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AGRO PRODUCTIVIDAD

First report of *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae) in apaxtleco chili (*Capsicum annuum* L.) cultivated in greenhouse

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Perenial ryegrass (*Lolium perenne* L.) yield as a response to fitoregulators produced in digestates

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ABSTRACT

Objective: To assess the effect on ryegrass (*Lolium perenne* L.) as a response to phytoregulators produced in digestates obtained from the anaerobic digestion of cow manure, at different initial pH.

Design/methodology: Anaerobic cow manure digestions were set up at different initial 5, 6.5, 7.5 and 8.5 pH values and 4, 8 and 20 days of digestion, from these, gibberellic acid (AG_3) and indole acetic acid (IAA) were quantified. The digestates were applied to ryegrass grown in pots: a) on 6 months pastures applying all the digestates and b) on 45 days pastures applying digestates at 4 days of digestion. The assessed variables were height, fresh and dry weight and number of stems. The control was developed on unfertilized soil.

Results: The initial pH of the digestion influenced the production of phytoregulators, being higher at pH 5.5 and 6.5; no IAA production was recorded at basic pH. The application of the digestates had a different effect depending on the pastures age, was greater on the leaf weight variable. In 6 months pastures the increase was between 21 and 24%, in young pastures from 48 to 115% respect to the control. Likewise, there were between 50 and 60% greater number of stems than in the control, applying digestate at 4 days of digestion.

Limitations/Implications: The study took place on ryegrass, it would be of interest in the area to evaluate it in other crops.

Findings/Conclusions: The initial manure pH has a higher effect on the digestates properties as well as the time of digestion. Digestates can be a fertilizer for ryegrass, its effect is better in young grasses. The digestate even with 4 days of digestion has a positive effect on ryegrass development.

Keywords: Lolium perenne, anaerobic digestion, phytohormones

INTRODUCTION

The importance of forage production lies in several aspects, for example they are food production systems for livestock, they influence the mitigation of climate change and serve as prevention and fire control. Forage yield relates to environmental factors and management practices. To increase the profitability of the agricultural sector, producers must make efficient use of the pasture resource, including intensifying forage production per area; as well as the search for forage species that meet the nutritional requirements of animals and establish a harvest system ensuring a constant production throughout the year (Araya-Mora and Bochini-Figueroa, 2005).

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An alternative to increase production is organic fertilizers, such as digestate. This is a by-product of the anaerobic digestion of organic solid waste, classified as a fertilizer due to its nutrient content and the presence of phytoregulators (Xin *et al*., 2016). The latter regulates physiological processes in plants and reduce the effects of biotic and abiotic stress (Vega-Celedon *et al*., 2016). The usage of plant hormones as growth promoters has increased (Sebastian *et al*., 2019); however, due to their high production cost, different production methods have been sought, for example, submerged fermentation or solid-state fermentation (Rodrigues *et al*., 2009), as well as via anaerobic digestion (Moller and Muller, 2012).

The perennial ryegrass (*Lolium perenne* L.) is one of the most used grasses for livestock production in temperate zones of Mexico, due to its high yields, nutritional quality, and ease of growing in different types of soil (Velasco-Zebadúa *et al*., 2002); however, there is little scientific information in the use of phytoregulators contained in digestates as promoters of plant growth in grasses. Therefore, in this research, the development and performance of ryegrass were evaluated as a response to phytoregulators produced in digestates, obtained from anaerobic digestion of cow manure, adjusted to different initial pH values and considering different days of digestion.

MATERIALS AND METHODS

The cow manure was donated by the Instituto Tecnológico del Altiplano de Tlaxcala, collected fresh at the bovine unit. For its characterization, its pH, C/N ratio, total solids and volatile solids were assessed. The evaluations were done following APHA normative (APHA, 2017).

Anaerobic digestions were established in 135 mL serological bottles, by triplicate, with 7% solids and initial pH of 5.5 (DA1), 6.5 (DA2), 7.5 (DA3) and 8.5 (DA4). The oxygen elimination was done by introducing nitrogen in the bottles, hermetically closed and kept for twenty days in incubation at 37 °C. At 4, 8, 12, 16 and 20 days, the pH, indole-3 acetic acid (IAA) and gibberellic acid (AG_3) were measured by high-performance liquid chromatography (HPLC Hewlett Packard) with a diode arrange sensor, an Eclipse XDB-C18 (4.6 mm ID × 250 mm 5) column was used following Teniza-García *et al*. (2015).

Two evaluations of the digestates were made in perennial ryegrass (*L. perenne*):

The first evaluation was in a 3×4 design, using digestates obtained at three different times of anaerobic digestion (4, 8 and 20 days) and at the 4 initial pH values for digestion (5.5, 6.5, 7.5 and 8.5), with five repetitions for each treatment. Ten grass seeds were sown in plastic containers with one kilogram of soil and kept in a greenhouse with irrigation. At 6 months, a uniformization cut was made at 5 cm and later fertilized by supplying 35 mL of digestate at the base of the tiller. Every 5 weeks of the development, a cutting was done again at 5 cm and then fertilized with digestates; this process was repeated every five weeks. The controls were only filled with soil.

The second evaluation was made with digestate at 4 days of the digestion process, and at the 4 initial pH values. The grass grown in plastic containers with one kilogram of soil was used, five grass seeds were sown and kept in the greenhouse with irrigation. After 45 days, a uniformization cut was made at 5 cm and later, 35 mL of the corresponding digestate was added to the base of the tiller. Every 5 weeks, a 5 cm cut was made again along with subsequent fertilization with digestate; this was repeated three times every five weeks. Five repetitions per treatment were made. In both evaluations, the height of the forage was weekly assessed. The forage yield was obtained by weighing the collected material on a Sartorius analytical balance, separating into green and senescent leaves, weighed fresh and later dehydrated in a forced air oven at 65 °C until constant weight, then

the value of the dry matter was recorded. At week five, the number of stems in each pot was counted.

The obtained results were analyzed with the PROC GLM procedure of the SAS® Statistical Software Version 9.0 for Windows®. Treatment means were compared using the Tukey test at a 5% significance level.

RESULTS AND DISCUSSION

Anaerobic digestions

Production of gibberellic acid. The measured amounts of gibberellic acid are shown in Figure 1. The modification of the initial pH changed the AG_3 production, this was higher in the DA1 and DA2 digestions, where digestion began in acidic pH values (5.5 and 6.5); in these digestions, this acid was detected from the beginning and until day 20. The maximum AG_3 production was registered in DA2 it was 85.3 mg/L. In all digestions, gibberellic acid was recorded four days after the digestion started, 38.01, 40.07 and 25.8 mg/L for DA2, DA3 and DA4, respectively. Xin *et al.* (2016) reported AG_3 production of 18 mg/L using chicken manure, 16 using cow manure and 47 mg/L with pig manure.

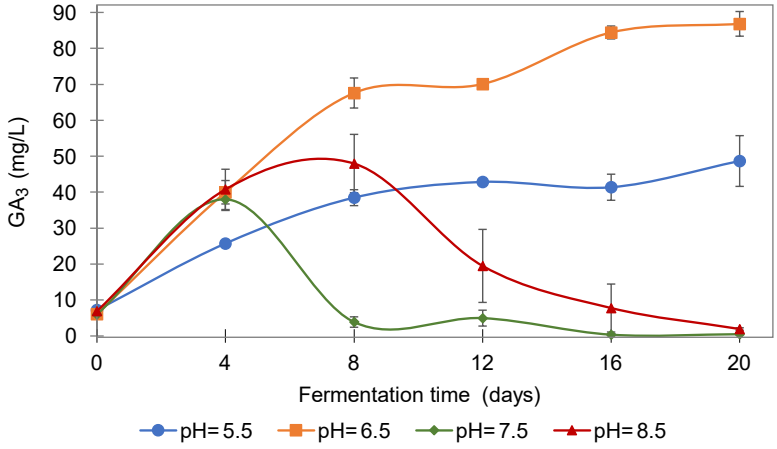

Figure 1. GA_3 quantification in the DA1 (pH=5.5), DA2 (pH=6.5), DA3 (pH=7.5) and DA4 (pH=8.5) digestions.

Production of indole acetic acid (IAA). In Figure 2 the values of IAA are shown, the variation of the initial pH affected the IAA production, this was registered only in the two digestions with initial acid pH (DA1 and DA2). In both, the maximum quantified was at day four after digestion started, 50.5 mg/L and 0.98 mg/L were quantified for DA1 and DA2 respectively. In the treatments that started with a basic pH value (DA3 and DA4) there was no IAA formation. Xi *et al.* (2016) performed digestions with different manures, and report IAA production of 12 mg/L, 22 and 21 mg/L using chicken, cow and pig manure respectively; these indicated that the nutritional characteristics of the digestate depend on the initial pH. Scaglia *et al.* (2015) reported 9.94 mg/L of IAA using pig manure.

Evaluation of digestates at 4, 8 and 20 days of fertilization. The average heights of the pasture are shown in Figure 3. Using the digestates obtained after 4 days of digestion in week 5, DA3 produced higher heights than that in the other treatments, these were 14% higher than the control. Fertilizing with the digestate obtained at 8 days of digestion, with DA2, DA3 and DA4, between 11 and 12.6% higher heights were recorded in week 5 compared to the negative control. While in the case of application of the digestate obtained at 20 days of digestion, with DA4 in week 5, they showed a significant difference ($p>0.05\%$), being 12% higher than those of the other treatments and the negative control.

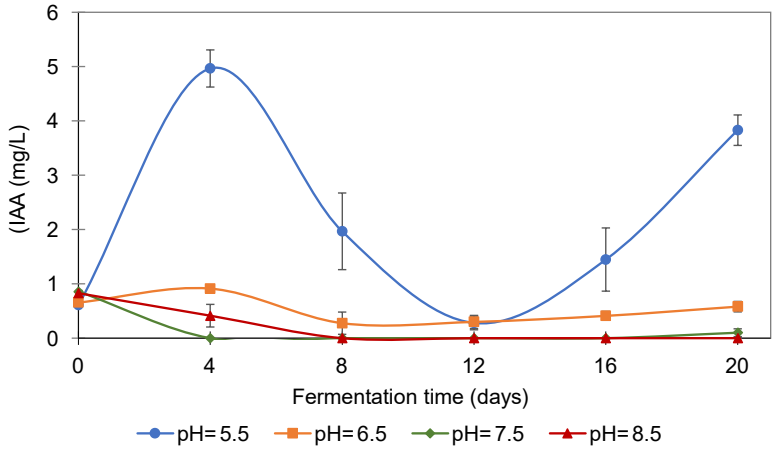

Figure 2. IAA quantification in DA1 (pH=5.5), DA2 (pH=6.5), DA3 (pH=7.5) and DA4 (pH=8.5) digestions.

Figure 4 shows the total accumulated biomass of the pasture, this was significantly higher ($p<0.05$) applying the digestates compared to the negative control, except using the digestate that was obtained after 4 days of digestion with an initial pH of 5.5 (DA1), in this case, the height equal to the control. The maximum biomass values were recorded in DA1, using digestate after 8 and 20 days of digestion, DA3 with digestates of 4 and 20 days of digestion and with DA4 fertilizing with the digestates obtained at the three times of digestion. Using these digestates increased between 21 and 24% respect to the negative control. In DA2, at all digestion times,

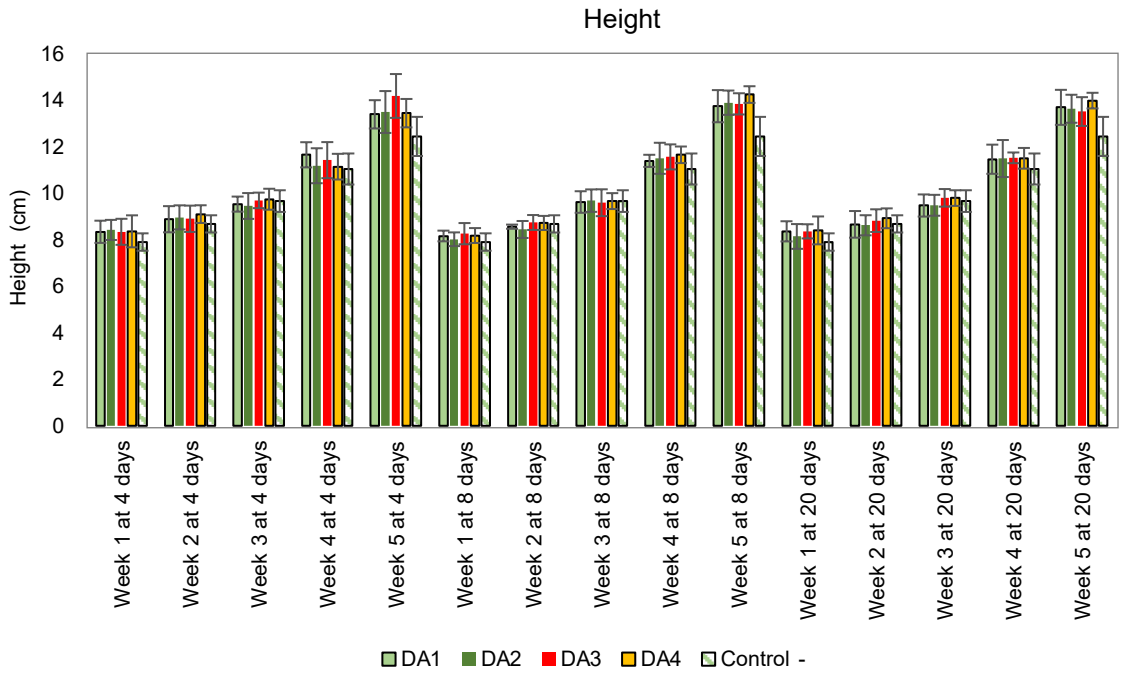

Figure 3. Average height of the grass applying digestate after 4, 8 and 20 days of digestion. Bars represent standard deviation.

lower heights were obtained than the above-mentioned treatments, but higher than with the negative control. This contrasts is observed in Figures 1 and 2: that in DA3 and DA4 at 20 days of digestion there was no AG_3 or IAA presence. This indicates that the digestate in addition to the phytoregulators contains other components that helped the development of the grass, as explained by Xin *et al*. (2016) who indicate that the positive effect of digestate on plants has been explained by its macronutrient content and the presence of phytoregulators. Likewise, Moller and Muller (2012) reported that digestates contain bioactive substances such as phytohormones with the potential to promote plant development.

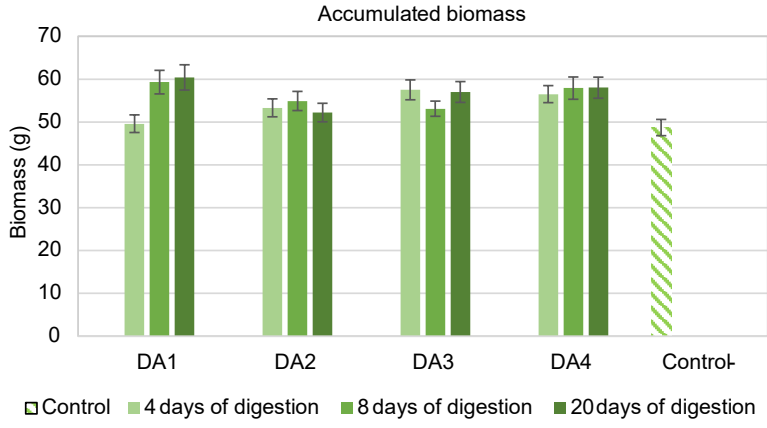

Figure 4. Total accumulated biomass applying the digestates DA1 (pH=5.5), DA2 (pH=6.5), DA3 (pH=7.5) and DA4 (pH=8.5) and in the control. Bars represent standard deviation.

The results concur with those reported by Tempere and Viiralt (2014) and Walsh and Rousk (2012) who found that when applying digestates, there was a higher yield in grasslands than were without no fertilization or chemical fertilizers.

According to the results in figures 3 and 4, 4 days of digestion was selected to make a second ryegrass fertilization evaluation, because fertilizing with the digestate obtained after 4 days of digestion had a similar effect in the development of the grass than using digestate of longer fermentation time (20 days).

Digestate evaluation at 4 days of fermentation

Figure 5 shows that during the second week, all the plants fertilized with digestate were statistically higher than the negative control; During the third and fourth weeks, the DA3 plants were statistically higher than those from the control; while in week 5, using the DA1, DA2 and DA3 digestates, taller plants were obtained compared to the negative control. This increase that was registered during week 5 was 12 to 16% higher than that of the control. This point that digestates, regardless of the digestion process, were useful to fertilize grasses. This is partially explained by that reported by Small *et al*. (2019), who indicate that gibberellins have positive effects on plant development

by controlling cell elongation. It is also observed that in the negative control there was no increase in height from week 4 to 5, while applying the digestate the growth was constant.

Table 1 shows the accumulated weight of fresh and senescent leaf and mean stem number. It is observed that there was a significant increase in biomass applying the four digestates, it was 115% applying DA2, 90% with DA1, 81% using DA3 and 48% with DA4 compared to the negative control. In the case of the dead leaves, a greater quantity was obtained in the negative control, between 56 and 69% more compared to the treatments where digestates were applied. It is to be noted that DA1, DA2 and DA3 digestates supply has between 50 and 60% higher stem production compared to the unfertilized control. These results concur with those reported by Eickenscheidt *et al.* (2014), who report that the application of digestate to a plot with grass increased its performance compared to the application of liquid manure.

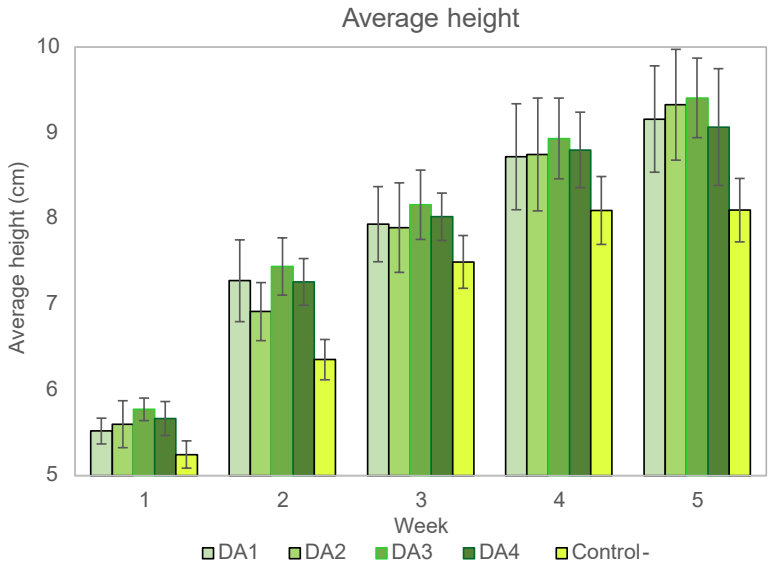

Figure 5. Average heights of the plants supplied with a digestate of 4 days of digestion DA1 (pH=5.5), DA2 (pH=6.5), DA3 (pH=7.5) and DA4 (pH=8.5).

For their part, Eich-Greatorex *et al.* (2018) report that the addition of digestates produced a similar yield in biomass production in two types of grass, Italian grass (*Lolium multiflorum*, var. Macho) and canary red grass (*Phalaris arundinacea*), compared to chemical fertilization; they also reported that the digestate contributed to improving the pH for plant development, reduced soil density of and increased water retention.

It has been reported that the regrowth capacity of a plant, after harvest or defoliation, is influenced, among others, by physiological factors such as the accumulation of carbohydrate reserves in the root, the remaining leaf area and the growth meristems activation (Pérez *et al.*, 2002). The increase in the number of stems when applying digestate is a crucial factor since it has been reported that the persistence of the pasture is directly determined by the combined effect of the seasonal pattern and stems mortality. In a perennial ryegrass meadow, both its persistence and forage production depend on the balance between the emergence rates and the death of the stems (Ramírez *et al.*, 2011).

As reported by Small *et al.*, (2019) the digestates in addition to increasing the grass yield to be used as forage helps grassland areas recovery.

CONCLUSIONS

The production of AG_3 and IAA in the digestates is influenced by the initial pH of the manure, the acid pH being better (5.5 and 6.5). The digestates obtained from the anaerobic digestion of cow manure can be used as fertilizer in perennial ryegrass. Its effect on the development of ryegrass applied in pastures with 6 months or 45 days of the establishment was different, the variable in which had the most influence was the leaf weight. It is shown that the obtained results using digestate after four days of digestion are like those obtained after eight or twenty days, reducing the digestion time to only four days, can have an important economic impact since the expenses to carry out the anaerobic digestion will be less than leaving the process for a longer time.

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Table 1. Weight of fresh and senescent leaf and number of stems of perennial ryegrass plants DA1 (pH=5.5), DA2 (pH=6.5), DA3 (pH=7.5), DA4 (pH=8.5) and the control.

	Green leaf weight (g)	Senescent leaf weight (g)	Average number of steams
DA1	15.47	0.11	38
DA2	16.80	0.16	37.8
DA3	14.20	0.14	38
DA4	11.60	0.16	35.6
control -	7.82	0.37	23.6

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Fertility in Cebú × brown Swiss cows treated with prostaglandins, progesterone and eCG

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ABSTRACT

Objective: To evaluate the application of one or two doses of prostaglandin F2α (PGF2α), the application of a progestogen on different days of the estrous cycle and the usage of eCG in the estrus synchronization and fertility of Zebu × Brown Swiss cows.

Design/methodology/approach: The study was based on three protocols. The first consisted of two treatments: PGI) 26 cows were injected with a single 25 mg dose of PGF2α and 10 cows with two 25 mg doses of PGF2α at a 14-day interval. Protocol 2 consisted of two treatments: NG14) 11 cows were implanted with 3 mg of Norgestomet on day 7 of their estrous cycle and NG7) 11 animals received the same dose on day 14. In protocol 3 all cows were implanted with 3 mg of Norgestomet for 9 days, 48 h before removing the implant, 25 mg of PGF2α was applied. Once the implants were removed, they were distributed into two treatments. Norgestomet (n=11) without eCG and Norgestomet + eCG (500 IU) (n=11).

Results: The application of PGF2α at two times had no influence ($p>0.05$) in the estrous percentages and conception. The NG7 achieved estrous synchronization in 81.8 % of the cows, in between 24 and 36 h, compared to 45.4 % of the NG14; however, the conception rate was lower ($p\leq0.05$). The eCG application synchronized 90.9 % of estrous between 24 and 36 h, compared with 36.4 % of the group with no eCG application.

Study limitations/implications: Transrectal ultrasounds are required to assess the ovarian structures present at the time of the estrus onset in a synchronization protocol.

Findings/conclusions: Cows that present *corpus luteum* do not require more than one injection of PGF2α, the pregnancy percentage increases when Norgestomet is implanted on day 14 of the estrous cycle, in addition the application of eCG increases the synchronization percentage of the heat between 24 to 36 h after the progestogen withdrawal.

Key Words: Estrus synchronization, Fertility, eCG, PGF2α, Norgestomet, Zebu × Swiss brown.

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INTRODUCTION

The use of estrus synchronization protocols (ESC) and artificial insemination (AI) in cows are common practices to improve fertility, reproductive parameters and accelerate genetic improvement of a herd. The objective of the estrus synchronization protocols is to control the life of the *corpus luteum*, as well as the cycle and emergence of follicular waves (Ayres *et al*., 2013). To achieve this, hormones as PGF2α, GnRH (Wiltbank *et al*., 2015), progestogens (Filho *et al*., 2009) and equine chorionic gonadotropin (eCG) (Lucy *et al*., 2004) are used. In tropical regions of Mexico, given their aptitudes for meat and milk production, Brown Swiss cattle and their crosses with zebu breeds, resistant to parasites and heat stress, are preferred by cattle ranchers; however, the reproductive efficiency of the herds in the tropics is usually low. For Mexico, a 14-month interval between births is reported (Hernández *et al*., 2001), which may be due to longer gestation periods, low postpartum body conditions and the calf's presence, which causes reduced frequency LH pulses in the cows (Filho *et al*., 2009). Consequently, the use of estrus synchronization protocols is particularly useful, as it guarantees that the cows are inseminated at detected estrus or under a fixed-time insemination scheme (IATF). Yet, the information regard to the use of synchronization and fertility protocols for Zebu × Brown Swiss cattle is limited. The objective here was to evaluate the application of 1 or 2 prostaglandins doses, the use of progestogen on a different day of the estrous cycle and the eCG application in the estrus synchronization and fertility of Zebu × Brown Swiss cows.

MATERIALS AND METHODS

Location: The study took place at the Experimental Unit "El Gargaleote", of the Universidad Autónoma Chapingo. The experimental unit is in Tamuín, San Luis Potosí, Mexico; between 21° 10' and 24° 29' N and 98° 20' and 102° 18' W, at 20 masl and with a 25.8 °C average temperature (García, 2004).

Animals and feeding: Zebu × Brown Swiss cows (n=83) from 3 to 6 years of age, with a live weight of 517±43 kg, healthy and with *corpus luteum* (CL) greater than 12 mm were used. The size of the CL was determined by transrectal ultrasonography (SONOVET 600®, Medison equipped with a 7.5 - Mhz transducer, Aloka 210, Corometrics Medical Systems, Wallingford, CT). The cows diet was based on forage, in a pasture of Star of Africa (*Cynodon nlemfuensis*) and Bermuda (*Cynodon dactylon*) grasses.

Protocols and experimental design

Three estrus synchronization protocols were evaluated.

Protocol 1: 36 cows were used, assigned to one of two groups, PGI and PGII. The PGI cows (n=26) received an I.M. shoot of 25 mg of dinoprost (Lutalyse, Pfizer), on a random day of their estrus cycle. The PGII cows (n=10) received two I.M. at 14-day intervals. The first injection was given at the same time as the PGI group cows.

Protocol 2: Twenty-two cows received a 25 mg dinoprost I.M. injection. The cows were monitored for estrus detection during the morning (06:00 to 10:00 h) and afternoon (16:00 to 19:00 h) for three days after the dinoprost application. All 22 cows showed estrus, considering estrus as day zero of their estrous cycle and were assigned to one of two groups: NG7 and NG14. The NG7 group cows (n=11) were subcutaneously implanted in the middle part of the ear with 3 mg of norgestomet (Crestar®, Intervet México, S.A.) on day seven after the estrus. The implant was removed nine days after placement and each received an injection of 25 mg of dinoprost. The cows of the NG14 group received the same treatment as those of the NG7, except that the application of the implant was carried out on day 14 of the estrous cycle.

Protocol 3: Cows (n=25) received a 25 mg dinoprost I.M. injection. The cows were monitored for estrus detection in the morning (06:00 to 10:00 h) and the afternoon (16:00 to 19:00 h) for three days, after the application of dinoprost. Estrus was detected in 23 of the 25 cows injected with dinoprost. The day of estrus was designated as day zero. Cows that presented estrus were assigned to one of two groups: Norgestomet (n=11) and Norgestomet + eCG (n=12). The cows of both groups received an implant with 3 mg of norgestomet (Crestar®, Intervet México, S.A.), on day 14 of the estrous cycle, by s.c. in the middle of the ear. The implant was removed nine days after its placement. The cows received a 25 mg injection of dinoprost seven days after implant application. On the implant removal day, the cows in the NG + eCG group received an I.M. of 500 IU of eCG hormone (500 IU; Folligon, Intervet México, S. A.), while those in the NG group received an injection of saline solution as a placebo.

In the three evaluated synchronization protocols, heat detection took place during the three consecutive days after the dinoprost application (protocol - 1), or after removing the implant (protocol 2 and 3), during the morning (06:00 at 10:00 a.m.) and afternoon (4:00 p.m. to 7:00 p.m.). Cows were then inseminated, following the a.m. - p.m. system, by the same inseminator, using conventional semen from a certified company. The pregnancy diagnosis was made via ultrasound 45 days after the artificial insemination.

The estrus percentage, distribution and conception percentage were evaluated. The estrus percentage was defined as the number of cows that presented estrus among the number of synchronized cows per hundred. The estrus distribution was defined as the number of cows that showed oestrus between 24 and 36 h and 37 to 48 h after the prostaglandins application (experiment 1) and the progestin withdrawal (experiments 2 and 3); while the conception percentage was defined as the number of pregnant cows among the number of inseminated cows.

Statistical analysis

The data were analysed with a model adjusted for a completely randomized design, where the incidence of estrus and percentage of conception variables were analysed using the Chi-Square test in the SAS statistical software for Windows version 9.3 (SAS, 2011).

RESULTS AND DISCUSSION

Protocol 1

Table 1 shows the estrus (84.6 *vs*. 100%) and conception rates (54.5 *vs*. 50%) under protocol 1. There was no statistical difference ($p \geq 0.05$) between treatments when applying one or two PGF2α doses, respectively.

Protocol 2

Prior to the treatment's application, all the cows had an average 14 mm *corpus luteum*. The PGF2α application achieved that all the animals presented estrus. After this, it was noted that the Norgestomet application on day 7 or 14 of the estrous cycle did had no influence on the number of cows that presented estrus ($p > 0.05$); although in 81.8% of the cows to which the progestogen was implanted on day 7 of the estrous cycle reported estrus between 24 and 36 h after the progestogen withdrawal, compared to 45.4% of the cows to which Norgestomet was implanted on day 14 of the estrous cycle ($p = 0.07$; Table 2). The remaining 18.2% of the cows implanted on day 7 of the estrus cycle presented estrus 37 to 48 h after the implant removal, compared with 54.6% of the cows implanted on day 14 of the cycle ($p = 0.07$). A higher conception percentage was observed in cows implanted with Norgestomet on day 14 of the cycle ($p < 0.05$; 81.8%) compared to those that were implanted on day 7 of the estrous cycle (36.4%).

Protocol 3

No effect was found of the application of 500 IU of eCG to the withdrawal of the progestin in the number of cows that reported estrus ($p > 0.05$); however, the eCG grouped 90.9% of cows in estrus between 24 and 36 h compared with 36.4% of cows without the eCG application ($p \leq 0.05$; Table 3). The 9.1 % of the remaining cows with the application of eCG showed estrus between 37 and 48 h after the removal of the implant compared to 63.6% of the cows without the application

Table 1. Estrus incidence and conception percentage in dual-purpose cows treated with one or two prostaglandins injections.

	PGI	PGII
Estrus percentage	22/26 (84.6%)[a]	10/10 (100%)[a]
Conception rate	12/22 (54.5%)[a]	5/10 (50%)[a]

[a,b] Means in the same row with the same literal are statistically different ($p < 0.05$). PGI: single injection of 25 mg prostaglandin (dinoprost) PGII: double injection of 25 mg of prostaglandin (dinoprost) with an interval of 14 days.

Table 2. Estrus distribution and pregnancy percentage in dual-purpose cows treated with Norgestomet on day 7 and 14 of their estrus cycle.

Estrus distribution	NG7	NG14
24-36 h	9/11 (81.8%) [a]	5/11 (45.4%) [a]
37-48 h	2/11 (18.2%) [a]	6/11 (54.6%) [a]
Total	11/11 (100%) [a]	11/11 (100%) [a]
Pregnancy percentage	4/11 (36.4%)[b]	9/11 (81.8%)[a]

[a,b] Means in the same row with different literals are statistically different ($p < 0.05$). NG7: Norgestment implant on day 7. NG14: Norgestment implant on day 14.

Table 3. Estrus distribution and percentage of pregnancy in dual-purpose cows treated with eCG at progestin withdrawal.

Estrus distribution	Norgestomet	Norgestomet + eCG
24-36 h	10/11 (90.9%)[a]	4/11 (36.4%)[b]
37-48 h	1/11 (9.1%)[b]	7/11 (63.6%)[a]
Total	11/12 (91.6%)[a]	11/11 (100%)[a]
Conception rate	9/11 (81.81%)[a]	6/11 (54.54%)[a]

[a,b] Means in the same row with different literals are statistically different ($p < 0.05$). T1: Norgestoment. T2: Norgestoment more 500 IU of eCG.

of eCG ($p \leq 0.05$). Although no statistical differences ($p > 0.05$) were observed in the percentage of conception, a numerical trend of 81.8 *vs*. 54.5% of cows that became pregnant with the application of eCG compared to the cows that did not receive an application of eCG, each.

A 100% of the cows that received double PGF2α application showed estrous compared to 84.6% in the cows that received a single dose. Previously, 100% of estrus in cows has been reported when applying two injections of prostaglandins at 11 days intervals (Sahatpure and Patil, 2008), although the fertility rate with the usage of prostaglandins is lower (Sales *et al*., 2011); likewise, the percentage of estrus were higher than the results reported by Ramana *et al*. (2013) who obtained 82% of estrus, when two injections of PGF2α were applied. PGF2α is effective when supplied between days 8 and 17 of the estrous cycle and when there is a functional *corpus luteum* in one of the two ovaries (Dejanette and Marshall, 2003). Using 35 mg of PGF2α after the application of GnRH (Pursley *et al*., 1997) can cause luteolysis, whereas using PGF2α in cyclic cows one day before and on the day of progestin insertion, reported no difference ($p > 0.05$) in the conception rate (Xu and Burton, 2000). At the same time, two PGF2α applications in Zebu cattle modify the conception rate by 67% (Ramana *et al*., 2013). The results in the present study show that in cows with the presence of a *corpus luteum* greater than 12 mm, two prostaglandins application doses are not necessary to increase the conception percentage; however, when the day of the estrous cycle is unknown, two PGF2α injections can be applied at 14 days intervals to increase the number of individuals in estrus.

The placing of the Norgestomet implant on day 7 or 14 of the estrous cycle had no influence the number of cows that showed estrus, however, it did modify the time of estrus onset, were 81.8% of the cows that were implanted on day 7 of their cycle showed estrus 24 to 36 h after the progestin withdrawal. In another study in Zebu cows implanted with Norgestomet, the estrus percentage was of 78 to 100%, with heats happening at 84.3% of the individuals 72 h after the removal of the implant (Singh *et al*., 1998). The application of PGF2α 48 h before withdrawing the progestogen also reports an increase in the pregnancy and delivery rate (Mialot *et al*., 1998). Due the fact that the conception percentage increased when inserting the progestogen on day 14 of the estrous cycle. The application of the eCG hormone was tested upon withdrawal of the progestin, reporting that the estrus presence did not change ($p > 0.05$). However, the eCG application when the progestin was withdrawn succeeded in synchronizing 90.9% of the cows between 24 and 36 h after withdrawal. Likewise, the percentage of conception increased from 54.5 to 81.8% with the application of eCG upon withdrawal of the progestogens. eCG hormone should be used when removing the implant (Baruselli *et al*., 2004) because it improves the ovulatory and conception rates (Duffy *et al*., 2004). In another study, on Zebu × Holstein cows treated with a Norgestomet implant for 9 days plus 500 IU of eCG, the presence of estrus during the first 26 h after the end of hormonal treatment was observed, and the conception rate was 61.5% (Soto *et al*., 2002), which is lower than the 81.8% found in the present study. In *Bos indicus* cows, the administration of 300 IU of eCG at the time of progestin withdrawal increased the conception rate by 76.6% compared to other hormonal protocols (Campos *et al*., 2013). Likewise, the results of the present study were superior to those reported in another research where the conception rate of zebu cows was 55.7% when 400 IU of eCG was supplied at the progestogen withdrawn (Villa *et al*., 2007); however, the higher conception percentage found in the present experiment could be because cycling cows were used. The administration of eCG at the end of the treatment with progestogens allows to advance and synchronize the estrus, so its usage is recommended to improve the pregnancy rate, because it increases the diameter of the dominant follicle and the concentration of progesterone (Dorneles *et al*., 2013).

CONCLUSIONS

Ovaries with active *corpus luteum* do not require two injections of PGF2α, while the use of Norgestomet in Zebu × Brown Swiss cycling cows is recommended to be applied on day 14 of the estrous cycle, but when greater estrus synchronization is required, the use of eCG is desirable at withdraw the progestogen.

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Bibliometric analysis of scientific research on biochar

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ABSTRACT

Objective: To identify the most relevant aspects of global scientific research on biochar in terms of number of articles published, main authors and publishing countries, citation, main issues of scientific journals, funding institutions and general trends.

Design/Methodology/Approach: A bibliometric study was carried out in the Scopus database. The word "biochar" was used in the search engine. The search was limited to articles and reviews published from 2009 to March 2020. The VOS viewer software was used to identify the main thematic axes and to glimpse the knowledge gaps that exist to date.

Results: A total of 11,444 documents were identified. The trend of work on biochar is on the rise. China and the United States are the countries with the highest number of publications on biochar. Jefferson Lehman and Stephen Josephs are the most cited authors on the subject. Global research on biochar focuses on the mitigating effect of climate change and good properties that biochar has to improve physicochemical properties of the soil. Research on biochar in Mexico is scarce.

Study Limitations/Implications: Biochar is a new technology that is not fully understood.

Findings/Conclusions: Interest in biochar as a multifaceted solution to agricultural and environmental problems is growing at a rapid rate both domestically and internationally.

Keywords: bibliometric analysis, biochar, literature review, Scopus, VOS viewer.

INTRODUCTION

Biochar is a solid material that is a product of the thermochemical conversion (pyrolysis) of biomass at over 250 °C in a total or partial absence of oxygen. The result of this process is a low density porous material, rich in carbon, with ample specific surface area, high CEC, generally alkaline, and very resistant to physicochemical and biological degradation (Lehmann and Joseph, 2009). Biochar is produced with the purpose of generating a soil enhancer and as a carbon sink.

Because of the nature of biochar, it possesses properties that can improve the physicochemical characteristics of agricultural land, such as higher moisture and nutrient retention, greater aeration and root penetration, it reduces the bioavailability of potentially toxic elements and is a niche for beneficial microorganisms (Liu *et al*., 2013; Zheng

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et al., 2013; Bruun *et al*., 2014; Hammer *et al*., 2014; Liu *et al*., 2017; Obia *et al*., 2016 Peng *et al*., 2018; Razzaghi *et al*., 2020), all of which increases soil fertility and productivity. The addition of this carbonaceous product to soil can be a palliative to soil fertility loss and the decline of physical, chemical and biological properties of soils. Besides, it enables the mitigation of greenhouse gas (GHG) emissions and is an option for the management of organic wastes (Jeffery *et al*., 2015).

Within the context of climate change, the production of biochar has been proposed as a promising and feasible strategy to mitigate the concentration of greenhouse gases in the atmosphere (Liu *et al*., 2014; Griscom *et al*., 2017, Woolf *et al*., 2018). Biochar has the potential to modify the fluxes of CO_2, CH_4 and N_2O in the soil, through changes in microbial activity and composition, soil pH and other biogeochemical processes (Van Zwieten *et al*., 2010; Song *et al*., 2016).

The greatest contribution to carbon reduction comes from the carbon stabilized within the biochar (40-50%) (Ibarrola *et al*., 2012). It is estimated that if only a fraction of all the biomass generated worldwide annually was transformed into biochar, it would have a great impact, since the annual capture of CO_2 by plants through photosynthesis is eight times greater than anthropogenic greenhouse gas emissions. In other words, if only 1% of net carbon sequestrated in plant biomass is transformed into biochar, this could mitigate 10% of all current carbon emissions (Woolf *et al*., 2010; Ennis *et al*., 2012).

The potential of biochar as a carbon sink is due to its recalcitrant nature (Singh *et al*., 2012; Han *et al*., 2018), which slows the speed at which C is emitted to the atmosphere. The average residence time of C the biochar in soils is variable. It is estimated that it has the potential to remain for 100-1000 years or even 10,000 years depending on the natural conditions (temperature, precipitation, topography, soil type, vegetation) of the place where it is incorporated (Zimmerman 2010; Cross and Sohi 2013).

While biochar has been the subject of many studies in the last decade. However to date there are still large gaps in knowledge that it would be advisable to address. Research on biochar and the technologies for its efficient implementation are in early development. However, interest in biochar as a multifaceted solution address to agricultural and environmental problems is increasing at an accelerated pace both nationally and internationally. The objective of this study was to identify the most relevant aspects of global scientific research on biochar in terms of the number of published articles, principal authors and publishing countries, citation, subjects of scientific journals, funding institutions, general trends; and to identify the knowledge gaps through analysis of bibliometric data and the creation of co-occurrence network maps.

MATERIALS AND METHODS

During the month of March, 2020, documentary research was undertaken in order to develop a bibliometric analysis on biochar. The Scopus database was consulted, with regards to online peer-reviewed scientific articles. Scopus is an exhaustive citation and summary database that includes millions of records from journals, books and conference proceedings. The word "biochar" was used as a search engine. This word is usually used in English and occasionally in Spanish in the title, abstract or keywords.

The search was limited to articles and reviews carried out within the period from 2009 to March 2020. With the help of intelligent tools to track, analyze and visualize the Scopus research, the annual production of studies published on biochar, the main authors, country of origin of the research, area of knowledge of the scientific journals where they were published and affiliate institutions, were identified. These data were registered in a database on Excel.

Using the VOSviewer software 1.6.14 (Van Eck and Waltman, 2010) the previously constructed database was analyzed and a co-occurrence network map was generated, as well as connectivity networks of keywords. This map allowed the visual identification of the key thematic axes of the research on biochar and highlighted the knowledge gaps that exist to date.

RESULTS AND DISCUSSION

A total of 11,444 documents were retrieved, both articles and reviews. An upward trend of studies on biochar was found (Figure 1). In the past five years alone, 75% of all publications were produced. It is estimated that in 2020 approximately 3110 papers on biochar will be produced.

According to the smart classification made by Scopus, the main sources of scientific journals where biochar research has been published are: bio-resource technologies, environmental sciences, environmental chemistry, science and environmental pollution research and environmental management, in that order (Figure 2).

China and the United States of America are the countries with the most papers on biochar. Both lead in publications on biochar with 63% of the total (Figure 3). In general, in these countries the publications on biochar are in the area of environmental sciences, agricultural and biological sciences. The studies are about the effect of biochar as an adsorbent agent for soil pollutants and as a retainer of nutrients in soil. In Mexico, the number of publications on biochar is scarce; only 41 articles were retrieved, which represents only 0.35% of the total of research papers. Research on biochar in Mexico is focused mainly on its use in the remediation of soils polluted with heavy metals and to retain nitrogen and phosphate nutrients. No papers on biochar were found relating to climate change or as mitigator of greenhouse gas emissions. (Figure 4).

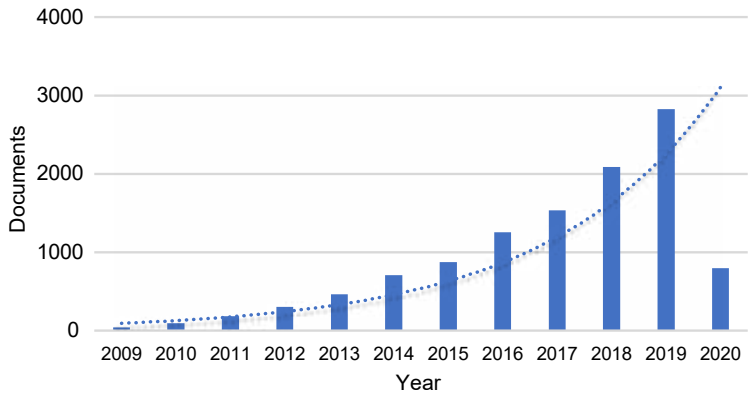

Figure 1. Worldwide production of scientific papers on biochar per year.

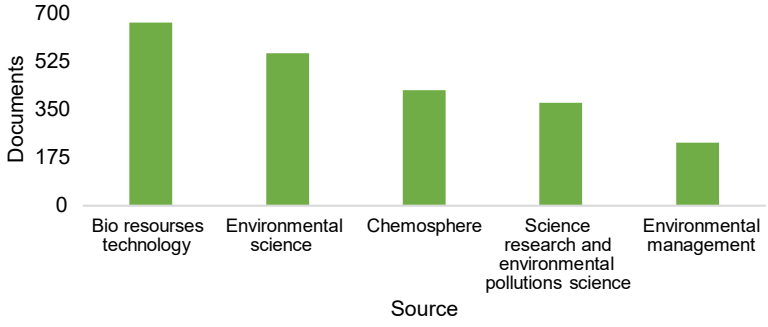

Figure 2. Papers in scientific journals per area of knowledge.

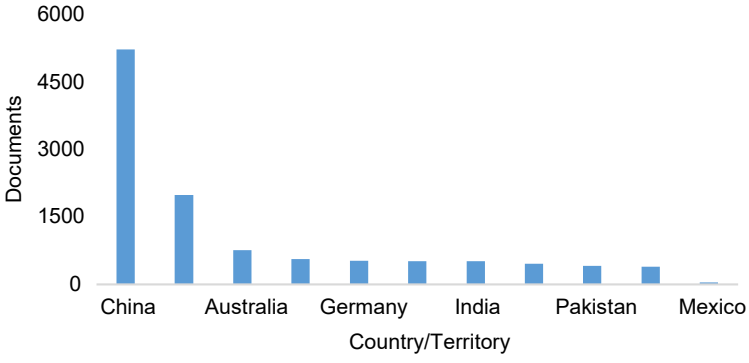

Figure 3. Scientific papers published on biochar by country.

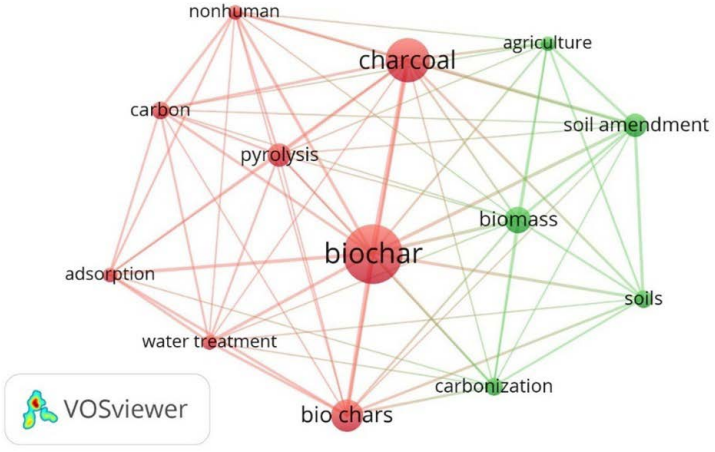

Figure 4. Co-occurrence map of keywords in scientific documents published on biochar in Mexico.

The sources of the publications with the greatest number of citations worldwide are China and the United States of America, followed by the United Kingdom, Australia and Germany. South Korea occupies fourth place with regard to production of scientific papers on biochar; however the papers are not cited as often as those from China, the United States or the United Kingdom (Figure 5). It is evident that publications in English have greater visibility worldwide in the scientific community.

The main authors that have published works on biochar are: Yong Sik Ok from Korea University with 248 studies; Daniel CW Tsang from Hong Kong Polytechnic University with 120 studies; and Bin Gao from University of Florida with 116 (Figure 6). However, Jefferson Lehman and Stephen Joseph are the classic authors, due to their widely recognized book "Biochar

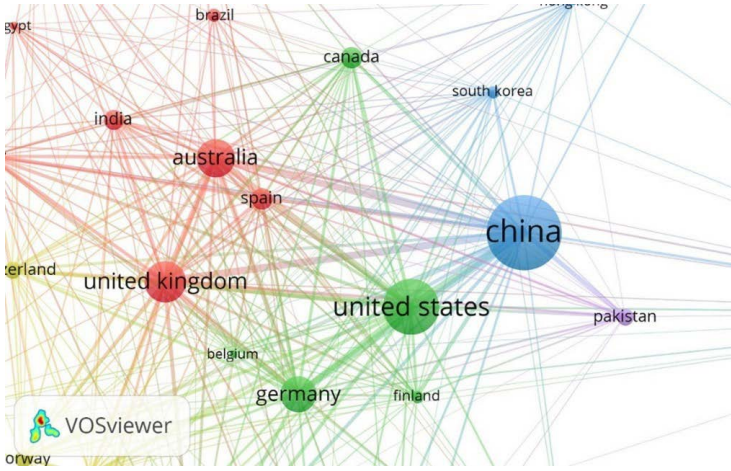

Figure 5. Network map of the citation level of the countries that produce scientific documents on biochar.

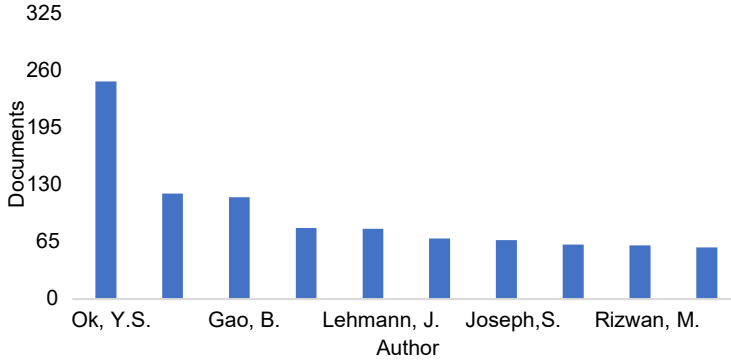

Figure 6. Main authors of research papers and studies on biochar worldwide.

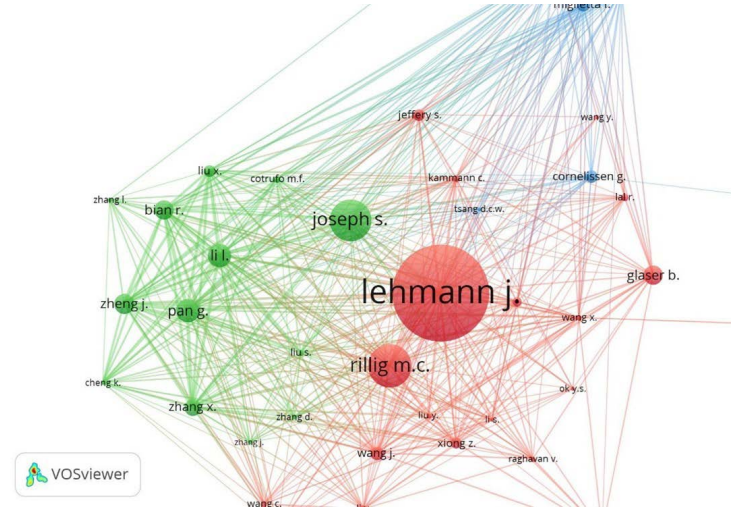

Figure 7. Degree of citation of authors of scientific papers on biochar worldwide.

for environmental management: science, technology and implementation", re-edited in 2015. These two authors, along with Bruno Glaser of Bayreuth University and Matthias C. Rilling, professor of plant ecology at Freie Universität in Berlin, Germany, are the most cited authors on biochar (Figure 7).

The Chinese Academy of Sciences, the Chinese Ministry of Education and the Ministry of Agriculture of the People's Republic of China are the three main institutions in the world that fund research on biochar (Figure 8).

The co-occurence map, using the key words in the 11,444 articles in the Scopus database, revealed the issues relationship between biochar research and other subjects of worldwide interest (Figure 9). The size of the circles is determined by the weight or relevance of the words within the network. That is, the bigger the circles, the bigger the concurrence or citation that the keyword had within the addressed data set. The distance between two circles represents affinity; the closer they are, the higher affinity. The lines represent the level of co-citation. It can be noted that the word "biochar" has great affinity with soil, climate change, fertility, soils, agriculture, soil amendment, carbon, pyrolysis, and biomass. Due to the size of the network it is not possible to see, but the green circles next to biochar correspond to carbon sequestration and climate change.

Zooming in on the network (Figure 10), "biochar" can be seen to have a strong relationship to words such as: climate change, carbon sequestration, soil amendment, agriculture, greenhouse gases, greenhouse effect, fertilizer, carbon footprint, biomass, agricultural waste. The largest green circle, which is found along with the word "biochar", is missing the word "climate change". This reaffirms that biochar is widely considered in the literature as a key element in climate change mitigation.

With the help of these graphic tools it is possible to identify the knowledge gaps or the subjects where more research is needed. On the edges of the network (Figure 11). It can be observed the keywords that are more segregated and therefore less developed. In this co-occurence network of all the keywords in published scientific papers on biochar it is clear that biological matters within the subject of biochar have not been concretely addressed; for example: soil microbiology, soil microfauna, bacteria, fungi, as well as issues of oxidation and mineralization of compounds. It can also be seen that soil temperature, soil respiration, cation exchange, economic analysis,

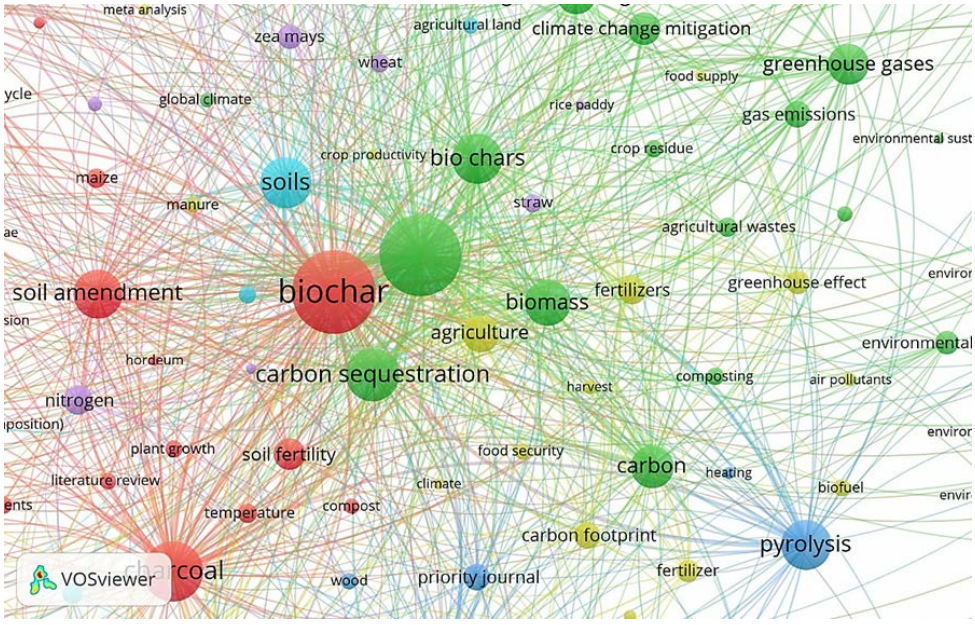

Figure 8. Main institutions that finance biochar research worldwide.

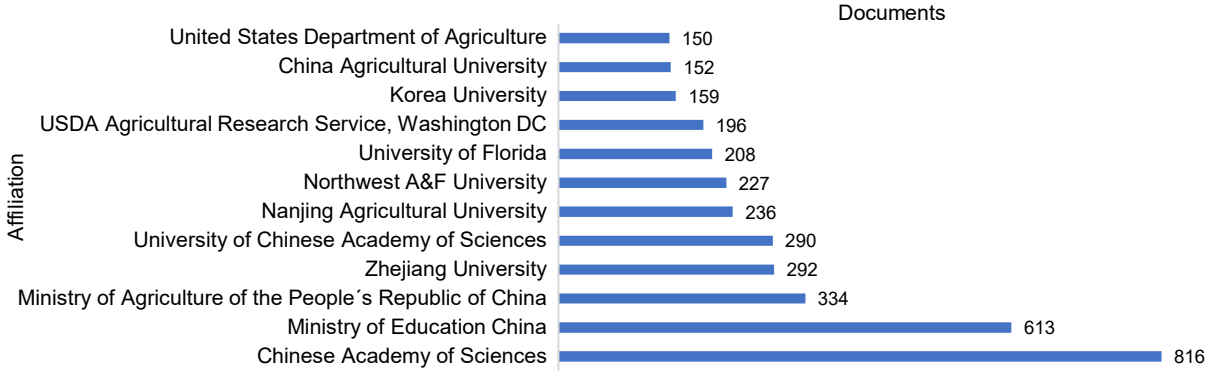

Figure 9. Co-occurrence of all keywords from published scientific papers on biochar.

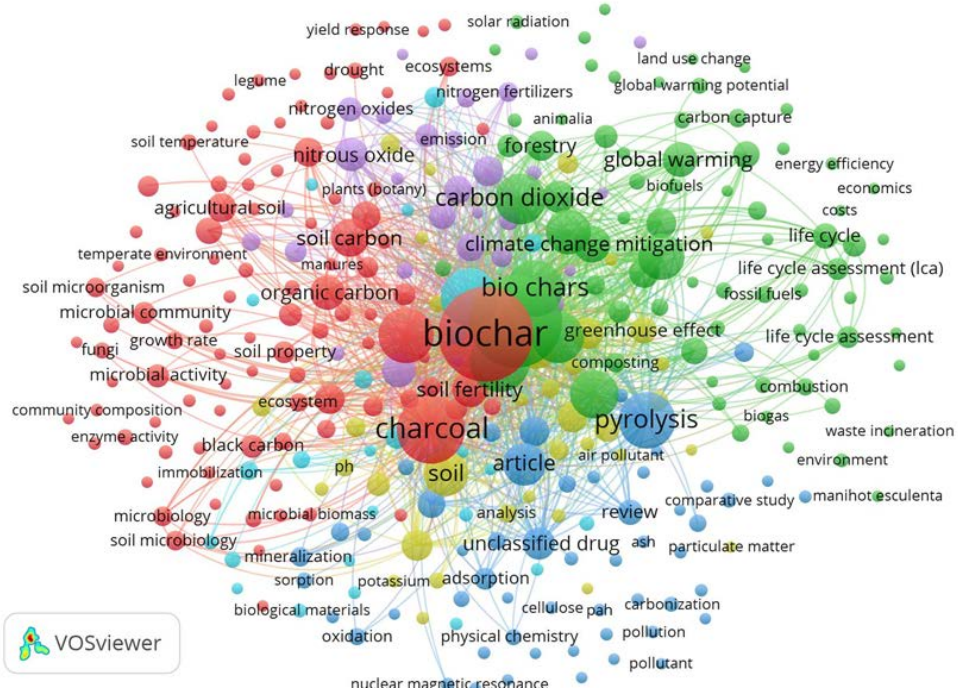

Figure 10. Close-up to the co-occurrence network of the biochar database.

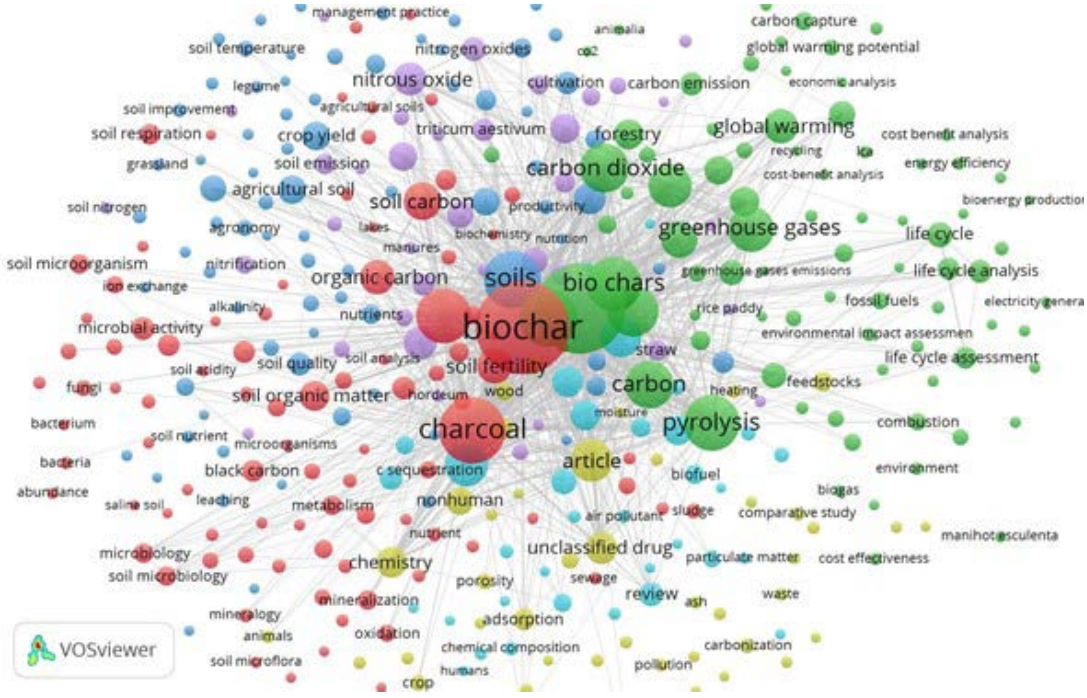

Figure 11. Network of co-occurrence of all keywords in scientific documents on biochar.

cost-benefit analysis, energy efficiency, bioenergy production, and pollution processing are areas where research efforts need to be focused.

CONCLUSIONS

The bibliometric analysis allowed identifying that during the past decade there has been a growing interest in carrying out research on biochar. China and the United States are leaders in research on biochar on a global level, not only in publications but also in citation of their studies. The three main authors on biochar focus their attention on its function to amend environment pollution with potentially toxic elements. Jefferson Lehman and Stephen Joseph are the most cited authors on the subject of biochar. Unfortunately, research on biochar in Mexico is still scarce.

In general, research on biochar worldwide focuses its attention on determining three mail areas: 1) The potential of biochar to stabilize carbon in residual biomass. 2) The capacity of biochar to modify the physicochemical properties of soil and its productivity. 3) The effect of biochar on retaining nutrients and potentially toxic agents in the soil. However, aspects such as biological interactions within the soil, economic and financial analyses, and their evaluation as part of a production system (agroecosystem) have not been completed addressed in research on biochar.

Much of the research on biochar and its effects on productivity and the capacity to mitigate GHG is developed under laboratory or greenhouse conditions. It is evident that research must be scaled up to determine the environmental, economic and social feasibility at farm level and producing biochar from agricultural residues or other types of raw materials. More research is needed about the optimization of the process to produce biochar and its use to sequester higher amounts of carbon. In other words, reactors and processes linked to the production of biochar need to be optimized, in addition to estimating financial feasibility, improving energy yields and promoting its portability and social adoption. The development of a reactor design scaled to a diversity of needs (family, intermediate or industrial) must be contemplated, with considerations for the recycling of potentially polluting or underutilized organic wastes.

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AP AGRO PRODUCTIVIDAD

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Structure and operation of the rabbit meat production chain, Texcoco, México

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ABSTRACT

Objective: The objective was to describe and to analyze the situation and interrelations among the of the rabbit meat production chain, in order to identify the main factors that determine competitiveness.

Design/Methodology/Approach: The methodology used in the research was mixed (qualitative and quantitative). The type of sampling we used was "Snowball". A survey was applied to 33 rabbit producers and a statistical analysis of the data was performed in SPSS (Statistical Package for the Social Sciences). Later, with the use of Geographic Information Systems, the farms were geo-located with Arcview® version 3.2.

Results: The results show the various stakeholders integrating the chain; the lack of communication among them, but highlight commercial relationship among producers, suppliers of equipment, feed and breeding stock; placing the producer as the weakest point in the chain, only as raw material supplier. Producers are heterogeneous and have mainly two types of farms; backyard farming and semi-technical. The spatial distribution map of 33 producers was obtained.

Limitations of the study/Implications: Although rabbit farming is an important complementary activity to food production, the study showed that in the area there are no links among the various agents that integrate the production chain. There is only the mere commercial relationship.

Findings/Conclusions: It is concluded that the null organization of producers keeps them excluded from the productive value chain.

Keywords: globalization, rabbit farming, value chain, product system.

INTRODUCTION

The production chain (Isaza, 2008; Kaplinsky and Morris, 2009) of rabbit meat is part of the chains identified as priority. Rabbit farming presents positive impact in 25 states of the Mexican Republic (the more active are: Puebla, Tlaxcala, Michoacán, Hidalgo, Southern CDMX and the state of Mexico). It is an activity for which there is no national

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information sufficient to determine its economic and social importance (Delgado, 2010; SAGARPA, 2014). The participation of the Municipality of Texcoco is highlighted with the highest level of regional production and consumption (SAGARPA, 2013; 2014).

The production chain of rabbit meat analyzes the interrelation among the stakeholders that are part of it. This chain is defined as a vertical set of companies that produce raw materials, intermediate products and final products. Each point is in charge of one activity such as marketing, research and development, sales and distribution of the final product to users, who in most cases are consumers (SAGARPA, 2004; Comité Sistema Producto Cunícola del Distrito Federal, 2012). The productive chain refers to production systems, which are a set of elements and concurrent agents of the productive processes of agricultural products, including the supply of technical equipment, productive inputs, financial resources, primary production, storage, transformation, distribution and marketing (FAO, 1996; Olivares, Gómez, Schwentesius and Carrera, 2009).

In this study, the production chain is defined as a tool to analyze relations among those stakeholders involved in the production of a commodity, who share the same market (Isaza, 2008; Kaplinsky and Morris, 2009). Therefore, the objective was to analyze roles and interrelations among the stakeholders in the rabbit meat production chain in order to identify the main factors that would allow it to develop competitiveness for the benefit of the participants and with emphasis on the producers.

MATERIALS AND METHODS

This research was conducted in the Zone of the Mountain of the Municipality of Texcoco (ZMMT), state of Mexico. ZMMT comprises 16 localities: Xocotlán, Santa Inés, Santa Cruz Mexicapa, San Dieguito Xochimanca, San Juan Tezontla, Villa San Miguel Tlaixpan, San Nicolás Tlaminca, San Joaquín Coapango, La Purificación Tepetitla, Santa María Nativitas, Tequexquinahuac, San Pablo Ixayoc, Santa Catarina del Monte, Santa María Tecuanulco, San Jerónimo Amanalco and Colonia Guadalupe Amanalco (Texcoco Municipality) (INEGI, 2009; 2010).

Methodology. A mixed (qualitative and quantitative) approach was used. Qualitative was based on Ethnographic exploratory observation. Whereas with the Quantitative, measurable data was obtained by applying a questionnaire (Hernandez, Fernandez and Baptista, 2010). The type of sampling we used was chained or in network ("Snowball") in which the key participants are identified. Then, they are asked if they know others who can provide information, once contacted they are included in the sample (Hernández, Fernández and Baptista, 2010). One questionnaire was applied to a producer, finding out about his links with other rabbit producers and this procedure ended when person declared no longer knew other producer. For the study, the sample size was defined as the total number of producers accessed in the mountain area, due to the lack of data on the actual number of producers living in the whole area. The size of the sample (n) was 33 producers and to collect data a 53-marks survey data, divided into eight sections, was applied to the producers. Statistical analysis was performed with SPSS (Statistical Package for Social Sciences). Later, with the use of GIS (Peña, 2006) the farms were geo-located, and a relational database was created in Arcview® v. 3.2. A map was created with the spatial location of producers' farms, by using a digital ortho-image at 1:10 000 scale.

RESULTS AND DISCUSSION

Operation of the production chain is composed of different stakeholders. Commercial relations that keep producers, suppliers of equipment, food and breeding stock in ZMMT are highlighted.

a) Equipment supplier. In the Municipality of Texcoco, two suppliers of rabbit farming equipment were located. The costs of items that they handle varied from $350 to $16,500; COMPROVET occupies 50% of the market. It is a company recognized and authorized by the Rabbit Production System of the state of Mexico. And the other 50% is occupied by INTEPEC, a company that offers imported equipment. These suppliers act in the free market or jointly with an agency that manages production projects, aimed at rabbit producers or people interested in participating in this activity.

b) Food supplier. Various companies were located to breed-balanced feed production. Four of them stand out (Purina, Malta Clayton, Albapesa and La Unión Tepexpan). A gross percentage (81.82%) of the market in the study area is held by Albapesa, followed by Unión Tepexpan and Purina, 9.09% each. Purina is the company that represents the feed link within the chain structure. However, for the producers of the ZMMT, this company handles higher prices, and they prefer to

purchase breed-balanced feed from another supplier at a more accessible price.

c) Provider of breeding stock. The producers acquire breeding stock with Chapingo Autonomous University and Granja la Esperanza (a link in the production chain). However, the practice of buying broods with other farms is present, causing inbreeding and health issues, since they do not know the sanitary measures to follow for the choice of broods.

d) Producer of rabbit meat. Rabbit farmers in the study area are characterized as rural producers; rabbit farming should be regarded as an economically important activity because it generates income, employment, and several activities related to rabbit breeding and the use of equipment from specialized distributors, feed factories and suppliers of breeding stock.

The participants of the production chain lack of communication among them, while maintaining a merest business relationship. This situation places the producer as the weakest point of the chain, solely as a provider of raw material.

Productive structure

Within each farm there are multiple factors that make their characterization difficult. However, the classification made by the FAO (backyard, semi-technical and technical) was used (FAO, 1996; 2011; 2015). Family farms that are sometimes difficult to classify within a system were considered. The producers are more heterogeneous and we worked with the characteristics of each farm visited in the field. In addition, the number of reproductive females in each farm was considered. Farms with 5 to 20 broods were considered as backyard farming; from 21 to 50 they are semi-technical, and with more than 51 they are technical. Of the visited farms, 72.73% were classified as backyard and 27.27%, as semi-technical.

Through the use of the GIS in ArcView 3.2, the spatial distribution of the 33 producers was depicted on a map of the study area (Figure 1). This information allows us to see the distance from one producer to another and the neighborhood of points. The spatial distribution shows that the producers are dispersed. This is one of the possible reasons that has prevented them from organizing.

Figure 2 shows the types of farms found in the study area. Backyard farms are characterized by using less technical equipment and having a minimum of 5 reproductive females; semi-technical farms have a more specialized equipment that allows them to implement the management more efficiently.

In the ZMMT there are 33 producers, with a total of 3,688 rabbits. This amount can vary due to mortality, sales, slaughter of animals or due to the closure of activities of

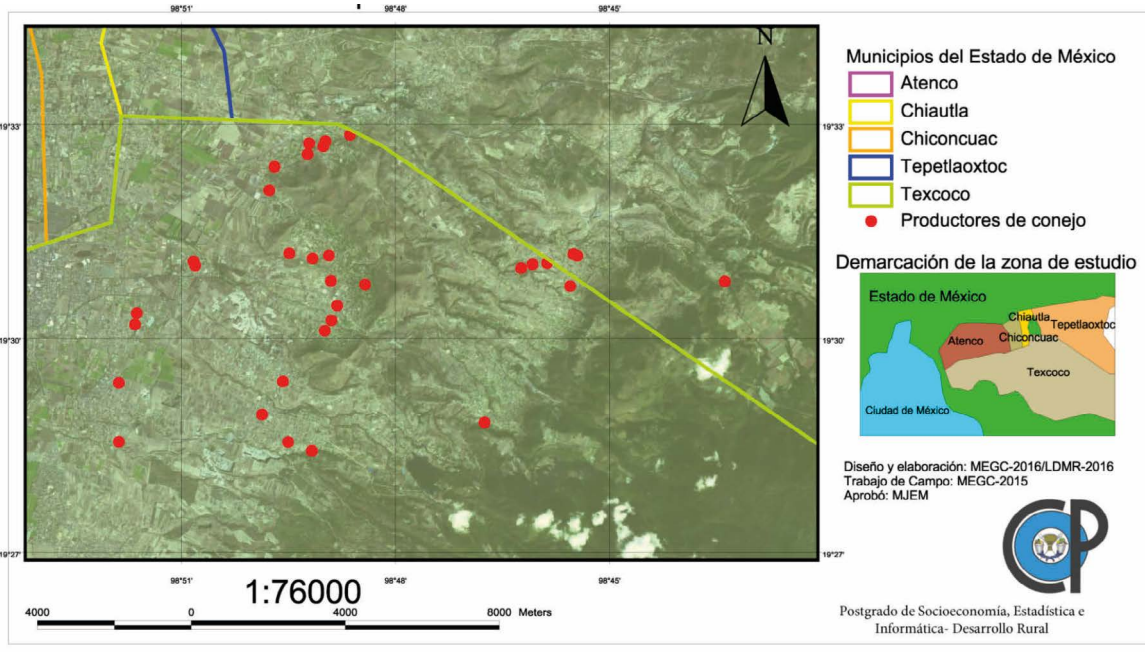

Figura 1. Spatial distribution map of rabbit farmers in the ZMMT, Texcoco, México.

Figure 2. Backyard farm (a) and semi-technical farm (b).

some farms. The towns of San Pablo Ixayoc, Santa María Nativitas and Santa María Tecuanulco did not present any record of producers.

Producers

Of the producers, 81.80% are male, and 18.20% female. The average age is 47 years old (minimum 28, maximum 68); 39.4% of producers finished junior High-school (total education, 9 years). Thus, ranking below the national schooling average of 9.7 years (INEGI. 2010); only 30.3% had a College degree, 18.2% studies in senior High-school and 12.1% finished the Elementary. The level of studies in the ZMMT is an important aspect, since the greater the degree of studies the producers the more open-minded to technological innovation in their rabbit farms, and they also adopt more recent technology in order to optimize their production.

Rabbit farming is a complementary activity among the producers of the ZMMT, and 33.3% stated that commerce is their main activity; another 30.3% is involved in services and self-employment such as plumbers, electricians, or taxi drivers.

Producers reported an average of 3.73 years performing rabbit farming. The minimum found was 1 year in the activity, while the maximum was 8 years. The latter figure, though, is justified because this person is an intermediary. Likewise, producers face problems that limit performance and yield. It is common to find producers without any training in the management of the species, and with little economic solvency that would allow them to stay actives in the market. These people is forced to participate as emerging producers; that is, depending on their income they enter and leave the market.

Marketing

The 85% of the production is sold at Texcoco; 11% to other municipalities; and the remaining 4% to another of the states as breeding stock. The 49.59% of all the stock is sold as breeding for productive projects, to intermediaries and to a single organization. While 50.41% is provided as raw material (cuts of meat) to restaurants, barbecue places, butchers, or direct consumers (family and friends).

Marketing margin

The main route of commercialization of rabbit meat is through the intermediary. Around 35.73% of the production is commercialized; 27.87% is sold to restaurants, in these places a diversity of dishes is prepared and their prices are higher. Restaurants obtain a much greater profit in relation to the producer.

Despite the producer holds 80% of the price of sells and the remaining 20% goes to the intermediary, this is only the raw production price. It seems that the producer keeps a higher profit; however, the production costs per rabbit have yet to be considered. Total production cost is calculated from the birth of the rabbit to the sacrifice (2 months), the calculated cost was $47.25 (2.40 USD). If the producer keeps $37.80 (1.90 USD) per sold rabbit, this is without considering the investment in equipment. This market exercise is segmented as follows, and it was calculated with prices and costs in December 2014. The estimation was that one rabbit eats an average of 5 kg of feed from birth to 2 months of age; the cost per kg of feed in 2013 was $6.25 (0.32 USD), then, total feed costs per rabbit (5 Kg) accounts for $31.25 (1.60 USD) plus $8 (0.41 USD) of labor, $2 water and $5 of averaged transportation caveat (0.36 USD, for both), the total production cost per rabbit is $47.25 (2.40 USD); the value said above which is higher than the profit per rabbit

(1.90 USD) held by the producer. Also, it is important to mention that in this calculation the investment in equipment was not considered, nor the transportation cost as a function of the distance to the selling location. The marketing margin was calculated with the minimum price paid by the consumer and it is a function of the product (Table 1).

Table 1. Marketing margin of rabbit meat.

Letter	Marketing	Price/Pieza ($ Pesos)	Percentage %
a	Producer Price	60	80
b	Channel Price (as raw meat)	65	87
c	Consumer price	75	100
d	Trade margin (b-a)	5	7
e	Marketing margin (c-b)	10	13
f	Total margin (c-a)	15	20

CONCLUSIONS

The production chain of rabbit meat is disjointed; the participants in it maintain solely a trading relationship. This situation forces each producer to develop their own market, by offering a product with little or no added value. This basic product usually does not comply with the required health standards, causing losses in their market position. Producers trade based on their own decisions, without knowledge of the general market information in relation to prices.

In such a way that, difficulties in trading, access to financing and markets, cause the farming activity to be emerging. It becomes difficult for the producers to maintain their farms while waiting for better conditions which may lead to increase their production. Thus, the greatest profit from the commercialization of rabbit meat goes to the hands of intermediaries, restaurants, and bulk sellers or transformers. These participants can provide added value to the product and raise the sale prices; which is why the producer is considered solely as an input (raw material) supplier.

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Productive response of creole lambs fed integral diets with *Samanea saman* (Jacq.) Merr. pods

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ABSTRACT

Objective: To evaluate the productive variables and the digestibility of the nutrients of an integral diet for lambs with increasing inclusion of *Samanea saman* pods.

Design/methodology/approach: 24 creole lambs (initial weight of 20.6±0.3 kg) distributed in a completely randomized experimental design were used. The treatments were: T1, 0%, T2, 12.5%, and T3, 25% of *Samanea saman* pod inclusion. The productive variables and the digestibility of the nutrients were evaluated and compared with the Tukey test (α=0.05); meanwhile, the response to the increasing content of *Samanea saman* was evaluated by orthogonal contrasts. The variables dry matter intake (DMI), daily weight gain (DWG) and feed conversion (FC) showed no differences (p>0.05) between treatments.

Results: Dry matter digestibility (DMD) and organic matter digestibility (OMD) increased (p<0.05) linearly, with increasing pod content. The digestibility of the neutral detergent fiber (DNDF) and acid (DADF) decreased (p<0.05) linearly as the inclusion of *Samanea saman* pod increased in the diets.

Limitations on study/implications: The substitution of soybean pulp by *Samanea saman* pod in integral diets does not affect the productive response of fattening lambs.

Findings/conclusions: The use of *S. saman* pod is proposed as a regionally available food alternative in the feeding of ruminants in the tropics.

Key words: pod, *Samanea saman*, lambs, productive response, tropic

INTRODUCTION

Sheep are economically important ruminants due to their meat, milk, and wool production (Galaviz *et al*., 2011). In tropical regions, grazing is the main sheep feeding strategy (Palma, 2005; Partida *et al*., 2013; Vélez *et al*., 2016). However, in these regions, the low availability of forage is an important limiting condition, specifically during the dry season. Therefore, it is essential to look for appropriate alternatives to meet the nutritional requirements of sheep (Pearson *et al*., 2008; Delgado *et al*., 2014).

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In the Mexican tropic, arboreal legume pods are produced during the dry season and have potential use in animal feed (Zamora *et al*., 2001). These pods represent an alternative feeding strategy that could reduce dependence on commercial concentrates in tropical regions (García *et al*., 2008; Clavero, 2013; Delgado *et al*., 2014). *S. saman* pods contain 14 to 18% of crude protein (CP) (Anantasook and Wanapat, 2012; Flores, 2016; Mazo et al., 2016), 60.5 to 93.1% of dry matter (DM), 1.3 to 5.0% of ashes (Ash), 29.2 to 53.0% of neutral detergent fiber (NDF), 23.7 to 42.0% of acid detergent fiber (ADF), 4.7 to 20.0% of lignin, 1.1 to 15.0% of ether extract (EE), 0.2 to 0.3% of calcium (Ca), and 0.2 to 0.3% of phosphorus (P) (Juárez *et al*., 2013; Delgado *et al*., 2014; Hernández-Morales *et al*., 2018). These pods also contain alkaloids, tannins, saponins, nitrogen compounds, glycosides, and mucilage (Juárez *et al*., 2013; Delgado *et al*., 2014).

Previous *in vitro* (Juárez *et al*., 2013; Hernández-Morales *et al*., 2018; Torres-Salado *et al*., 2018) and *in vivo* (Pirela *et al*., 2010) studies have indicated the relevance of using *S. saman* pods as a supplement in ruminant feeding. Therefore, this study aimed to evaluate the productive variables of lambs and the digestibility of the nutrients of an integral diet with increasing concentrations of *S. saman* pods.

MATERIALS AND METHODS

This study was carried out in the School of Veterinary Medicine and Zootechnics No. 2 of the Universidad Autónoma de Guerrero, under the supervision of the Academic Committee. The university is located in km 197 of the Acapulco-Pinotepa Nacional highway, Cuajinicuilapa, Guerrero (16° 58' N and 98° 45' E, at 30 m).

Experimental diets

Diets were made with regional ingredients (Table 1) and adjusted according to the sheep's growth rate and physiologic stage (NRC, 2007). In the Spring of 2016, the physiologically mature pods of selected wild *S. saman* trees were collected. Pods were ground in a hammer mill with and in-built blower (Azteca No. 20, Mexico).

A sample of each diet was dehydrated at 60 °C until constant weight in a forced-air oven (Felisa® FE-293A, Mexico). All samples were ground using a 1 mm sieve in a Thomas-Wiley Mill (Thomas Scientific®, Swedesboro, NJ, USA). Samples were analyzed using the methods described by the AOAC (2005) to determine the dry matter (DM), organic matter (OM), crude protein (CP), and ash (Ash) content. The neutral detergent fiber (NDF) and acid detergent fiber (ADF) content were determined following the ANKOM Technology® method, as per Van Soest *et al*. (1991), and the acid-insoluble ash (AIA) with the Van Keulen and Young (1977) method.

Animals

Creole lambs (n=24, initial weight of 20.6±0.3 kg) were housed in individual 2 m^2 feedlots provided with shadow and equipped with feeding and drinking troughs; clean and fresh water was freely available. Before the experiment, lambs were weighed (kg) and received prophylactic treatment with 5% Closantel (Panavet®; 10 mg kg^{-1} orally) and ADE+B12 vitamins (Polivit®; 5 mL intramuscularly); lambs were also immunized (Ultrabac® 7; 2.5 mL subcutaneously). Animals were handled following the internal bioethics and well-being regulation of the Universidad Autónoma de Guerrero, which is based on the Official Mexican Standard NOM-062-ZOO-1999.

Feeding and productive response

Lambs had an adaptation period of 15 days to feeding and handling conditions. During the experiment, daily

Table 1. Ingredients and chemical composition of the diets.

Ingredients, g kg^{-1} DM	T1	T2	T3
Corn	550	550	490
Urea	10	20	20
Soybean meal	100	-	-
S. saman pods	-	125	250
Molasses	70	70	70
Pangola hay	240	205	140
Mineral premix°	20	20	20
Sodium bicarbonate	10	10	10
Bromatological analysis (g kg^{-1} MS)			
DMI	883	875	875
OM	848	851	835
CP	128	124	126
NDF	349	262	273
ADF	153	106	125

T1, integral diet with 0% of *Samanea saman* pods; T2, integral diet with 12.5% of *Samanea saman* pods; T3, integral diet with 25% of *Samanea saman* pods. Mineral premix P, 6.0%; Ca, 15.0%; Na, 6.8%; Cl, 10.2%; Zn, 3500 ppm; Cu, 500 ppm; Fe, 1800 ppm; I, 12 ppm; Co, 6 ppm; Mg, 1000 ppm; Se, 12 ppm; Mn, 2000 ppm. DMI, dry matter; OM, organic matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber.

dry matter intake (DMI) and feeding trough readings were recorded to assure that the offered feed was 10% higher than the feed consumed the previous day (Harris, 1970). Feed was supplied at 08:00 and 17:00 h; water was freely accessible. The fattening period was 56 d; every 15 d weight changes were recorded using an electronic scale (Rhino, BAR-9TX®). Feed conversion (FC) was calculated according to the procedure described by McDonald *et al*. (2011).

Nutrient digestibility

Five days before the end of the fattening period, feces from each lamb were collected directly from their rectum. Feces were dehydrated at 60 °C until constant weight in a forced-air oven (Felisa® FE-293A, Mexico) for 48 h; these samples were then ground in a Thomas-Wiley mill (Thomas Scientific®, Swedesboro, NJ, USA) using a 1 mm sieve. DM, TP, Ash, OM, NDF, ADF, and AIA were determined. The digestibility of DM, OM, NDF, ADF, and CP was calculated using the equations described by Church (1988) and Van Keulen and Young (1977).

Experimental design

The experiment followed a completely randomized design (CRD), with eight repetitions per treatment and each repetition an experimental unit. The productive variables and digestibility of the nutrients were analyzed with the GLM procedure. Average values were compared using the Tukey test ($p \leq 0.05$); the linear and quadratic orthogonal contrasts were performed with the CONTRAST option in SAS® (2013).

RESULTS AND DISCUSSION

The DMI, DWG, and FC remained the same ($p > 0.05$) with the different treatments (Table 2). The average DMI of 945 g d^{-1} was 71.8 and 5.2% higher than that reported by Álvarez *et al*. (2003) and Peralta *et al*. (2004). These researchers used lamb diets with up to 30% of *Enterolobium cyclocarpum* pods. Velázquez *et al*. (2011) used diets with 40% of *Acacia farnesiana* and reported a DMI 53.9% lower than the one observed in this study. These variations in DMI could be attributed to the type of forage included in the diets, the physiologic age, and the breeds used in each experiment (Abu-Hafsa *et al*., 2017). Peralta *et al*. (2004) reported a DWG of 160 g d^{-1} in lambs fed a diet with 20% of *Enterolobium cyclocarpum*, similar to the DWG observed in this study with the diet including 25% of *S. saman* pods. However, Velázquez *et al*. (2011) reported a DWG of 77.8 g d^{-1} in lambs fed a diet with 20% of *Acacia farnesiana*, which is lower than the results reported in this study. The differences in DWG are attributed to the diets' ingredient composition; protein sources were different in each study. These results were reflected in the FC. Peralta *et al*. (2004) and Velázquez *et al*. (2011) reported a FC of 3.9 and 7.7, respectively. These results were higher and lower than those observed in lambs fed a diet with 25% *S. saman* pods.

The increasing content of *S. saman* pods in the integral diets linearly increased ($p < 0.05$) dry (DMD) and organic matter digestibility (OMD) (Table 3).

Nitrogen compounds have been shown to improve microbial growth in the rumen (Mendoza *et al*., 1993; McDonald *et al*., 2011), and the CP content of *S. saman* pods is an estimated 16% (Anantasook and Wanapat, 2012; Hernández-Morales *et al*., 2018), which explains the higher DMD and OMD observed in this study. Moreover, these pods contain 43% of soluble carbohydrates (Pizzani *et al*., 2006) that, when combined with nitrogen

Table 2. Effect of increasing levels of *Samanea saman* pods on the productive variables of creole lambs fed an integral diet.

Variables	Treatments			SEM	Effect°	
	T1	T2	T3		linear	quadratic
Starting weight (kg)	20.89	20.16	19.86	0.49	0.41	0.84
Final weight (kg)	31.86	29.13	29.02	0.93	0.22	0.52
CMS (g d^{-1})	1030	837	968	0.05	0.59	0.11
GDP (g d^{-1})	202	148	189	0.01	0.63	0.06
CA, CMS/GDP	5.48	5.52	4.88	0.17	0.16	0.34

Means with different letters in the same column indicate significant difference ($p < 0.05$). °Probability of a significant effect with the increasing dose of *S. saman* pods (linear and quadratic effect).
DMO, dry matter intake; DWG, daily weight gain; FC, feed conversion; SEM, standard error of the mean; T1, integral diet with 0% of *Samanea saman* pods; T2, integral diet with 12.5% of *Samanea saman* pods; T3, integral diet with 25% of *Samanea saman* pods.

compounds (Pirela *et al*., 2010), could synergistically increase the digestibility of DM and OM.

Additionally, *S. saman* pods contain 2.3-7% kg MS^{-1} of condensed tannins (Ukoha *et al*., 2011), which decrease ruminal protozoa and economize ruminal nitrogen (Hu *et al*., 2005; Ukoha *et al*., 2011; Wang *et al*., 2011). It should be noted that these tannins can form covalent links with the proteins in the diet (Obasi *et al*., 2010; Pirela *et al*., 2010; Delgado *et al*., 2012), which improves the passage rate of non-degradable nitrogen compounds in the rumen (Ho *et al*., 1989; Stienezen *et al*., 1996).

Digestibility of neutral detergent fiber (DNDF) and acid detergent fiber (DADF) linearly decreased ($p<0.05$) with increasing concentrations of *S. saman* pods (Table 3).

This decrease in digestibility could be attributed to the tannin and saponin content in the *S. saman* pods (Ukoha *et al*., 2011; Millán *et al*., 2017). The antimicrobial effect of these compounds could potentially affect the hemicellulolytic and cellulolytic bacteria and thus reduce the degradation of the structural carbohydrates in the diet.

The apparent digestibility of crude protein (CPAD) was the same in all treatments ($p>0.05$). The CPAD was 513 g kg^{-1} (Table 3), which is why the CPD remained unchanged after substituting the soybean meal for urea and *S. saman* pods as the protein source in lamb feed. Abreu *et al*. (2004) reported similar results using a lamb diet supplemented with 25% of *Sapindus saponaria*. In contrast, the CPAD in this study was 17.42% lower than that reported by

Table 3. Effect of increasing levels of *Samanea saman* pods on the digestibility of creole lambs fed an integral diet.

Variable (g kg^{-1})	Treatment			SEM	Effect°	
	T1	T2	T3		linear	quadratic
DMS	578[b]	666[a]	629[a]	10.5	0.01	0.00
DMO	502[b]	608[a]	555[ab]	12.5	0.03	0.00
DFDN	436[a]	355[b]	322[b]	13.7	0.00	0.27
DFDA	402[a]	195[b]	178[b]	23.6	0.00	0.00
DPC	498	518	518	12.3	0.21	0.13

[a,b] Means with different letters in the same row indicate significant difference ($p<0.05$). °Probability of a significant effect of increasing concentrations of *S. saman* pods (linear and quadratic effect).
SEM, standard error of the mean; DMD, dry matter digestibility; OMD, organic matter digestibility; DNDF, digestibility of neutral detergent fiber; DADF, digestibility of acid detergent fiber; CPD, crude protein digestibility; T1, integral diet with 0% of *Samanea saman* pods; T2, integral diet with 12.5% of *Samanea saman* pods; T3, integral diet with 25% of *Samanea saman* pods.

Roa *et al*. (2012) and Ben Salem *et al*. (2005); they reported a CPD of 64.8% in integral diets with up to 20% of *Acacia cyanophylla*, and, according to Roa *et al*. (2012), 64% of CPD at 72 h in an *in situ* test with 70% of forage and 30% of legume leaves.

CONCLUSION

In integral diets, the complete substitution of soybean meal with *Samanea saman* pods does not affect the productive response of fattening lambs. Therefore, this study recommends using this regionally available product as sheep feed in tropical regions.

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Genetic transformation of *Paulownia elongata* S. Y. Hu., mediated by *Agrobacterium tumefaciens* and biolistic system

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ABSTRACT

Objective: The most appropriate conditions for genetic transformation through direct (bioballistic) and indirect (*Agrobacterium tumefaciens*) transformation systems in *Paulownia elongata* were established.

Design/methodology/approach: Starting from *in vitro* propagation through both direct and indirect organogenesis, internodal stem segments with 0.5 to 1 cm length were determined as the best explant. The optimum dose for selection media was determined to be 15 mg L^{-1} of kanamycin. It was possible to obtain transgenic plants under both transformation systems. In the case of *Agrobacterium tumefaciens*, two hours of incubation, 48 h of co-cultivation, and optical density of 0.9 were used; while for bioballistics, the best conditions were 120 PSI of shot pressure, shot height at level 6 (16 cm), and vacuum pressure of 22 Hg mm, with particle inflow gun system (PIG).

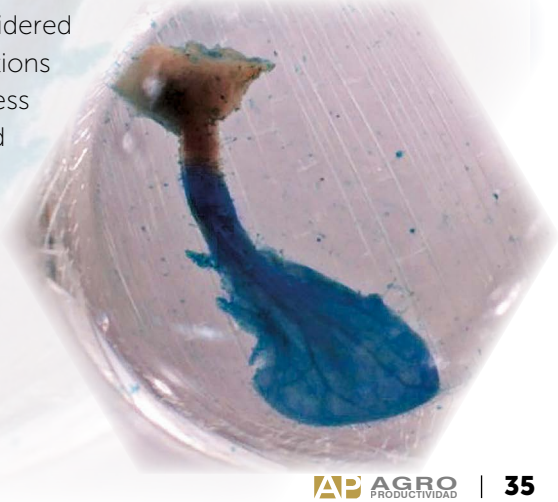
Results: Both systems produced complete transformants, chimeras, as well as those confirmed by histochemical X-GLUC and PCR analysis, producing a total of 14 positive plants by *A. tumefaciens* transformation from 26 trials and ten positive plants by the bioballistic system from 30 trials; a construction with chitinase and glucanase, NPT II selection gene and the GUS reporter gene were used.

Findings/conclusions: So far, this has been the first report including integration of chitinase and glucanase genes.

Keywords: (BA) 6-bencil-adenine, (ANA) naphthalenic acid, micro-propagation.

INTRODUCTION

Paulownia elongate (Scrophulariaceae) originates in China, from where it extended to countries like Japan, Australia, Brazil and the U.S.A. in the 20th century, and to Mexico at the end of the 20th century, considered as a species potential for production. It is a forest species of rapid growth and considered an alternative for the establishment of timber-yielding commercial plantations (Tang *et al*., 1980). Paulownia wood is of low density, which provides lightness and allows its use in manufacture of furniture, crafts, musical instruments, and interior decoration. Because of their quick growth all the species of genus *Paulownia* have great potential for reforestation and improvement of poor soils (Zhu *et al*., 1986, Melhuish *et al*., 1990), they are drought-tolerant and



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adapt to different types of soil, which increases their economic potential (Tang *et al*., 1980). These qualities, however, make them especially vulnerable for the distribution in plantations under agro-climatic conditions other than those of their original habitat, which may lead to failure, furthermore, susceptibility to soil pathogens such as *Rhizoctonia* has been reported in different *Paulownia* species (Mehrotra, 1994; 1997).

The aforementioned, may be moderated by the improvement of their adaptation qualities through genetic transformation. Genetic transformation is a technique that consists in the integration of strange genes to the genome of an individual through the techniques of recombinant DNA, the incorporated genes can improve plant characteristics by direct methods such as bioballistics, which is the acceleration of particles with DNA of desirable features (Vain *et al*., 1993), or indirectly by *Agrobacterium tumefaciens* through the *Ti* plasmid (Herrera-Estrella *et al*., 1983), thanks to advances in in vitro propagation by different organogenic pathways in species of the *Paulownia* genus, such as propagation by shoot tips (Song *et al*., 1990; Sharma *et al*., 2003), or direct organogenesis from different explants (Song et al., 1991; Castillo *et al*., 2012); such as by direct embryogenesis (Ipecki and Gozukirmizi, 2003) or indirect embryogenesis (Ipecki and Gozukirmizi, 2004), laying the foundation for the initial processes to carry out research aimed at genetic engineering.

With the purpose of improving the characteristics in *Paulownia elongata* and the adequate conditions for genetic transformation of this species, studies have been carried out designed to determine the susceptibility of *P. elongata* to *Agrobacterium tumefaciens* and *Agrobacterium rhizogenes*, which demonstrated that inoculating shoots generated under *in vitro* conditions with the strains 542, A281, and C58 of *A. tumefaciens*, the formation of crown gall is possible with a frequency by 83%. Other plants have promoted quick calli proliferation and root formation in damaged shoot parts, when inoculated with strain R1601 of *A. rhizogenes*. The molecular analyses showed the presence of opinae, the same as Southern blot tests, which confirmed the existence of the transformation plasmid (T-DNA) in callus as well as in roots (Bergmann *et al*., 1999). In other species like *P. fortunei*, callus was obtained, transformed after co-cultivating petiole segments with *A. tumefaciens* strain LBA 4404 with a binary vector (pB121), that included the GUS reporter genes and those of NPT II selection, resistant to kanamycin, and the transformation was confirmed by histochemical tests of the GUS gene expression and the detection of incorporation of the selection gene to the genome of transformed calli (Mohri *et al*., 2003). Transformation studies, already approached to gene integration with the purpose of conferring some characteristics have been carried out in a hybrid of *P. tomentosa* × *P. fortunei*, which was transformed via *A. tumefaciens* with vector p43 8PRSI, containing the *shiva-1* gene, which codes a bactericide peptide controlled by the promoter gene called CaMV35S. The molecular analyses like PCR, Southern blot, showed the successful integration of the *shiva-1* gene in the plant genome, confirming the gene transcription through RT-PCR (Tao *et al*., 2005).

On the other hand, with the objective to generate resistance to root diseases, studies have been conducted with chitinase genes (RCH10) and β-1-3-glucanase (AGLU) genes, having generated genotypes with tolerance to pathogens like *Rhizoctonia solani* in *Oryza sativa* L. (Mao *et al*., 2003; Li *et al*., 2004). The aforementioned allows considering the viability of application of these techniques and incorporating them to forest species para generar arboles resistententes a enfermedades como reportaron en Populus deltoides (Zou *et al*., 2006); therefore the objective of this study was integrating chitinase-glucanase genes through genetic transformation by *Agrobacterium*-mediated and bioballistic systems in *Paulownia elongata* (S.Y.Hu.).

MATERIALS AND METHODS

Genetic Transformation

The tests of *Agrobacterium tumefaciens*-mediated transformation were established based on the principles created by Horsch *et al*. (1985), utilizing *Agrobacterium tumefaciens* strain LBA4404, which contains the plasmid pCAMBIA 2301-CHIT-GLU with chitinase and glucanase genes, the GUS reporter gen, and the NPTII selection gen (resistance to kanamycin) with a total size of 11621 base pairs. In order to conduct the transformation assays, internodal segments of 0.5 m length were used generated in MS medium (Murashigue and Skoog, 1962), cultivated for 35 days to favor the greatest possible internode elongation. Within the culture recipient, shoots of 8 cm length on average were produced, and the dissected segments were placed in liquid MS medium before their inoculation with the *Agrobacterium*

tumefaciens strain. Different transformation conditions were created through variations in bacteria incubation times, bacterium concentration, measurement through culture optical density, and times of co-incubation.

Transformation by Particle Acceleration

The particle acceleration system utilized was a PIG (Particle Inflow Gun), (Vain *et al*. 1993) which operates with helium gas and tungsten particles as projectile agents and carrying plasmids at low pressure (40-120 *psi*) utilizing a vacuum pump (25-30 *psi* Hg). Calli were used for transformation by particle acceleration, which were obtained from internodes cultured in MS medium supplemented with 4 mg L^{-1} of TDZ, 3% of sucrose, 0.7% agar, and pH of 5.8. Its growth period took until reaching 1 cm^2 at two weeks of 26 °C under conditions of darkness. Different transformation conditions were established, such as: variations in utilized pressure, regulating helium gas outlet and shot speed, keeping constant plasmid concentration in particles, and shot distance.

Selection of Transformed Segments and Shoot Evaluation

The segments of the transformation assays were transferred to a selection medium made up of MS medium, added with 4 mg L^{-1} of BA, 0.2 mg L^{-1} of ANA, 15 mg L^{-1} of kanamycin, 3% sucrose, 0.7% agar, and pH of 5.8, the stem segments as well as calli remained for a period of 35 days, time when shoot formation was initiated. The shoots longer than 1.5 cm were dissected, taking a 2 mm segment in order to conduct the histochemical XGLUC test and observe the expression of the GUS gene in the tissues, the rest of the shoot was used for DNA extraction required for PCR analysis confirming the integration of the genes of interest for the genome of the transformed shoots.

RESULTS AND DISCUSSION

Genetic Transformation

A total of 26 assays were carried out representing the management of 7,984 explants, submitted to *Agrobacterium*- mediated transformation process. Only 37 shoots were obtained from all of them (Table 1), which produces a mean transformation frequency of 1.12 of the total of shoot assays; each shoot-yielding assay, however, had its particular percentage. It is important to emphasize that the highest transformation frequency of independent experiments was obtained with two hours of bacteria incubation, more than 40 h of co-cultivation, and optical density from 0.8 to 1.1, since it is quite probable that higher optical density coincides with the stationary phase of bacterial growth, leaving less activity to strains and thus lower transformation efficiency; likewise, it is probable that the strain working with low optical density (>0.5) in the phase of growth lag and the time of co-cultivation, may be not sufficient for the bacterium to infect the tissues properly. Therefore, the obtained results partially agree with those reported by Mohri *et al*. (2003), who after 48 h of co-cultivation found better response in segments of leaves, stem, petiole, and root, but at incubation times not longer than 30 min, and maximum optical density of 0.5 in petioles. Even though in the present research we worked with internodes determined by direct organogenesis response, it is considered that this way is the most appropriate for *P. elongata*, taking into account that the studies previous to trans- formation with species of genus *Paulownia* spp. had led to obtaining of calli and roots, not of shoots or complete plants, this factor being the most important of the expected results. Even when it had been necessary to utilize a great many initial explants, in the total of assays as well as in each of them particularly, in none of the cases fewer than 250 stem segments were used. At first we expected to obtain a larger number of shoots, starting from the selected organogetic route; however, this did not happen, partly due to the fact that although the kanamycin dose was not higher than 15 mg L^{-1} it is probable that it would have indirect effects on diminishing the capacity of forming shoots, even in the transformed tissues.

Internode and Calli Transformation by Particle Acceleration

Shoot formation were evaluated in each experiment with the possibility of having a transformants in a total of 20 assays, which represented the management of 6,474 explants submitted to the transformation process by particle acceleration through (PIG). Nineteen shoots were obtained (Table 1), generating a mean transformation percentage of 1.19 of the total of shoot assays; however, putting these results on a level with similar studies on *P. elongata* presents difficulties as a species, since so far, genetic transformation through particle acceleration has not been examined yet. Nevertheless, there is information of other forest species of the coniferous family, such as *Pinus roxbughii*, where direct biolistc transformed on mature embryos was made in order to incorporate bar gene, obtaining transformed plants from epicotyls and adventitious shoots. This approaches the system used in the present study, due to the fact that after biolistic

Table 1. Response of assays with stem and callus samples of *P. elongata*, in X-GLUC substrate and by PCR analysis.

No. of trials	Terms	No. of SPROUTS	Positive in X-GLUC	Positive by PCR for the chitinase gene	Full or partial expression
6 A	H1=2 H2=40 OD=1.1	4	2	2	partial
9 A	H1=2 H2=48 OD=0.9	3	1	--	partial
10 A	H1=2 H2=48 OD=0.9	1	--	--	----
11 A	H1=1.5 H2=40 OD=0.8	2	2	2	partial
16 A	H1=2 H2=48 OD=0.9	6	3	3	total
17 A	H1=1.5 H2=48 OD=1.1	7	2	2	partial
18 A	H1=2 H2=48 OD=0.8	1	1	1	partial
22 A	H1=2 H2=48 OD=0.8	2	--	--	----
23 A	H1=2 H2=48 OD=0.9	5	4	2	partial
24 A	H1=2 H2=48 OD=0.7	3	--	--	----
25 A	H1=1.5 H2=48 OD=0.8	2	2	2	partial
Subtotal with *A. tumefaciencs*		36	17	14	
8 Bio	PSI= 120/ A=6 /PL 1	1	--	--	----
9 Bio	PSI= 120/ A=6 /PL 1	5	3	3	total
10 Bio	PSI= 120/ A=6 /PL 1	4	4	2	partial
17 Bio	PSI= 120/ A=6 /PL 1	2	1	1	partial
20 Bio	PSI= 120/ A=6 /PL 1	1	1	1	partial
3 Biocall	PSI= 120/ A=7 /PL 1	5	1	1	partial
6 Biocall	PSI= 120/ A=7 /PL 1	7	6	2	partial
7 Biocall	PSI= 120/ A=7 /PL 1	2	2	--	partial
10 Biocall	PSI= 120/ A=7 /PL 1	4	--	--	----
Subtotal with Biobalistics	PSI= 120/ A=7 /PL 1	31	18	10	
TOTAL		67	35	24	

Footnote: A: Transformed by *Agrobacterium*
Bio: Transformed by bioballistics
Biocall: Callus transformed by bioballistics
H1: Hours of co-incubation with bacterium in liquid medium
H2: Hours of co-culture in solid medium
OD: Optical density of bacterial culture
PS1: Shot pressure
A7: Shot height
PL1: Plasmid with genes of interest **contained plasmid**
-- Without response

even when starting from sexual tissue in the beginning, finally the transformants are recovered from organogenesis; however, the usually preferred way for transformation by particle acceleration in conifers by 70% has been through somatic embryogenesis (Tang & Newton 2003). The aforesaid is mainly due to an increment of bombed cells in calli of embryogenic type, which increases the possibility of transformation, once the somatic embryos derived from the transformed cells are being produced, since the organogenic way is associated to genetic changes by somaclonal variation. This would be in this particular case- of high risk, starting from the fact that the original material is a hybrid of high plasticity and yield, for which it is not desirable to alter these characteristics; since the objective and the point only was integrating the genes of interest without changing the original condition of *P. elongate*.

The calli were transferred at half strength recuperation media without antibiotics for seven days were sub cultivated in selection medium (MSSEL) during 30 days, and shoot production was evaluated in each experiment. The total number of conducted assays was 10 with an average of 24 calli for each; but with the highest transformation percentage to the order of 21.21, which is related to the fact that even though this has to do with a reduced number of

calli each callus had 1.5 cm diameter on an average with greater impact surface for the tungsten particles, which in turn represented a larger number of possibly transformed cells, even when the way of indirect organogenesis was followed. It is important to emphasize that in the case of transformation by particle inflow gun system in forest species such as conifers, somatic embryogenesis is of 70% (Tang & Newton 2003). The use of calli and genetic transformation in *P. elongata* with *Agrobacterium*-mediated systems has been reported (Mohri *et al.*, 2003) achieving transformed tissue and deriving in callus lines. On the other hand, Bergmann *et al.* (1999) report callus and root formation, after transforming with *Agrobacterium rhizogenes*; however, despite these advances, up to date, complete and stable transformation in *P. elongata* by bioballistic systems have not been reported, since there are only records of preliminary assays with temporary expression, like those carried out by Mohri and Shinohara (1996), who utilized a transformation system by particle inflow gun, in order to insert the luciferase gene in *P. fortunei*; however, they do not report stable expression neither complete plant development.

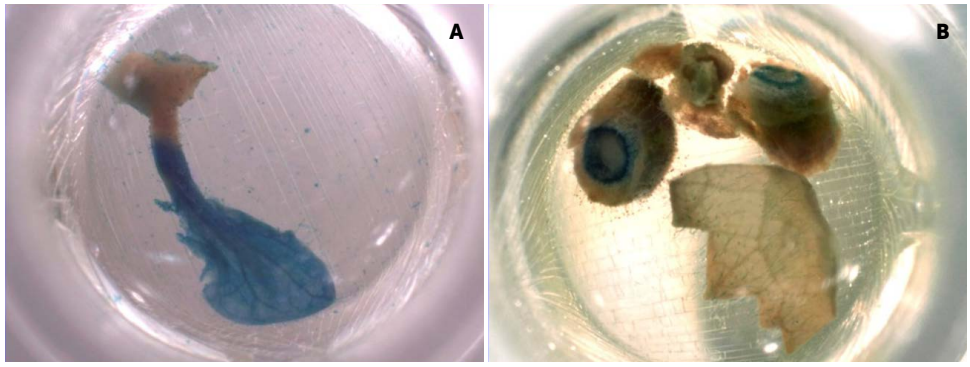

Figure 1: GUS expression in X-GLUC substrate of transformed tissues (A) total response, (B) Partial response

Histochemical Evaluation and PCR Analysis of Shoots

Tissue samples in X-GLUC substrate allowed evaluating GUS gene expression, blue coloration in tissues being observed with positive response to the expression of this gene. As a disadvantage that this may be a destructive test. At the end of the evaluation, it could be observed that only 35 out of the 67 shoots obtained and kept in selection medium resulted positive for GUS, 6 of these with total expression and 29 partially (Table 1, Figure 1); the fact of achieving shoots with partially transformed tissue indicates the majority presence of chimeras, whose transformed tissues can be identified- even though they are not fully transformed individuals - and starting from them, complete individuals can be generated. Mohri *et al.* (2003) report calli with total expression for the case of *P. fortunei*, whereas in the present study, all the calli obtained had partial expression. It must yet be clarified that *P. fortunei* was transformed with *Agrobacterium* (species) and in the present case *P. elongata* was transformed by particle inflow gun system. Most of the calli kept in selection medium resulted positive, at least partially. In this sense, mature *Pinus roxbughii* embryos, for example, were transformed by particle inflow gun system, generally obtaining partial transformants, from which complete individuals were identified and generated from such transformed tissues, inducing adventitious shoots (Parasharami *et al.*, 2006). Therefore, shoots and calli from *P. elongata* were kept in selection medium, in order to subsequently identify the transformed tissues and generate complete individuals, which could be identified through histochemical X-GLUC analysis.

Molecular analysis by PCR allowed assessing the incorporation of the genes of interest in the transformants, presence of bands being observed in the shoots with positive response or band absence in non-transformed shoots. We emphasize the fact that 24 out of the 67 assessed elements showed positive response (Table 1, Figure 2), 35% of the total. Interesting situations could be observed, like shoots having had partially positive response for GUS and negative response for PCR; or on the contrary, materials negative for GUS and positive for PCR. One of the main causes of chimeric effects is the fact that mostly partially transformed materials (chimeras) were obtained by *Agrobacterium* as well as by bioballistics, which brings about that tissue parts are taken for the tests, that may contain or not the transformed portions, which produces such responses in the moment of analysis.

CONCLUSIONS

It was possible to genetically transform *P. elongata* by the direct method of ballistics or indirectly by *Agrobacterium tumefaciens*-mediated transformation in order to incorporate genes of interest. Complete as well as partial transformants could be achieved in both

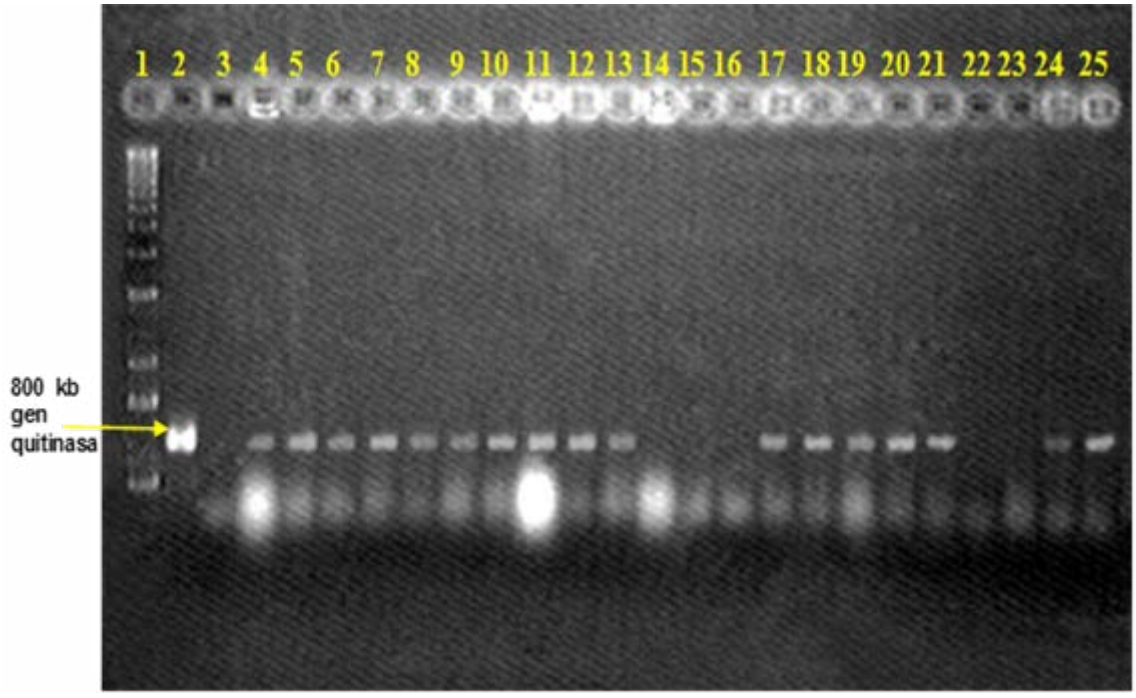

Figure 2: PCR test for chitinase gene integration to transformed *P. elongata* materials by direct and indirect transformation systems, in agar gel of 1.5%, showing the results of Rail 1, stairs 1Kb, rail 2 positive control for chitinase gene, rail 3, negative control, rail 4-25 DNA tissue samples obtained in selection media.

systems, via direct organogenesis and indirectly for the case of transformed calli.

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Asexual reproduction: an alternative for the propagation and conservation of papaya (*Carica papaya* L.) native to Guerrero, Mexico

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ABSTRACT

Objective: Evaluate the effect of three types of substrate and different shoot lengths on the rooting of *Carica papaya* L. shoots.

Design/methodology/approach: This experiment followed a completely randomized design with a 2×3 factorial arrangement. Shoots of 30 and 40 cm of length were collected in March 2018 from the lateral branches of papaya plants (*Carica papaya* L.) native to various regions of Guerrero. Shoots were placed in different substrates: 1) sand, 2) Peat Moss®, and 3) sand and Peat Moss® mixture (70:30, v/v). Sixty days after planting, rooting percentage, root length, plant height, stem diameter, number of leaves, crown diameter, biomass fresh weight, biomass dry weight, root fresh weight, and root dry weight were evaluated. Data were analyzed through an analysis of variance and a mean difference test (Tukey, $p \leq 0.05$).

Results: The rooting of 40- and 30-cm shoots was 60 and 50%, respectively. Plants with the highest height, number of leaves, root length, and crown diameter derived from 40-cm shoots. Sand was the best substrate for rooting, where plants with higher fresh biomass were significantly developed.

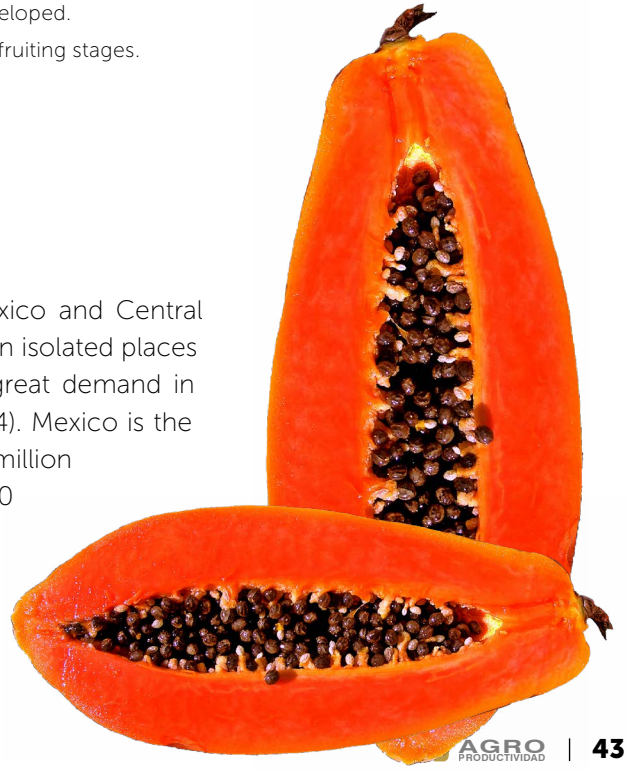
Study limitations/implications: Continue study during the flowering and fruiting stages.

Findings/conclusions: This protocol allows the rooting of papaya shoots.

Keywords: *Carica papaya*, rooting, shoots, root length, substrate.

INTRODUCTION

Papaya (*Carica papaya* L.) originated in southern Mexico and Central America. It is possible to find wild populations in isolated places in these regions. Papaya is an economically important crop with great demand in the national and international markets (Fuentes and Santamaría, 2014). Mexico is the third country worldwide with the highest papaya production, one million 40 thousand tons/year, and the first exporter with more than 163,000 tons/year (FAOSTAT, 2019).



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Several researchers around the world work on the preservation of genetic variability. More than 30 *Carica* sp. collections exist worldwide to preserve, characterize, and evaluate germplasm (Dantas *et al*., 2002).

The most used propagation method is by seed; this is an easy and inexpensive approach that takes advantage of the fact that fruits generally have hundreds of seeds. However, this method has some disadvantages, such as genetic and phenotypic heterogeneity (Jiménez, 2002). Papaya is a dioecious species with female and hermaphrodite flowers in different plants. In the market, the preferred fruits come from hermaphrodite plants. Since sex can only be determined by the plant's flowers, which appear after the fifth month of germination, propagation by open-pollinated seeds decreases production value (Giampan *et al*., 2005). Moreover, the sarcotesta, the mucilaginous external layer of the seeds, can cause dormancy, which results in dehydration-sensitive seeds that easily lose viability (Tokuhisa *et al*., 2007). This problem could be overcome by vegetative propagation of shoots and grafts; these simple, fast, and inexpensive techniques preserve the genotypic characteristics of mother plants (Giampan *et al*., 2005; Hartmann *et al*., 1990).

Papaya can be propagated by the rooting of shoots; these asexually propagated plants show greater uniformity, shorter fruiting time, shorter fruiting height, and higher yield per productive cycle (Ruíz-López, 2016). This propagation method allows the selective multiplication of plants with desired characteristics, such as hybrids, hermaphrodite plants free of pests and diseases, among others (Allan, 1990; Giampan *et al*., 2005). Additionally, the plants obtained using this propagation method start flowering shortly after plantation; thus, fruiting occurs earlier than in the plants obtained by seed propagation (Giampan *et al*., 2005; Grana, 2000; Reuveni and Shlesinger, 1990). A common problem of asexual propagation is shoot rooting (Grana, 2000).

Hidaka *et al*. (2008), Ruíz-López (2016), and Yu *et al*. (2000) reported that shoot length affects the vigor of the generated plant. Additionally, solutions containing auxins, such as indole-3-butyric acid (IBA), or combinations of stimulating hormones significantly affect the rooting and survival of papaya shoots.

It is possible to secure higher shoot rooting percentages and plant quality through the evaluation and control of different factors, such as type of substrate, age of the mother plant, shoot length and type, and the dose of growth regulators (Hartmann *et al*., 2002; Rivera-Rodríguez *et al*., 2016). Biotechnology could be an alternative tool for the rapid propagation of elite papaya plants, as well as the commercial scale introduction of new varieties or hybrids (Posada-Pérez, 2016). Therefore, this study aimed to evaluate the effect of three types of substrate and different shoot lengths on the rooting of *Carica papaya* L. shoots.

MATERIALS AND METHODS

Collection. The vegetative material (shoots) was collected from the lateral branches of papaya plants (*Carica papaya* L.) native to several Guerrero regions. Shoots were stored in properly labeled plastic bags and kept in a cooler during their transportation to the planting site. Twenty-four hours after collection, shoots were processed in the nursery and fruit propagation laboratory (18° 20' 39.4", N 99° 30' 09.7" W).

Propagation material. Shoots of 30 and 40 cm and a diameter of 1.5 cm were collected. Leaves were cut with a previously disinfected scalpel; only a couple of them were left at the apical part. The material was washed with running water and immersed for 5 min in a 1.5 g L^{-1} fungicide solution (Mancozeb 80 WP) to reduce contaminating microorganisms. Subsequently, shoots were submerged for 10 min into a Rootex® solution (2.0 g L^{-1}); Rootex® is both a fertilizer and a rooting agent. These prepared shoots were then planted in the substrate 5 cm deep in 59×19×16 cm disinfected plastic pots with three 2-cm diameter perforations at the bottom. Three types of substrates were used: 1) sand, 2) Peat Moss®, and 3) sand and Peat Moss® mixture (70:30, v/v). Substrates were sterilized with steam for 30 min.

Pots were kept in a nursery at field capacity and irrigated every three days. Additionally, Rootex® (a combination of rooting promoting amino acids, organic acids, and nutrients) was applied three times (2.0 g L^{-1}), at 24 h, 8, and 16 days after planting. During the experiment, the nursery temperature was 25±4 °C, relative humidity ranged from 70-80%, and plants were subjected to a 12±1 h photoperiod. These variables were recorded every two hours using a Hobo® U12 data logger.

Evaluated variables. Sixty days after establishing the experiment, rooting percentage (RP), root length (RL),

plant height (PH), stem diameter (SD), number of leaves (NL), crown diameter (CD), biomass fresh weight (BFW), biomass dry weight (BDW), root fresh weight (RFW), and root dry weight (RDW) were evaluated.

Experimental design and statistical analysis. This experiment followed a completely randomized design with a 2×3 factorial arrangement. The studied factors were: three types of substrate (sand, Peat Moss®, and mix of both) and two shoot lengths (40 and 30 cm), as well as the interaction of both factors. Three shoots (replicates) per pot were placed using only one substrate type (factor 1) and shoot length (factor 2). Thus, six combinations were studied, with four repetitions (pots) per combination, 24 experimental units in total. The experimental units were randomized using the "design.ab" procedure in the statistical program "R" to ensure the independence of the observations. Error normality was determined using the Shapiro-Wilk test, and Barttlet's test was used to evaluate the homogeneity of variances. An analysis of variance and a Tukey mean difference test ($p \leq 0.05$) were carried out using the statistical software SAS® V.9.4 for the study factors and evaluated variables.

RESULTS AND DISCUSSION

Rooting. There were no significant differences in the rooting of papaya shoots between the different substrates ($p \leq 0.05$). However, the rooting of the 40-cm long shoots was significantly higher (Figure 1).

Moreover, all the experimental units had a positive response to the treatments (number of roots, root length, and biomass dry and fresh weight). This demonstrates that asexual propagation is a viable option for establishing mass reproduction and conservation units of papaya plants. This could be due to the positive action of the rooting agent Rootex® as an exogenous growth regulator. Under natural conditions, auxins intervene in root formation (Cuisance, 1988). The use of auxins is important in species with rooting difficulties (Hartmann *et al.*, 2002). Vargas-Simón *et al.* (1999) reported higher rooting percentages, number of roots, and root length in icaco plants (*Chrysobalanus icaco* L.) treated with 10 000 mg·L^{-1} of indole-3- butyric acid (Radix 10 000®). González-Pulido *et al.* (2019) evaluated the effect of the cutting type, treatment with a rooting agent (Radix 10 000®), and fertilization in *Acer negundo*. Their results indicated differences between the different cutting types; the use of the rooting agent resulted in larger buds and higher dry and fresh weights. The rooting agent/fertilizer interaction had a positive synergistic effect on the different evaluated variables.

Similar to our results, Ruíz-López (2016) reported that shoot length significantly affects plant vigor. Furthermore, he mentions that using a rooting agent, such as IBA, is critical for successfully establishing papaya shoots. Yu *et al.* (2000) reported that low IBA concentrations (2.5 Mm) result in satisfactory rooting percentages. Schmildt *et al.* (2016) reported significant rooting results in papaya cv. Calimán shoots treated with 1.5 to 2 ppm of IBA.

Root length, plant height, and crown diameter. The plants originated from 40-cm shoots were statistically taller, with a higher number of leaves, wider crown diameter, and longer root length (>20 cm) (Table 1).

Plants from the Sand*40-cm shoot interaction had the highest plant heights, number of leaves, crown diameter, and root lengths (Table 2). These results suggest collecting shoots of at least 40 cm long and

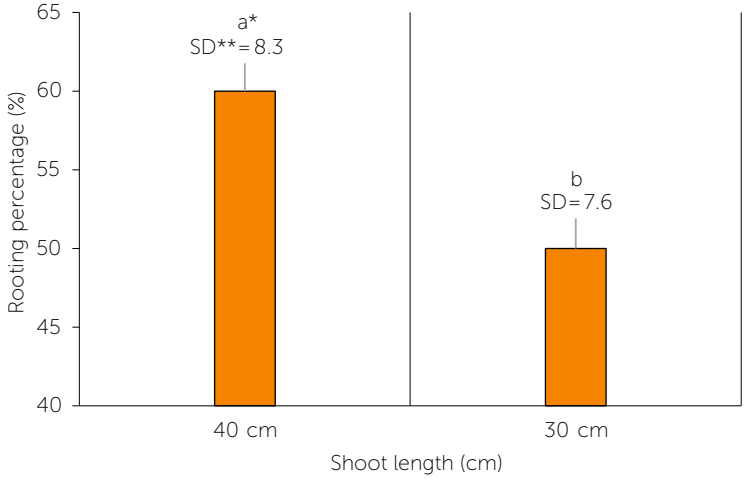

Figure 1. Rooting percentage of *Carica papaya* L. shoots according to shoot length. *Means with different letters are statistically different according to the Tukey test ($p \leq 0.05$). ** SD=Standard Deviation.

Table 1. Effect of shoot length in *Carica papaya* L. plants.

Shoot length (cm)	PH*	SD	NL	CD	RL
40	45.58 a**	12.83 a	6.83 a	27.62 a	27.72 a
30	35.33 b	11.62 a	4.16 a	6.66 b	4.83 b

*PH=Plant height, SD=Stem diameter, NL=Number of leaves, CD=Crown diameter, RL=Root length. **Means with different letters in the same column are statistically different according to the Tukey test ($p \leq 0.05$).

Table 2. Effect of the multiple interactions of different substrates and shoot lengths on the plants developed from *Carica papaya* L. shoots.

Interaction (cm)	PH*	SD	NL	CD	RL
Sand - 40	46.50 a**	12.50 a	8.25 a	31.87 a	31.66 a
Sand - 30	35.00 b	13.25 a	3.75 b	7.12 bc	5.25 b
Peat Moss®- 40	44.75 a	13.00 a	7.50 ba	23.50 bac	27.50 a
Peat Moss®- 30	35.50 b	11.87 a	4.00 b	5.25 c	4.25 b
Mixture- 40	45.50 a	13.00 a	4.75 ba	27.50 ba	24.00 a
Mixture- 30	35.50 b	9.75 a	4.75 ba	7.62 bc	5.00 b

*PH=Plant height, SD=Stem diameter, NL=Number of leaves, CD=Crown diameter, RL=Root length. **Means with different letters in the same column are statistically different according to the Tukey test ($p \leq 0.05$).

carefully considering the type of rooting substrate used for propagation.

Rivera-Rodríguez *et al*. (2016) evaluated the effect of different substrates (perlite *vs*. peat and vermiculite mix), the mother plant age (12, 18, and 24 months), and the dose of IBA (0, 5000, and 10 000 ppm) on the rooting of *Pinus patula* shoots. They observed the highest rooting percentage with perlite; however, they obtained the highest survival, root length, and callus percentage in non-rooted shoots using the peat-vermiculite mix. Galindo-García *et al*. (2012) reported the rooting of poinsettia shoots using indolebutyric acid (IBA). IBA is particularly recommended in warm climates or when propagation is delayed.

Root, dry biomass, and fresh biomass weight. The statistical analysis and factorial design determined that sand was the best substrate; the fresh biomass weight was significantly higher using this substrate (Table 3).

Furthermore, this study determined that the 40-cm shoots resulted in plants with higher fresh and biomass weights (Table 4). The latter was confirmed in the factor interaction analysis (Table 5).

These results are like those reported by Cachique *et al*. (2011), who evaluated two substrate types, five IBA doses, and three shoot lengths to optimize the asexual propagation of sacha inchi (*Plukenetia volubilis* L.). Their factor interaction analysis indicated that the use of intermediate texture sand, 0.2% of IBA, and 8-cm shoots was the best combination for high rooting percentages and plant quality. Similarly, Boschini-Figueroa and Rodríguez (2002) reported the significantly ($P \leq 0.01$) successful rooting and establishment of white mulberry

Table 3. Effect of different substrates on the plants developed from *Carica papaya* L. shoots.

Substrate	BFW*	BDW	RFW	RDW
Sand	24.91 a**	5.19 a	3.21 a	0.63 a
Peat Moss®	17.87 ba	5.39 a	2.87 a	0.55 a
Mixture	16.00 b	3.98 a	2.43 a	0.46 a

*BFW=Biomass fresh weight, BDW=Biomass dry weight, RFW=Root fresh weight, RDW=Root dry weight. **Means with different letters in the same column are statistically different according to the Tukey test ($p \leq 0.05$).

Table 4. Effect of shoot length in *Carica papaya* L. plants.

Stem length (cm)	BFW*	BDW	RFW	RDW
40	24.33 a**	6.25 a	3.75 a	0.73 a
30	14.86 b	3.45 b	1.93 b	0.36 b

*BFW=Biomass fresh weight, BDW=Biomass dry weight, RFW=Root fresh weight, RDW=Root dry weight. **Means with different letters in the same column are statistically different according to the Tukey test ($p \leq 0.05$).

Table 5. Effect of the multiple interactions of different substrates and shoot lengths on the plants developed from *Carica papaya* L. shoots.

Interaction (cm)	BFW*	BDW	RFW	RDW
Sand - 40	29.50 a**	7.02 a	4.12 a	0.77 a
Sand - 30	20.33 ba	3.36 a	2.30 a	0.50 a
Peat Moss®- 40	23.25 ba	5.85 a	4.00 a	0.77 a
Peat Moss®- 30	12.50 b	4.93 a	1.75 a	0.32 a
Mixture- 40	20.25 ba	5.90 a	3.12 a	0.65 a
Mixture- 30	11.75 b	2.06 a	1.75 a	0.27 a

*BFW=Biomass fresh weight, BDW=Biomass dry weight, RFW=Root fresh weight, RDW=Root dry weight.
**Means with different letters in the same column are statistically different according to the Tukey test ($p \leq 0.05$).

(*Morus alba*) shoots using IBA, compared to untreated shoots.

CONCLUSIONS

This protocol allows the rooting of *C. papaya* shoots and their conservation by establishing mass production units. The 40-cm shoot and sand substrate interaction showed the highest rooting percentages. The proper management of propagation material during collection and the different intervening factors, such as substrate type, age of the mother plant, and use of growth regulators, are crucial for obtaining satisfactory results.

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Characterization of fruit and vegetable waste as an alternative ruminant feed in Pachuca, Hidalgo, Mexico

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ABSTRACT

Objective: Measure and characterize the fruit and vegetable waste generated during a working day in a commercial juice and fruit cocktail establishment in Pachuca, Hidalgo, Mexico, for four weeks.

Design/methodology/approach: The total amount of fruit waste generated during a working day in a commercial juice and smoothie establishment in Pachuca, Hidalgo, Mexico, was collected Monday through Saturday for four weeks in September and October 2019. Waste was weighted and separated daily into the different types of fruit. We then analyzed their dry matter (DM), crude protein (CP), and ash (Ash) content. The experimental design was completely randomized.

Results: The amount of fruit and vegetable waste was constant during the four collection weeks; the total amount of waste collected was 465 kg; orange peel was the most abundant waste (75%). The moisture content ranged from 75 to 94% based on the type of fruit. The CP concentration ranged from 4.33 to 6.95%, except for the papaya peel, which had a CP content of 11.55%.

Limitations/implications: An alternative to avoid landfilling this type of organic waste is to subject it to a silage-making process; this would reduce negative environmental impacts, generate value-added products, and reduce the pressure on natural resources.

Findings/conclusions: The amount of fruit and vegetable waste produced every week was constant for four weeks. Although their dry matter content is low, these wastes, mixed with cereal straw and subjected to a silage-making process, can result in value-added products, and avoid landfilling.

Keywords: fruit peel, organic waste, sustainability, landfills.

INTRODUCTION

Feeding strategies for an economical, safe, and quality-based production are among the main objectives of animal production (Makkar, 2016). The use of unconventional products, such as fruit and vegetable waste and agroindustrial subproducts, as ruminant feed, represents an important recycling strategy (Almaraz *et al*., 2012). This alternative use reduces greenhouse gas emissions into the atmosphere (Almaraz *et al*., 2012), production costs, competition for food between

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animals and humans (Dellomonaco *et al*., 2010), and the destruction of forests and biodiversity resulting from agricultural practices (Torres *et al*., 2020). In Mexico, orange is the most important fruit used for preparing juices and fruit cocktails (Bautista-Mayorga *et al*., 2020); significant amounts of organic waste correspond to orange peel and pulp, which are rich in fiber, vitamins, and minerals (Gómez and Schwentesius, 1997; Cedillo-Portugal and Anaya-Rosales, 2018). Fruit and vegetable landfill wastes represent an important source of greenhouse gas emissions (CH_4 and CO_2 mainly) and promote pest development (Environmental Protection Agency [EPA], 2020). Data on the amount of fruit and vegetable waste generated by food processing, such as in juice and cocktail making, is limited to zero. Plazzotta *et al*. (2020) estimate that fruit and vegetable waste represent 60% of the annual food waste generated worldwide; this amount is continuously increasing and requires implementing proper management strategies and alternative uses to obtain value-added products, such as biogas.

Thus, this study aimed to estimate the amount of fruit waste produced daily in a commercial juice and fruit cocktail establishment in Pachuca, Hidalgo, Mexico, and its dry matter and protein content as a potential alternative for ruminant feed.

MATERIALS AND METHODS

Materials and Methods. The total amount of fruit waste generated during a working day in a commercial juice and smoothie establishment in Pachuca de Soto, Hidalgo, Mexico, was collected Monday through Saturday for four weeks in September and October 2019. Wastes were weighted and separated daily by fruit type: carrot (*Daucus carota*) and beetroot (*Beta vulgaris*), orange peel (*Citrus* × *sinensis*), banana peel (*Musa* × *paradisiaca*), pineapple peel (*Ananas comosus*), papaya peel (*Carica papaya*), and vegetable waste containing mixtures of lettuce (*Lactuca sativa*), spinach (*Spinacia oleracea*), celery (*Apium graveolens*), and others. In the end, the amount of waste was grouped by day to identify the weekly trend.

Chemical analysis. Chemical analyses were carried out in the Animal Nutrition and Reproduction Laboratory of the Universidad Autónoma del Estado de Hidalgo. Partial dry matter was obtained by weighting representative samples (5-10% of each fruit waste) and placing them on aluminum trays for dehydration in a drying oven (Riossa, Mexico) for 48 h at 55 °C. Samples were then processed to determine total dry matter (DM, method 934.01), crude protein (CP, method 954.01), ashes (Ash, method 942.05), and organic matter (OM) according to the Association of Official Analytic Chemists (AOAC, 1990).

Statistical analysis. The statistical analysis was performed with the Microsoft® Excel® Solver Add-in data analysis tool (2013). The experimental design was completely randomized, and means were compared with the function "Two-factor analysis of variance with various samples per group" adjusted to a significance level of α=0.05.

RESULTS AND DISCUSSION

Orange peel was the most abundant waste during the collection period (Figures 1 and 2), representing 75% of the total waste (351 kg from Monday to Saturday for four

Figure 1. Fruit and vegetable waste generated in the commercial establishment "Sandwichon" in Pachuca, Hidalgo, Mexico.

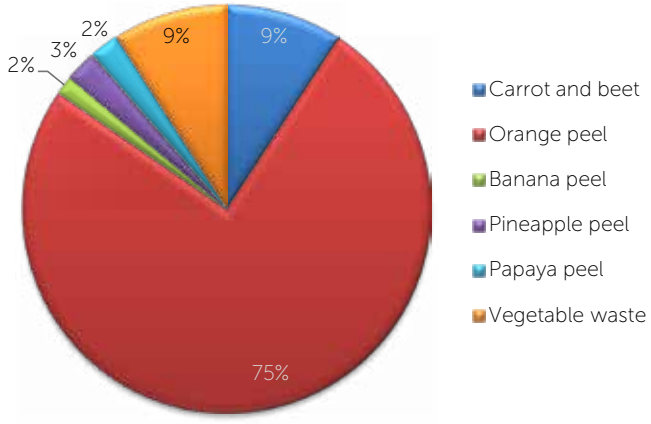

Figure 2. Proportion of fruit and vegetable residues generated in a commercial juice and fruit cocktail establishment in Pachuca, Hidalgo, Mexico.

weeks, Table 1). The second most abundant waste was carrot and beetroot (9%); the remaining waste represents 2 and 3%.

These results coincide with previous reports by Gómez and Schwentesius (1997) and Bautista-Mayorga *et al*. (2020) that indicate that orange is the most consumed fruit in Mexico. Banana peel was the least produced waste; pineapple and papaya peel were generated in similar amounts ($p>0.05$, Table 1). The amount of waste per day was similar throughout the week ($p>0.05$), which implies that the consumption of these products and the expenses necessary for their transportation and disposal are also constant throughout the week.

A total of 465 kg of waste was collected during the four-week collection period in a commercial juice and cocktail fruit establishment. These results indicate the need to generate proper sustainable management strategies in the commercial establishments of Pachuca and the main urban areas, as proposed by Inthapanya et al. (2013) and Yen et al. (2017). The DM content (Table 2) ranged between 5 and 24%, depending on the fruit type.

Yen *et al*. (2017) and Plazzotta *et al*. (2020) mention that this type of waste generates significant environmental problems due to its high moisture content and biodegradability, complicating its transportation. Despite the extensive and constant research on the management of fruit and vegetable waste, their main destination is represented by landfills, composting, anaerobic digestion, and carbonization (Yen *et al*., 2017; Dos Santos *et al*., 2020; Plazzotta *et al*., 2020). Based on the DM, OM, and CP content of the fruit and vegetable waste analyzed in this study (Table 2) and considering that this type of waste is rich in fiber, vitamins, and minerals (Gómez and Schwentesius, 1997; Cedillo-Portugal and Anaya-Rosales, 2018), subjecting it to a silage-making process represent a valuable strategy with environmental and economic advantages (income from the sale of value-added products). According to the EPA (2020), recycling food waste is one of the best organic waste management strategies. In Mexico, food waste production is constant and expected to increase due to the importance of fruit production as a source of economic resources. Mexico is a country with a great ecological, climatic, and soil diversity that promotes a great variety of fruits (Cedillo-Portugal and Anaya-Rosales, 2018).

Table 1. Fruit and vegetable waste generated from Monday to Saturday for four weeks in a commercial juice and fruit cocktail establishment in Pachuca, Hidalgo, Mexico.

	Fruit type (kg, wet basis)						
Day	Carrot and beetroot	Orange peel	Banana peel	Pineapple peel	Papaya peel	Vegetable waste	SEM
Monday	1.87^{aA}	15.00^{bB}	0.26^{cC}	0.46^{dD}	0.44^{dD}	2.15^{aA}	1.12
Tuesday	1.80^{aA}	12.50^{bB}	0.30^{cC}	0.49^{dD}	0.43^{dD}	1.77^{aA}	0.94
Wednesday	1.55^{aA}	14.00^{bB}	0.36^{cC}	0.57^{dD}	0.43^{dD}	1.80^{aA}	1.04
Thursday	1.55^{aA}	14.50^{bB}	0.28^{cC}	0.50^{dD}	0.43^{dD}	1.22^{aA}	1.09
Friday	2.02^{aA}	16.75^{bB}	0.29^{cC}	0.46^{dD}	0.46^{dD}	1.70^{aA}	1.26
Saturday	1.92^{aA}	15.00^{bB}	0.26^{cC}	0.42^{dD}	0.47^{dD}	1.92^{aA}	1.11
SEM	0.08	0.61	0.15	0.02	0.01	0.13	

^{abcd} Means in the same column with a different letter are statistically different ($p<0.05$). ^{abcd} Means in the same row with a different letter are statistically different ($p<0.05$). SEM=standard error of the mean.

For a more efficient silage-making process, forage moisture content must range between 60 and 70%, or 30 and 40% of DM. Considering the proportion of fruit and vegetable waste (Figure 1) and their DM content (Table 2), the DM concentration of the waste mixture would be 18%, which could difficult the aerobic stage of the silage-making process. However, to increase the proportion of DM up to more than 30% (Table 3), a viable option is to add cereal stubble (wheat, oats, barley, corn, or sorghum, which have a DM content of 85-90%) and maintain an 8:2 ratio of waste: stubble. Therefore, the moisture content is adjusted to what Cobos (s/a) recommends limiting the aerobic stage and obtain good silage.

Table 2. Nutrient concentration (%) in fruit and vegetable waste.

Substrate	DM	Moisture	CP	Ash	OM
Carrot	9.98	90.02	5.98	6.76	93.24
Banana	12.81	87.19	6.50	16.95	83.05
Pineapple	14.13	85.87	6.20	4.87	95.13
Papaya	12.23	87.77	11.66	9.91	90.09
Orange	24.14	75.86	4.33	8.85	91.15
Vegetable waste	5.59	94.41	6.95	8.94	91.06

DM=Dry matter; CP=Crude protein; Ash=Ashes; OM=Organic matter.

Table 3. Proposal to make silage from fruit and vegetable waste and increase the proportion of DM.

Substrate	As feed (%)	DM (%)	CP (%)
Carrot	7.20	0.72	0.43
Banana	1.60	0.20	0.10
Pineapple	2.40	0.34	0.15
Papaya	1.60	0.20	0.19
Orange	6.00	12.68	2.60
Vegetable waste	7.20	0.40	0.50
Cereal stubble	20.00	17.20	1.20
Total	100.00	31.74	5.38

CONCLUSIONS

The amount of fruit and vegetable waste generated in a commercial juice and cocktail fruit establishment is constant throughout the week, with a dry matter and protein content lower than 20% and close to 7%, respectively. This waste, added with cereal stubble, create value-added products for ruminant feeding that could decrease their disposal to landfills.

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Analysis of the productive and immune response of lambs infected with gastrointestinal nematodes and fed with saccharin

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ABSTRACT

Objective: Determine the productive and immune response of Blackbelly lambs infected with gastrointestinal nematodes (GIN) and fed with saccharin.

Design/ methodology/ approach: A total of 18 Blackbelly lambs, with an initial live weight (LW) of 13.9 + 3.2 kg, were randomly assigned to three different treatments (T): T1, anthelmintic treatment + basal diet (CTah); T2, basal diet without anthelmintic treatment (STah); and T3, grazing lambs without anthelmintic treatment (STPS). This experiment followed a completely randomized design with repeated measures over time; mean values were compared using Lsmeans. The parameters evaluated included live weight (LW), fecal egg count per gram (FEC), packed-cell volume (PCV), plasma protein (PP), white blood cell differential count (LEU), and IgA concentration by ELISA with *Haemonchus contortus* and *Trichostrongylus colubriformis antigens*.

Results: STah and CTah lambs showed higher FEC (885 ± 142) and LW (29.73 ± 5.06 kg). Grazing lambs (STPS) had lower PCV (26.4 ± 0.5%) compared to the STah and CTah lambs (27.4 to 28.4%) due to the high prevalence of *H. contortus*. The IgA concentration in grazing lambs ranged from 20.2 to 24.5% of the positive standard serum titer. The feedlot lambs (STah and CTah) showed values close to 5%.

Study limitations/implications: Due to anthelmintic resistance problems, it was impossible to maintain grazing lambs free of infection; therefore, this group was not included.

Findings/conclusions: Saccharin increases sheep resilience and achieves adequate weight gains in parasitized lambs.

Keywords: *Haemonchus contortus*, *Trichostrongylus colubriformis*, humoral immunity, cellular immunity.

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INTRODUCTION

Gastrointestinal nematodes (GIN) affect the health of grazing sheep and reduce flock productivity (Roeber *et al*., 2013). Anthelmintics have been used as the only control strategy, but their continued use has decreased effectiveness and developed anthelmintic resistance (AR). Different control methods have been used to avoid AR (Bishop, 2012). In lambs, an alternative is feedlot fattening; this avoids parasitic effects and increases productivity. However, these lambs are susceptible to infection with AR GIN, even after receiving prophylactic treatment, with detrimental effects on their health.

Feeding strategies have been used to increase immunity and reduce parasitic effects (Torres-Acosta *et al*., 2012). Sugarcane has stood out in ruminant feeding due to its high sugar concentration, which decreases feeding costs. Its processing as saccharin enriched with energy-protein compounds meets the nutritional requirements of sheep (Godínez *et al*., 2017), improving productivity and promoting the immune system; thus, controlling the infection with established parasites. Therefore, this study aimed to determine the productive indices and the immune response of Blackbelly lambs infected with GIN and fed with saccharin.

MATERIALS AND METHODS

Location, animals, and feeding

This study was performed in 2016 in Salto de Agua, Chiapas (17° 34' N and 92° 29' W, at 85 masl). This region has a tropical climate with year-round rainfall, with annual rain precipitation of 3,346 mm and an annual mean temperature of 26.5 °C (CONAGUA, 2017). A total of 18 Blackbelly lambs, with an initial live weight (LW) of 13.9 ± 3.2 kg, were randomly assigned to three different treatments (T). In T1, lambs were dewormed every two months with albendazole and levamisole (10 and 7.5 mg kg^{-1} LW) and housed in individual cages with feeding and drinking troughs. Animals were fed an integral saccharin-based diet (CTah), formulated as indicated by Godínez-Juárez (2017). In T2 (STah), lambs did not receive anthelmintic treatment during the entire study. Animals were fed as those in T1 (Table 1). Lambs were subjected to a 15 d-adaptation period in which they were fed twice a day with amounts ranging from 3.0 to 3.5 kg in total. Rejection of at least 30% of the offered feed was allowed due to big sugarcane particles that the animals rejected. T3 included lambs fed by grazing of *Urochloa brizantha* cv. Humidicola CIAT-679 (Rendle) Schweickerdt, eight hours daily. These animals did not receive anthelmintic treatment (STPS). Lambs were locked up at night in a galley and provided with water and mineral salts.

Dry matter intake (DMI, kg d-1) was calculated daily by measuring the difference between the offered and rejected feed. Daily weight gain was also determined (DWG, kg d^{-1}), and feed conversion (FC) was calculated as the DMI/DWG ratio. Dry matter (DM), crude protein (CP), and true protein (TP) content were determined following the methodology indicated by the AOAC (AOAC, 2005).

Parasitological and hematological sampling

During the five-month study period, fecal samples were collected by rectal stimulation every 21 d; nine collections in total. Samples were collected in plastic bags and kept in ice until further processing. In the laboratory, samples were subjected to the McMaster technique, with a sensitivity of 50 FEC (Cringoli *et al*., 2004). Fecal cultures were established to isolate the larvae and perform morphological identification (Van Wyk and Mayhew, 2013).

Additionally, two blood samples (3 mL) were collected from the jugular vein using Vacutainer needles. The first sample was collected in tubes containing an anticoagulant (BD Vacutainer); the second sample was collected in serum separator tubes, without anticoagulant (BD Vacutainer). PCV was determined by the microhematocrit technique (Weiss and Wardrop, 2010), and a refractometer was used to measure PP (g dL^{-1}).

Table 1. Ingredients and chemical composition of the diet.

Content	Percentage	Bromatological composition		
		Chemical composition	Mean	Standard Deviation
sugar cane	74	Dry matter (%)	48.7	7.7
soybean paste	4	Crude protein (%)	14.3	2.4
ground sorghum	20	Ash (%)	2.8	0.7
Minerals	0.5	Organic matter (%)	97.2	0.8
Urea	1	Neutral Detergent Fiber (%)	28.9	6.7
Magnesium sulfate	0.5	Acid Detergent Fiber (%)	16.1	4.1
		Hemicellulose (%)	15.3	0.8

IgA determination by ELISA

Serum samples were stored in Eppendorf tubes at −20 °C until IgA analysis. Following the methodology described by González-Garduño *et al.* (2017), IgA concentration was determined using an indirect ELISA with antigen obtained from the crude extract of *H. contortus* and *T. colubriformis* adult nematodes. The optical density (OD) of each serum was obtained by subtracting the blank, which represented the non-specific binding of the conjugate. The IgA activity was expressed as percentages of the positive standard serum, based on the equation indicated by Cardoso *et al.* (2013).

Statistical analysis

Obtained data were analyzed using the SAS MIXED procedure (SAS, 2017) for a completely randomized model with repeated measures over time. Log transformation of FEC was performed [Log (HPG+1)] to approximate the model to a normal distribution.

The statistical model used in this experiment was the following:

$$Y_{ijk} = \mu + \gamma_i + \zeta_j + \gamma^*\zeta_{ij} + \delta_k + \varepsilon_{ijk}$$

Where: Y_{ijk} = Response variable, μ = General mean, γ_i = Treatment effect (i=CTah, STah, STPS), ζj = Sampling day effect, $\gamma^*\zeta_{ij}$ = Treatment and sampling day effect, δ_k = Random effect of the animal, ε_{ijk} = Random error ~(0, σ2).

RESULTS AND DISCUSSION

Productive indices

DMI was higher (p≤0.05) in STah lambs compared to the CTah group (1.04±0.24 kg *vs* 0.94±0.28 kg). FC and DWG were the same in both groups. The DWG of feedlot lambs was 95 and 99 g, which is 102% higher than the DWG of grazing lambs (49 g) (p≤0.05; Table 2).

Godínez-Juárez *et al.* (2017) reported that a DMI of 1.2 kg d^{-1} results in DWG higher than 90 g in feedlot animals. STah and CTah lambs maintained similar DWGs and intakes. Contrary to that observed by Cardia *et al.* (2011), who reported that animals infected with *Trichostrongylus* spp. showed a 37% weight loss due to their lower nutrient absorption capacity (McRae *et al.*, 2015). This was not observed in this study, probably because feedlot lambs' nutrient requirements were met through 14% CP diets, necessary for achieving DWG higher than 100 g (NRC, 2007; Table 1), even with parasitosis. Growth differences between the feedlot and grazing lambs were evident when comparing their DWG (p≤0.05); grazing animals had the lowest final weight of both groups. Although STah lambs showed higher FEC counts than the CTah group, their DWG were similar (p>0.05), which indicates lambs develop some degree of resilience when fed properly (Torres-Acosta *et al.*, 2012).

Parasitological and hematological variables

The FEC was lower (p≤0.5) in CTah lambs (146±31.0) than in grazing (502±71) and STah lambs (885±143; Table 3).

The PCV of feedlot lambs (STah and CTah) was higher (27.4 to 28.4 %) than that of grazing lambs (p≤0.05; 26.4±0.5 %); this may be related to the nutritional level. A previous study reported that the anemia induced by *H. contortus* was affected by nutrition (Cériac *et al.*, 2017). The highest PP value (p≤0.05) was observed in grazing lambs (6.9±0.1 g dL^{-1}). STah (6.4±0.1 g dL^{-1}) and CTah (6.3±0.1 g dL^{-1}) had similar values (p>0.05). Additionally, grazing lambs showed the highest (p≤0.05) IgA percentage with values ranging from 20.2 to 24.5% of the positive standard titer. STah and CTah lambs behaved similarly (p<0.05; 2.9 to 4.0%) against

Table 2. Feed consumption, live weight, and feed conversion changes in Blackbelly lambs fed with saccharin.

Variable	Stabling		Grazing without anthelmintic GWT
	Without anthelmintic SWT	With anthelmintic STA	
Initial body weight (kg)	14.05±2.6[a]	12.16±3.6[a]	15.5±2.7[a]
Final body weight (kg)	29.73±5.06[a]	27.32±6.56[a]	23.38±1.02[b]
Change weight (kg)	15.68	15.16	7.88
Days	159	159	159
Food intake (kg d^{-1})	2.50±0.59[a]	2.28±0.67[b]	
Dry matter intake (kg d^{-1})	1.04±0.24[a]	0.94±0.28[b]	
Daily gain weight (kg d^{-1})	0.099[a]	0.095[a]	0.049[b]
Food conversion	10.51	9.89	

Means with different literal in each row are significantly different (P<0.05).

Table 3. Parasitological and hematological variables in Blackbelly lambs, with and without anthelmintic treatment.

Variable	Stabling		Grazing without anthelmintic GWT
	Stabling without anthelmintic SWT	Stabling with anthelmintic STA	
PCV (%)	27.4±0.6[a]	28.4±0.5[a]	26.4±0.5[b]
PP (g dL^{-1})	6.4±0.1[b]	6.3±0.1[b]	6.9±0.1[a]
EPG	885±813[a]	146±182[b]	502±493[a]
Log (EPG+1)	5.9±1.4 [a]	3.1±2.4[c]	5.1±2.2[b]
IgA (%) - *H. contortus*	3.7±0.5[b]	4.0±0.8[b]	20.2±5.3[a]
IgA (%) - *T. colubriformis*	2.9±0.5[b]	3.6±0.7[b]	24.6±8.2[a]

PCV. Packed cell volume. PP. Plasma protein, EPG. Eggs per gram of faeces. IgA (%) regarding to positive standard (RPS) using two crude antigens of adult nematodes. Averages with different literals in same rows are significantly different (P<0.05).

the *H. contortus* and *T. colubriformis* antigens (Torres-Acosta *et al*., 2012).

After larval identification, it was found that the most prevalent species was *H. contortus* (>33% of the identified larvae). *T. colubriformis*, *Cooperia curticei*, and *Oesophagostomum colombianum* were also abundant, and *Strongyloides papillosus* in low proportion (Figure 1).

The fecal egg count in STah increased 35 d after starting the confinement period. Subsequently, the FEC lowered and remained stable with similar values to those reported for grazing animals (Figure 2). Notably, STah animals had the highest FEC because parasites continue their life cycle.

The increase in FEC and its subsequent reduction in STah lambs has been reported in other studies as a long-term immunological effect. Infection levels were not high enough to a show statistical difference in the DWG of infected and non-infected animals (p≤0.05). However, there were differences in the immunological parameters when compared to grazing lambs. Therefore, it may be inferred that constant reinfection triggers the immune response since grazing lambs had higher IgA levels (Aguilar *et al*., 2011).

After infection, PCV values decreased in all experimental groups. However, these values returned to normal 35 days post-infection, especially in the STah group. The recovery in grazing lambs was slower (Figure 3).

PCV values were affected by the feeding practices; the lowest values were observed in grazing lambs infected with *H. contortus*. Moreover, due to the constant infection with GIN, the PP of grazing lambs was higher (p≤0.05) than in CTah and STah lambs; this was associated with the higher concentration of IgA observed in grazing lambs. After starting the study, IgA levels ranged from 15 to 30% of the positive standard titer. Later, these levels

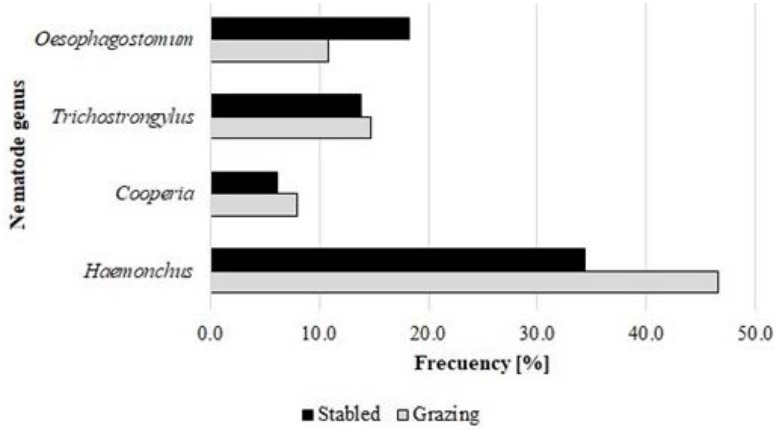

Figure 1. Gastrointestinal nematode genera found in Blackbelly lambs according to the production system.

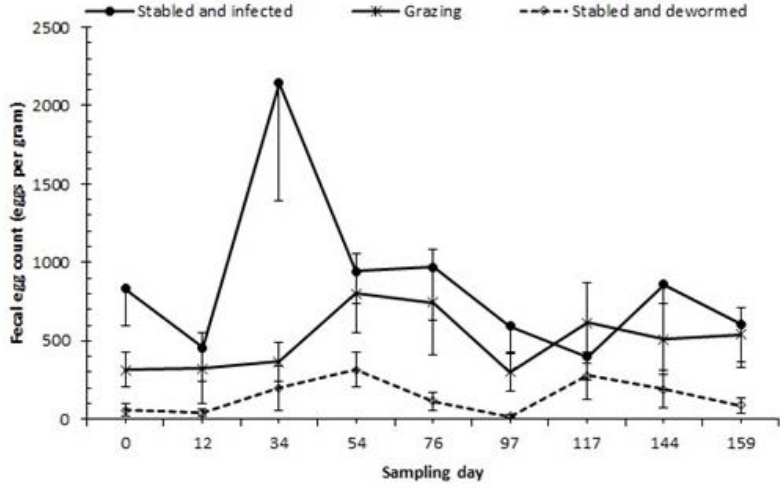

Figure 2. Fecal egg count per gram in Blackbelly feedlot (CTah and STah) and grazing lambs.

increased to 35%. Feedlot lambs (CTah and STah) showed very low levels of IgA (Figure 4). In grazing lambs, the host-parasite interaction after reinfection promoted higher levels of PP and IgA as an acquired immune response. Feedlot lambs (CTah and STah) had similarly low IgA levels (3.72±0.47 and 4.02±0.83% of the positive standard titer, respectively) against the *H. contortus* antigen.

Due to the lack of immunological stimulus, STah lambs could not maintain high levels of IgA. Strain and Stear (2001) reported that animals with properly supplemented diets had small nematodes and produced more IgA than lambs with non-supplemented diets. However, this did not occur in the present study; feedlot animals had no immunity-promoting reinfections (Santos *et al.*, 2014). Therefore, it is assumed that IgA production is stimulated by the infection degree (McRae *et al.*, 2015).

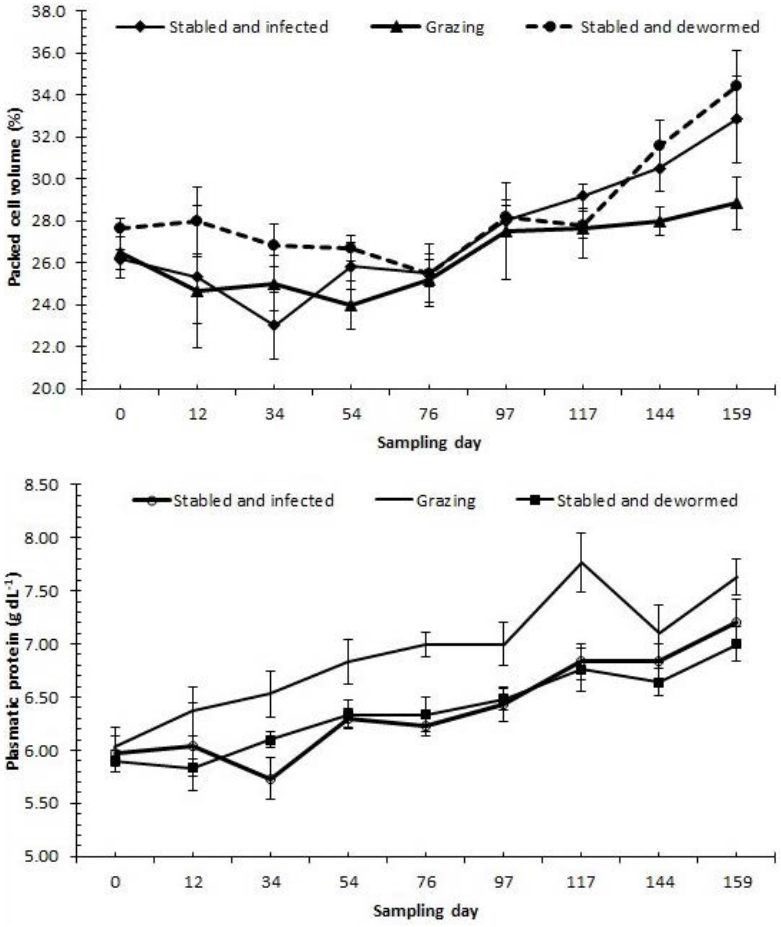

Figure 3. Packed-cell volume and plasma protein in Blackbelly lambs by sampling day.

CONCLUSIONS

Feedlot lambs with an established gastrointestinal nematode infection and without anthelmintic treatment showed higher fecal egg counts and lower IgA levels than grazing lambs; this implies that the reinfection continues during grazing and stimulates the development of the acquired immune response.

Growing lambs fed an integral saccharin-supplemented diet had higher packed-cell volumes and weight gains than grazing lambs; this allowed animals to express resilience by tolerating the established nematode infection and obtaining the highest productive parameters. However, their elevated fecal egg count indicates that animal resistance did not increase.

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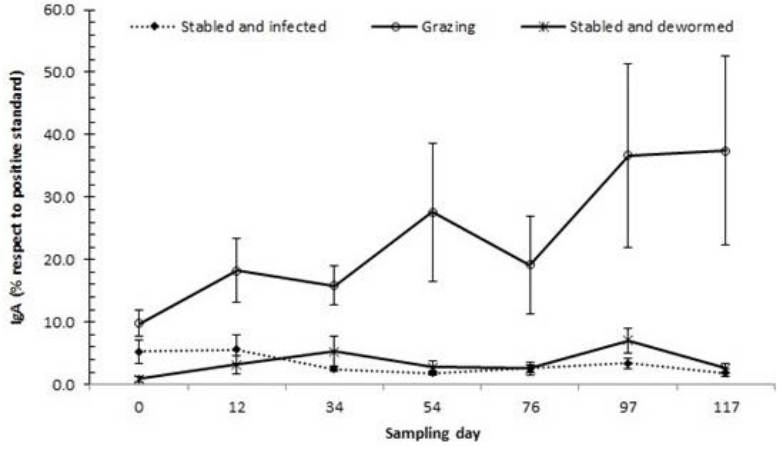

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Productive performance of rabbits fed alfalfa (*Medicago sativa*)- or white clover (*Trifolium repens*)-based diets

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ABSTRACT

Objective: Evaluate the productive performance of rabbits fed alfalfa- and white clover-based diets.

Design/methodology/approach: Rabbits can consume high-fiber diets. Therefore, this study evaluated a white clover-based diet (Diet 1) and an alfalfa-based diet (Diet 2). The experiment was carried out during the Fall-Winter season of 2018. This study used 111 rabbits (males and females), weaned at 31 days of age. Diets were randomly assigned to 20 cages, four to six animals per cage. In total, 56 rabbits received Diet 1 and 55 Diet 2. All animals had free access to feed and water during the 35-day growth period. The weekly recorded response variables were live weight (LW), daily weight gain (DWG), and feed conversion (FC). At 67 days of age, hot carcass yield (HCY) and cold carcass yield (CCY) were determined.

Results: At the end of the fattening period (31 to 66 days of age), there were no significant differences between the two experimental groups. The results for Diet 1 and 2, respectively, were the following: LW=2012±36 and 1960±37 g, DWG=32.2±1.57 and 28.4±1.60 g/animal, FC=3.3±0.2 and 3.4±0.2 g/g, HCY=48.0±0.5 and 48.1±0.5%, and CCY=55.9±0.6 and 55.8±0.7%.

Limitations/implications: Due to the lack of significant differences (P≥0.05), further studies are required to better understand these legumes.

Findings/conclusions: White clover could completely replace alfalfa in rabbit diets.

Keywords: white clover, alfalfa, growing rabbits.

INTRODUCTION

Rabbit is a viable food alternative for humans. Its meat is rich in protein (Guevara-Hernández et al., 2014), with values ranging from 19 to 25%; protein content in chicken meat ranges from 12 to 18% (Gómez, 2017). However, in Mexico, concentrate-based feeding is expensive. In September 2019, the price per kilogram of balanced feed from the

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main brands fluctuated between 8.05 and 9.38 Mexican pesos. Therefore, it is necessary to evaluate alternative rabbit feeding ingredients that promote a more profitable production (Ogbuewu *et al*., 2017). Rabbits require fiber-rich diets (De Blas, 1989); thus, ingredients, such as alfalfa flour, are commonly used in their diets (Pond *et al*., 2012). These ingredients can constitute 100% of the ration when high weight gains are not intended (De Blas, 1989). Sánchez-Laiño *et al*. (2018) studied the use of *Morus alba*, *Erythrina poeppigiana*, and *Tithonia diversifolia* in rabbits. However, no information was found regarding the use of white clover instead of alfalfa. Therefore, this study aimed to evaluate the productive response of rabbits fed a white clover-based diet compared to an alfalfa-based diet.

MATERIALS AND METHODS

Localization

The experiment was carried out at the rabbitry unit, annex to the Experimental Poultry Farm of the Department of Zootechnics of the Universidad Autónoma Chapingo, located at 19° 29' 13.7" N and 98° 53' 48.0" W, at an altitude of 2278 m, in Chapingo, Texcoco, Mexico State. This location has a "Cb (w0) (w)" climate, the driest of the Cw type, which corresponds to a humid subtropical climate with fresh and rainy summers and an annual mean temperature of 12-18 °C (García, 2004).

Experimental development

The experimental phase comprised the Fall-Winter period of 2018. This study used 111 rabbits (males and females), weaned at 31 days of age. These rabbits resulted from crosses of the following breeds: New Zeland, California, Chinchilla, Mariposa, and Azteca Negro. Two rabbit groups were used; the first had an average initial weight of 725±21 g and the second of 793±21 g. Subjects in group 1 were fed with Diet 1 (white clover), those in group 2 with Diet 2 (alfalfa). Animals were housed in metallic commercial fattening cages (650 cm^2/animal) equipped with hopper feeders and water bottles.

Treatments

Two diets were evaluated, a white clover-based diet (Diet 1) and an alfalfa-based diet (Diet 2). The composition of diets 1 and 2, respectively, included: white clover flour (41.42 and 0.00%), alfalfa flour (0.00 and 66.82%), wheat bran (15.95 and 10.16%), ground corn (17.93 and 18.37%), sunflower shells (21.38 and 0.00%), vegetable oil (21.38 and 0.00%), molasses (0.00 and 1.21%), threonine (0.12 and 0.04%), L-lysine HCl (0.41 and 0.18%), DL-methionine (0.38 and 0.14%), dicalcium phosphate (1.53 and 0.00%), minerals and vitamins premix (0.28 and 0.27%). Both diets included 0.10% of robenidine and 0.50% of sodium chloride. Moreover, both diets were subjected to a proximate and Van Soest analysis according to the AOAC (1990) and Van Soest (1994). The chemical fractions determined as feed in diets 1 and 2, respectively, were: dry matter (88.8 and 88.5%), crude protein (17.5 and 16.0%), ether extract (2.6 and 2.6%), crude fiber (17.8 and 14.2%), ashes (9.9 and 9.7%), neutral detergent fiber (33.7 and 27.8%), cell content (66.3 and 72.2%), acid detergent fiber (25.3 and 18.8%), hemicellulose (8.4 and 9.0%), cellulose (19.1 and 14.9%), lignin (5.8 and 3.6%), calcium (1.1 and 1.7%), phosphorus (0.7 and 0.3%). The concentration of digestible energy in diets 1 and 2 was 2,224 and 2,228 kcal kg^{-1} of feed, respectively. Live weight (LW), feed intake, daily weight gain (DWG), and feed conversion (FC) were evaluated every seven days until reaching 35 days of fattening (66 days of age). Hot carcass yield (HCY) and cold carcass yield (CCY) were determined at 36 days of fattening (67 days of age). The clean, hot carcass, without its head, hind and forelegs, and viscera (except kidneys), was weighed at the end of the slaughter. The hot carcass was immediately placed in iced water (12 h at 2-8 °C), drained for 12 h, and weighed. HCY and CCY were expressed as a fraction of the LW.

Statistical analysis

This experiment followed a completely randomized design. For the LW and DWG variables, each rabbit was considered a replicate (55 and 56 replicates, respectively, for diets 1 and 2). For feed conversion (FC), each cage was considered a replicate (10 replicates per treatment, 4-6 rabbits per replicate). For HCY and CCY, each carcass was considered a replicate (41 and 36 replicates for diet 1 and 2, respectively). To compare the two diets, statistical models were run weekly for all the variables, except for HCY and CCY, for which the statistical models were only run once at slaughter. Means (of each diet) were compared using the F test ($P \leq 0.05$) of the analysis of variance (SPSS, 2011).

RESULTS AND DISCUSSION

Live weight was the same in both treatments ($P > 0.05$), except at the beginning of the experiment (Table 1).

However, despite their lower initial live weight (68 g lower), rabbits fed a white clover-based diet reached the same final weight as those fed with alfalfa ($P > 0.05$). They even showed higher final weights (52 g higher) than those

fed with alfalfa. In a hypothetical example with 1000 rabbits, the 52 g difference would represent 52 kg of LW at slaughter.

There were no weekly differences between treatments (P>0.05) for DWG and FC (Table 2). Total weight gain (1-35 days) was not statistically different between treatments (P>0.05).

Table 1. Weekly mean live weight (g) of fattening rabbits.

Days on experiment	Diet 1 (White Clover)	Diet 2 (Alfalfa)
1	725±21[a]	793±21[b]
7	1118±29	1147±29
14	1295±31	1303±31
21	1509±37	1481±37
28	1814±42	1821±42
35	2012±36	1960±37

[a, b] Different letters in the same row indicate statistical differences (P≤0.05).

The HCY and CCY of rabbits fed with white clover (48.0±0.5 and 55.9±0.6%, respectively) were not statistically different (P>0.05) from the values observed in animals fed with alfalfa (48.1±0.5 and 55.8±0.7%, respectively).

Composition of the experimental diets

The white clover-based diet (41.4%) contained wheat bran (15.9%) and sunflower shells (21.4%) as fiber-rich ingredients (78.7% in total). The alfalfa-based diet (66.8%) also contained wheat bran (10.2%), 77% of fiber-rich ingredients. Thus, the white clover-based diet had a higher content of fiber-rich nutrients (1.7%). Moreover, its crude protein content was 1.5% higher than that of the alfalfa-based diet. High levels of fiber decrease feed intake and growth (Gidenne, 2015). Therefore, the similar productive response observed with both diets was probably due to the higher protein content of Diet 1 (white clover) compared to Diet 2 (alfalfa).

Chemical determinations

The CP content of the white clover-based diet (17.5%) was higher than that of the alfalfa-based diet (16.0%). Both diets met the nutrient requirements of fattening rabbits: 14% to 16% of CP (NRC, 1977; Cheeke, 1987; De Blas and Wiseman, 2010). Moreover, these diets had 17.8% and 14.2% of CF, respectively, which corresponds to the requirements reported by Clíment (1979), Cheeke (2002), Maertens (1998) and De Blas and Wiseman (2010). These authors indicate a minimum content of 14 to 15% of CF for fattening or more than 17% (Gidenne, 2000) for ADF.

Both diets met the FDA requirements, and only the white clover-based diet met the NDF requirements. According to De Blas and Wiseman (2010), ADF and NDF requirements are 18-20% and 33-35%, respectively.

Live weight

Jiménez (2005) reported LWs of 2053, 1969, and 1949 g after 35 days of fattening with different commercial feeds (Purina, Unión Ganadera Regional de Guanajuato, and Albapesa, respectively). These values are similar to those obtained in rabbits fed with white clover (2012±36 g) and alfalfa (1960±37 g). Additionally, Gómez (2017) observed that 50% supplementation with mata ratón legume (*Gliricidia sepium*) resulted in an average LW of 2771 g at 66 days. The lowest LW occurred with the control diet (concentrate). The 75% supplemented concentrate and the Mexican sunflower-based diet (*Tithonia diversifolia*) showed average values of 2184 g and 1979 g, respectively. Due to the high protein content of *T. diversifolia* (28.5%) and *G. sepium* (23%), these diets outperformed the experimental diets evaluated in this study.

Daily weight gain

In rabbit production, the DWG during fattening ranges from 30 to 40 g/day. The most frequent values are 35-38 g d^{-1}. These values depend on the breed and feeding conditions (Méndez, 2006). There were no significant differences in DWG (P>0.05) between treatments. Rabbits fed white clover-based diets showed DWG of 32.2 g; those fed with alfalfa had DWG of 28.4 g.

Nieves *et al.* (2009) reported DWG of 29.5, 21.9, and 26.0 g

Table 2. Daily and total weight gain (g) and weekly and total feed conversion (g/g) of fattening rabbits.

Days on experiment	Daily weight gain		Feed conversion	
	Diet 1 (White Clover)	Diet 2 (Alfalfa)	Diet 1 (White Clover)	Diet 2 (Alfalfa)
1 - 7	56.1±2.1	50.6±2.1	1.89±0.11	2.18±0.11
8 - 14	31.9±2.0	35.3±2.1	3.68±0.39	3.68±0.39
15 - 21	38.9±1.6	36.3±1.6	3.56±0.44	3.10±0.45
22 - 28	37.9±3.1	46.3±3.3	3.46±0.34	3.15±0.39
29 - 35	33.1±3.0	37.6±3.7	5.61±0.82	5.61±0.87
1 - 35	32.2±1.6	28.4±1.6	3.33±0.15	3.41±0.15

[a, b] Different letters in the same row indicate statistical differences (P≤0.05).

with granulated *Leucaena leucocephala*, *Trichanchera gigantea*, and *Morus alba*, respectively. These values are lower than the ones obtained in this experiment with both experimental diets.

Gómez (2017) reported a DWG of 30.0 g when feeding diets supplemented with 50% *Gliricidia sepium*. The lowest DWG was observed when supplementing diets with 75% of *Trichantera gigantea* and 75% of *Tithonia diversifolia*, with average values of 22.8 g and 20.6 g, respectively. These values were lower than those obtained in this study with Diet 1 and 2.

Feed conversion

No significant differences (P>0.05) were observed between diets, weekly or from 0 to 35 days. Méndez (2006) suggests that FC significantly increases with age and weight.

The FC with Diet 1 was similar to Diet 2 because of its higher CP, CF, NDF, and ADF contents, which induced intestinal transit and feed intake (Gidenne, 2015).

Sánchez-Laiño *et al*. (2018) evaluated commercial feed, *M. alba*, *Erythrina poeppigiana*, and *T. diversifolia* and reported FC values of 2.93±0.27, 3.34±0.28, 3.23±0.19, and 4.08±0.45 g/g, respectively. According to Méndez (2006), FC values must range from 3.35 to 3.45 g/g, similar to the average values observed in this experiment (3.33±0.15 g/g for white clover-based diet and 3.41±0.15 g/g for the alfalfa-based diet).

Carcass yield

Roca (2009) defines HCY as the percentage relationship between the commercial carcass weight and the animal live weight. Oteku and Igene (2006) reported a HCY of 46.7±0.1% in 70-day-old rabbits fed a corn, soybean meal, and wheat bran diet. This result was lower than the one observed in this study: 48.0±0.5 and 48.1±0.5% for Diet 1 and 2, respectively. Sánchez-Laiño *et al*. (2018) reported a higher HCY in fattening rabbits fed a balanced feed and *Tithonia diversifolia* diet: 49.6±0.6%.

Furthermore, Hernández-Bautista *et al*. (2015) reported a HCY and CCY of 47.4 and 47.3%, respectively, in New Zeland × California rabbits fed with a commercial feed. These values are lower than those obtained with the white clover (HCY: 48.0±0.5% and CCY: 55.9±0.6%) and alfalfa diets (HCY: 48.1±0.5% and CCY: 55.8±0.7%).

CONCLUSION

White clover can completely replace alfalfa in diets for fattening rabbits.

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Scarification treatments in chepil seeds (*Crotalaria longirostrata* Hook. & Arn.) used to improve their germination

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ABSTRACT

Objective: The objective was to evaluate different scarification treatments to improve germination in chepil seeds (*Crotalaria longirostrata* Hook. & Arn.).

Design/Methodology/Approach: The study was established in the School of Veterinary Medicine and Zootechnics N. 2 of the Universidad Autónoma de Guerrero. The chepil seeds were weighed and counted; 2 experiments were established through a CRD with 4 treatments of 4 repetitions each. Imbibition and germination were evaluated. The data were analyzed with the statistical software package SAS® 9.0.

Results: The use of water at different temperatures and times presented positive results in imbibition and increased the germination percentages. The treatment with water at 100 °C until cooling reached a germination of 80%, and the control of 12.3%.

Study Limitations/Implications: Chepil is a wild species that has seeds with physical dormancy, which is something that requires more research in order to accelerate and increase the germination percentages.

Findings/Conclusions: The imbibition and germination was affected by the treatments applied. Chepil seeds presented physical or superficial dormancy that may be eliminated with the use of heat treatments; however, evaluations still need to be performed to accelerate and find a higher percentage of germination.

Keywords: legume, emergence, nutrition, quality.

INTRODUCTION

Endemic or vulnerable species must be conserved, and for this purpose, it is necessary to deepen knowledge and seek adequate techniques for their multiplication (Camarillo-Castillo and Mangan, 2020; Zapata *et al*., 2017). The germplasm of small or shrub species that develop in the tropics is strongly affected by environmental conditions and factors such as felling, overgrazing, burning, etc., which increases the possibility of extinction (Javed *et al*., 2013; Solh and van Ginkel, 2014); therefore, their conservation is necessary.

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Chepil, chipil or chipilín (*Crotalaria longirostrata* Hook. & Arn.) is a legume of the Fabaceae family, originally from southern Mexico and Central America; it is used for both human and animal diets (Arias *et al*., 2003). Species of the *Crotalaria* genus develop throughout the year, in tropical and subtropical zones (Casimiro *et al*., 2013; Gómez Sosa, 2000). Chepil is a wild plant that contains a large amount of protein (Laguna, 2016), with functional properties for animal nutrition; however, there is scarce bibliographical information recorded about its agronomic behavior, other than its leaves substituting the scarcity of grasses during the drought season (Sanabria *et al*., 2003).

On the other hand, huaje (river tamarind, *Leucaena leucocephala*), parota (guacanaste, *Enterolobium cyclocarpum*), carob (*Ceratonia siliqua*), chepil (*C. longirostrata*), crotalaria (*C. Juncea* L.), etc., are very important alternatives for shepherding communities, because they improve the quality of the diet and contribute nitrogen to the soil (Boschi *et al*., 2016; Sevillal and Fernández, 1991), whether cultivated as sources of protein or in associations (Benítez-Bahena *et al*., 2010; Castillo *et al*., 1993). To achieve the implantation of a fodder legume species, it does not only depend on management practices, but rather on the form of propagation and the availability of genetic material (botanical or vegetative seed); in addition, the hardness of the seed depends on the species (Zimmermann *et al*., 2003), since the seeds do not always generate a plant quickly and in some cases they take up to four months or more to germinate (Sevillal and Fernández, 1991).

Legumes are propagated primarily by seed, which in some cases presents viability; however, the so-called dormancy makes the germination percentages small, because even when there are favorable conditions, they are incapable of absorbing and emerging (Doria, 2010; Sanabria *et al*., 2003). Latency is a mechanism for defense and survival; it can be reduced by using mechanical, physical or chemical scarification, which allows accelerating the process of germination (Kimura and Islam, 2012). Studies carried out about germination of legume seeds show that physical scarification is positive when germination is minimal (Juárez and Lagunes, 2014; Sánchez-Paz and Ramírez-Villalobos, 2006; Villagra *et al*., 2004); in addition, the use of treatments with hot water is effective for some species (Navarro *et al*., 2010; Zapata *et al*., 2017). The hypothesis of this study is that chepil seeds present dormancy, which must be overcome with some pre-germination treatment. Therefore, the objective of this study was to evaluate different scarification treatments to improve germination in chepil seeds (*Crotalaria longirostrata* Hook. & Arn.), in the dry tropics.

MATERIALS AND METHODS

The study was carried out in the fodder area of the School of Veterinary Medicine and Zootechnics N. 2, located in Cuajinicuilapa, Guerrero, Mexico, at 16° 28' 28'' LN and 98° 25' 11.27'' LW, at an altitude of 46 m; this is a zone with dry tropical climate and summer rains, average precipitation of 1,129 mm, which is distributed in the months of June to October and mean annual temperature of 28.4 °C (García, 2004).

The experiment was established in January, 2020. Prior to this, dry chepil fruits (*Crotalaria longirostrata* Hook. & Arn.) were harvested, the pericarps opened, and seeds were obtained; they were left under the shade at room temperature for 3 days and conserved in a closed glass container at 4 °C, for 15 days.

The seeds were counted and weighed in an electric-analytical scale (Scientech® ZSA 120), and 4 treatments were carried out with 4 repetitions of 250 each, using a Completely Random Design (CRD). Each repetition was placed in a cloth (tulle) bag, for treatments: 1) the bags were submerged in water at 100 °C until cooling; 2) they were submerged in boiling water (100 °C) for one minute, and after that time they were dried and placed in water at room temperature (cold) for 3h (during which time all the treatments were at room temperature); 3) consisted in placing the seeds in water at 70 °C until reaching room temperature; and 4) control treatment that consisted in intact seeds. All the treatments were carried out by duplicate, using one for imbibition and the other for germination.

For imbibition, each repetition was placed on cotton inside Petri dishes, they were moistened with 25 ml distilled water, without flooding, covered and placed on a table at room temperature. The seeds were weighed at 5, 12, 24, 36 and 48 h, moment when the emergence of the radicle began, the number of emerged seeds was counted. The imbibition rate was calculated by subtracting the initial weight (g) from the final weight (ISTA, 2003).

For the germination, 4 l plastic trays were used, they were labeled and 3 l of a mixture of sand, peat and soil (1:1:1 v/v/v) were added, they were watered to field capacity. The seeds from each repetition were dispersed homogeneously according to the treatment established, and they were covered with 0.5 cm of the mixture used. Irrigation was applied every 2 days, to field capacity, during the whole cycle of the experiment. The variables evaluated were the weight of 1, 100 and 1,000 seeds, percentage of germination $\left(PG = \frac{NTSG \times 100}{NTSS}\right)$ which consisted in the rate of the total number of germinated seeds (NTSG) divided by those sown (NTSS), and the speed of germination $\left(VG = \sum(ni/t)\right)$ with *ni* being the number of germinated seeds on day *i* in relation to the time (*t*) of germination, performed from the moment when the first plant emerged and, later, counted every 3 and 5 days.

For the analysis, the percentages of the data were transformed with the function ARCSIN $\sqrt{X/100}$, then they were subjected to analysis of variance (ANOVA) and means comparison (Tukey α=0.05), using the statistical package SAS® 9.0 (SAS, 2009). The graphs were made with the Microsoft Excel 2010® software.

RESULTS AND DISCUSSION

The different treatments applied to the chepil seeds showed significant differences (α=0.05), in the imbibition rate and the number of emerged seeds (Figure 1). It was found that the control treatment presented lower rates of absorption, remaining minimal; this is considering that it was constant during the 48 h of evaluation, but without exceeding or equaling the other treatments.The emergence of the radicle began in some seeds during this time; however, the control presented 98.7% of intact seeds and the use of water at 100 °C until 81.4% cooling, finding at plain sight that the seeds presented physical or superficial latency. This agrees with studies performed by Ramorinoa giraloe, where they mention that the physical dormancy present in the seeds restricts the process of imbibition (Zapata *et al*., 2017).

Lastiri *et al*. (2007) evaluated the emergence and germination of chickpea (*Cicer arietinum*) and alfalfa (*Medicago sativa* L.) in saline conditions, and they found that each species absorbs differently, the result was reflected in the germinating capacity; in addition, when seeds present dormancy, they do not absorb even when the conditions are adequate (Sanabria *et al*., 2003) which agreed with this study, since the control (without heat treatment) showed low absorption rates.

On the other hand, the treatment where the seeds were submerged in water at 70 °C until cooling presented better absorption; however, it remained constant during the whole process, which shows that these treatments accelerate germination and decrease dormancy; in addition, the physical treatments in seeds increase the number of plants. Studies in different species show that the volume of the seeds increases discretely and the imbibition increases considerably (Méndez *et al*., 2008; Monroy-Vázquez *et al*., 2017; Souza-Pavia *et al*., 2006); this coincides with the present study.

The water absorbed increased according to the time of imbibition, situation that can be observed in this study; in this sense, in the treatment where water was used at 100 °C until cooling there was higher absorption. Each seed seemingly absorbed 0.01 g; however, some did not show an effect from water absorption while others drastically increased their size (they swelled) (Bonner, 1974), and therefore they presented timely emergence. The use of hot water decreased the physical latency, which depends on time because when placing the seeds for 1 min in water at 100 °C, 95.3% of them did not absorb.

This study culminated at 48 h, due to the emergence of the radicle of the seeds, in all the imbibition treatments; when using water at 100 °C until cooling, 186 (18.6%) emerged seeds were found, treatment that had the greatest effect.The others were lower, according to the treatment applied, where seeds were placed during 1 min in the water at 100 °C; the emergence was 4.7% and the control barely reached 1.3% (Bar in Figure 1).

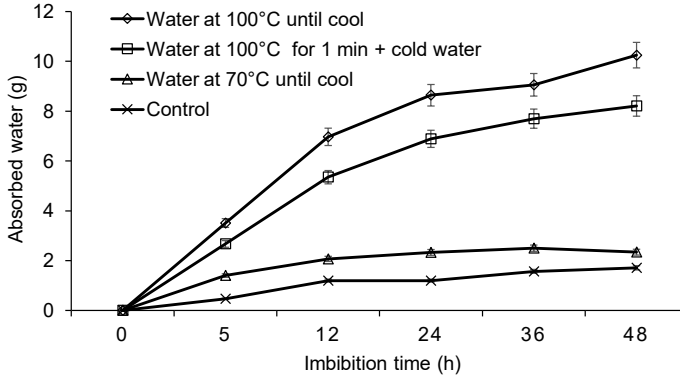

Figure 1. Water absorbed over time and onset of emergence of chepil seeds (*Crotalaria longirostrata* Hook. & Arn.), with heat treatment.

Chepil seeds are orthodox, since they are conserved and dispersed when they have low percentage of humidity (Camacho, 1994; Doria, 2010); in addition, they present physical dormancy, which prevents germination from taking place under low success conditions for development, and at the same time, this causes for seeds not to germinate in some cases, even when the conditions are adequate and favorable, because there are times when they lose their ability to emerge (Chong *et al*., 2002), situation caused by the latency they present.

The water applied in the different treatments increased the absorption and germination percentages (Table 1). At 30 days after the start of germination, the treatment with higher amount of germinated seeds was 1, with 80.0%, followed by treatment 2 with 69.2%, treatment 3 with 29.1%, and lastly, the control with 12.3%, corroborating that the species evaluated presents waterproof testa (Juárez and Lagunes, 2014); in addition, Zuloaga *et al*. (2011) mention that the heat shock at 100 °C breaks the dormancy and increases the germination percentages of the species.

The germinated plants in treatments 1, 2 and 3 showed the first true leaves, rolled, small and deformed; meanwhile, those from the control were normal, probably because subjecting them to heat treatments accelerated and improved the germination, but showed a negative effect in the initial development of the seedling.

The average weight of a chepil seed is 0.0096 g, 100 seeds weigh 0.9 to 1 g and 1,000 9.6 g, respectively (α=0.05). Dormancy was observed clearly in the control treatment (Figure 2), which presented lower germination percentages. This shows that the wild seeds of this species require scarification treatments to accelerate and increase the emergence. The control sown in trays began germination at 9 days, with 1.2%, while treatments 1, 2 and 3 began at 3 days after the experiment was established, with 12, 9.3 and 0.3 %, respectively; at 6 days, treatment 2 presented an accumulated value of 23% and 1 of 28%; at the same time, treatment 3 reached 1.9%. Studies performed by Zapata *et al*. (2017) in tree-like legumes mentioned that the use of hot water significantly improves the germination; however, when it is compared with mechanical scarification, more time is required in order to reach 70% of germination.

Table 1. Imbibition and germination of chepil (*Crotalaria longirostata* Hook. & Arn.) seeds with heat treatment.

Treatments	Imbibition (g)	Germination (%)
1 Water at 100 °C until cool	10.25 a	80.0 a
2 Water at 100 °C for 1 min + cold water	8.21 b	69.2 ab
3 water at 70 °C until cool	2.34 b	29.1 b
4 Control	1.71 b	12.3 b

Equal letters do not differ significantly from each other, according to Tukey's test (α=0.05).

It is important to mention that there were differences in the time of emergence of the radicle at the moment of absorbing water in the Petri dishes, compared to germination in the trays with a mixture of soil, sand and peat. In the imbibition of seeds from all the treatments, the emergence began at 2 days, while in the trays the onset of the germination happened at different times, at 3 days in treated seeds and in the control it extended until 9 days. This could be due to the availability of water.

CONCLUSIONS

Chepil seeds presented physical or superficial dormancy, the testa is waterproof; however, the physical treatments accelerated and increased the percentages of water absorbed and of germination.

The use of water at 100 °C until cooling showed better imbibition rates; this treatment presented

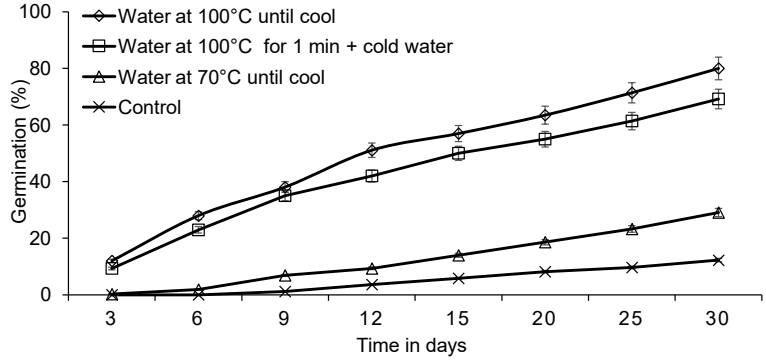

Figure 2. Germination percentages, accumulated, in chepil seeds (*Crotalaria longirostrata* Hook. & Arn.).

80.0% of germination. In addition, it began at three days in treatments 1, 2 and 3, but the first true leaf was deformed and rolled. On the other hand, in the control the emergence began at 9 days, but the plants were normal.

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Morphological characteristics of okra fruits [*Abelmoschus esculentus* (L.) Moench.] cultivated in the dry tropic

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ABSTRACT

Objective: The objective was to evaluate the morphological characteristics of okra fruit [*Abelmoschus esculentus* (L.) Moench.], an endemic crop of the Afro-Mexican region, in the dry tropics.

Design/Methodology/Approach: Materials were collected in Cuajinicuilapa, in the state of Guerrero in Mexico. Using a completely randomized design (CRD), 4 repetitions of 100 fruits were selected, and each was evaluated for the following: weight, number, size and shape of fruits and seeds. Data were analyzed using measures of central tendency, utilizing SAS.

Results: The fruits are heterogeneous and some are deformed, they have a long and fluted shape, with an average weight of 10.4 g and 95 seeds each weighing 0.05 g. The seeds are round with a conical micropyle, the testa is dark grey, and the embryo white.

Study Limitations/Implications: It is necessary to keep studying the morphological characteristics of okra fruit for a longer period of time and to establish farming in order to widen the outlook of decision making.

Findings/Conclusions: Okra fruits from backyard farming have better quality and potential for fresh or processed consumption. There is a lack of management and improvement of this crop.

Keywords: Congo coffee, shape index, morphological quality, Mazorquita coffee.

INTRODUCCIÓN

Okra [*Abelmoschus esculentus* (L.) Moench.], also known as Congo coffee or Mazorquita coffee, is consumed by people of African descent. It is a species of the Malvaceae family, native to tropical Africa (Díaz *et al*., 2007), where it has been grown for more than 4,000 years (Ayyub *et al*., 2013). It is an annual crop which develops in tropical and subtropical areas all over the world (Saifullah and Rabbani, 2009), mainly in India, Nigeria, Pakistan, Ghana and Egypt, where temperatures range above 26 °C. In Mexico it is a non-traditional vegetable, between 4,000 and 7,000 ha are sown per year, in the states of Morelos, Michoacán, Guerrero and Tamaulipas, with an average yield of 10 t ha^{-1} (Díaz *et al*., 2007).

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It is a profitable crop for developing countries (Tamura and Minamide, 1984), it generates currency and it is an important source of employment, okra as a vegetable is mainly exported to the United States (Adiger *et al*., 2011).

Okra can be grown year round; however, it is mainly produced during the summer, due to the demand of certain climatic factors such as light, temperature and water. It completes its productive cycle with an average volume of 665 mm (Tiwari *et al*., 1998), and it is also known to have germination problems. Studies have shown that the whole plant is edible (Fekadu *et al*., 2015), the fruit is mucilaginous, and it can be used as a fresh or cooked vegetable, or added to salads or soups. It can be stored in a freezer or as seeds (Ayyub *et al*., 2013), which are rich in unsaturated fatty acids (Kumar *et al*., 2013); they provide water, proteins, carbohydrates and fiber (Saifullah and Rabbani, 2009); and they have high content of polysaccharides, which gives them their medicinal properties (Sengkhamparn *et al*., 2009).

The plant is a shrub, with a height of 1 to 2 m, ramified, with large leaves ranging from 20 to 40 cm in length and 3-7 lobes each; they have cross pollination, the seeds are round, large, and grey in color (Akinyele and Osekita, 2006). Studies indicate that the number of fruits per plant has a direct effect on the seeds yield, followed by the plant's height, branching and bloom time (Kumar and Reddy, 1982), the weight of the fruit is directly related to the yield (Ariyo, 1987). In Mexico there are limited scientific studies on okra cultivation (Díaz and Ortegón, 1996; Díaz *et al*., 2003; Díaz *et al*., 2007), aimed toward crops with a potential for fresh consumption and dried seeds are processed in order to make a drink similar to coffee.

In the Costa Chica region in the states of Guerrero and Oaxaca in Mexico, inhabited by people of African descent, okra was farmed in the past, yet due to pests and climate factors this species has become a backyard crop in these municipalities, and its seeds are used to make a beverage to substitute coffee (Cuata and Manzaneda, 2018) which is commonly known as Congo coffee or Mazorquita coffee. Due to its color and aroma it is often called "chocolate de los negros" (black-men's coffee). Recent studies have shown that it contains pectin and lecithin which act as substitutes of chocolate (Datsomor *et al*., 2019). Therefore, the objective of this study was to evaluate the morphological characteristics of okra [*Abelmoschus esculentus* (L.) Moench.] fruits and seeds, as an endemic crop in the Afro-Mexican region with commercial potential for Mexico.

MATERIALS AND METHODS

This research was carried out in June, 2019, in the Fodder Laboratory of the School of Veterinary Medicine and Zootechnics No. 2, in Cuajinicuilapa, Guerrero, Mexico. This municipality is located at 16° 28' 16'' LN and 98° 24' 55'' LW at an altitude of 45 m, with an average annual temperature of 27 °C and dry tropical climate, with up to 1200 mm per year of summer rains (INEGI, 2012). Okra fruits [*Abelmoschus esculentus* (L.) Moench.] were harvested from backyards, from visibly healthy plants, associated with maize, hibiscus and sesame.

In order to determine the number of fruits per plant, these were trimmed and placed in transparent plastic bags, they were labeled and taken to the laboratory where they were counted and the healthy and complete ones were selected. Using a completely randomized design (CRD) four repetitions of 100 fruits were chosen, in order to evaluate morphological quality. The collection of fruits was carried out according to field availability, taking into account characteristics of variation and production based on backyard cultivation tasks. The polar and equatorial diameters were measured with the use of a Vernier (Truper Stainless® Steel); for the polar diameter the apical end was measured until the base, and the middle section was measured for the equatorial diameter. These data generated the shape index, by dividing the polar diameter by the equatorial diameter (Alia-Tejacal *et al*., 2012; Maldonado *et al*., 2016).

The dry weight of fruits and seeds, per fruit and individual, was obtained using an analytical balance (Scientech ZSA120, Boulder, USA). The number of channels, seeds per channel, and seeds per fruit were counted, as well as the hollow seeds. A Vernier was used to measure the polar and equatorial diameter of the seeds and the shape index was determined. The color of the seeds was determined using a colorimeter (Chroma Meter Cr-400, Ramsey, USA) which registers luminosity values (L*), hue angle (H*) and chromaticity (C*).

The results from the variables were analyzed using Measures of Central Tendency with the statistical program SAS® 9.2 (SAS, Institute, 2009).

RESULTS

Okra fruit is elongated and originates individually on the axil of the leaf; it is exposed in an erect manner, perpendicular to the stem, joined to the plant by a small pedicle, with an ellipsoid basal end and a conical apical end; each plant developed an average of 10.4 fruits (Table 1), with 7 to 14 mature fruits.

The average weight of dried fruits was 10.4 g, with a polar diameter of 11.48 cm, and an equatorial diameter of 3.99 cm; the plants used in this study have never been genetically modified, which makes them resistant and allows for heterogeneity in the fruits. In addition, the fruits found near the apex had an arched shape, probably due to pollination or because the first fruits usually are of better quality. Because of this, the fruits in this study are considered of large size, fluted, they have between 6 and 9 channels each, and an average of 12.58 seeds.

The fruit has between 53 and 118 viable seeds, with few or no hollow seeds, with a shape index of 2.93 giving it its elongated shape, typical of this species.

The seeds have semi-hard testa; the average color showed a luminosity (L*) of 45.48, chromaticity (C*) of 5.31 and a hue angle (H*) of 10.20, respectively, which gives it a dark grey almost black color with a touch of green. Each seed weighs an average of 0.05 g, with a polar diameter of 0.54 and an equatorial diameter of 0.49 cm. The seeds shape index is on average 1.14, considering they are round, but with a micropyle with a conical apex (Figure 1); inside, they have a large, round, white embryo. It is worth mentioning that dehydrated seeds are toasted on a skillet and ground, resulting in a powder with aroma similar to coffee used to make a comparable drink.

DISCUSSION

Okra fruits are heterogeneous in size. Atijegbe *et al.* (2014) evaluated the okra crop and reported between 2 to 10 fruits per plant; in this study a higher number of fruits were reported, considering that being a backyard crop it did not receive any specific treatment and the production period was seasonal during the raining season. Akinyote *et al.* (2011) applied nitrogenous fertilizer and found better fruit yield when compared to the control. Akinyele and Temikotan (2007) mentioned that okra plants that grow in sandy soils present fruits with lower weight and size compared to those grown in

Table 1. Description of quantitative characteristics and statistical parameters of okra [*Abelmoschus esculentus* (L.) Moench.] fruits and seeds.

Variables evaluated	Minimum	Maximum	Average	Range	CV	EE
Fruit						
Number of fruits per plant	7	14	10.4	7	20.79	2.16
Nug weight (g)	7.29	15.31	10.4	8.02	16.52	1.72
Fruit polar diameter (cm)	8.32	14.36	11.48	6.04	16.55	1.9
Fruit equatorial diameter (cm)	2.85	5.28	3.99	2.43	17.68	0.71
Number of carcasses per fruit	6	9	7.6	3	16.2	1.23
Number of seeds per carcasses	8.83	15.33	12.58	6.5	13.07	1.64
Number of seeds per fruit	53	118	95	65	16.57	15.74
Number of vain seeds	0	4	0.6	4	174.38	1.05
Fruit shape index (Polar diameter/Equatorial diameter)	2.03	4.3	2.93	2.27	19.15	0.56
Seeds						
Seed weight (g)	0.04	0.07	0.05	0.03	17.34	0.01
Polar diameter of the seed (cm)	0.45	0.61	0.54	0.16	7.42	0.04
Equatorial diameter of seed (cm)	0.42	0.55	0.49	0.13	6.73	0.03
Seed shape index	0.98	1.31	1.14	0.33	7.77	0.09
Seed color						
Brightness (L*)	36.70	51.40	45.48	14.70	10.15	4.62
Chromaticity (C*)	1.70	8.60	5.31	6.90	50.33	2.67
Hue (H*)	8.60	13.00	10.20	4.40	12.83	1.31

N:400 fruits; Range: Range of Variation; CV: Coefficient of Variation; SE: Standard Error.

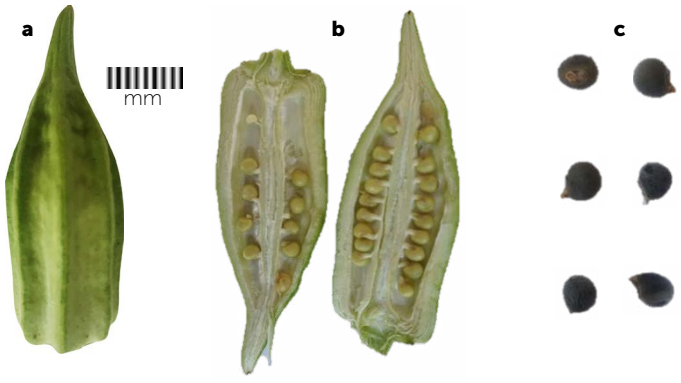

Figure 1. Fruits and seeds of okra [*Abelmoschus esculentus* (L.) Moench.]. a) complete fruit in physiological maturity, b) fruit split longitudinally with arranged seeds, c) dehydrated seeds in different positions, with the micropyle exposed.

loam clay soils; likewise, the number of seeds also varied, with a lower count in fruits with lower weight and size.

The average fruit weight was 10.4 g and in the region no fruits as large were found, which contradicts the research reported by Cuata and Manzaneda (2018), but agrees with figure 10 which is reported in their study; in addition, this study reports similar diameters to those reported in their study.

Studies carried out in northeastern Mexico, evaluating the quality of okra fruit as a vegetable in different cultivars, found an fruit yield of 9.8 up to 18.1 t ha^{-1}, tender fruits are different tones of green, depending on the cultivar and their quality, which also depended on the materials used (Díaz *et al*., 2007); these authors also mention that in the state of Guerrero this crop was abandoned due to pests, which coincides with what is mentioned by the farmers.

Fekadu *et al*. (2015) studied okra and found diverse qualities, focusing on health benefits and referring to okra as a food able to reduce malnutrition for the whole community, which is not uncommon in regions of African descent; mentioning once more that the seeds are toasted, ground, and used as a coffee substitute (Moekchantuk and Kumar, 2004).

Okra seeds have been used for consumption, but they are rarely studied; however, the use and description of their morphological traits can improve our knowledge about its production and the relationship which exists with the environment (Luck *et al*., 2012). Cuata and Manzaneda (2018) reported similar values to those reported in this study in terms of number, weight, and size of the seeds.

Considering that the seed is the most representative part of a plant and that in Afro-Mexican regions this crop has been preserved for generations, without improvemewnt or records of production, as a result this is the first study on the subject in the region and it is unknown if there have been changes in the yield or quality of the fruits. In this study a heterogeneous number of total seeds per fruit were found, but shape and color were homogeneous, and the majority was complete and healthy. Huayamave and Maldonado (2002) and Cuata Manzaneda (2018) found that the color of the seeds is grey with tendency toward green, which is consistent with this study.

In Mexico few advances have been made on okra cultivation, while in other countries this crop has been studied, resulting in a wide range of health benefits, nutritional, pharmaceutical, etc. Therefore, it is necessary to give more importance to this crop and to seek alternative production options for fresh consumption, dehydration or processing, in addition to having useful characteristics for national and international sales.

CONCLUSION

Okra fruits can be consumed fresh or processed; on average they measure 10.4 cm in length, and weigh 10.4 g, they have 9 channels and in each one an average of 12.58 seeds. In total they have 95 seeds, each weighing 0.05 g, round, with a semi-hard dark grey testa and a white embryo.

In the Cost Chica region, okra is a backyard crop, which should be taken into account for commercial farming; however, there is a lack of research and improvements in order to increase the quality and homogenize the fruits.

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Productive Performance of Sheep in an Agropastoral System on the Coast of Oaxaca, Mexico

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ABSTRACT

Objective: To determine the prolificacy and weight of lambs at birth (BW) in two genotypes of hair sheep within an agropastoral system on the coast of Oaxaca, Mexico, and to evaluate the effect of two sources of selenium on the performance of lambs from said system during the fattening phase.

Design/Methodology/Approach: The study was carried out in an agropastoral system (coconut palm and *Cynodon dactylon* grass) in Río Grande, Oaxaca. Two experiments were conducted; the first studied 58 ewes distributed in a completely randomized design (CRD) with factorial arrangement of 2×2×3, and focused on the following fixed effects: genotype, number of births, and body condition (BC). The measured variables were prolificacy and BW. The second experiment studied 23 lambs of 4 months in age that were offspring of the females in experiment 1. They were distributed in a CRD with three treatments: T1, without selenium provided; T2, with barium selenate provided; T3, with selenomethionine provided. The dependent variables were daily weight gain (DWG), dry material consumption (DMC), and feed conversion (FC).

Results: The Pelibuey ewes with one and two births and the Black Belly ewes with one birth demonstrated the highest averages in prolificacy ($P \leq 0.05$). The lowest averages ($P \leq 0.05$) were detected in ewes with BC 4 irrespective of number of births. The BW was affected ($P \leq 0.05$) by genotype; Black Belly lambs weighed 680 g ($P \leq 0.05$) more that Pelibuey lambs. The DWG, DMC and FC behaved similarly ($P \geq 0.05$) between treatments and their general averages were 0.114 kg day^{-1}, 0.679 kg day^{-1}, and 6.18, respectively.

Study Limitations/Implications: In the agropastoral system, the BC of ewes limits their reproductive performance. The weight of the lambs at the start of fattening determines their performance during fattening.

Findings/Conclusions: In the agropastoral system, Pelibuey and Black Belly females present adequate prolificacy averages and BW; however, it is very important that they remain within BC 3. Neither of the two sources of selenium improves DWG, DMC, and FC of lambs fattened in pens in the agropastoral system.

Keywords: Pen finishing, tropical livestock systems, selenium.

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INTRODUCCIÓN

In agro-silvo-pastoral, silvo-pastoral, and agropastoral systems, the use and management of resources are directed toward sustainability (Russo, 2015) and they show the importance of livestock production under sustainable models in tropical regions (Ibrahim *et al*., 2006). Although these systems began in Latin America three decades ago, they have not been fully adopted despite the multiple goods and services that they offer (Clavero and Suárez, 2006). Thanks to their ethological behavior, sheep are the animal species that most easily and rapidly adapts to those sustainable production systems.

In 2017, sheep farming in Mexico produced 4,903 t of meat. Oaxaca contributed 3.36% (SIAP, 2018); this low production is attributed to extensive sheep farming management; in this regard, Hernández *et al*. (2017) characterized sheep production units and found that 62.3% are for subsistence, 33% are in transition, and only 4.7% are for business. These systems have developed under an extractive base with grass monocultures, large changes in land use, and desertification, which limit the environmental benefits of the biodiverse systems in the tropics (Alonso, 2011). In tropical regions, extensive livestock production systems are characterized by their low yield and negative environmental impact (Bacab *et al*., 2013). Martínez *et al*. (2011) mention that low profitability in sheep farming is mainly due to scarce application of technology. The Ministry of Agriculture and Rural Development, or SAGARPA (2011) considers it necessary to increase inventory, as well as to classify and integrate farmers to increase yields.

The alteration of ecosystems has resulted in the loss of vegetative cover, which could be recovered with the establishment of food systems where the tree-cover component is included in association with a variety of multi-purpose species (Bulgarín, 2012). In this respect, Milera (2013) reports that silvo-pastoral systems (SPS) that are agro-ecologically managed with a diversity of species and development patterns guarantee self-sufficiency and resilience to climate change. Agropastoral systems are an option for guiding livestock farming toward sustainability, and they are a productive alternative for small farmers (Febles and Ruiz, 2008). The study objective was to determine the prolificacy and weight of lambs at birth in two hair sheep genotypes within an agropastoral system on the coast of Oaxaca, Mexico, as well as to evaluate the effect of providing two sources of selenium on the performance of lambs from this system during the fattening phase.

MATERIALS AND METHODOS

The study was carried out in an agropastoral system located in the community of Río Grande, Villa de Tututepec, on the coast of Oaxaca, located between the coordinates 97° 26' 1.57'' LW and 15° 59' 27.2'' LN, and at 16 masl (Trejo, 1999). The climate was Aw1, warm dry, with a temperature greater than 26 °C and annual precipitation oscillating from 1,175 to 1,550 mm (INEGI, 2018). The soil is eutric regosol, with a pH of 5.5, not gravelly, deep and with medium fertility. The agropastoral system was established on a surface area of 10 ha and consisted of a 30-year-old coconut palm (*Cocus nucifera*) plantation, laid out in rows in a square system (5×8 m), and three-year-old forage grass (*Cynodon dactylon*).

Experiment 1

Fifty-eight (58) ewes were submitted for study (29 Pelibuey breed and 29 Black Belly breed) with an average of 31 months in age and an average weight of 32.6±2 kg.

Experimental design

The 58 ewes were distributed in a completely randomized design with a factorial arrangement of 2×2×3, having as fixed effects the genotype (Pelibuey and Black Belly), the number of births (1 and 2), and the body condition (2, 3, and 4; according to the scale described by Russel, 1984).

Feeding strategy

Zacate grass (*Cynodon dactylon*) was offered through daily grazing, in a timeframe from 8:00 to 16:00 h. Feeding was complemented by providing, in feedlots, a concentrate with 18% crude protein concentrate and 2.7 mcal EM day^{-1}. Each head was given 50g/day during the whole reproductive cycle.

Period and type of mating

It was done by natural mating in a 45-day period, during the months of March and April, 2018, using two studs.

Evaluated variables

Prolificacy. Determined by recording the number of lambs born alive, at the time of birth, for each of the ewes in the study.

Individual weight at birth. The weight of each one of the live-birth

lambs was registered, 4 h after birth, in both groups of ewes; the study used a digital Torrey[MR] scale with a 20 kg capacity.

Weight of the litter at birth. Obtained by adding up the weights of each lamb born alive in the litter.

Data analysis

The data obtained was subjected to variance analysis through a completely randomized model. The fixed effects were genotype with two levels (Pelibuey and Black Belly), the number of births with two levels (1 and 2), and the body condition with three categories (2, 3, and 4); in addition, double and triple interactions were considered. To determine the difference between means, the least mean squares test was used, using $\alpha=0.05$.

Experiment 2

Twenty-three (23) weaned males were studied, a cross between Pelibuey and Black Belly with 4 months of age that were offspring of the ewes evaluated in experiment 1.

Experimental design

The 23 male lambs were distributed in a completely randomized design, with three treatments. Treatment one (T1) was a control group with eight repetitions, without selenium provided. Treatment 2 (T2) had eight repetitions, where each animal was administered an intramuscular dose of 1 mg/kg of live weight of barium selenate (Selenate, L.A. 50 mg/ml). Treatment 3 (T3) consisted of seven repetitions and 0.2 mg/kg of live weight of selenomethionine (Bioways selenium 2000 ppm[MR]) was provided orally. The experimental unit was one lamb in an individual pen.

The pens had an area of 1.95 m^2, with an individual drinking and feeding trough. The study had a duration of 125 days divided in three stages: initial (42 days), intermediate (42 days), and final (41 days).

Feeding strategy

All the lambs were fed with a wholegrain ration, formulated with 16% protein and 2.6 Mcal EM kg^{-1}, in accordance with the nutritional requirements suggests by the NRC (2007); Table 1 shows the proportions used of each of the ingredients. The ration was offered *ad libitum* with feeding recorded twice per day, at 8:00 a.m. and 4:00 p.m.

Table 1. Proportion of inputs used in the elaboration of the integral ration offered to lambs from an agropastoral system during the fattening period.

Ingredient	Percentage of dry matter
Rolled corn grain	44.00
Soybean paste	18.30
Corn stubble	26.64
Urea	1.00
Common salt	2.00
Molasses	8.06

Evaluated variables

Weight gain. The lambs were weighed every 42 days and weight gain per period was calculated using the difference. The result was divided by number of days to calculate daily weight gain. The study used a digital hanging Crane[MR] scale, with a 300 kg capacity.

Food consumption. The amount of food offered daily was regulated using feeding trough records. The rejected food was weighed every seven days. Food consumption was calculated by the difference of food offered minus food rejected.

Feed conversion. Estimated based on the consumption of dry material and the weight gain per day; it was expressed in kilograms of dry material per kilogram of live weight generated.

Information analysis

The data were analyzed using variance analysis under a completely randomized model. The fixed effect was the treatment, and the covariable was initial live weight. To determine the difference between averages, the least mean squares test was used, using $\alpha=0.05$.

RESULTS AND DISCUSSION

Experiment 1

Prolificacy was affected ($P\leq0.05$) by two double interactions, genotype × number of births, and body condition × number of births. Figure 1 shows the prolificacy averages in the two genotypes according to the number of births per ewe. It can be seen that the Black Belly ewes with two births presented the lowest average ($P\leq0.05$) in prolificacy. The Pelibuey ewes with one and two births, and the Black Belly ewes with one birth presented the highest averages ($P\leq0.05$); between them, the averages were similar ($P\geq0.05$).

Figure 2 shows the prolificacy averages in ewes with different number of births and body condition, the highest average ($P\leq0.05$) was seen in first-birth sheep with body condition 3, followed by those that presented body condition 2 with two births. The lowest averages

(P≤0.05) were detected in ewes with body condition 4 irrespective of number of births, in ewes with body condition 2 with one birth, and in ewes with body condition 3 with two births.

Martínez *et al*., (2011) report that prolificacy in the Mexican dry tropics, in semi-extensive systems and for said genotypes, is 1.55±0.8; these values are close to those found for first-birth ewes, due mainly to the farming conditions. In other studies performed by Dickson *et al*. (2004) and Macedo and Alvarado (2005), data were compiled from established extensive systems in Mexico and they found averages of 1.23±0.49 and 1.2±0.39. The described values are below those reported in the present study.

For the Black Belly genotype, Andrade *et al*. (2015) report that the prolificacy in a semi-extensive system in Campeche was 1.74±0.06 lambs. Rojas and Rodríguez (1995) report values of 1.4±0.08 for yearling Black Belly sheep (1-2 years), value lower than that of juveniles (2-4 years) with 1.67±0.06, adults (4-7 years) with 1.75±0.07, and seniors (8-10 years) with 1.80±0.29. Lastly, Hinojosa *et al*., (2015) indicate that the F1 crosses of Pelibuey and Black Belly generate values of 1.23±0.03 in lamb prolificacy within a tropical system in Tabasco. Comparing the averages found in the present study with those described in the literature, it can be said that the prolificacy averages in Pelibuey and Black Belly ewes in the agropastoral system compete with those observed in semi-extensive systems in the tropics.

The individual weight of lambs at birth was only affected (P≤0.05) by the genotype. Figure 3 shows the averages obtained by Pelibuey and Black Belly ewes. The highest value (P≤0.05) was shown by lambs from Black Belly mothers; those lambs weighed 680 g more than the lambs from Pelibuey mothers.

The litter weight at birth was affected by the interaction of body condition and number of births of the ewe. The lowest averages were

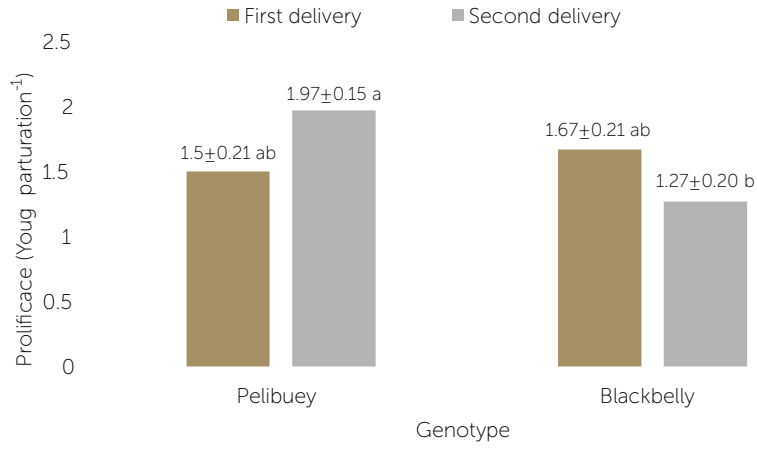

Figure 1. Prolificacy averages of ewes of two genotypes and different number of calvings, in an agropastoral system established in a warm dry climate on the coast of Oaxaca, Mexico. Different letters on the columns indicate significant statistical differences (P≤0.05).

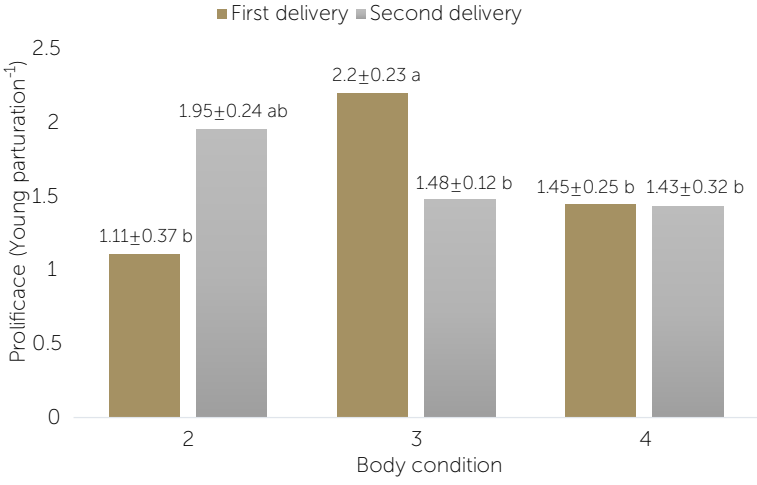

Figure 2. Prolificacy averages in ewes of different body conditions and number of calvings, in a warm climate agropastoral system on the coast of Oaxaca, Mexico. Different letters on the columns indicate significant statistical differences (P≤0.05).

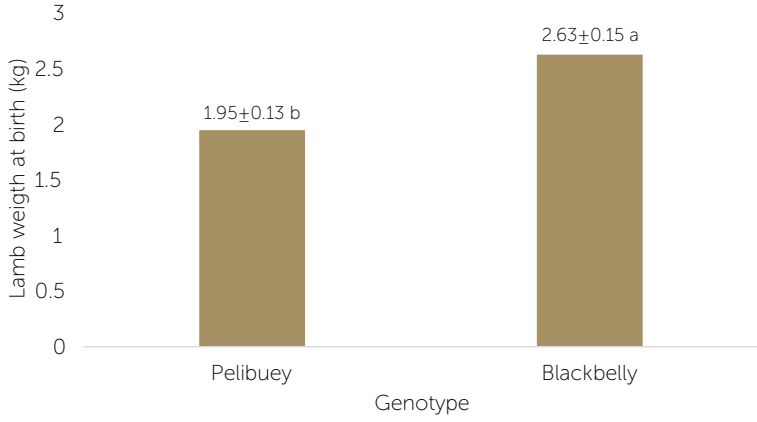

Figure 3. Averages of birth weight of Pelibuey and Black Belly ewes, in a warm climate agropastoral system on the coast of Oaxaca, Mexico. Different letters on the columns indicate significant statistical differences (P≤0.05).

found in lambs from mothers with body condition 4, irrespective of number of births, followed by lambs from ewes with body condition 2 and first birth. The highest averages were for lambs from ewes with one birth and body condition 3 (Figure 4).

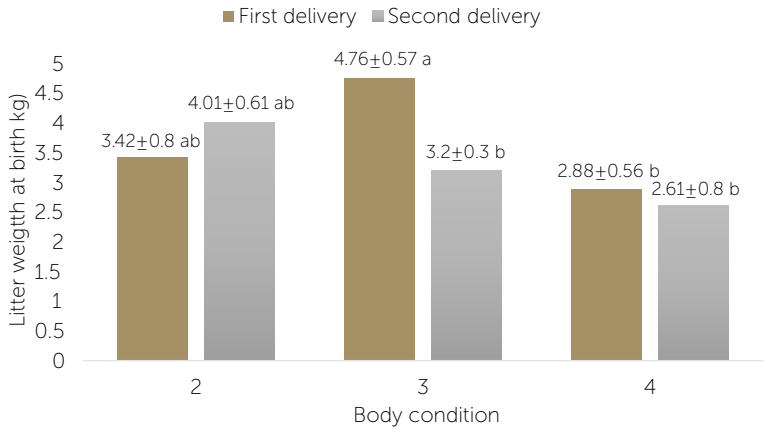

Figure 4. Averages of litter weight at birth in ewes with different body conditions and number of farrowings, in an agropastoral system established in a warm dry climate on the coast of Oaxaca. Different letters on the columns indicate significant statistical differences (P≤0.05).

González *et al.* (2002) report averages of 2.7±0.06 kg in lambs of the Black Belly genotype, which are similar values to those described by Ríos *et al.* (2014), who report averages of 2.6±0.07 kg in lambs of the same genotype and of 2.7±0.08 kg for cross-breed lambs. The average obtained in the present study for Black Belly lambs is similar to those described by other authors. The Pelibuey ewes showed values lower than those found in other studies; it is possible that the Black Belly breed adapts more easily to the agropastoral system.

In a study conducted by Macedo and Arredondo (2008), they found litter weights of 6.42 kg in ewes with double births and 6.39 kg for triple births, within an intensive farming system. The averages are above those described in the present study, and the difference is due to a lack of control in number of lambs per birth and to the farming system.

Experiment 2

Table 2 shows the averages in weight gain, food consumption, and feed conversion of lambs in the fattening phase treated with two sources of selenium (organic and inorganic). The variance analysis showed that the selenium source affected (P≤0.05) two of the three variables in the study.

In the intermediate stage, the administration of sodium selenite decreased (P≤0.05) daily weight gain by 33%, compared with the averages obtained by the animals in the control treatment and the selenomethionine treatment. In the other two stages (initial and final) and the total period, the daily weight gain averages were similar (P≥0.05); hence, only the general averages per stage are reported: initial, 0.106 kg day^{-1}; final, 0.117 kg day^{-1}; and total, 0.114 kg day^{-1}.

Food consumption was not affected (P≥0.05) by the administration of a different source of selenium in any of the three evaluated stages. The general averages were 0.506 kg day^{-1} in the initial stage, 0.584 kg day^{-1} in the intermediate stage, 1.039 kg day^{-1} in the final stage, and 0.679 kg day^{-1} in the total period.

Concerning feed conversion, in the intermediate and final stages, animals from the control and selenomethionine treatments showed the best averages (P≤0.05), while sheep in the sodium selenite treatment showed the least efficient averages. Despite this tendency, the total averages were similar (P≥0.05) in the three treatments.

Weight gain averages obtained in the present study are lower than those described by other authors. Macedo and Castellanos (2004) found weight gain averages of 182 g animal day^{-1} with commercial diets, Pérez *et al.* (2011) determined averages of 163 g animal day^{-1}, and Salinas *et al.* (2013) reported increments of 234 g animal day^{-1} on diets with polished rice. The animals used in the present study had a low live weight at the start of fattening (13.86 kg), due to the fact that most lambs were born in double births, and as described by Macedo and Arredondo (2008) in their study, the superiority in growth and live weight of lambs from single births, compared to multiple births, is maintained after weaning. Another factor that influenced this outcome was the genotype; as it is known, animals bred with

meat breeds demonstrate better performance than the Black Belly and Pelibuey breeds (Quintana, 2018).

With regard to feed conversion, values ranging from 4.2 to 8 are reported. Concerning this, INIFAP (2011) reported FC of 4 in whole-grain based diets. Salinas *et al*. (2013) describe averages of 4.23, 5.12, and 5.41 FC in sheep with diets where polished rice was included, at 0, 11 and 22% respectively. This means that feed conversion is unfavorably affected as the percentage of fiber increases in the ration.

Results obtained in this study for feed conversion are similar to those obtained by Berumen et al. (2003) by fattening confined male F1 Katahdin-Pelibuey lambs for 90 days, with a live weight of 15 kg. Regarding the experiments performed with selenium, Reséndiz *et al*. (2012) observed an increase in weight gain when using doses of 0.90 mg/kg (Biotecap®) added with chromium as well (1.4 mg/kg). However, Rodríguez *et al*. (2011) did not find differences in weight gain, dry material consumption, and feed conversion ($P>0.05$) when supplemented with premixes of selenium and chromium with final concentrations of 0.3 mg/kg and 0.4 mg/kg in final-stage sheep. Domínguez *et al*. (2013) mention that adding organic selenium (0.3 mg/kg) and chromium (0.25 or 0.35 mg/kg) to a finishing diet for sheep does not affect the productive variables, but when combined, there is an interaction between them ($P<0.05$) since food consumption and feed conversion decrease, while weight gain and carcass characteristics rise when chromium is increased.

It is possible that lambs from agropastoral systems do not require selenium administration at the start of fattening because they begin consuming green forage containing selenium during the nursing stage.

CONCLUSIONS

In the agropastoral system, Pelibuey and Black Belly ewes present adequate averages in prolificacy; nevertheless, it is very important that they maintain body condition 3, since any change in category affects prolificacy unfavorably.

In the agropastoral system, the Black Belly breed produces heavier lambs than the Pelibuey breed; however, it is necessary to monitor the body condition of the ewe, since litter weight decreases in underweight and obese ewes.

Ewes managed in the agropastoral system present better averages in prolificacy and weight in newborn lambs when compared to extensive farming systems.

The addition of organic or inorganic selenium does not improve weight gain, food consumption, and feed conversion in pen-fattened lambs within an agropastoral system.

Lambs from agropastoral systems present low weight at the time of weaning, which results in a reduction in weight gain and food consumption during the fattening period. Despite this behavior, the averages in feed conversion are efficient; thus, it is necessary to prolong the fattening period in order to obtain an optimum live weight for slaughter.

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Table 2. Averages (± standard error) of weight gain, food consumption, and feed conversion of sheep finished in pens with different sources of selenium, from an agropastoral system established in a warm dry climate on the coast of Oaxaca, Mexico.

Variables	Treatment		
	Witness	Barium selenate	Selenomethionine
Weight gain (kg día^{-1})			
Initiation	0.100±0.015	0.133±0.014	0.084±0.014
Intermediate	0.158±0.015 a	0.100±0.0144 b	0.144±0.014 ab
Ending	0.115±0.017	0.103±0.017	0.134±0.016
Global	0.126±0.011	0.103±0.011	0.113±0.011
Food consumption (kg de MS día^{-1})			
Initiation	0.527±0.030	0.480±0.028	0.511±0.020
Initermediate	0.602±0.035	0.568±0.033	0.582±0.033
Ending	0.977±0.062	1.113±0.062	1.028±0.58
Global	0.670±0.031	0.696±0.031	0.670±0.295
Feed conversión (kg kg^{-1})			
Initiation	8.921±1.558	6.593±1.578	10.883±1.458
Intermediate	4.038±0.583 b	5.903±0.583 a	5.210±0.545 b
Ending	6.119±0.856 ab	10.754±0.856 a	5.672±0.800 b
Global	5.499±0.511	6.950±0.511	6.088±0.478

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First report of *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae) in apaxtleco chili (*Capsicum annuum* L.) cultivated in greenhouse

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ABSTRACT

Objectives: To generate information about a new host of the *Polyphagotarsonemus latus* (Banks) mite, and to understand the damages caused by the cultivation of apatlexco chili pepper (*Capsicum annuum* L.) in the northern region of the state of Guerrero.

Design/Methodology/Approach: The identification of the mite was carried out through taxonomic keys and the damages caused in vegetative shoots, mature leaves and flower buds were described.

Results: The *P. latus* mite was identified as causing important damage to the crops of aplaxtleco chili pepper grown in greenhouses in the state of Guerrero, Mexico, and this is the first report of this mite in the Aplaxtleco chili pepper crop grown in greenhouses in the state of Guerrero, Mexico.

Findings/Conclusions: Economic income is obtained from the cultivation of aplaxtleco chili peppers, a characteristic crop of the municipality of Aplaxtla, in the northern zone of Guerrero; therefore, knowing the identity of the *P. latus* mite in the aplaxtleco chili crop will help to suggest effective control methods to obtain higher yields.

Keywords: *Polyphagotarsonemus latus*, aplaxtleco chili pepper, mite, damage, description.

INTRODUCTION

Chili pepper (*Capsicum annuum* L.) is one of the crops with greatest agricultural importance at the global and national level, due to its high consumption, uses and benefits. Mexico is the second producer of this vegetable in the world, with a cultivated surface of 149 thousand hectares (SIAP, 2019). Additionally, in recent years, chili production in Mexico in its different varieties reached 3,379,289 t in the year 2018 (Panorama Agroalimentario, 2019).

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On the other hand, there are various types of landrace chili peppers that are consumed broadly in different sectors of the Mexican population, and they have the advantage of being accepted by consumers (Mena *et al*., 2007); among the numerous types of landrace chili peppers there is apaxtleco chili, which is characteristic and of great economic importance in the municipality of Apaxtla de Castrejón, in the northern region of the state of Guerrero, México (Moreno *et al*., 2007; Mena *et al*., 2007; Aguilar-Rincón *et al*., 2010). Its traditional cultivation on small surfaces, of which a low percentage of fruits are harvested to be destined to auto-consumption and 90% of the production sold to mole paste makers of the region, representing an important source of income for the producers who grow it (Vázquez-Casarrubias *et al*., 2011).

However, since it is a regional species and of broad use, it is affected by the presence of various pest insects and mites (Aarwe *et al*., 2019, Tirkey *et al*., 2019), harming the development of plants and decreasing production (Patrock and Schuster, 1992; López *et al*., 2003). Among the most important pests there are mites, and among them the white mite (*P. latus*) stands out, which causes severe damages to chili pepper crops (Garza, 2000).

This mite was discovered for the first time in terminal shoots of mango plants in a greenhouse in Washington, USA (Denmark, 1980; Banks, 1904); in addition, it is known as a polyphagous species of temperate and subtropical areas (Fasulo, 2007; Peña and Campbell 2005). *P. latus* has several hosts, among them the chili crop (Garza, 2000; Brown and Jones, 1983). In Mexico it has been reported in serrano and jalapeño chili peppers (Garza, 2000), causing important damages by suctioning the sap; the leaves roll downwards, giving the appearance of "an inverted spoon, a brown cork-like tissue between the nervation is formed on the underside, the leaves and the flowers are deformed causing a reduction in the photosynthesis and instability of the water potential" (Black *et al*., 1993; King and Saunders, 1984; Baker 1997).

Based on the problem described before, the objective of this study was to generate information about a new host of the *P. latus* (Banks) mite, and to understand the symptoms of the damages of the apaxtleco chili pepper (*C. annuum*) grown in greenhouses in the northern region of the state of Guerrero.

MATERIALS AND METHODS

This study was carried out in the facilities of the Master's in Agricultural and Livestock Sciences and Local Management of the Universidad Autónoma de Guerrero, located on the Iguala-Tuxpan Highway km 2.5, Iguala de la Independencia, Guerrero, México on geographic coordinates 18° 20' 57'' latitude N and 99° 28' 43'' longitude W, at an altitude of 757 m. Apaxtleco chili pepper seeds were used, from the municipality of Apaxtla de Castrejón, Guerrero, Mexico (18° 8' 00'' N; 99° 56' 05'' W, at 1182 m altitude). Later, the seeds were sown on 10/05/2018 on a propylene tray with 200 cavities filled with moist peat at field capacity, three seeds were placed per cavity and they were covered with the same substrate. Right away the tray was covered with black polyethylene to maintain the moisture and the temperature and to favor germination; then, it was placed in a tunnel-type greenhouse covered with milky white polyethylene plastic of 700 mm and anti-aphid mesh on the sides. Once the seedlings emerged, the first 15 days two watering events were carried out per day with tap water and then Steiner's (1984) universal nutritional solution (SN) was added at 20% of its original concentration, until the seedlings reached an average height of 20 cm and they presented five to six true leaves. At 39 days the transplant was carried out to black polyethylene bags of 12 L, filled with pumice stone with particle size of 1 to 5 mm, placing a seedling per bag (pot), which were distributed inside the greenhouse described previously, with a total of 126 pots. The crop was irrigated manually every day with three watering events per day, the first in the morning (8:00 h) with the indicated nutritional solution for the seedling stage, adjusting it in agreement with the phenological stage of the crop, with pH of 5.5 and electrical conductivity that was modified gradually from 0.5 to 2.0 dS m^{-1} according to the phenological stage of the crop, the second (14:00 h) and third (18:00 h) watering events were done with tap water.

Symptoms of *P. latus* appeared at 144 days, and samples were taken; for this purpose, an identification transect of damage and symptomatology present in the plant's organs was used, to later describe and obtain the mites, cutting three leaves on each cardinal point from ten plants, which were washed under a strong stream of water and sieving with different size meshes, following the methodology described by Southwood (1978). Next, the samples from the specimens collected were processed and mounted

between slides for their taxonomic determination; the mites were identified based on the determination made by Peña and Campbell (2005). Image capture was made with a scanning electron microscope brand JEOL, model IT-300LV, in the Scanning Electron Microscopy and Microanalysis Laboratory of the Earth Sciences School at Universidad Autónoma de Guerrero. The taxonomy of the specimens was determined with images from it, and the identification was made according to the description performed by Peña and Campbell (2005).

RESULTS AND DISCUSSION

According to the taxonomic keys and characteristics mentioned by Brown and Jones (1983), and Peña and Campbell (2005), the *P. latus* mite was determined (Figure 1) which was present in the apaxtleco chili pepper crop (*C. annuum*), and the first report of this species in the crop is reported. This mite has been reported in serrano and jalapeño chili peppers (Garza, 2000, Qureshi and Kostyk, 2020), sweet pepper (Raudez-Centeno and Jiménez-Martínez, 2018, Naituku *et al.*, 2017); however, there is no report in the apaxtleco chili pepper crop.

The characteristics that led us to its identification agree with those described by Peña and Campbell (2005), authors who determined the white mite *P. latus*. The adults were observed with a white to light yellow color, the females and the males had the same color (Figura 1 E, F), but with a clear difference in their morphology (Figure 1 C, D). The male is faster and of smaller size

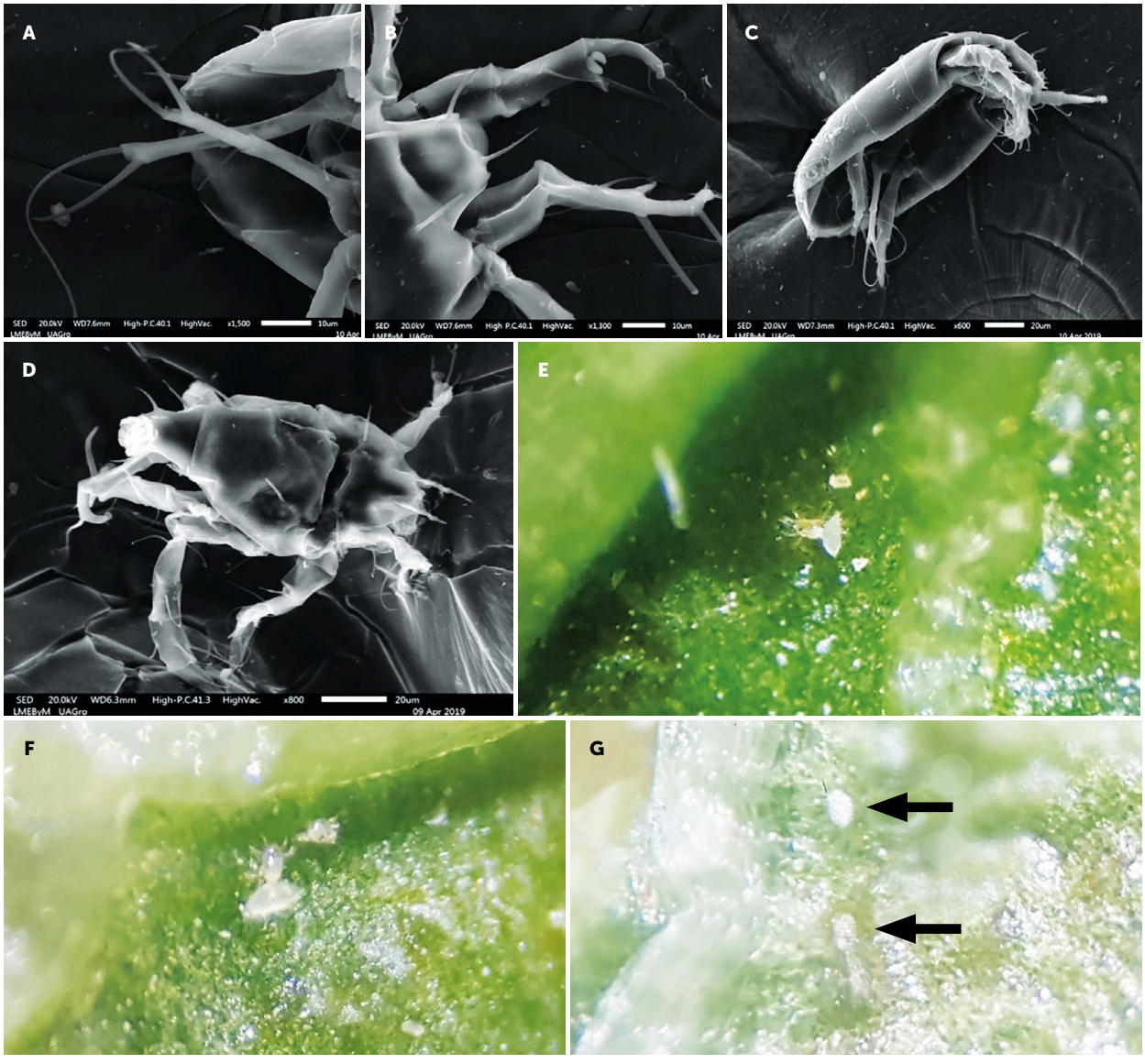

Figure 1. IV pair of legs from the female of *P. latus* (A), IV pair of legs from the male of *P. latus* (B), lateral female view (C), and dorsal male view (D) of *P. latus* in the scanning electron microscope. Male and female (E, F), eggs (G) of *P. latus* in stereoscopic microscope. (Initials based on Spanish terms).

compared to the female. The male, with robust back legs used to lift and carry the female in nymph state for their later mating (Figure 1 B, D, E, F). The back legs of the female were smaller in size, assimilating the shape of whips (Figure 1 A, C) present a very thin white line longitudinally and on the posterior part it forks close to the end (Brown and Jones, 1983; Peña and Campbell 2005). The eggs are transparent and slightly flattened (Baker 1997), the surface with five to six lines of tubers, which make it different from other mite eggs (Lavoipierre, 1940) (Figure 1 G).

Symptoms caused by the attack from *P. latus* on the apaxtleco chili pepper crop

The presence of damage to the crop from the high impact of pests was observed at 144 days after transplant (dat). The most severe symptoms were observed in the vegetative shoots and young leaves, which did not develop normally, becoming irregular and distorted in shape, the same as was described by Baker (1997), except contrary to what Garza (2000) mentions, where it is indicated that the leaves of tender shoots remain narrow and threadlike, some shoots present downward rolled leaves giving the shape of inverted spoon, as mentioned by Black (1993), in addition to finding some withering on the new leaves that did not allow them to reach maturity, but rather that lasted very few days adhered to the bud and fell after a few days, in the same way that Garza (2000) observed (Figure 2 A, B). The attacks were also seen in mature leaves, where they reached high populations of the white mite, which caused withering, rolling in leaves, and falling in few days; however, they did not have cork-like appearance as mentioned by Garza (2000).

An increase in the populations was seen when the flowering period took place, which caused abortion or falling, and in severe attacks the whole inflorescence withered and fell, in addition to atrophy in the development of the plant as mentioned by Denmark (1980), Gerson (1992), and Rai *et al*. (2007) (Figure 2 C).

CONCLUSIONS

The *P. latus* mite was found in the apaxtleco chili pepper; this mite is considered of great agronomic and economic importance because of the damages that it causes this crop, reducing the production. The apaxtleco chili pepper is endemic and characteristic of the region of Apaxtla de Castrejon, in the northern region of the state of Guerrero, which demands more research to maintain the production and to propose effective control methods that will help them to obtain a higher yield.

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We thank M.C. Jazmin López-Diaz and Dr. Oscar Talavera, for providing the scanning electron images and performing the EDS analyses in the Scanning Electronic Microscopy and Microanalysis Laboratory of the Universidad Autónoma de Guerrero (CONACyT, grant 231511)".

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Figure 2. Symptoms of damage in vegetative shoots (A), mature leaves (B) and flower buds (C) in apaxtleco chili pepper.

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AGRO PRODUCTIVIDAD

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Mycelial disturbance stimulates the formation of sporomes of edible ectomycorrhizal fungi associated with two neotropical pines

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ABSTRACT

Objective: To determine the effect of mycelial disturbance on the formation of sporomes of two edible ectomycorrhizal fungi of great biocultural relevance in Mexico (*Laccaria laccata* and *Hebeloma leucosarx*) associated to two Neotropical pines with economic and ecological importance, *Pinus greggii* and *P. montezumae*.

Design/Methodology/Approach: Spore inoculum was produced using ground pilei of the evaluated ectomycorrhizal fungi; each pine plant was inoculated with 10^7 to 10^8 fungal spores. A completely randomized experimental design was used with four treatments and six replicates per treatment for each pine species, having a total of 48 experimental units, each one consisting in an inoculated tree. During two years the sporome production was evaluated in the treatments with and without mycelial disturbance. The duration of the experiment, since seed germination, was 5 years.

Results: The mycelial disturbance originated a higher formation of sporomes in both fungal species, regardless of the associated tree species. The highest sporome formation was recorded in plants inoculated with *H. leucosarx* compared to those inoculated with *L. laccata* in both pine species. Mycelial disturbance, originated a higher number of sporomes in *Pinus greggii* compared to *P. montezumae*.

Study Limitations/Implications: The evaluation of factors influencing sporome formation in edible ectomycorrhizal fungi requires long term experiments.

Findings/Conclusions: This study shows for the first time that mycelial disturbance increases sporome formation in Neotropical ectomycorrhizal fungi. Additionally, a differential influence of the fungal and tree species on the number of produced sporomes was found. These findings shed some light on potential cultivation methods for edible ectomycorrhizal mushrooms.

Keywords: spore inoculum, ectomycorrhizal symbiosis, wild edible fungi, mycelial disturbance.

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INTRODUCTION

Ectomycorrhiza is a mutualistic symbiosis of paramount structural and functional importance in forest ecosystems. The edible ectomycorrhizal mushrooms *Laccaria laccata* and *Hebeloma leucosarx* have great biocultural and economic relevance in Mexico and they are commercialized in numerous Mexican markets (Pérez-Moreno *et al*., 2008; Pérez-Moreno *et al*., 2019). The genus *Hebeloma* has been reported as a toxic fungus in other latitudes (De Bernardi *et al*., 1983; Liu *et al*., 2002). However, in central Mexico, large amounts of diverse species of that genus are consumed and commercialized (Montoya *et al*., 2008; Pérez-Moreno *et al*., 2008). Worldwide there is high demand for the production of edible ectomycorrhizal fungi which represent a potential agroindustry of high economic and environmental importance, due to their relevance in successful reforestation programs and given their nutritional quality and medicinal use. However, despite their importance, the factors that originate the sporome formation of edible ectomycorrhizal fungi have been scarcely studied. Previously, it has been reported that the light quality is a factor that influences the formation of ectomycorrhizal sporomes (Villegas-Olivera *et al*., 2017). In addition, it has also been shown that the application of electrical impulses stimulates the formation of sporomes both in saprotrophic and ectomycorrhizal fungi, including: *Pholiota nameko*, *Lentinula edodes*, *Lactarius deliciosus*, *Laccaria laccata* and *Tricholoma matsutake* (Guerin Laguette *et al*., 2000; Ohga *et al*., 2011; Takaki *et al*., 2009; Ohga *et al*., 2012). However, it is unknown whether the rupture of hyphae is a factor that stimulates the formation of edible ectomycorrhizal fungi sporomes. This study assessed the hypothesis that mycelial disturbance increased the sporome formation of two edible ectomycorrhizal fungi associated with two Neotropical pines of great economic and ecological importance, *P. greggii* and *P. montezumae*.

MATERIALS AND METHODS

Fungal material and preparation of the inoculum

The fungal material used was acquired in the Ozumba market, Estado de México, and collected in pine forests adjacent to this region. The inoculum was obtained from fresh sporomes of *H. leucosarx* and *L. laccata*, separating the pileus from the stipe. The pilei were dehydrated at 35°C (JERSA drier, Cuatitlán Izcalli, Mexico). Later these pilei were ground and sieved in a mesh with opening of 1.19 mm to homogenize the particle size. The spore concentration was evaluated, by using a Neubauer chamber (Marienfeld, Lauda-Königshofen, Germany), which was estimated to be of 10^7-10^8 spores per cm^3 of inoculum. The final inoculum was stored in vials of 1.5 cm^3 at 5 °C, until it was used.

Establishing the experiment

Seeds of *P. greggii* and *P. montezumae* were collected in a natural forest of Xochicoatlán, Hidalgo, and Cofre de Perote, Veracruz, respectively. Previous to sowing, the seeds were soaked in running water for 24 hours, and then they were disinfected with H_2O_2 at 30% for 20 minutes and rinsed 4 times with sterile distilled water, under aseptic conditions. Sowing consisted in placing three seeds at a depth of 2 cm from each pine species in black plastic forest tube containers with a volume of 140 cm^3. These containers had 150 g of substrate made from a mixture of river sand, pine bark and forest soil, in proportion 2:2:1. The substrate was sterilized previously with vapor at a pressure of 1.3 kg cm^2 and a temperature of 125 °C for 3 h, then it was left resting for two days and, on the fourth day, it was sterilized again for 2 h. Once the seeds germinated, and when the plants had their first true leaves, each plant was inoculated with 10^7 to 10^8 spores of *L. laccata* and *H. leucosarx*. The inoculum was placed at three centimeters depth; then covered with a layer of substrate until filling the container, and a layer of sterilized volcanic rock was placed on their surface. The plants were kept in a greenhouse with irrigation every third day with sterile distilled water during 3 years (Figures 1 a-c).

Mycelial disruption

To induce mycelial disruption, 3-year-old *P. greggii* (Figura 1a) and *P. montezumae* (Figura 1b) plants were selected, grown in forest tubes of 130 cm^3 volume, following the methodology previously described, with mycorrhization percentages of at least 90%. These plants were transplanted to PVC tubes (polyvinyl chloride) with capacity of 6 kg (Figure 1c). Five months after the transplant, perforations of 5×5 cm^2 were made at three heights of the tube, in the superior, middle and inferior parts. As inducer of mycelial disruption, soil was extracted with roots with the aim of provoking the split of hyphae. Finally, the perforations were covered with a mesh with opening of 0.3 cm. The same number of perforations was made on the PVC in the case of the experimental units of the control treatments and the

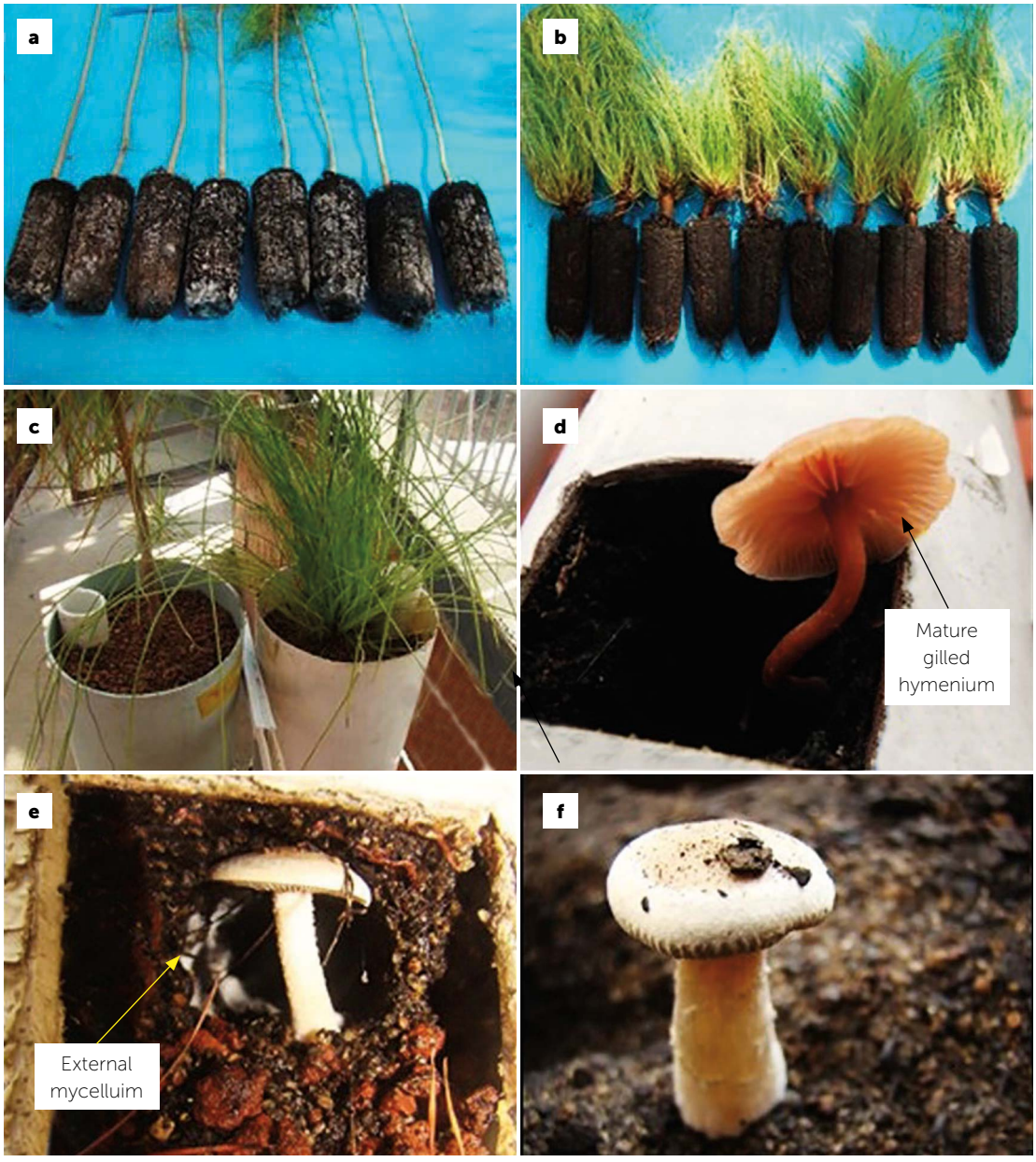

Figure 1. General aspects of the bioassay. a) *Pinus greggii* root balls showing abundant external hydrophobic mycelia of *Hebeloma leucosarx* (Hl); b) *P. montezumae* inoculated with *L. laccata* (Ll), showing hydrophilic mycelia in the root balls; c) General view of the experiment in PVC tubes; to the left *P. gregii* and to the right *P. montezumae*; d and e) Formation of mature sporomes of Ll (d) and Hl (e) in PVC containers, with mycelial disturbance. F) Mature sporome of Hl without mycelial disturbance.

mesh was placed, although the soil was not extracted so the mycelial disturbance was not made. All the plants were maintained during two more years to carry out the evaluations in the greenhouse, so the total duration of this experiment was 5 years.

Experimental design and statistical analysis

The experimental design used was completely randomized with 4 treatments and 6 repetitions for each pine species (*P. greggii* and *P. montezumae*). Then, there were 48 experimental units in total, each one consisting of an inoculated tree. The treatments were the following: i) Inoculated plants (IP) with *H. leucosarx* (Hl) without mycelial disturbance; ii) IP with Hl with mycelial disturbance; iii) IP with *Laccaria laccata* (Ll) without mycelial disturbance; and iv) IP with Ll with mycelial disturbance. A variance analysis was carried out and then Tukey's test was used out to compare means with $p \leq 0.05$ (SAS, 2002).

Sporome record and mycorrhization

Mycelial disturbance was carried out at the beginning

of April and the formation of sporomes was recorded daily in the PVC containers three months later, when the sporomes began to appear. This record was made during July to August, for 2 consecutive years, 2018 and 2019. The formation of sporomes was registered photographically (Figure 1d-f) with a Full HD 1080 digital camera (SONY Corporation, Japan). Only those sporomes that reached their state of maturity were counted, for which a slide was placed to collect the spores and carry out a microscopic evaluation of these (Figure 2c, f). The spores were photographed in a microscope Olympus BX51 model U-LH100H. At the end of the experiment a morphoanatomical evaluation of the mycorrhizal root tips in all of the experimental units was carried out in order to assess the presence of mycorrhizae belonging to the evaluated mycobionts, finding a minimal mycorrhization of 90% in all cases (Figure 2a, b, d, e).

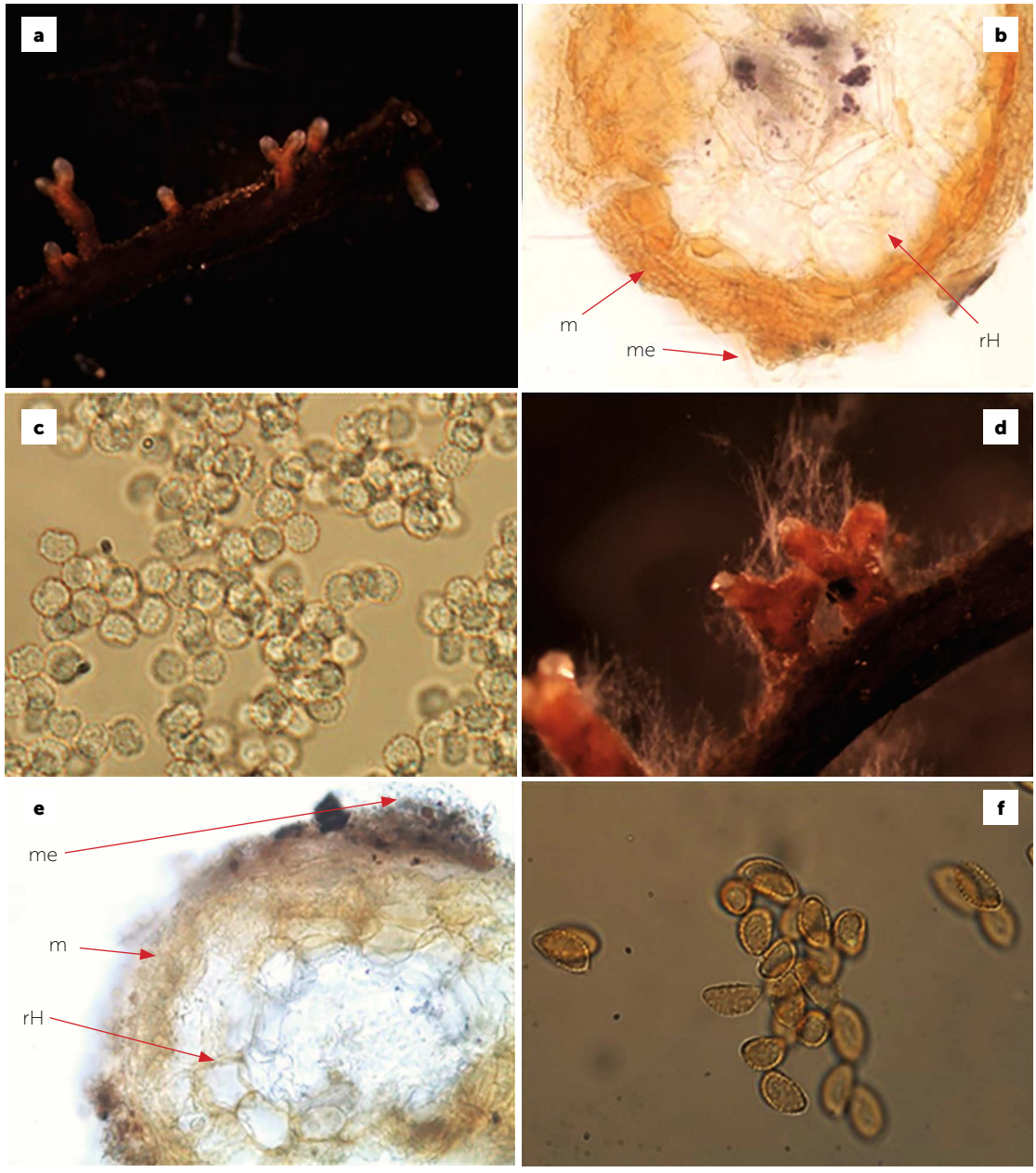

Figure 2. Microscopy of ectomycorrhizae of *Pinus greggii* with *Laccaria laccata* (Ll) and *Hebeloma leucosarx* (Hl) and morphology of mature spores. (a) Simple and dichotomous Ll mycorrhizal roots; (b) Cross section of Ll ectomycorrhiza showing Hartig net (rH), mantle (m) and external mycelium (me); (c) Echinulate globose spores characteristic of Ll produced by mature sporomes in the bioassay; (d) Hl mycorrhizal root with abundant loose emanating hyphae, white in color; (e) Cross section of Hl ectomycorrhiza showing abundant external mycelia (me), mantle (m) and Hartig net (rH); (f) Amygdaliform spores with verrucose ornamentation collected from mature sporomes of Hl produced in the bioassay.

RESULTS AND DISCUSSION

Mycelial disturbance and sporome number

The mycelial disturbance induced the sporome formation of *L. laccata* and *H. leucosarx*, regardless of the host plant (Figures 1d-f). The sporome number of *H. leucosarx* was twice higher when the plants were exposed to the mycelial disturbance compared to those that did not have mycelial disturbance, in *P. montezumae* and *P. greggii*, respectively. Meanwhile, in the case of *L. laccata* the sporome number recorded was three times higher (Figure 3).

Likewise, mycelial disturbance promoted a higher formation of sporomes of *L. laccata* (7%) and *H. leucosarx* (81%) compared to the experimental units without mycelial disturbance. It was observed that in the first year of evaluation, sporomes were formed with completely exposed lamellae from 90 to 150 days after the transplant to PVC tubes. From the mature sporomes, 57% and 43% corresponded to *H. leucosarx* and *L. laccata*, respectively. *P. montezumae* produced 20 sporomes, while *P. greggii* produced only 15 sporomes. The first sporomes of *H. leucosarx*, associated to *P. montezumae* were recorded 90 days after the transplant. In the second year after the mycelial disruption, 110 mature sporomes of *L. laccata* and *H. leucosarx* with completely exposed lamellae were found. Seventy-one percent corresponded to *H. leucosarx* and 29% to *L. laccata*. Sixty five sporomes were formed associated with *P. greggii*, and in contrast only 26 were formed with *P. montezumae*.

The sporome longevity, from a visible globose primordium of at least 3 mm of diameter until senescent sporomes, was on average 18 days. In contrast, the time elapsed from the beginning of the mycelial aggregates of 500 μm until the senescence of sporomes was 36 days. The sporomes of *L. laccata* and *H. leucosarx* produced abundant spore prints white in color 10 and 14 days, respectively, after the appearance of visible primordia. The microscopic analysis of these spores confirmed the identity of the fungal evaluated species. All the primordia of *L. laccata* and *H. leucosarx* were developed in the orifices that were made on the PVC tubes, with or without mycelial disturbance. However, there were clear differences in the sporome number between the experimental units with or without mycelial disturbance. All the sporomes presented positive geotropism, and they reached heights of 5 to 7 cm, which are similar to their heights recorded under natural conditions in forest areas (Figure 1d,e).

Diagnostic description of the sporomes of *H. leucosarx* and *L. laccata*

The sporomes of *H. leucosarx* pilei were cream-colored, sub-globose to hemispheric, changing to flat-convex with age, lamellae were concolorous with the pilei and turned light brown color when mature. The spores had amygdaliform shape (Figure 2f). The morphological characteristics of the spores of *H. leucosarx* recorded in this study agree with those described by Villegas-Olivera (2017). Meanwhile, the sporomes of *L. laccata* presented convex pilei salmon pink in color to brown orange, and cylindrical stipes concolorous with the pileus or slightly darker, with free, pale pink lamellae. The spores had globose shape and echinulate ornamentation (Figure 2c). The characteristics of the sporomes of *L. laccata* agreed with those described by Mueller (1985).

The factors that promote the sporome formation by ectomycorrhizal fungi associated to their plant symbionts have not been fully understood. However, it is reasonable to hypothesize that these factors are differential among the huge diversity of ectomycorrhizal fungi known worldwide. In this study it was found that the mycelial disturbance promoted a higher formation of sporomes of *L. laccata* and *H. leucosarx*. A greater

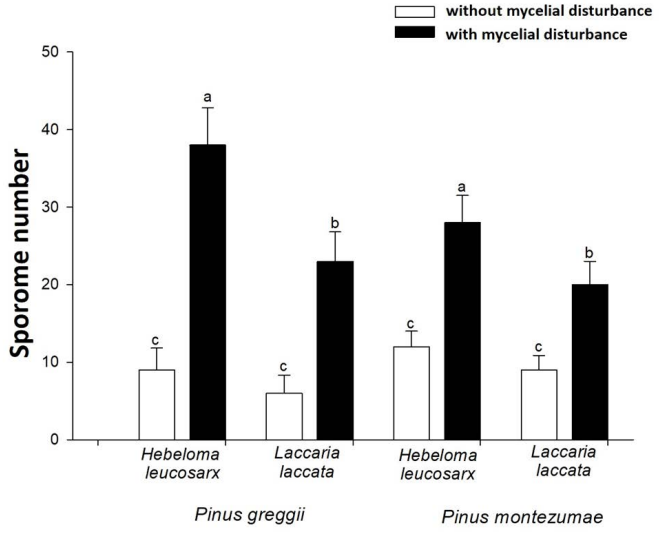

Figure 3. Production of sporomes during a 3-month period (from July to August) of two edible ectomycorrhizal fungi associated with 2 species of 5-year old Neotropical trees, without mycelial disturbance (hollow columns) and with mycelial disturbance (full columns). The bars represent average values and the lines over them the standard error of the mean. n=6. Bars with the same letter for each tree species are equal according to Tukey's means comparison test ($p \leq 0.05$).

induction of the formation of sporomes of *L. laccata* and *H. leucosarx* was observed with *P. montezumae,* compared to *P. greggii*, under the same environmental conditions. Previously, Godbout and Fortin (1990) had found that *L. bicolor* formed more sporomes with *P. strobus* than with *P. taeda* and *Picea glauca*. In addition, it has been found that environmental conditions such as temperature, carbon dioxide concentrations in the environment and salinity are determinant in the formation of sporomes by ectomycorrhizal fungi (Kües and Liu, 2000). The influence of the fertilization regimes and of the relative humidity in the formation of sporomes have also been demonstrated (Godbout and Fortin 1990). It has also been shown that the application of electrical impulses promotes the formation of sporomes, probably associated to the fact that the cracks that they generate in the mycelium modify the enzymatic activity, mainly that of laccases and proteases (Ohga *et al*., 2001; Ohga and Ida, 2001; Islam *et al*., 2013; Takaki *et al*., 2014). Ohga *et al*. (2001) recorded the formation of sporomes of *L. laccata* associated with *P. densiflora* when using electrical impulses, and Islam *et al*. (2013) and Ferzana and Shoji (2012) found the same phenomenon when applying electrical discharges in *T. matsutake*, associated to *P. densiflora* in the field. Similarly, it has been shown that the application of electrical impulses stimulates a greater formation of sporomes of edible saprotrophic fungi such as *Lentinus edodes*, *Pholiota nameko* and *Lyophyllum decastes* (Ohga *et al*., 2001; Takaki *et al*., 2014). In this study an interesting observation was the difference in the sporome size that had been previously found in bioassays in forest tubes in various species of *Laccaria* and *Hebeloma* (Pérez-Moreno *et al*., 2020), compared to those recorded in this study in the containers with larger volume. The sporomes in forest containers of 140 cm^3, are maximum 3 cm in height, while the sporomes formed in the PVC tubes of 6 kg were up to 8 cm in height. A reason that could explain the reduced size of sporomes in the containers of smaller size might be a reduced supply of nutrients and water, as pointed out by Godbout and Fortin (1990).

CONCLUSIONS

This study demonstrates for the first time that mycelial disturbance stimulates the formation of sporomes of Neotropical ectomycorrhizal fungi. Two- to three-fold increase in the number of sporomes of *H. leucosarx* was recorded when the plants were exposed to mycelial disturbance compared to those that did not have hyphal ruptures, both in *P. montezumae* as in *P. greggii*. In addition, there were differences in the sporome number produced when comparing the species of fungi and trees evaluated. *H. leucosarx* produced a higher amount of sporomes compared to *L. laccata* when their mycelia were disturbed regardless of the associated phytobiont. The sporome number produced by *P. greggii* was higher when compared to *P. montezumae*. This study shed some light on the comprehension of the factors involved in the formation of edible ectomycorrhizal fungi, which is valuable information for the domestication and production of wild edible fungi.

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