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DUNG REMOVAL BY BEETLES EXPOSED TO FECES OF SHEEP FED *Guazuma ulmifolia* Lam.

Gylia Sylene **Toledo-Zárate**[1], Silvia **López-Ortiz**[1*], Lucrecia **Arellano**[2], Jesús **Jarillo-Rodriguez**[3], Eusebio **Ortega-Jiménez**[1], Ponciano **Pérez-Hernández**[1]

[1]Colegio de Postgraduados Campus Veracruz. Postgrado en Agroecosistemas Tropicales. Carretera Xalapa-Veracruz km 88.5, Manlio Fabio Altamirano, Veracruz, Mexico. C. P. 91690.
[2]Instituto de Ecología A. C., Red de Ecoetología. Carretera Antigua a Coatepec 351, Colonia El Haya, Xalapa, Veracruz, Mexico. C. P. 91073.
[3]Universidad Nacional Autonoma de México. Facultad de Medicina Veterinaria y Zootecnia. Centro de Enseñanza, Investigación y Extensión en Ganadería Tropical. Carretera Martínez de la Torre-Tlapacoyan km 5.5, Tlapacoyan, Veracruz, Mexico. C. P. 93650.
* Author for correspondence: silvialopez@colpos.mx

ABSTRACT

This study examines the relationship between the phenolic content (total phenols and tannins) of three diets for sheep (*Ovis orientalis aries* L.) containing *Guazuma ulmifolia* Lam. (Gu0: 0 %, Gu30: 30 %, and Gu60: 60 %) and the removal of feces by dung beetles fed on sheep excreta. The study was conducted in two stages: in the first, nine Pelibuey sheep (40 ± 4 kg live weight), randomly assigned to one of three groups, were fed the experimental diets (Gu0, Gu30, and Gu60). For 9 d, feces were collected and stored at 8 °C. In stage two, three dung beetle species (*Canthidium pseudopuncticolle*, *Canthon leechi*, and *Canthon chiapas* [Coleoptera: Scarabaeinae]) were captured, artificially paired in couples, and placed in terraria to evaluate dung removal, during 29 d. Sheep preferred the Gu30 diet, of which they consumed 33.5 ± 1.7 g of dry matter (DM) kg^{-1} of live weight (LW). All diets had similar chemical composition, except Gu60, which maintained a higher concentration of total tannins (3.5 ± 0.3 mg g^{-1} DM). In feces, Gu0 showed the highest concentration of phenols (0.507 ± 0.3 mg g^{-1} DM; $p \leq 0.05$) and total tannins (0.317 ± 0.1 mg g^{-1} DM; $p \leq 0.05$). *Canthon leechi* removed the highest amount of feces (249.6 g) and had the strongest day × diet interaction effect ($p \leq 0.05$). Adding up to 30 % *G. ulmifolia* forage in the diet did not compromise dry matter intake of sheep and maintains good nutritional quality; the concentration of phenolic compounds in the diet and feces changed according to the proportion of *G. ulmifolia* in the diet. Although the beetles removed less excreta from sheep that consumed *G. ulmifolia* (Gu30 and Gu60), it is not possible to conclude whether phenolic compounds are responsible for this response.

Keywords: excreta, phenols, tannins.

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INTRODUCTION

Dung beetles inhabit a wide range of environments, from temperate to tropical climates. They use organic matter for food and nesting (Martínez-Morales *et al*., 2015).

Dung beetles are important for grasslands as they contribute to the removal and decomposition of livestock manure (Martínez-Morales and Lumaret, 2022). Removal is the action of relocating the dung away from the source, either by rolling it or burying it in galleries (under the source) to elaborate masses or nest balls, to lay eggs, or simply to feed on organic matter (Tonelli, 2021). Telecoprid or rolling species elaborate dung balls that they transport away from the source using their hind legs, while paracoprid or burrowing species elaborate galleries (tunnels) and feeding chambers where they deposit food masses and nest balls, finally dweller species feed and breed inside the food mass (Martínez-Morales *et al*., 2015).

Through feces removal, dung beetles provide important ecosystem services such as pasture cleaning, dung dispersal and degradation, as well as soil improvement, secondary seed dispersal, and burial of invertebrate organisms that parasite herbivores and affect their health (Basto-Estrella *et al*., 2016). However, dung beetle communities are sensitive to environmental changes, such as the destruction of primary vegetation (Halffter and Arellano, 2002), changes in land use, landscape fragmentation (Tec-Pardillo *et al*., 2024), and the increase of invasive species (Morales-Trejo *et al*., 2024).

New techniques for feeding livestock include silvopastoral systems with native or introduced woody forage species. These forage species contain a diversity of secondary compounds that provide benefits to the livestock diet (Villalba *et al*., 2014). *Guazuma ulmifolia* Lam. is a deciduous forest species widely used in silvopastoral systems due to its adaptation to tropical areas with long dry seasons and its forage attributes (Manríquez-Mendoza *et al*., 2011), although it contains phenolic compounds in stems, leaves, and fruits (López *et al*., 2004; Márquez and Suárez, 2008; Rafi *et al*., 2020).

The biological benefits that secondary compounds have to herbivores have recently been recognized. For example, phenols and saponins have antiparasitic and rumen fermentation-regulating properties that reduce methane gas emission (Sepúlveda-Vázquez *et al*., 2018; Pereira *et al*., 2019). The study of secondary compounds in ruminant diets has focused mostly on phenolic compounds as they bond with proteins, protecting them from ruminal degradation and facilitating their absorption at the duodenum ~~level~~ (Torres-Acosta *et al*., 2008). On the other hand, phenols also inhibit fungal growth and can deter nematodes, mites, and insects (Nava-Pérez *et al*., 2012).

It is currently unknown whether dung beetles are affected during their nesting process and removal activities when they break down feces of herbivores fed on forages with a high concentration of phenolic compounds. Therefore, in this study, feces removal by dung beetles was measured when they fed on sheep feces containing varying amounts of *G. ulmifolia*.

MATERIALS AND METHODS

Location and experimental site description

This research was conducted at a site located in Manlio Fabio Altamirano municipality, in central Veracruz, Mexico. The territory has an Aw_1'' (w)(i) g (warm-dry-regular) climate based on the Köppen classification (García, 2004), having a long dry period and abundant rainfall from June to September. The experimental site is located at 63 masl, with an average temperature of 26 °C and average annual rainfall of 1191 mm (SMN, 2010).

Research stages

The research was conducted in two stages. The first phase involved cultivating and harvesting forage to create three distinct diets for nine young females of the Pelibuey sheep breed (n = 3 sheep in each diet) (40 ± 4 kg live weight, LW), kept in confinement to collect dung, for a period of 8 d. During the second stage, dung beetles were captured and maintained in conditions similar to those prevailing in the area. Three species that were suited to eating sheep dung were selected. A couple of each beetle species was assigned to each treatment to evaluate dung removal and their behavior.

Stage 1. Sheep diet and feces collection

At this stage, three experimental diets containing two concentrations of *G. ulmifolia* (Gu30: 70 % grass with 30 % *G. ulmifolia* and Gu60: 40 % grass with 60 % *G. ulmifolia*) and a control diet (Gu0: 100 % grass) were prepared using 70-d regrowth green foliage of *G. ulmifolia* and 60-d regrowth green grass *Megathyrsus maximus* Jacq. cv. Cuba 22). Each diet was analyzed for crude protein (CP) using the AOAC (1990) method. Neutral detergent fiber (FDN), acid (FDA), and lignin content were determined using the filter bag method (ANKOM Technology). The concentration of total phenols and tannins was calculated according to the FAO (2000) methods.

From September 3 to 15, 2021 (12 days), fresh forage from *G. ulmifolia* and *M. maximus* was harvested every day, and the amounts required for each experimental diet were chopped. The amounts offered daily to each sheep were calculated on a dry basis for an intake of 3 % LW but were mixed and offered on a wet basis. The amount required by each sheep was offered throughout the day to ensure a constant supply of forage; rejected forage was collected daily to determine the sheep's voluntary intake based on the difference between the offered and rejected forage. Both offered and rejected forage were corrected for dry matter before calculating the difference to account for weight loss caused by spontaneous water loss throughout the day.

During this period, feces were collected every day between 8:00 and 17:00 h and stored at 8 °C for 10 to 27 days until use. Samples for phenolic compounds analysis were dried in a forced air oven at 40 °C during 72 h and kept at room temperature until analysis.

Stage 2. Diet-related behavior of the beetles

At this stage, pit fall traps baited with different types of excreta (cattle, sheep, and human feces) were placed to capture beetles. These traps were made of 1 L plastic containers with a partially perforated lid (triangle-shaped perforation that allows beetles to enter) and a plastic plate suspended upside down over the trap to prevent direct exposure to light or rainwater. The traps were placed from August 13 to September 4, 2021, in sites where cattle and sheep were grazing. All captured dung beetles were identified using taxonomic keys and kept in temporary terraria under conditions like those of their environment, feeding them with feces from grazing sheep. The beetle species *Canthon leechi*, *Canthon chiapas*, and *Canthidium pseudopuncticolle* were chosen as they displayed constant use of sheep dung in the terraria; the three species showed good adaptation to environmental conditions (humidity and temperature) and had enough individuals to form couples for each type of diet.

Canthidium pseudopuncticolle is a small (4 to 6 mm long), paracoprid, coprophagous, and diurnal species (Rivera-Cervantes and Halffter, 1999). On the other hand, *C. chiapas* is a medium-sized (7 to 13 mm) and widely distributed species in tropical climates, which withstands high temperatures, direct sunlight, and low humidity (Martínez-Morales and Montes de Oca, 1994), making it abundant in grasslands (Basto-Estrella *et al*., 2014). Finally, *C. leechi* is a small-sized (3.5 to 6 mm), habitat generalist, and diurnal species (Rivera-Cervantes and Halffter, 1999). The preference of dung beetle communities (which include these species) for sheep dung is known (Correa *et al*., 2013), and their presence has been associated with environments where sheep forage (Ortega-Martínez *et al*., 2021; Ríos-Díaz *et al*., 2021); however, it is not known yet whether these species use sheep dung for nesting.

Terraria were prepared using 1 L rectangular-shaped plastic containers (21 × 13 × 7 cm) with perforated sides and lids to allow air circulation. Each terrarium was filled with sterilized soil to 2/3 of its capacity. The terraria were placed horizontally for the telecoprid or rolling species, (*C. leechi* and *C. chiapas*), and vertically for the paracoprid or burrowing species (*C. pseudopuncticolle*). Forty-two terraria were set, 27 for *C. leechi* (nine per treatment), nine for *C. pseudopuncticolle* (three per treatment), and six for *C. chiapas* (two per treatment). The number of terraria for each treatment corresponded to the number of couples formed with the captured individuals.

Initially, 10 g of manure were placed in the center of each terrarium, according to every treatment (Gu0, Gu30, or Gu60). Terraria were checked on a daily basis throughout the experiment (September 13 to October 12). Each time, the remaining dung on the surface was weighed to determine whether it was removed by burial or displacement (by weight difference), and more feces were added when all had been removed or there was fungal contamination. Terraria were kept moist by spraying water with a spray bottle when the soil was dry. Throughout the experimental period, temperature (ranging from 23 to 30 °C) and relative humidity (ranging from 62 to 90 %) were recorded daily under the prevailing environmental conditions.

The behavior of the couples was monitored to assess whether the diet they received (feces from sheep that consumed *G. ulmifolia*) affected the survival and behavior of the species. Every day (for 3 hours), the surface of each terrarium was checked, and seven activities performed by the beetles were recorded: male on surface, female on surface, couple on surface, presence of balls, entrance to galleries (holes), dead male, and dead female. Additionally, the state of the food was checked (dung with fungi, disintegrated dung, and buried dung). Finally, the terraria were opened and checked for buried dung. The frequency of these activities was recorded.

Statistical analysis

The variables dry matter intake by sheep, chemical composition, and concentration of phenolic compounds (total phenols and tannins) of the diets were analyzed with a completely randomized design using the analysis of variance (ANOVA) procedure of SAS/STAT (SAS Institute Inc., Cary, NC, USA) and the Tukey test to compare means between treatments, with an alpha of 0.05. Feces removal by dung beetles was analyzed with the MIXED procedure of SAS/STAT; the model included treatment (n = 3 types of feces from sheep fed three different diets), day of the experiment (1 to 29 days), as well as the treatment × day interaction as a fixed effect, and species as a random effect. An autoregressive AR(1) covariance structure was used for autocorrelating values. Treatment means were compared with the LS-means test, and X^2 contingency tables were built to analyze whether activities performed by dung beetles and dung condition were independent of diet (treatments); these analyses were performed for each species separately. To analyze the relationship between the amount of dung removed and temperature and relative humidity, Kendall correlations were performed. The last two analyses were performed using the PAST program version 2.13 (Hammer *et al*., 2001).

RESULTS AND DISCUSSION

Chemical composition of experimental diets

The nutritional value of the experimental diets did not vary among treatments ($p \geq 0.05$). Total phenolic compound concentrations were consistent across all diets ($p \geq 0.05$); however, the total concentration of tannins was higher in diets that included *G. ulmifolia* ($p \leq 0.05$) (Table 1). The grass treatment (Gu0) had low concentrations of tannins; as this group of plants is adapted to herbivory, they do not need chemical defenses to protect themselves from herbivores and pathogens (Provenza *et al*., 1996).

Table 1. Nutritional value (%) and phenolic compound concentration (mg g-1 DM) of diets Gu0 (100 % grass [*Megathyrsus maximus* Jacq. cv. Cuba 22]), Gu30 (70 % grass and 30 % *Guazuma ulmifolia* Lam.), and Gu60 (40 % grass and 60 % *G. ulmifolia*) fed to ewes (*Ovis orientalis aries* L.)

Variables	Gu0	Gu30	Gu60
CP (%)	6.17 ± 0.27	6.24 ± 0.27	7.35 ± 0.27
NDF (%)	68.10 ± 0.67	70.16 ± 0.67	70.00 ± 0.67
ADF (%)	42.20 ± 1.37	46.06 ± 1.37	51.20 ± 1.37
Lig (%)	7.80 ± 1.55	11.83 ± 1.55	19.43 ± 1.55
TP (mg g^{-1} DM)	5.48 ± 0.29	5.88 ± 0.29	6.10 ± 0.29
TT (mg g^{-1} DM)	1.36 ± 0.29^{b}	4.11 ± 0.29^{a}	3.51 ± 0.29^{a}

$^{a, b}$ Mean values having different superscript letter within a row differ ($p \leq 0.05$). DM: dry matter; CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; Lig: lignin; TP: total phenols; TT: total tannins.

Diet intake by sheep

Dry matter intake by sheep differed by the effect of the amount of *G. ulmifolia* foliage in the diet ($p \leq 0.05$). Those fed Gu60 (27.4 ± 1.7 g kg^{-1} LW) had a lower intake than those fed Gu30 (33.5 ± 1.7 g kg^{-1} LW) or Gu0 (33.8 ± 1.7 g kg^{-1} LW). Livestock ingests tannin-containing plants as part of a diverse diet (Clemensen *et al*., 2020); however, individuals can regulate their intake to use the nutrients these plants contain without compromising their health. It is estimated that domestic ruminants can include between 5 and 30 % of tannin-containing foliage in their diet with no negative effects on intake (Márquez and Suarez, 2008), so the sheep in treatment Gu60 may have been regulating the amounts consumed from the mixture offered.

The dry matter intake of the sheep in this study is greater than that of grazing sheep supplemented with varying amounts of *G. ulmifolia* (Sosa-Rubio *et al*. 2004) and penned sheep exposed to both Taiwan grass and *G. ulmifolia* foliage offered simultaneously (unpublished data). Grazing sheep consumed between 2.9 and 3.2 % LW (approximately 32 g DM kg^{-1} LW), whereas housed sheep consumed 107.9 to 123.6 g MS kg^{-1} LW. The consumption of both forages was 1:1 (nearly 50 % of each forage) at the end of the period evaluated in the second study, which contrasts with the behavior of the animals in this experiment, as their limit was the diet with 30 % *G. ulmifolia* foliage, possibly because both forages were integrated. This may be because the sheep evaluated in those studies were younger. On the other hand, the animals exposed to free choice could make more homogeneous use of both forages over time.

García *et al*. (2008) offered *G. ulmifolia* to goats in a cafeteria test (free choice among several forage types offered simultaneously) and classified it as a moderately accepted foliage (0.164 kg DM day^{-1}), and associated the chemical composition of the forage with the concentration of secondary compounds and other possible deterrent properties. This could explain why the intake in Gu0 is statistically different from Gu60, due to the concentration of phenolic compounds in the diet. In addition, the rumen environment could be modified and affect the intake. In contrast, the maximum inclusion of 30 % *G. ulmifolia* (Gu30) in the diet did not affect sheep intake ($p \geq 0.05$) in this study.

Total phenols and tannins in sheep feces

Total phenols ($p \leq 0.05$) and total tannins ($p \leq 0.05$) in feces differed as an effect of different amounts of *G. ulmifolia* in the diet; Gu0 had the highest concentrations of phenols and tannins (Table 2). Phenolic compounds in food are not completely metabolized in the gastrointestinal tract of organisms; the majority is broken down into low molecular weight phenolic acids and accumulates in the organism, exerting physiological effects such as regulating bacterial communities or as gastrointestinal prebiotics (i.e., *Clostridium perfringens, C. difficile*, and *Bacteroides* spp.) (Lee *et al*., 2006). Chen *et al*. (2018) fed tea polyphenols to rats (Pure Herbal Remedies, Pte Ltd., Singapore) and observed the benefits of these compounds in moderating bacterial populations in the organisms. During their metabolism, phenols underwent drastic changes, with 4-hydroxyphenylacetic acid being the main phenolic compound found in feces and

Table 2. Total phenolic and tannin concentration in feces of ewes (*Ovis orientalis aries* L.) fed on diets Gu0 (100 % grass [*Megathyrsus maximus* Jacq. cv. Cuba 22]), Gu30 (70 % grass and 30 % *Guazuma ulmifolia* Lam.), and Gu60 (40 % grass and 60 % *G. ulmifolia*).

Variables	Gu0	Gu30	Gu60
Total phenols (mg g^{-1} DM)	0.507 ± 0.3^{a}	0.395 ± 0.3^{ab}	0.345 ± 0.3^{b}
Total tannins (mg g^{-1} DM)	0.317 ± 0.1^{a}	0.273 ± 0.1^{ab}	0.221 ± 0.1^{b}

[a, b] Mean values having different superscript letter within a row differ ($p \leq 0.05$). DM: Dry matter.

plasma. Lower amounts of phenols in the diet are excreted, so it is reasonable to find traces in feces. The concentration of phenolic compounds in feces could depend on the changes in chemical structure that these compounds undergo through the digestive tract and the way they are metabolized. It is likely that a fraction is absorbed at the blood level or that not all compounds read in the excreta correspond to phenols.

The differences in the amounts of phenolic compounds in feces could suggest that the compounds excreted are not the same as those contained in the feed because their chemical structure is modified as they are metabolized through the digestive tract (Vollmer *et al.*, 2018), which could have interfered with the readings of total phenols and tannins. Another explanation is that tannins create bonds with proteins depending on the pH of their environment, and there is a possibility that the pH of the feces and the bonds created with proteins did not allow a correct reading of the phenolic compounds sought. The background on the study of phenolic compounds in feces is limited, but most of it is focused on the analysis of phenols in forages and feeds or testing methodologies for their estimation.

Chen *et al*. (2018) analyzed phenolic compounds in rat eces after digestion of diets containing high and low fats. They used gas chromatography to detect metabolites derived from phenolic compounds. This method could be better method for detecting phenols and metabolites in herbivore feces. Our findings are inconclusive and require further analysis to relate them to dung beetle behavior. However, it is possible to relate the forages and chemical composition of diets fed to sheep to dung beetle activity without looking for a chemical explanation.

Dung removal by the beetle species

The amount of feces removed by dung beetles differed by effect of the diet consumed by sheep ($p \leq 0.05$) and the dung beetle species ($p \leq 0.05$). Beetles removed more dung ($p \leq 0.05$) from Gu0 (0.727 ± 0.03 g d^{-1}) and Gu30 (0.716 ± 0.03 g d^{-1}) than Gu60 (0.567 ± 0.03 g d^{-1}). *Canthidium pseudopuncticolle* removed 0.294 ± 0.03 g d^{-1}, *C. leechi* removed 0.319 ± 0.02 g d^{-1}, while *C. chiapas* removed 1.396 ± 0.04 g d^{-1}, a higher amount than the first two species ($p \leq 0.05$), perhaps because it was the largest species in size (Nervo *et al*., 2014). However, it was also observed that the amount of dung removed by *C. leechi* depended on the treatment × day interaction ($p \leq 0.05$), manifested in the removal peaks that the couples made in all treatments, mainly between days 1 and 12 of the experimental period (Figure 1). There were removal peaks in all three treatments, but on different days (days 1, 6, 8, and 10 in Gu0; days 1, 6, 10, and 12 in Gu30; and days 1, 3, and 6 in Gu60). Toward the end of the period, dung beetles in Gu0 removed more uniformly than in Gu30 and Gu60.

Canthidium pseudopuncticolle and *C. chiapas* also showed removal peaks (Figures 2 and 3), apparently in greater synchrony between treatments, as no interaction was observed ($p \geq 0.05$). These removal peaks shown by the dung beetles, regardless of an interaction, may be due to the consumption, nesting, and reproduction habits of each species, but also to natural fluctuations that individuals may show during their processes, for example, removing one day and then spending some time manipulating, feeding, or nesting in the removed dung mass (Martínez-Morales and Lumaret, 2022) to start again.

The time the dung beetle species were in the laboratory may not have been enough to fully observe their habits, because the nesting periods of these species vary. For example, *C. chevrolati*, a closely related species with similar habits and size to *C. chiapas*, has a 30 to 40 d pre-nidification period, and the first egg laid occurs approximately 10 days after the first mate (Martínez-Morales and Lumaret, 2022). These times are longer than the observation period in our research, so it was not sufficient to observe the nesting stage, assuming that this had not taken place before capture.

The activities of each beetle species and the condition of the dung provided during the experimental period were affected by the diets (treatments) (Table 3). The behavior

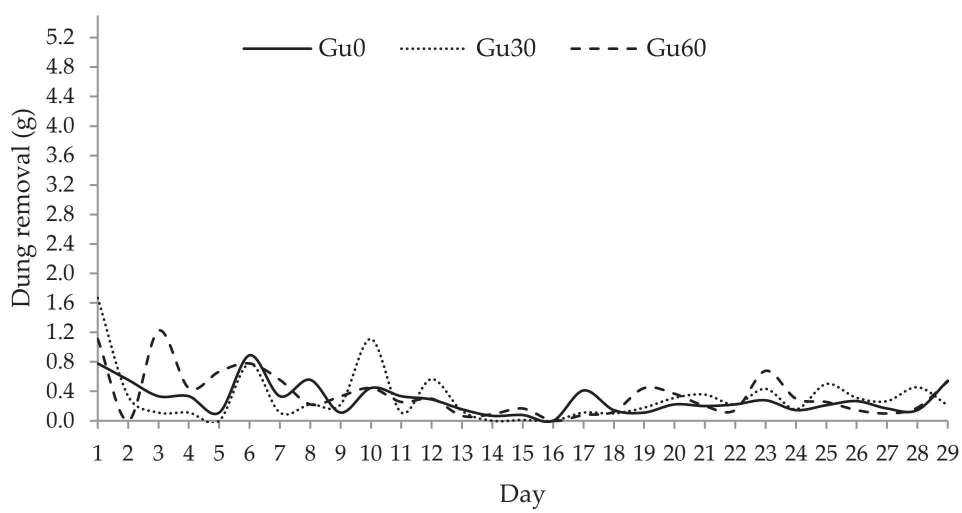

Figure 1. Dung removal (g d^{-1}) by *Canthon leechi* in treatments Gu0 (100 % grass [*Megathyrsus maximus* Jacq. cv. Cuba 22]), Gu30 (70 % grass and 30 % *Guazuma ulmifolia* Lam.), and Gu60 (40 % grass and 60 % *G. ulmifolia*).

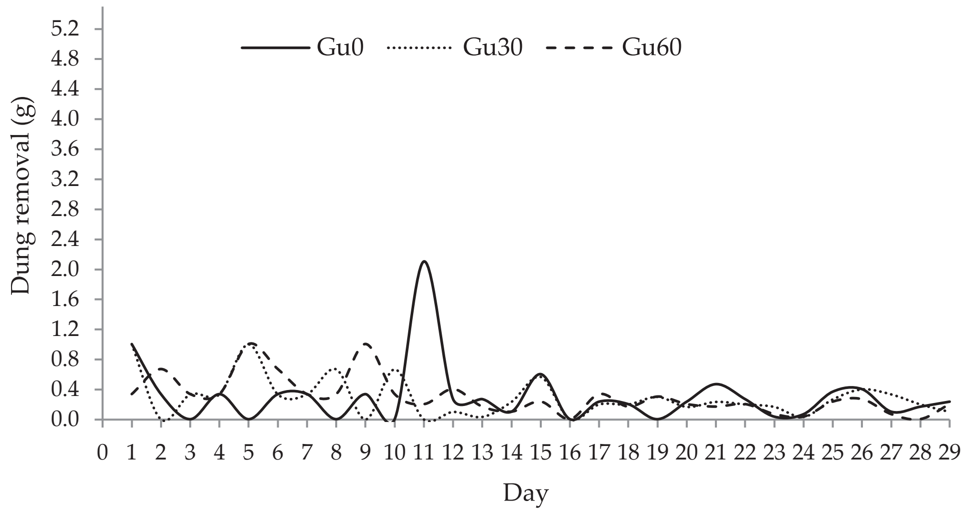

Figure 2. Dung removal (g d^{-1}) by *Canthidium pseudopuncticolle* in treatments Gu0 (100 % grass [*Megathyrsus maximus* Jacq. cv. Cuba 22]), Gu30 (70 % grass and 30 % *Guazuma ulmifolia* Lam.), and Gu60 (40 % grass and 60 % *G. ulmifolia*).

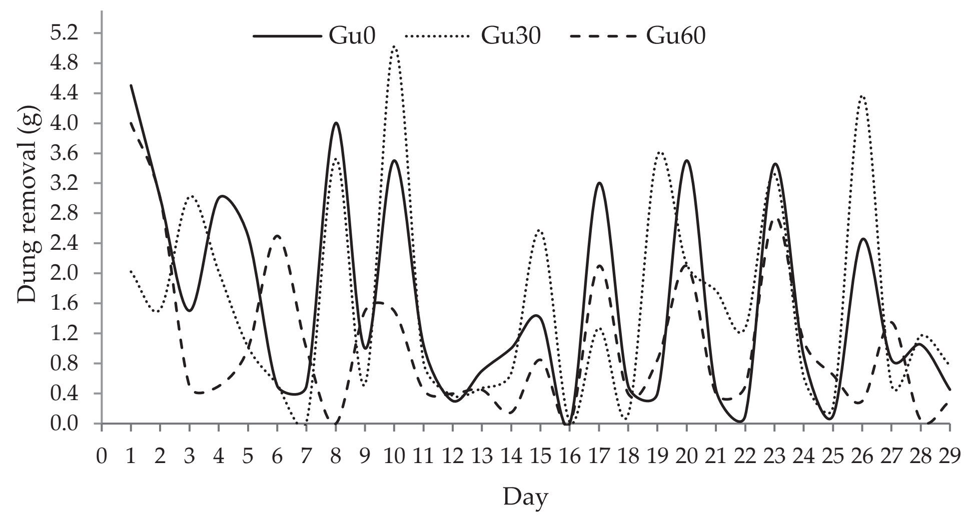

Figure 3. Dung removal (g d^{-1}) by *Canthon chiapas* in treatments Gu0 (100 % grass [*Megathyrsus maximus* Jacq. cv. Cuba 22]), Gu30 (70 % grass and 30 % *Guazuma ulmifolia* Lam.), and Gu60 (40 % grass and 60 % *G. ulmifolia*).

of *C. leechi* (df = 12, X^2 = 22.9, p = 0.028) was not independent of the type of manure removed because couples, males, and females, were seen interchangeably several times on the surface; only on the Gu60 diet were males seen on the surface fewer times than males in the other treatments. The presence of dung balls and gallery entrances

Table 3. Frequency of activities performed by the dung beetle species *Canthon leechi*, *Canthidium pseudopuncticolle*, and *Canthon chiapas* assigned to treatments Gu0 (100 % grass [*Megathyrsus maximus* Jacq. cv. Cuba 22]), Gu30 (70 % grass and 30 % *Guazuma ulmifolia* Lam.), and Gu60 (40 % grass and 60 % *G. ulmifolia*).

Type of observation	*C. leechi*			*C. pseudopuncticolle*			*C. chiapas*		
	Gu0	Gu30	Gu60	Gu0	Gu30	Gu60	Gu0	Gu30	Gu60
Frequency of activities performed by the dung beetles									
MS	21	14	4	1	3	3	1	0	0
FS	38	61	49	10	7	6	4	4	0
CS	28	22	24	0	1	5	6	2	0
PDB	3	5	3	0	2	1	0	1	1
ET	14	9	16	4	5	2	3	1	8
DM	5	3	1	1	2	2	0	0	0
DF	1	3	1	0	1	2	0	0	0
Frequency of dung condition in the terraria									
DFu	29	38	47	0	0	0	0	0	0
DD	50	59	39	2	16	10	1	5	0
BD	17	25	21	7	7	2	16	10	15

MS: male on the surface; FS: female on the surface; CS: couple on the surface; PDB: presence of dung balls; ET: entries to tunnels (holes on the surface); DM: dead male; DF: dead female; DFu: dung with fungi; DD: dispersed dung; BD: buried dung.

throughout the terraria was higher in Gu30. This observation could denote that those beetles were preparing for nesting using available feces. The presence of fungi in the food offered to this species was highly frequent, and disintegrated and buried dung was observed; however, the condition in which dung was found was independent of the diet (df = 4, $X^2 = 6.9$, $p = 0.144$).

The behavior of *C. chiapas* was not independent of diet (df = 12, $X^2 = 19.3$, $p = 0.013$), but a greater participation of females on the surface in treatments Gu0 and Gu30 and of couples in Gu0 was observed. There was a scarce presence of dung balls, more entries to galleries in Gu60, and no dead individuals. No fungi were observed in any treatment; however, dung condition is not independent of treatment (df = 2, $X^2 = 8.6$, $p = 0.013$). While there was more disintegrated manure in G30, more buried manure was observed in Gu0 and Gu60, using all the manure offered.

Finally, the activity of *C. pseudopuncticolle* was independent of the type of manure consumed (df = 12, $X^2 = 14.5$, $p = 0.272$). Although there was a greater presence of females on the surface in very similar frequencies among treatments, few dung balls, entries into galleries, and deaths were observed. The manure provided to this species did not develop fungi, but broken-down and buried dung were high (df= 2, $X^2 = 9.03$, $p \leq 0.01$).

Laboratory environmental conditions influenced removal behavior in all studied dung beetle species. The behavior of *C. leechi* and *C. pseudopuncticolle* was negatively correlated with temperature (τ = -0.32, p = 0.015 and τ = -0.29, p = 0.03, respectively) and positively correlated with humidity (τ = 0.33, p = 0.01 and τ = 0.43, p = 0.001, respectively); that is, their removal activity decreased as temperature increased but improved with increased relative humidity. This is because these species are native to tropical dry forests and have adapted to thrive in humid environments. On the contrary, the removal of *C. chiapas* showed a marginal negative correlation with temperature (τ = -0.24, p = 0.06) and did not have a significant correlation with humidity, which could be explained by the fact that this species is mostly present in pastures where solar radiation is high.

CONCLUSIONS

Guazuma ulmifolia foliage can be included up to 30% in sheep diets when offered integrated with other components of the diet, as it is well accepted by sheep and does not compromise voluntary intake. When integrated into the grass-based diet, the chemical-nutritional content of the mixture increased compared to the diet containing only grass. Although *G. ulmifolia* was used as a source of secondary metabolites for the purpose of this research, further evidence of the potential of this species to be integrated into sheep production systems was generated.

The results on the presence of phenolic compounds in feces are inconclusive because quantification must be done with other more appropriate methods that allow separation of these compounds from other products of ruminant digestion. The activity of dung beetles varied when exposed to sheep manure fed different proportions of *G. ulmifolia*. *Canthon chiapas* showed higher removal activity in all treatments, although it made greater use of the grass-only diet because it is a species adapted to consume the dung of cattle that feed on grass.

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