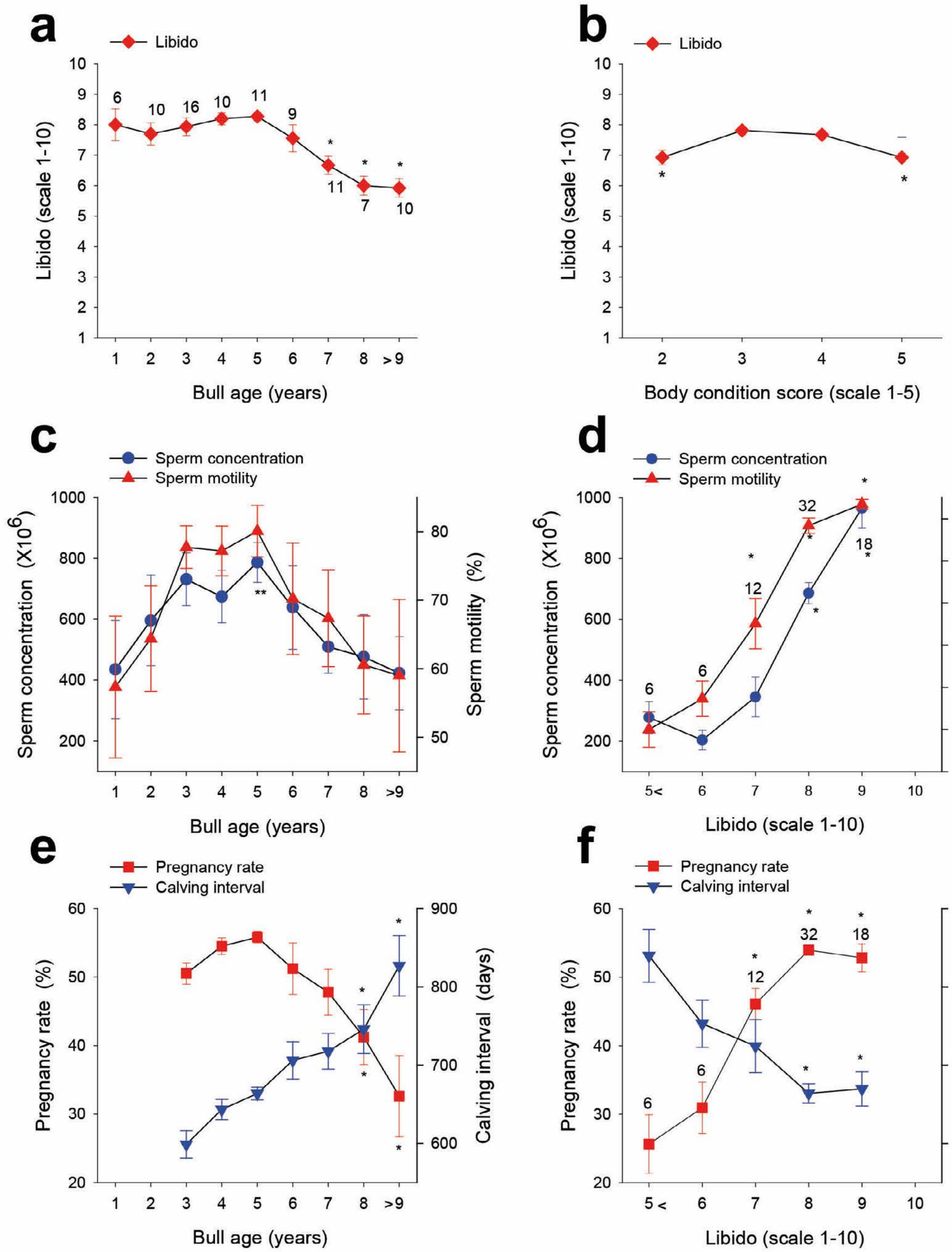

Figure 3. Analysis of main effects of Bull Breeding Soundness Evaluation in Charolais and Charbary bulls (age, body condition and libido) on sperm concentration and sperm motility, as well as herd efficiency: pregnancy rate and calving interval. a. Bull libido at different ages: dashed line shows the average libido. b. Body condition score on bull libido: blue dashed line shows average libido. c. Sperm concentration (left) and Sperm motility (right) in bulls of different ages (years). d. Sperm concentration (left) and sperm motility (right) in bulls with different libido values. (e, f) Herd reproductive parameters, pregnancy rate (left) and calving interval (right) in bulls of different ages (e) and libido value (f). Numbers above the error bar show the number of bulls analyzed. (*) Indicates significant statistical differences ($p<0.05$). 1 and 2 year old bulls, no data on pregnancy rate shown. No bulls with a libido 10 vale are reported.

Multivariate analysis of the BSE of Charolais and Charbray bulls on herd reproductive efficiency

Multiple regression (Forward Methodology) was performed with Libido as response variable, since it was found to be associated with BSE variables; the model obtained was: $Y_i = \alpha + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \varepsilon_i$ (Eq. 3). Where: Y=Libido (scale 1-10), α=intercept (0.9749±2.71), β_1=(−0.175±0.047), X_1=Age (1-11 years), β_2=(0.845±0.336), X_2=Body condition (scale 1-5), β_3=(0.140±0.070), X_3=Scrotal circumference (cm), ε=error (1.15), R=0.469, adjusted R^2=0.193 ($p<0.05$); (Figure 4a and 4b). The pregnancy rate was the variable best associated with BSE, the multiple regression model obtained was as follows: $Y_i = \alpha + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \varepsilon_i$ (Eq.4). Where: Y=Pregnancy rate (%), α=intercept (22.745±7.89), β_1=(−1.116±0.432), X_1=Age (1 to 11 years), β_2=(2.450±0.972), X_2=Libido (scale 1-10), β_3=(0.206±0.083), X_3=Sperm motility (%), β_4=(−0.001±0.004), X_4=Sperm concentration ($X10^6$), ε=error (7.39), R=0.733, adjusted R^2=0.510 ($p<0.05$); (Figure 4c and 4d).

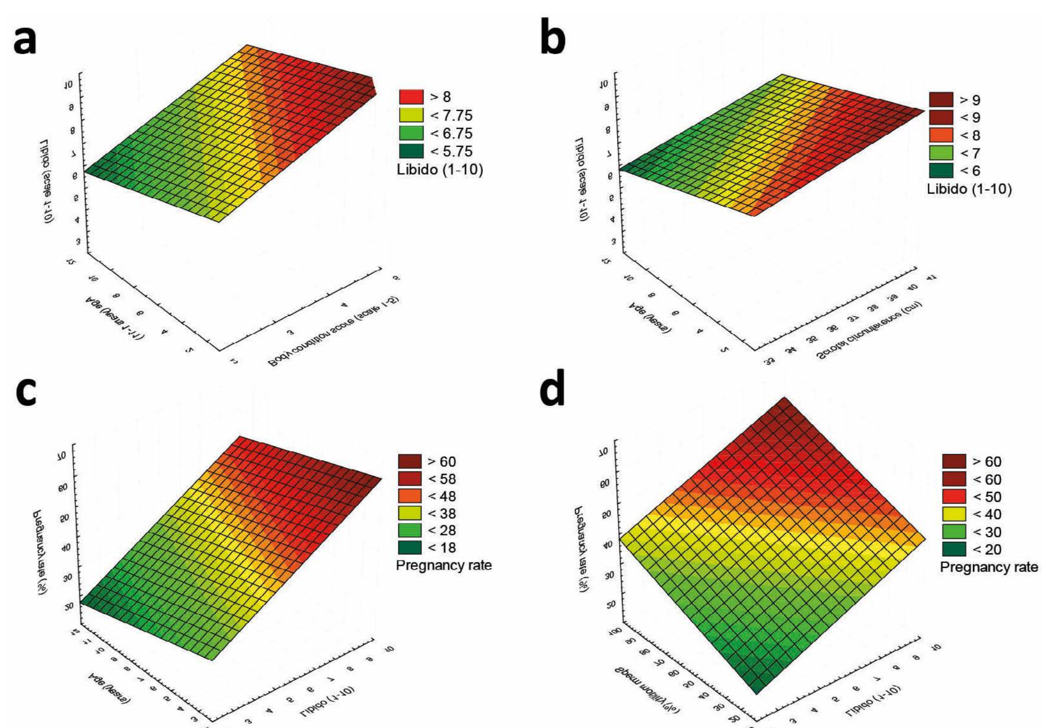

Figure 4. Multiple correlation analysis of BBSE on the variables Libido and Pregnancy rate. a. Relationship between libido scale, body condition score and bull's age. b. Relationship between libido scale, scrotal circumference and bull's age. c. Relationship between pregnancy rate, age and libido scale. d. Relationship between pregnancy rate, libido scale and sperm motility. The intensity of the color indicates an increase in the dependent variables (libido and pregnancy rate).

Finally, a multivariate analysis was performed, and Figure 5a shows how the variables individual motility and sperm concentration are linked to the libido variable, and these variables that describe the reproductive capacity of the bull are associated with the pregnancy rate. It is worth mentioning that the bull's age is associated with the calving interval. By means of a correspondence analysis (Figure 5b) the dimensions (degree of inertia, variation) on herd efficiency (pregnancy rate and calving interval) were plotted with bull characteristics (libido, body condition and age); semen variables (concentration and motility) show lower degree of inertia (association), remoteness.

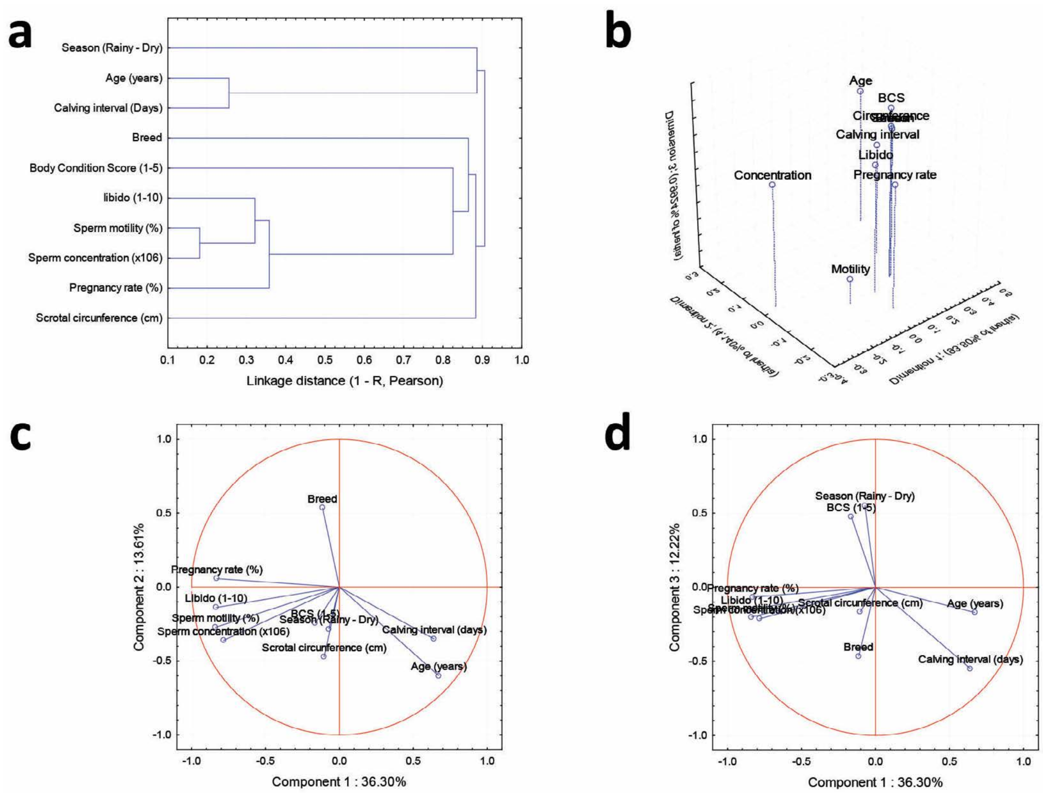

Figure 5. Multivariate analysis of BBSE of Charolais and Charbray bulls on herd efficiency. a. Clustering analysis of the BBSE and herd efficiency variables, the linkage distance was Pearson's 1-R. b. Correspondence Analysis, the dimensions that best explain the association between variables (Inertia, variability) are plotted in 3D. c. Principal components analysis of BBSE and herd efficiency, Components 1 *vs* 2 are plotted (Total variability 49.91%). d. Principal components analysis of BBSE and herd efficiency, components 1 *vs* 3 are plotted (Total variability 48.52%).

Finally, the principal components analysis shows the degree of association and the direction of the vectors (variables) by plotting components 1 *vs* 2 (Figure 5c) and components 1 *vs* 3 (Figure 5d). Figure 5c shows that the gestation rate has association with libido, motility and sperm concentration; body condition is associated with season of the year; bull age shows association with inter-calving interval; bull breed is not associated with any variable; they are equal. Figure 5c shows similar results of Figure 5b as components 1 *vs* 3 are plotted.

The climatology of the zone analyzed shows significant variations throughout the year, which can influence the reproductive performance of domestic species; two predominant constants are high temperature and relative humidity, which, when combined, cause the animals to lose the ability to dissipate the heat they produce, causing heat stress (Hansen, 2009). High values of temperature and humidity are observed in the rainy season, and with increased rainfall there is biomass available for bulls in extensive systems (Casagrande *et al.* 2018). The semen variables of Charolais and Charbray bulls analyzed under these environmental conditions show values similar to those reported by other researchers (Torres-Aburto *et al.*, 2020a), without marked seasonal modifications; thus showing that the Charolais breed and its crosses have adapted to the climate of the area. Dance *et al.* (2015) and Bourgon *et al.* (2018) describe a positive relationship between the quality of the pasture produced at different times of the year and the sperm concentration; in this sense, the breeds analyzed are similar between seasons, where differences in the quantity and quality of biomass available for grazing are marked (Cruz-Hernández *et al.*, 2017). Bulls of European origin show a lower reproductive performance than Zebu bulls and their crosses in a warm sub-humid environment (Jiménez-Severiano, 2002); in the results obtained, there were no differences in libido between the two breeds; they have adapted to the warm sub-humid environment. Bull libido involves two components: (1) its ability to identify females in estrus and (2) its ability to mount them; apparently, libido is not related to semen quality or to scrotal circumference; therefore, it is possible to obtain an excellent semen sample in bulls with low libido (Galina *et al.*, 2007), which is why it is important to include it in the BSE. Age plays a decisive role in BSE; libido, sperm concentration and motility show high values in young and mature animals, as can be seen in the results. Khan *et al.* (2018) report a decrease in reproductive ability as the animal ages. Regardless of the breed analyzed, once sexual maturity is reached (3 years), the indicators are stabilized for a period of 2 years and then decrease as the bull ages, causing a decrease in the reproductive efficiency of the herd (da Silva *et al.*, 2020). Bulls aged 3 to 5 years show better performance in extensive systems on herd reproductive parameters than bulls older than 5 years, demonstrating that bulls have a useful life in extensive systems regardless of their breed (Byrne *et al.*, 2018).

Although libido evaluation and mating ability are not sufficient to predict reproductive success, it is prudent to use bulls that passed all stages of BSE, including libido (Menegassi *et al.*, 2015). Lastly, multivariate analyses have been used in the breeding soundness evaluation of bulls with the aim of defining a degree of reliability higher than 95% for its inclusion and permanence in the herd; likewise, the effects of breed, age, season, on the sperm variables (Felton-Taylor *et al.*, 2020) have been analyzed, and this type of analysis allows associating intrinsic (inherent to the bull) and extrinsic (management, nutrition, environment) factors that can affect the reproductive performance of the bull, and with it, explaining the effects as a whole that simple analyses cannot determine. The best association of the inter-calving interval with BSE variables was found with age; as the animal ages, the number of open days increases, where the reproductive performance of the herd presents a negative correlation in relation to the age of the bulls and cows, which leads to a negative effect on the profitability of the livestock production unit in extensive systems (Torres-Aburto *et al.*, 2020b).

CONCLUSIONS

Charolais and Charbray bulls show similar reproductive efficiency in herds under the hot sub-humid climate of Veracruz.

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Recommendation of Choline Inclusion in Lambs' Diet

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ABSTRACT

Objective: To establish an inclusion recommendation for choline (herbal or synthetic) in fattening lambs' diet based on productive performance and blood metabolites.

Design/Methodology/Approach: A literature review was carried out of herbal and synthetic choline supplementation in the diet of fattening lambs.

Results: The addition of protected choline chloride (50% purity) in the lamb's diet should be less than 2.5 g/kg DM in order not to cause a detrimental effect on the productive performance. On the other hand, the inclusion of herbal choline in lambs' diet should be greater than 6 g/kg DM to have positive effects on body gain and feed conversion.

Study Limitations/Implications: It is necessary to establish the requirement for choline in fattening lambs because studies where choline is supplemented show that the contribution of conventional ingredients is insufficient.

Findings/Conclusions: The inclusion of herbal (6 g/kg DM) and synthetic (2.5 g/kg DM) choline in lambs' diet improves body weight gain and modifies energy metabolites.

Keywords: choline chloride, phosphatidylcholine, herbal, sheep, synthetic.

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INTRODUCTION

Choline is a water-soluble vitamin of the B complex that is required in lambs' nutrition, and its metabolites are important for the synthesis of proteins, phospholipids, acetylcholine, and the metabolism of hepatic fats (NRC, 2007). The choline obtained from the feed ingested is degraded significantly in the rumen and very little escapes ruminal degradation (Baldi and Pinotti, 2006). Although there are sources of protected choline (PC) available, they are generally not included in the diet because the requirement has not been established clearly, though its inclusion in the diet can improve the productive performance (NRC, 2007).

Protected choline chloride (PCC) from ruminal degradation obtained synthetically has been the source of choline most frequently evaluated for small ruminants (Bryant *et*

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al., 1999; Godinez-Cruz *et al.*, 2015; Habeeb *et al.*, 2017), and lower doses of 2.5 g/kg of DM showed beneficial effects in the productive performance of lambs (Li *et al.*, 2015).

Another alternative to add choline to the diet is the use of plants with high content of choline conjugates that resist ruminal degradation. The results from studies show that the addition of herbal choline in the lamb's diet (Godinez-Cruz *et al.*, 2015; Martínez-Aispuro *et al.*, 2019) and in the production ewe's milk (Alba *et al.*, 2020) make production more efficient. One of the advantages of the inclusion of herbal choline is that it contains primarily phosphatidylcholine, which enters directly to the animal's metabolism (Godinez-Cruz *et al.*, 2015; Crosby *et al.*, 2017).

Therefore, the objective of this literature review is to attempt to establish a recommendation of choline inclusion (synthetic or herbal) in fattening lambs' diets based on productive performance and blood metabolites.

Choline in ruminants

Choline is a water-soluble vitamin from the B complex that must be synthesized endogenously, unlike other water-soluble vitamins. Choline is an essential nutrient for mammals, especially when methionine and folic acid are limited in the diet (Zeisel *et al.*, 1991; Zeisel and Holmes-McNary, 2001). Choline, methionine, betaine, folic acid and vitamin B12 in the diet contribute to the choline requirements.

Choline occupies a key position between the energy and protein metabolism in mammals. Choline per se plays an important role in the metabolism of lipids, particularly in lipid transport, as lipotropic agent, optimizing the balance between fat retained and fat metabolized by the liver (Baldi and Pinotti, 2006). Choline is a critical component for the synthesis of phospholipids (phosphatidylcholine), which has structural functions in the biological membranes and in the use of lipids by the tissues. Choline is also a component of neurotransmitters (acetylcholine), supporting the structural integrity and the signaling functions in cell membranes (Zeisel and Holmes-McNary, 2001). Choline is an important source of unstable methyl groups for the biosynthesis of other methyl compounds via betaine for methylation reactions, as in the cases of the formation of methionine from homocysteine and creatine from guanidinoacetic acid (NRC, 2007). Based on this last function, choline and methionine are exchangeable, as sources of methyl groups (Pinotti, 2012). The choline demand as methyl donor is probably the main factor that determines the speed with which choline induces metabolic inefficiency (Zeisel *et al.*, 1991).

In ruminants, choline from the diet is thoroughly degraded in the rumen, which is why its availability is low, while the demand from methyl compounds is particularly high, and it is likely that choline and methionine are scarce, as well as the other sources of methyl groups (Pinotti, 2012).

From the point of view of animal nutrition, the relatively high sources of choline are soybean, soybean meal, rapeseed meal, fish meal and dry yeast, although its bioavailability is considered "moderate". This is why it is thought that choline from the diet contributes insignificantly to the body reserve in adult ruminants (Pinotti *et al.*, 2002). Studies in sheep show that 76% of the choline injected in the rumen is degraded as methane, while 15% was accumulated as trimethylamine. Under such conditions, less than 10% of the choline escapes the degradation from incorporation, such as phosphatidylcholine, in the structural membranes of ciliated protozoa (Neill *et al.*, 1979).

The data reviewed in this document are consistent with these assumptions and support the need of supplementation with choline protected from ruminal degradation for fattening lambs, which can improve the balance of the metabolism of methyl groups and the state of other nutrients.

Protected choline chloride (PCC) in lambs

Several feedstuffs used in ruminant nutrition contain choline, but this natural choline is degraded rapidly in the rumen, so it must be offered as choline protected from ruminal degradation in ruminant species (Kawas *et al.*, 2020). In this review, the use of choline chloride is one of the main sources used to supplement choline within the diet of ruminants, although choline chloride is not well absorbed in the intestine and most of it is degraded in the rumen (Sharma and Erdman, 1989). Therefore, the incorporation of this choline must be protected from ruminal degradation, thus increasing the availability for intestinal absorption (Baldi and Pinotti, 2006). Other PCC problems are that commercial products differ in their choline content and degradability in the rumen (Kung *et al.*, 2003; Brusemeister and Sudekum, 2006); in addition, it is only absorbed 61% of the choline chloride that reaches the duodenum (Veth *et al.*, 2016).

The evaluations with PCC products have improved milk production in cows (Sales *et al.*, 2010; Jayaprakash *et al.*, 2016), weight gain in beef cattle (Pinotti *et al.*, 2009), and feed conversion and meat quality in fattening goats (Habeeb *et al.*, 2017; Tu *et al.*, 2020). Li *et al.* (2015), when supplementing different PCC concentrations at 50% purity (0.25, 0.50 and 0.75%) in fattening lambs, observed a quadratic response in gain weight and feed conversion concluding that the best level was 2.5 g/kg of PCC, since with the levels of 0.50 and 0.75% the productive response had a detrimental effect. Bryant *et al.* (1999) observed that the ADG was reduced when using 2.5 and 5 g/kg DM of PCC (25% purity) in the lamb's diet, but a similar response to the control treatment was obtained with 10 g/kg DM of PCC. Kawas *et al.* (2020) evaluated different levels (0, 0.1, 0.2 and 0.3%) of a product with a concentration of 25% PCC in fattening lambs' diet. The addition of PCC in the diet was not associated with the feed intake, weight gain, feed conversion, body weight, and carcass yield although more dorsal fat was obtained with higher levels of RPC.

Herbal choline in lambs

There is a commercial herbal additive (herbal choline) that contains phosphatidylcholine which shows natural resistance to ruminal degradation in sheep with the potential of replacing the PCC products (Godinez-Cruz *et al.*, 2015; Crosby *et al.*, 2017). Studies show that the inclusion of herbal choline in ruminants can improve milk production and general health (Gutiérrez *et al.*, 2019; Mendoza *et al.*, 2020).

Martínez-Aispuro *et al.* (2019), when evaluating three levels of herbal choline (3, 6 and 9 g/kg MS) in the diet, observed that the ADG and feed conversion improved with a linear response when increasing the additive, although a better productive response was observed when adding 6-9 g/kg of herbal choline. In another study (Rodríguez-Guerrero *et al.*, 2018), the inclusion of 4 g/kg DM of herbal choline in the diet did not affect the growth response of fattening lambs, although it did affect the feed digestibility and the weight of the carcass. In an attempt to increase the availability of methyl compounds, these same authors (Rodríguez-Guerrero *et al.*, 2018) combined herbal choline with protected methionine but they did not find any effect on the growth variables.

Herbal choline is a product based on herbs that concentrates only 16 g/kg of total choline conjugates, and in addition it contains other metabolites that could modify the growth performance and ruminal fermentation (Martínez-Aispuro *et al.*, 2019; Mendoza *et al.*, 2020). Crosby *et al.* (2017) observed that supplementation of ewes with 4 g/day of herbal choline from 30 days before and until 30 days after the birth increased the lamb's birth weight, the milk production, and the content of oleic fatty acid in the milk. Roque-Jiménez *et al.* (2020) hypothesized that the active compounds of herbal choline have epigenetic properties that could impact the fetal development and growth of the offspring of ewes supplemented during the entire gestation with 4 g/day; however, they only observed increments in milk production, quality of colostrum, and milk.

Godinez-Cruz *et al.* (2015) attempted to compare the synthetic and herbal choline in fattening lambs without clear results, since when supplementing with 4 g/kg DM from both sources in sheep they found null effects on the productive response.

Effect of choline in lambs' health

Studies have shown that phosphatidylcholine and choline could stimulate the immune response (Lewis *et al.*, 2015). Supplementation with herbal choline (5 g/day) in sheep in the transition period improved milk production (persistence in the peak of lactation and production) and general health (Alba *et al.*, 2020). Goats growing under heat stress showed an increase in globulins when supplementing with PCC (Habeeb *et al.*, 2017). The general health status in response to the intake of herbal choline was reflected in the increase of globulins (Martínez-Aispuro *et al.*, 2019; Alba *et al.*, 2020) and enzymes related to the immune system (Alba *et al.*, 2020).

Effect of supplementation with protected choline on lambs' metabolism

The increments in the concentration of choline in blood confirm that the supplementation of herbal choline is effective to incorporate this metabolite into the organism of sheep (Crosby *et al.*, 2017; Martínez-Aispuro *et al.*, 2019) and the PCC in goats (Habeeb *et al.*, 2017). In humans, the levels of choline in plasma were reduced through the restriction of choline in the diet (Zeisel *et al.*, 1991). Meanwhile, experiments in rats showed that phosphatidylcholine in the diet was effective to increase the level of choline in blood (Lewis *et al.*, 2015).

Studies in lambs reported that the inclusion of PCC or herbal choline in the diet increased the concentrations of non-esterified fatty acids (Bryant *et al.*, 1999; Rodríguez-Guerrero *et al.*, 2018; Guo *et al.*, 2020), cholesterol (Rodríguez-Guerrero *et al.*, 2018), glucose, (Rodríguez-Guerrero *et al.*, 2018; Martínez-Aispuro *et al.*, 2019), low density lipoproteins (Li *et al.*, 2015; Martínez-Aispuro *et al.*, 2019; Guo *et al.*, 2020), and reduced high density lipoproteins (Li *et al.*, 2015) and triglycerides (Rodríguez-Guerrero *et al.*, 2018; Kawas *et al.*, 2020).

Choline could act by altering the intracellular signaling of the energy metabolism, and supplementation with choline to insulin resistance mice reduced the use of glucose for the synthesis of fatty acids and triglycerides, and increased muscular glycogen (Taylor *et al.*, 2017). Choline is associated with the receptors activated by peroxisome proliferators that regulate adipogenesis and lipogenesis (Yu *et al.*, 2003), as well as adiponectin, which performs an important role in the regulation of metabolism of fatty acids and glucose. The change in the concentration of phosphatidylcholine in the organism can influence the biological activity and expression of genes that regulate the production of high and low density lipoproteins (Cole *et al.*, 2012). In addition, choline in the liver through phosphatidylcholine is necessary for packaging and export triglycerides in the very low density lipoproteins (Noga and Vance, 2003).

In experiments with lambs, Li *et al.* (2015) showed that the expression of several genes related to lipogenesis varied between the levels of PCC. However, the variables of the carcass and fat depots did not change in response to the levels of PCC (Bryant *et al.*, 1999; Li *et al.*, 2015) despite the lipotropic effect of choline (Piepenbrink and Overton, 2003) and the fact that the oxidized form of choline (betaine) reduces fat depots in other species (Eklund *et al.*, 2005).

Although herbal choline has a lower concentration of choline byproducts, it can offer similar results to PCC because herbal choline contains phosphatidylcholine (Godinez-Cruz *et al.*, 2015; Crosby *et al.*, 2017). Phosphatidylcholine is the main phospholipid of ruminants, essential for the absorption and transport of lipids, maintenance of the cell membrane structures, cellular signaling, and lipoprotein synthesis (Zeisel and Holmes-McNary, 2001). The metabolic path of phosphatidylcholine in the organism differs from that of free choline, since phosphatidylcholine requires less energy expense and does not require several metabolic processes to be available for the cells (Fagone and Jackowski, 2013).

CONCLUSION

The inclusion of herbal (6 g kg^{-1} DM) and synthetic (2.5 g kg^{-1} DM) choline in lambs' diet improves body weight gain. The inclusion of choline in the diet modifies the energy metabolites, which in turn make more efficient the productive response. Protected synthetic or herbal choline is resistant to rumen degradation, since this metabolite is present in the animal's organism.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Factors Associated with the Onset of Ovarian Activity of Cattle Commonly Reared in the Huasteca Veracruzana, Mexico

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ABSTRACT

Objective: To identify the factors that affect the onset of ovarian activity in prepuberal heifers in the Huasteca Veracruzana of Mexico.

Design/Methodology/Approach: Samples were taken from animal production units of the municipality of Tantoyuca, Veracruz. The onset of ovarian activity was determined by the presence of ovarian follicles and corpus luteum by ultrasonography technique. Zoometric indices and body condition were calculated at the same time, and the zootechnical practices that were applied to the animals in the last six months before the sampling day were registered. A survey was applied to characterize the management of animal production units; the main topics in the survey were: health, reproduction, nutrition, and suckling. All animals available within selection criteria in the production units were sampled. A multiple linear regression (Statistical Analysis Software Version 9.4) and cluster analysis (R Studio Version 1.1.419) were used to identify the main categorical variables that affected the model.

Results: The onset of ovarian activity depends mainly on the anatomical development of the heifers. The biostimulation (male effect) on the heifers has an important effect on reducing the age at puberty.

Study Limitations/Implications: The study was developed during a long drought period in the region.

Findings/Conclusions: The genetic mosaic of the evaluated animals in the production units did not affect the onset of ovarian activity of the heifers; however crossbred heifers (*Bos taurus* × *Bos indicus*) begin the ovarian activity younger.

Keywords: Male effect, reproductive efficiency, zoometrics.

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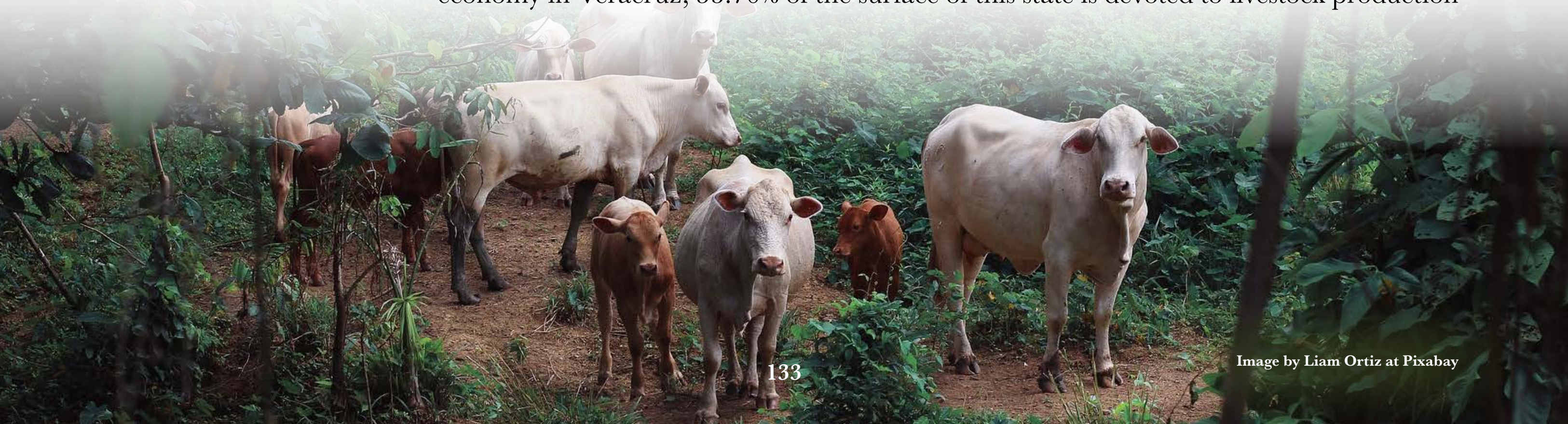
INTRODUCTION

The dual purpose cattle production system (DPCPS) is developed in most of the tropical regions of Latin America, and in this system extensive grazing is the most widely used (Vilaboa and Díaz, 2009). Meat and milk production depends on this system in this region (Orantes *et al.*, 2010). The DPCPS in Veracruz contributes to the national total of around 739 thousand tons of standing livestock and carcass and 745.30 million liters of milk annually. Therefore, livestock production is one of the activities that contributes most to the economy in Veracruz; 53.70% of the surface of this state is devoted to livestock production



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(PSAV, 2019). The DPCPS is characterized by having low productive and reproductive indices compared to the specialized animal production systems (beef and dairy cattle). One of these indices is the age at puberty, which can be defined as the moment when females physiologically start the capacity to reproduce (Estill, 2014). The onset of puberty is determined by age, and this process also depends on an undefined order of physiological conditions that result from a specific critical weight (Kenny *et al.*, 2018), for others, body development and nutrition are more important (Grajales *et al.*, 2006). The beginning of ovarian activity is a parameter that affects the fertility of the herd and this in turn affects the productive level of a livestock system (Osorio-Arce and Segura-Correa, 2002; Teyer *et al.*, 2003; Parra-Bracamonte *et al.*, 2005). Given the importance of this physiological event, this study identifies some factors that affect the beginning of the ovarian activity in heifers bred in DPCPS in the Huasteca Veracruzana (HV).

MATERIALS AND METHODS

The research work was carried out in the municipality of Tantoyuca, Veracruz, located in the mountainous zone of the HV, in coordinates 98° 14' Longitude West and 21° 21' Latitude North, at an average altitude of 140 meters above sea level. Its climate is warm-extreme with an annual average temperature of 23 °C; the mean annual rainfall fluctuates between 1,000 and 1,500 mm (INEGI, 2010).

The study was performed in the last six months of the year 2019, and 73 prepuberal heifers (12-24 months of age) were included, from nine livestock production units devoted to DPCPS in the municipality of Tantoyuca, Ver. The genetic groups of the animals sampled were: I. *Bos indicus*, II. *Bos taurus*, III. *B. indicus* × *B. Taurus* Cross, IV. Predominance of *B. indicus*, and V. Predominance of *B. taurus*. The dependent variables evaluated were: right ovary follicular activity (ROFA), left ovary follicular activity (LOFA), right ovary size (ROS), left ovary size (LOS), right ovary shape (ROSh) and shape of the left ovary (LOSh). The ROFA and LOFA were determined with the presence of follicles and *corpus luteum*. Three categories were established for ROS and LOS: I. (<1 cm), II. (1-2 cm), and III. (>2 cm). These variables were measured with ultrasound techniques (Mindray DP 10-VET). The ROSh and LOSh were determined through the rectal palpation technique, establishing three categories: I. Amorphous, II. Oval plane, and III. Oval cylindrical. In addition, the following values were found: body condition (Edmonson *et al.*, 1989), biometric measurements of the heifer's trunk (Fernandes *et al.*, 2010; Dubuc, 1991; Contreras *et al.*, 2011), proximity to the male (males and females together or separate), number of technological-productive practices implemented in herd management (nutrition-health index), and zoometric indices of anatomical development (Contreras *et al.*, 2011).

Data analysis

The systematization of information was digitalized in the Microsoft Excel 2016 software. The data were processed through a multiple linear regression analysis with the statistical package SAS version 9.4 and the REG procedure, in order to explain the possible relationships between independent and dependent variables. The independent or categorical variables were: ranch (X_1), race (X_2), age (X_3), body condition (X_4), proximity of male (X_5), nutrition-health index (X_6), thoracic index (X_7), body index (X_8), lateral body index (X_9), and transverse-pelvic index (X_{10}). Meanwhile, the dependent variables corresponded to: ROSh, ROS, ROFA, LOSh, LOS, and LOFA. A cluster analysis was carried out, which allowed identifying the management differences between the livestock production systems studied, and which in turn impacted the beginning of the ovarian activity; this was done through the statistical package R Studio Version 1.1.419.

RESULTS AND DISCUSSION

The regression equations that showed significant impact ($p<0.0001$), of the categorical variables (ranch, race, age, bodily condition, proximity to the male, nutrition-health index, thoracic index, bodily index, lateral body index, transverse-pelvic index) on the response variables were: LOS, LOFA, ROFA and ROS. Multiple determination coefficients were obtained with these equations, with values higher than 42%. The equations related to the response variables LOFA and ROFA showed a value of R^2 of 47%, which indicates a moderate adjustment of the data of the model designed for these equations only under LOS, which for these same models was significant ($p<0.0001$). This result could be consequence of the genetic mosaic found in the bovines studied and the variability in the nutritional and sanitary management that is given to the animals in the different livestock production units where the study was performed; in this regard, Heslin *et al.* (2020) found that heifers who had daily weight gains of 1 kg during 150 days began puberty at a younger age, and this is because a better diet allows a greater anatomical development and favors the physiological maturation as a result of higher contribution of energy and protein in the diet.

The model for the variable LOSh obtained an R^2 of 17% ($p=0.24$), and these results are the ones of lowest value of all the equations developed. The data related to this equation are not adjusted to the model and therefore lack practical importance in the conditions under which this study was carried out. For the case of ROSh, the model was significant with an R^2 of 30% ($p=0.0081$). This indicates that data adjustment regarding the model is not conclusive (Table 1).

The modelled variables ROFA and LOFA were affected primarily by the categorical variables, body index (BI) ($p=0.0002$), lateral body index (LBI) ($p=0.0009$), thoracic index (TI) ($p=0.001$) and age (A) ($p=0.01$). These two models were affected moderately by the categorical variable PM ($p=0.13$). The rest of the categorical variables showed a non-significant impact toward the models.

The onsert of the ovarian activity of the heifers raised in the dual-purpose system in the Huasteca Veracruzana is influenced by the anatomical development and chronological age, and concerning this Cardoso *et al.* (2021) mention that body weight and physical development are the main factors that determine the age at puberty, and they also indicate that they are closely related with the weight at birth, which can be compromised by nutritional deficiencies of the mother during gestation. Maternal nutrition is directly related to the functioning of the neuroendocrine system so that it can modulate the hypothalamus routes that control the secretion of the gonadotropin liberating factor.

Table 1. Multiple regression equations of ROFA, LOFA, ROS, LOS, ROSh and LOSh, R^2 and significance level.

Variable	Equation	R^2	Significance
ROSh	$y=5.43-0.004x_1-0.19x_2+0.05x_3-0.11x_4+0.93x_5+3.39x_6-0.007x_7-0.03x_8-0.02x_9-0.07x_{10}$	0.30	0.0081
ROS	$y=16.84+0.11x_1-0.009x_2+0.03x_3+0.33x_4+0.24x_5-1.91x_6-0.05x_7-0.08x_8-0.03x_9+0.02x_{10}$	0.42	0.0001
ROFA	$y=28.68+0.003x_1-0.001x_2+0.02x_3+0.01x_4-0.16x_5-0.46x_6-0.06x_7-0.13x_8-0.07x_9-0.009x_{10}$	0.47	0.0001
LOSh	$y=16.72+0.001x_1-0.11x_2+0.004x_3-0.25x_4+0.76x_5+1.95x_6-0.04x_7-0.07x_8-0.04x_9-0.02x_{10}$	0.17	0.2412
LOS	$y=40.12+0.13x_1-0.07x_2+0.02x_3+0.28x_4+0.47x_5-2.08x_6-0.11x_7-0.17x_8-0.09x_9-0.02x_{10}$	0.52	0.0001
LOFA	$y=28.68+0.003x_1-0.001x_2+0.02x_3+0.01x_4-0.16x_5-0.46x_6-0.06x_7-0.13x_8-0.07x_9-0.009x_{10}$	0.47	0.0001

Dependent variables: ROFA=right ovary follicular activity, LOFA=left ovary follicular activity, ROS=right ovary size, LOS=left ovary size, ROSh=right ovary shape and LOSh=left ovary shape. Independent variables: X_1=ranch, X_2=racial group, X_3=age, X_4=Body condition score, X_5=Proximity of male, X_6=nutrition-health index, X_7=thoracic index, X_8=body index, X_9=lateral body index, X_{10}=transverse-pelvic index.

The stimulating effect of the males' presence in the females' lots (Male effect) showed a positive impact on the onset of the ovarian activity. The fact of keeping females and males in the same lot of animals proved that the onset of the ovarian activity can be stimulated ($p=0.13$), in the bovines bred under the dual purpose system in the Huasteca Veracruzana (Table 2). In this regard, Diskin and Kenny (2014) reported that prepuberal heifers exposed to bulls advanced puberty; although the physiological mechanism is not well defined, the possibility has been proposed that the reduction in sensitivity to estradiol caused by masculine pheromones increases the secretion of the gonadotropin liberating factor and the luteinizing hormone.

Table 2. Significance of the parameters of the multiple regression equations of the categorical variables for the modeled variables ROFA, LOFA, ROS, LOS, ROSh and LOSh in bovines older than 12 months raised in the dual-purpose system in the Huasteca Veracruzana.

Variable	Categorical variables									
	R	RG	Ag	BCS	PM	NHI	TI	BI	LBI	TPI
ROSh	0.84	0.005	0.02	0.43	0.0009	0.0005	0.87	0.71	0.68	0.002
ROS	0.0002	0.9149	0.33	0.07	0.48	0.11	0.35	0.45	0.62	0.42
ROFA	0.68	0.95	0.01	0.75	0.13	0.22	0.001	0.0002	0.0009	0.36
LOSh	0.95	0.07	0.85	0.05	0.002	0.02	0.28	0.34	0.36	0.23
LOS	0.0001	0.38	0.41	0.10	0.15	0.07	0.05	0.09	0.12	0.46
LOFA	0.68	0.95	0.01	0.75	0.13	0.22	0.001	0.0002	0.0009	0.36

Modeled variables: ROFA=right ovary follicular activity, LOFA=left ovary follicular activity, ROS=right ovary size, LOS=left ovary size, ROSh=right ovary shape and LOSh=left ovary shape. Categorical variables: R=ranch, RG=racial group, Ag=age, BCS=Body condition score, PM=Proximity of male, NHI=nutrition-health index, TI=thoracic index, BI=body index, LBI=lateral body index, TPI=transverse-pelvic index.

Choudhary *et al.* (2020) researched the biostimulating effect of the presence of the male in Sahiwal heifers of 14 months of age in confining conditions, and they found that biostimulated females began ovarian activity five months before those that were not biostimulated ($p<0.05$). In addition, they reported that the contact with males affected positively the serum concentrations of progesterone (2.0 ng/mL^{-1}), in comparison to the control group with concentrations lower than 1 ng/mL ($p<0.05$). Rekwot *et al.* (2000) showed that biostimulation (exposure to vasectomized males) reduced the age at puberty in Bunaji and Friesian × Bunaji heifers managed in grazing; they found that biostimulated heifers began puberty at an age of 23.10±0.4 months, while the females that did not receive biostimulation began puberty at 26.40±0.4 months of age ($p<.05$). Fiol *et al.* (2010) found a higher proportion of cyclic animals at the end of the exposure period in Aberdeen Angus × Hereford crossed heifers (Age 11 months, under grazing conditions), exposed to androgenized steers, in comparison to females that were kept isolated from the males; they also determined that the age at puberty of females exposed to males was lower compared to those not exposed (428 *vs* 441 days) ($p=0.06$). Therefore, biostimulation would be efficient to reduce the age at puberty in heifers of different genetic characteristics and under different management conditions.

Oliveira *et al.* (2009) showed a reduction in age at puberty, age at first season of mounting, age at pregnancy, and higher rate of pregnancy in Nelore heifers supplemented and not supplemented exposed to the bull at prepuberal phase. Thus, the degree of development, the nutritional status, and the rates of gain have a direct relationship with the age at puberty in meat heifers (Diskin and Kenny, 2014). The significance value of the categorical variable racial group (RG) was $p=0.95$ (Table 2), which indicates that in this study the genetic characteristics did not have an impact on the onset of the ovarian activity. These results are set against what was reported by Rekwot *et al.* (2000), who found that the F1 Friesian × Bunaji heifers bred in grazing began puberty at 23.6 months of age, while the lot of pure breed Bunaji females began puberty at an age of 26.1 months ($p<0.05$).

The modelled variables ROSh and LOSh were influenced significantly by the categorical variables, nutrition-health index (NHI) ($p=0.0005$), proximity to the male (PM) ($p=0.0009$), transverse-pelvic index (TPI) ($p=0.002$), GR ($p=0.005$) and A ($p=0.02$). The rest of the categorical variables showed low relevance on the modelled variables. This could indicate that the shape of the ovaries depends on the nutritional and health management that they receive since their breeding stage, until the beginning of their reproductive stage, and that this is all directly related with its anatomical development and physiological maturity. The modelled variable LOS was affected significantly by the categorical variable TI ($p=0.05$), followed by the effect of the categorical variable NHI ($p=0.07$) and with lower effect the categorical variable BI ($p=0.09$); however, it was found that the size of the ovary was not significantly affected by the PM variable ($p=0.15$). These results are contrary to those reported by Choudhary *et al.* (2020) who mention that biostimulation affects the number of small follicles present in Sahiwal heifers of 14 months of age under confinement conditions. These authors found that the groups of females not exposed to males produced a lower number of small follicles in comparison to the groups of biostimulated females with the presence of the male ($p<0.05$).

The cluster analysis formed two main groups (I and II). Group II showed the best behavior of the modelled variables while group I presented a low behavior for these same variables (Figure 1). Within group II, the best behavior corresponded to livestock production with animals of the racial group *Bos taurus* × *Bos indicus*, 14 months of age, body condition of 2.75, and the females with ovarian activity were concentrated in this group. Meanwhile, in group I livestock of low behavior was for individuals with amorphous ovaries, of smaller size and absence of ovarian activity (Figure 1).

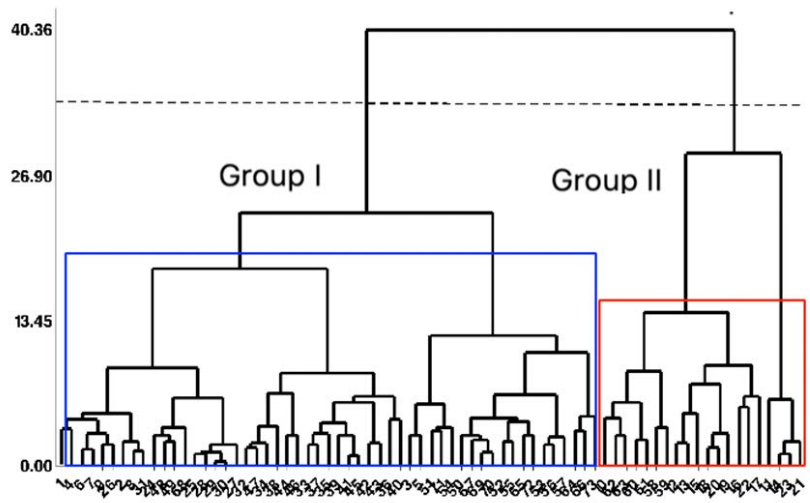

Figure 1. Dendrogram of dependent variables (shape, size and follicular activity of right and left ovary) and categorical variables (ranch, racial group, age, body condition score, proximity of male, nutrition-health index, thoracic index, body index, lateral body index, transverse-pelvic index) for the dual-purpose bovine system in the Huasteca Veracruzana.

CONCLUSIONS

The heifers reared in the dual-purpose system in the Huasteca Veracruzana could begin the ovarian activity at a younger age when their anatomical development, expressed in zoometric indexes, are of higher value and when the chronological age is over 14 months. The biometric measurements of heifers are a useful tool as parameter for the selection of replacements when the intent is to improve the sexual precocity of the herd. The husbandry practices designed to reduce the age at puberty should be directed at improving the quality of the diet. There is a need to research alternatives and practices of viable nutritional management (ecologically and economically) for replacement females in the DPCPS, and with this to guarantee an anatomical development in agreement with their genetic potential. The genetic improvement programs designed to reduce the age of puberty could lack effectiveness if the breeds or racial groups evaluated in this research are used. Biostimulation (presence of the male) is a useful tool to reduce the age at which the ovarian activity of heifers begins.

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