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Ingestion of *L. ferrea* and *S. spectabilis* Seeds from the Caatinga by Goats and Sheep Enhances Germination and Vigor

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HIGHLIGHTS

- Ecological methods for overcoming dormancy in seeds
- Spatial and temporal distribution of Caatinga seeds
- Dispersal of caatinga seeds by goats

Abstract: Endozoochory in dry tropical forests is a phenomenon that ensures species perpetuation, due to its positive effects on seed germination and plant establishment. However, the mechanisms behind this germinative success are poorly understood. This study aimed to analyze the ingestion of *Libidibia ferrea* and *Senna spectabilis* seeds by goats and sheep and the effect on dormancy and morphophysiological qualities. Four hundred seeds of each species were provided in the feed base of goats and sheep, followed by an evaluation of the recovery rate, germination (%), germination speed index, mean germination time, shoot and root length, and seedlings dry mass. The recovery of seeds from sheep occurred more rapidly (within 48 hours for both species) in the first hours after ingestion, while for goats, this process occurred slowly and over a more extended period, which may potentially favor the perpetuation of the species within the habitat. To elucidate the study, it was noted that vigor variables, dry mass, and germination speed index contribute most to the data explanation for *L. ferrea* seeds, whereas for *S. spectabilis* seeds, the germination showed intermediate contribution, suggesting its relevant role in this species. Dormancy of *L. ferrea* and *S. spectabilis* seeds can be broken when ingested by goats and sheep, indicating that the passage of the seeds through the digestive system enhances their physiological quality in comparison to those that were not ingested by these animals.

Keywords: Endozoochory; Seed coat dormancy; Fabaceae; *Ruminants.*

INTRODUCTION

Invasive plants, associated with anthropogenic activities and global environmental changes cause ecological disturbances, resulting in a reduction in the biodiversity of various biomes, including the Caatinga, causing significant changes in its ecosystem functions [1]. In seeds of species occurring in Seasonally Dry Tropical Forests (SDTF), development and survival after germination occur mainly in seedlings that, after overcoming dormancy, find favorable conditions for the entire life process [2].

Strategies aimed at reducing the impacts of degrading activities can mitigate the effects on the environment. Seed dispersal plays a crucial role in the establishment of many species [3], contributing to increased biodiversity and the maintenance of the ecosystem [4]. An effective form of seed dispersal occurs by animals via ectozoochory and endozoochory [5].

Thus, understanding the effectiveness of seed dispersal by different animals can enable low-cost strategies for restoration projects in disturbed environments [6], such as the Caatinga biome.

The effect of endozoochorous dispersal on seed viability generally depends on morphological aspects [7], such as size, shape, permeability, seed coat thickness, or type of dormancy [8], as well as animal characteristics, such as body condition score, digestion type (ruminant or non-ruminant), and dietary preferences [9]. In addition, chewing intensity during the feeding process may also vary among different animals, affecting seed dispersal 3].

After seed ingestion, the passage through the digestive system, friction, contact with gastrointestinal juices, and seed morphological aspects, contribute to different effects on germination [10]. Most species of the Fabaceae family are physically dormant, and the percentage of seed germination increases significantly after being ingested by some animals [11].

Several naturally occurring species in the Caatinga, such as *Libidibia ferrea* (Mart. ex Tul.) L.P. Queiroz [12], and in tropical and subtropical zones, such as *Senna spectabilis* (DC) HS Irwin and RC Barneby [13], are cultivated as forage sources due to its well palatable fruits to small ruminants [14].

Thus, it is suggested that: (i) the percentages of seed recovery of the two forest species might differ after excretion by sheep and goats due to seed morphological aspects and structural/functional aspects of the digestive system of these animals, in addition to their distinct dietary patterns; (ii) Seed morphology and germination percentage are affected differently due to differences in the structure of the digestive system of goat and sheep such as, pH, and digestive enzymes.

Therefore, this study aimed to analyze the ingestion of *Libidibia ferrea* and *Senna spectabilis* seeds by goats and sheep and their effect on seed dormancy and morphophysiological traits.

MATERIAL AND METHODS

Seed collection and fruit characterization

The fruits of L. *ferrea* were randomly collected from a natural environment at the experimental farm Chã-do-Jardim, located at the Centro de Ciências Agrárias of the Universidade Federal da Paraíba (Brazil, 06° 58' 12" S and 35° 42' 15" W). The fruits of *S. spectabilis* were collected in a biome fragment of the Caatinga located in the municipality of Alagoa Grande – Paraíba (Brazil, 07° 08' 12.55" S 35° 36' 50.65" W). For each species, 10 matrices were selected based on good visible phytosanitary aspects, well-shaped canopies, and high fruit productivity, collected in August 2021. The fruits were sent to the Seed Analysis Laboratory (LAS), for processing. The seeds were placed in closed containers and stored in a dry, dark place at ±20 °C until the beginning of the experiment in November 2021. The seed moisture content was determined by the oven method at a temperature of 105 ± 3 ºC for 24 hours, with four replications of 50 seeds [15]. The seeds of each species were measured (length, width, and thickness) using digital calipers, also in four replications of 50 seeds, totaling a sample of 200 (intact seeds), with results expressed in millimeters. The thousand seed weight was obtained by weighing eight subsamples of 100 seeds on an analytical scale with a sensitivity of 0.001 g; the thousand seed weight was then calculated by multiplying the average weight obtained in the subsamples by 10 [16].

Seed recovery after intestinal passage

Eight adult goats (Moxotó) and eight adult sheep (Santa Inês), both weighing approximately 24 kg with similar ages, were housed in individual metabolic cages (1.5 × 1.5 × 0.5 m), in the Caprine Sector of the Animal Science Department, Universidade Federal da Paraíba, (Brazil, 618 m altitude, 05° 57' 48'' S and 35° 41' 30'' W). Four hundred (400) seeds of *L. ferrea* and *S. Spectabilis* were fed to the animals combined with the ration consisting of 39.05 kg of corn bran, 7.99 kg of soybean meal, 49.99 kg of Tifton grass hay, 1.79 kg of mineral supplement, and 0.40 kg of limestone. The seeds were amended with molasses and mixed with the ration to facilitate ingestion and were provided at once [16]. The animals, remained confined, and were fed with concentrated feed, equivalent to 3% of the body weight of each animal, divided into two times a day, one part in the morning (06:00 am) and the other in the afternoon (5:00 pm) based on their body weight to meet the nutrition requirement of the animals, according to NRC (2007). The feces were recovered from each animal every 24 hours after ingestion for a total period of 456 hours for *L. ferrea* seeds (0, 24, 48, 72, 96, 120, 144, 168, 192, 216, 240, 264, 288, 312, 336, 360, 384, 408, 432, and 456 hours) and up to 312 hours for *S. spectabilis* seeds (0, 24, 48, 72, 96, 120, 144, 168, 192, 216, 240, 264, 288, and 312 hours). For each animal and time interval, all feces were manually macerated on the collection day and placed in plastic trays in the caprine sector. The seeds of each forest species were counted separately and then sent to the Seed Analysis Laboratory (LAS) for germination and vigor tests.

Seed Germination after Intestinal Passage

The germination of seeds recovered after passage through the digestive system was compared with the germination of non-ingested seeds, used as controls. The seeds of each forest species consumed by the different animal species were removed at the same time intervals and homogenized for germination and vigor experiments. After digestion, the recovered seeds of *L. ferrea* were classified into 19 periods and those of *S. spectabilis* into 13 periods, along with the control treatment (non-ingested seeds), included for each species. All seeds were disinfested by immersion in a 1% sodium hypochlorite solution for 3 minutes and thoroughly rinsed with sterile distilled water (10 minutes) before setting up the germination experiments. Seeds recovered by forest species, animal, and time interval were placed to germinate on germitest® paper substrate moistened with distilled water at a ratio of 2.5 times its dry weight. After placement of seeds, the paper rolls were incubated in a Biological Oxygen Demand (B.O.D.) germination chamber for 20 days at 30 °C for *L. ferrea* seeds, and for 14 days at 25 °C for *S. spectabilis* seeds [17]. The evaluated variables were: (a) Recovery Rate; (b) Germination (G%) – (c) germination speed – determined by the Germination Speed Index (GSI), obtained from daily counts of seedlings with primary roots (>2 mm), and calculated according to the formula proposed by [18]; (d) Mean Germination Time (MGT), evaluated daily from root emergence, and calculated according to the formula proposed by [19]; (e) shoot and root length of seedlings, and (f) Dry Mass of shoots and roots of germinated seedlings (DM), [20].

Data Analysis

A completely randomized statistical design was used, with a 2 × 20 factorial scheme for *L. ferrea*, consisting of two animal species (goats and sheep) and 20 recovery periods after being ingested (0, 24, 48, 72, 96, 120, 144, 168, 192, 216, 240, 264, 288, 312, 336, 360, 384, 408, 432, and 456 hours). For *S. spectabilis*, a 2 × 14 factorial scheme was obtained, characterized by two animal species (goats and sheep) and 14 recovery periods after being ingested (0, 24, 48, 72, 96, 120, 144, 168, 192, 216, 240, 264, 288, and 312 hours). Data were subjected to the Shapiro-Wilk residual normality test (1% probability level). The numbers of recovered seeds were plotted in box plots, with the mean values of the variables subjected to Principal Component Analysis (PCA). Heatmaps were constructed using cluster analysis (UPGMA) with Gower distance. Subsequently, a simple Pearson correlation (rp) was performed between all variables at a 1% probability level by the Scott-Knott test ($p < 0.05\%$). All analyses were performed using the R statistical softwared [21].

RESULTS

Table 1 shows the morphological characteristics of *S. spectabilis* and *L. ferrea* seeds. *S. spectabilis* had a water content estimated in 10.5% while *L. ferrea* had 10.6%. For the external morphological characteristics (length, width, and thickness), *S. spectabilis* seeds measured 6.56 ± 0.43 mm in length, 4.11 ± 0.32 mm in width, and 1.60 ± 0.33 mm in thickness (Table 1). The average weight of a thousand seeds was 4.064 g.

Table 1. Morphological characteristics (mm), weight of a thousand seeds (g), and water content (%) of *S. spectabilis* and *L. ferrea seeds.* Values represent mean values (n=30-35 seeds).

Species	Len.	Wid.	Thi.	weight/10^3	WC
	---------- mm ----------			g	%
S. spectabilis	6,56±0,43	4,11±0,32	1,60±0,33	4,064	10,5
L. ferrea	7,58±0,55	4,79±0,16	3,33±0,12	12,071	10,6

Len = length; Wid= width; Thi = thickness; weight/10^3 = thousand seeds; WC = water content

The *L. ferrea* seeds, showed higher values in all physical aspects, with a length of 7.58 ± 0.55 mm, width of 4.79 ± 0.16 mm, and average thickness of 3.33 ± 0.12 mm and the average weight of a thousand seeds was 12.071.

No significant difference in the accumulated recovery rate of *L. ferrea* seeds collected after 456 h, with values of 2,008 and 1,682 seeds in goats and sheep, respectively as shown in the boxplots in Figures 1A.2 and 1B.2. For *S. spectabilis,* 1,019 seeds were recovered after 312 h from goats and 1,439 from sheep, with a significant difference in the recovery rate for each species in relation to the dispersing animals.

It is possible to observe that 5 out of 19 periods (48, 144, 168, 192, and 216 h) showed a significant difference in the number of *L. ferrea* recovered seeds between the animals (Figure 1A.1).

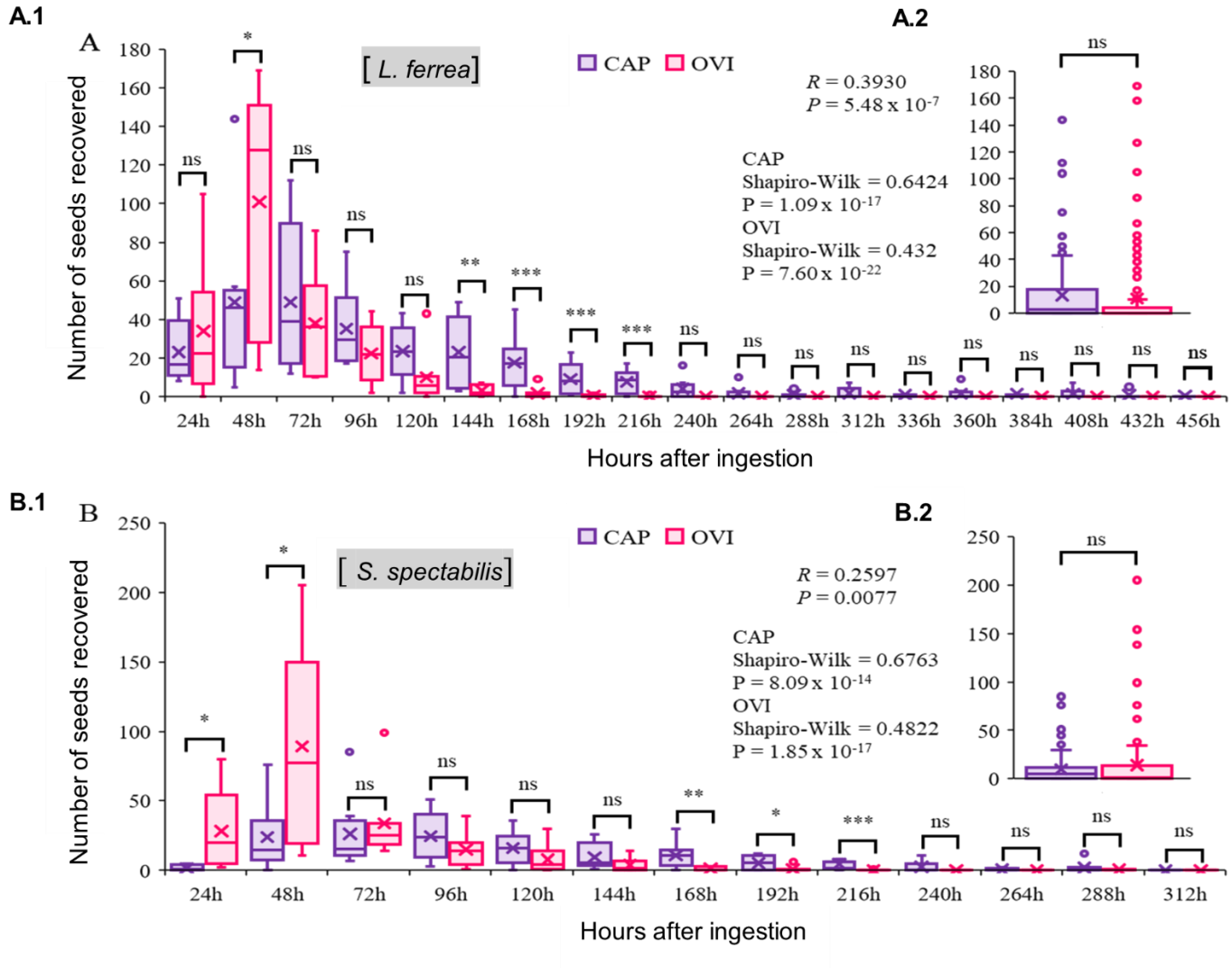


Figure 1. A. Number of *L. ferrea* seeds recovered based on different excretion periods (A.1) and cumulative recovery (A.2) after passage through the digestive system of goats and sheep. ns, *, **, ***, not significant, significant at 5%, 1%, and < 1%. B. Number of *S. spectabilis* seeds recovered based on different excretion periods (B.1) and cumulative recovery (B.2) after passage through the digestive system of goats and sheep. ns, *, **, ***, not significant, significant at 5%, 1%, and < 1%.

The Shapiro-Wilk test (1% probability level), showed a normal distribution of the recovery rate data of *L. ferrea* and *S. spectabilis* seeds after ingestion by goats and sheep (Figures 1A and 1B). It is also observed that the highest recovery rate occurred for *L. ferrea*, after 48 hours when the seeds were ingested by sheep (169 seeds), with an average mean trend of 101, and only one outlier point (144), justified by the higher daily recovery rate in goats. In the other periods where there was a difference in the recovery number between the animal species, higher values were observed in seeds recovered from goats in the periods of 144, 168, 192, and 216 h (52, 50, 25, and 20 seeds, respectively).

A significant difference was observed in 5 out of the 13 periods regarding the number of excreted seeds of *S.spectabilis* (Figure 1B.1). From that, the highest recovery rate of seeds was recovered after 24 and 48 hours after ingestion by sheep (80 and 205 seeds, respectively). In the remaining periods, there was a significant difference in the number of recovered seeds in the periods of 168, 192, and 216 h after being ingested by goats, with an average recovery rate of 11; 6, and 5 seeds/period, respectively, superior to that observed in sheep.

The mean clustering analysis among the treatments(periods/animals), revealed a formation of groups with similar seed viable and vigor characteristics, which are visualized in heatmaps and correlation maps (Figure 2.A and Figure 3A).

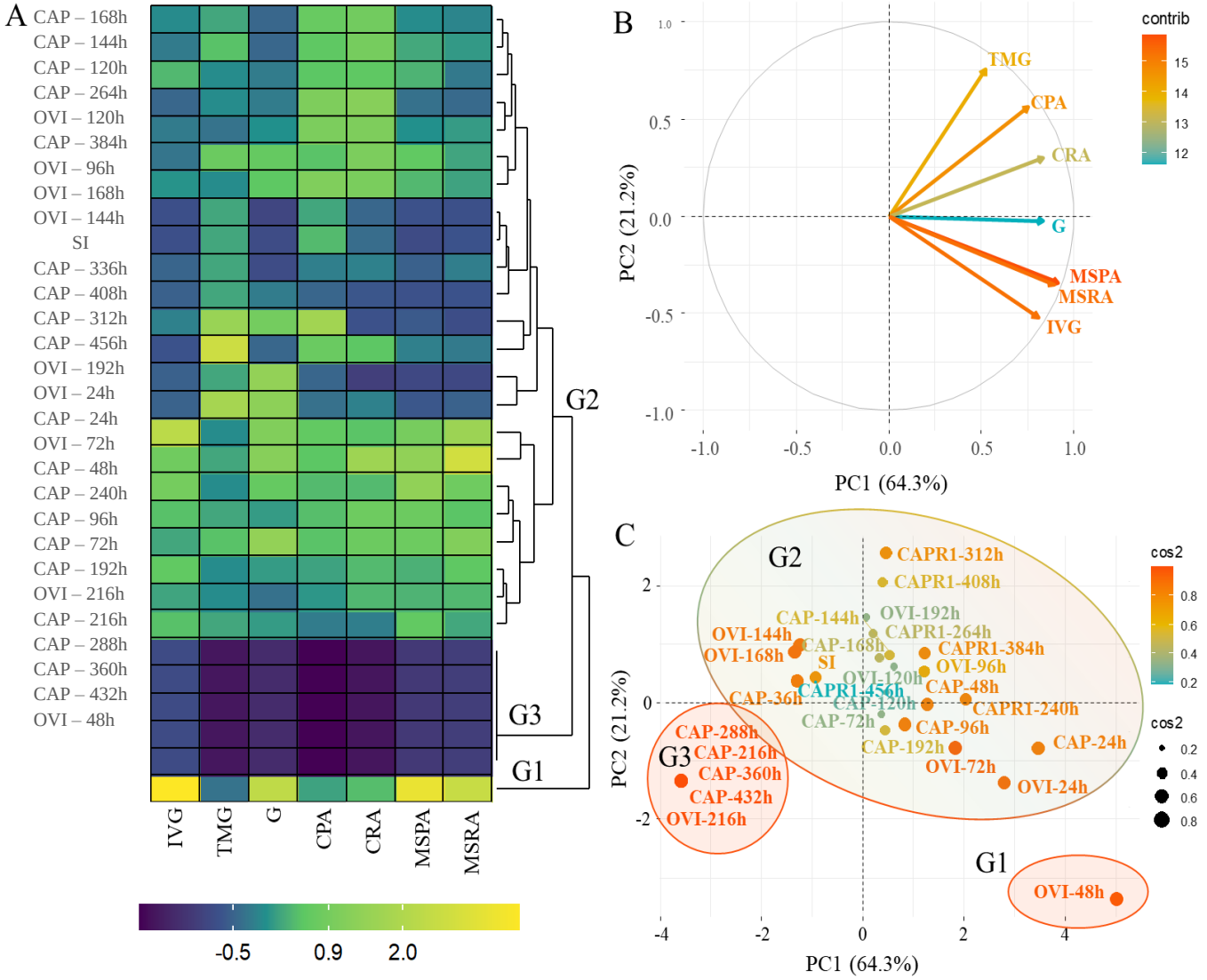


Figure 2. Heatmap showing the relationship between treatments (periods/animals) and the variables analyzed in L. ferrea seeds (A), analysis of variable contribution (B), and principal component analysis (PCA). Germination speed index (GSI), mean germination time (MGT), germination (G), shoot length (SL), root length (RL), shoot dry mass (SDM), and root dry mass (RDM). GO- Goat; SHE - Sheep.

For the *L. ferrea* seeds, three groups were formed: Group I, represented by only one treatment (SHE- 48 h), which contained seeds with a higher percentage of viability and vigor, differing from the other treatments. Group II, comprised of treatments: GO- 168; GO- 144; GO- 120; GO- 264; SHE- 96; SHE- 168; SHE- 144; SI (control); GO- 336; GO- 408; GO- 312; GO- 456; SHE- 192; SHE- 24; GO- 24; SHE- 72; GO- 48; GO- 240; GO- 96; GO - 72; GO - 192 h), composing an intermediate group. Group III, was composed of treatments: SHE- 216, GO - 216, GO- 288, GO- 360, and GO - 432 h, containing low values of vigor and viability components (Figure 2A).

Data obtained from the *L. ferrea* seeds under the different treatments were used for the multivariate analysis of principal components (Figure 2B and 2C). The two components (PC1 and PC2) show a variability of 85.5%, allowing the explanation of the effect of treatments on the two components.

Germination stands out with a lower contribution (12%) in the SHE - 120, GO - 120, SHE - 192, and GO - 456 h treatments compared to the other variables used. Vigor, dry mass, and germination speed index had the most contribution (total of 45%), presented in groups 1 and 3, and some from group 2, including SHE - 24, SHE - 48, SHE - 96, SHE - 240, SHE - 312, SHE - 360, SHE - 384, GO - 24, GO - 72, GO - 144, and GO - 168, better explaining the results obtained in the study (Figure 2B).

In the case of *S. spectabilis* seeds, three distinct groups were observed in terms of formation. Group I consists of the treatments SHE - 24, SHE - 48, and GO - 48 h, which showed higher percentages of viability (33, 28, and 35%) and vigor, with higher dry mass performance (0.0433, 0.0279, and 0.0309 g) respectively, compared to the other treatments and intact seeds (16% and 0.0210 g). Group II encompasses the treatments SI (control), SHE - 120, GO - 96, SHE - 96, GO - 120, GO - 144, GO - 72, and GO - 168 h, revealing intermediate levels of viability and vigor. Finally, Group III consists of the treatments SHE - 144, SHE - 72, GO - 24, GO - 216, GO - 192, GO - 240, SHE - 168, GO - 264, and GO - 288 h, which presented lower values in viability, vigor, and performance for these components (Figure 3A).

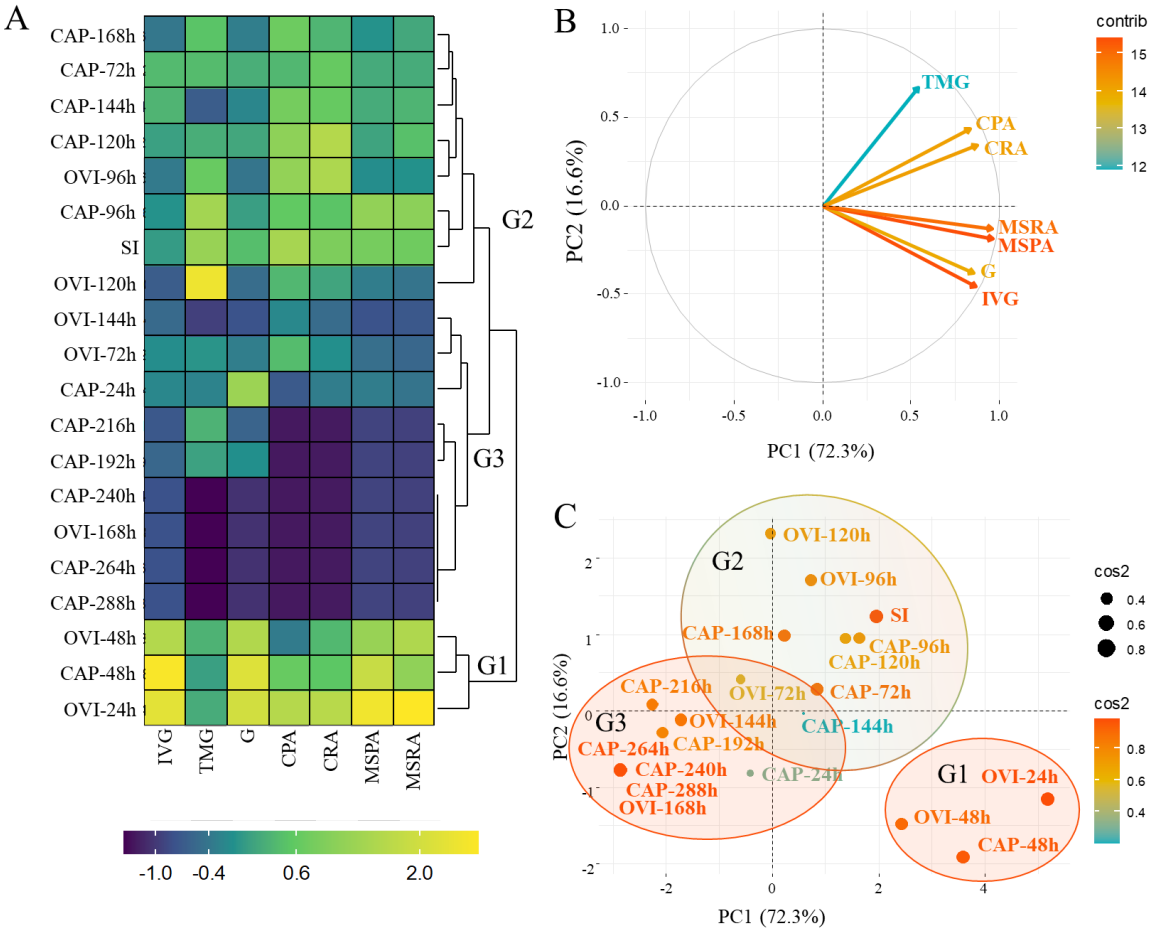


Figure 3. Heatmap showing the relationship between treatments (periods/animals) and the variables analyzed in *S. spectabilis* seeds. Analysis of variable contributions(A) and principal component analysis (PCA)(B). Germination speed index (GSI), mean germination time (MGT), germination (G), shoot length (SL), root length (RL), shoot dry mass (SDM), root dry mass(RDM). GO- Goat; SHE – Sheep.

For the *S. spectabilis* multivariate analysis of principal components (Figures 3B and 3C), two principal components were formed, PC1 and PC2, which together explained 88.9% of the total variability in the data. The mean germination time (TMG) had the lowest contribution (12%) compared to the other variables, while the germination rate showed an intermediate contribution (13.5%), suggesting that this variable may have a relevant contribution (Figure 3B). The dry mass and germination speed index stood out as the most contributing variable factors in the analysis, with a significantly higher contribution (45%) to better explain the effect of the passage of seeds through the digestive system of sheep and goats (Figure 3B).

DISCUSSION

In our study, we showed that *S. spectabilis* and *L. ferrea* require different periods to pass through the digestive system. These results are mainly attributed to their morphological characteristics. The *S. spectabilis* seeds have smaller weight, length, width, and thickness compared to *L. ferrea* seeds (Table 1), which allowed faster digestion by the animals, particularly in sheep.

The passage of seeds through the digestive system of small ruminants is dependent on morphological characteristics, such as small seed size (<3 mm), spherical shape, and weight [22, 23]. This was also reported for *Mimosa tenuiflora* by Nóbrega and coauthors [24], where smaller seeds passed faster through the digestive system of goats and sheep. Differently, [25]. stated that seeds of *Prunus spinosa* L. are more efficiently dispersed by goats due to their large size and the resistance of the seed coat during passage through the digestive system.

The faster digestion of seeds by sheep, in terms of flow kinetics, indicates shorter retention times in the rumen compared to goats [26], thus allowing faster food passage, contributing to the dispersal process of viable seeds after ingestion.

Differences in recovery rates between ruminant species are supposedly influenced by the morphology of their teeth, the type of digestive system, and the chemical properties of their intestines [27]. These factors may lead to the opening of new fissures in the outer layer of the seed coat and, in some cases, the disintegration of denser layers of the seed coat during contact with the chemical components present in the digestive system.

The number of seeds recovered after passage through the digestive system is also attributed to some physical and morphological factors of the seeds, such as hardness and length [23]. Seeds longer than 4 mm tend to be recovered from the feces of goats with damage, and over longer periods, which can affect the germination rate by possibly damaging the embryo, causing abnormalities in seedlings, in some cases resulting in seed death [28].

Seeds of *S. spectabilis* and *L. ferrea* were recovered in higher quantities, i.e., occurring mainly between 24 and 48 hours after ingestion, especially from sheep. These results are similar to previous reports with seeds of Fabaceae species, such as *Prosopis juliflora* (Sw.) DC, in which the maximum recovery occurred on the first day after ingestion by goats [29, 30].

Although a higher seed recovery rate was observed from sheep shortly after ingestion, our results demonstrated that goats can contribute more efficiently to the perpetuation of the studied species by distributing seeds over long periods and larger territorial areas, helping in the maintenance of environments with seasonal rainfall (Figure 1).

In addition to common responses, passage through the digestive system of goats and sheep exerted different effects on the forest species. The seeds of *L. ferrea* have a very thick palisade layer, composed of lignin, suberin, and cutin, which contributes to a denser coating of the membranes, thereby preventing water entry and consequently the resumption of metabolic processes for embryonic [31]. These structures protect and prepare the seeds for dispersal through endozoochory [32]. The passage through the digestive system of goats and sheep effectively contributed positively to the dry mass of the shoots and roots and the germination speed index. In addition, the effects of the seed ingestion were also reflected in the viability of *L. ferrea* seeds, which, although attenuated, resulted in higher percentages of the variables evaluated compared to intact seeds (SI).

Previous studies proposed an interaction between physical dormancy and animal dispersal [33]. In the present study, the animal ingestion of *L. ferrea* and *S. spectabilis* seeds improved the germination compared to intact seeds (control), consistent with other studies aimed to improve germination through dormancy overcoming methods [34].

The ability of *S. spectabilis* and *L. ferrea* seeds to survive the passage through the digestive system of sheep and goats, associated with an improvement in seed germination, suggests the significant importance of these small ruminants in dormancy overcoming processes and dispersal of these species, which occur naturally in dry tropical forests along grazing routes [35].

The evaluation of physiological aspects in seeds after passage through the digestive system of small ruminants can significantly contribute to the recovery of degraded areas and contribute to the perpetuation of some plant species, especially in areas with grazing activity.

Understanding the relationships between animals and plants is necessary to mitigate the negative effects caused by anthropic activities in the environment. Studies elucidating the role of small ruminants in seed dispersal can help in the recovery of degraded areas and contribute to the perpetuation of endemic species.

Field experiments are essential to confirm the effects of passage through the digestive system of goats and sheep on seeds with tegument hardness in addition to the different performances of these animals.

CONCLUSIONS

The seed dormancy of *L. ferrea* and *S. spectabilis* is overcome when passed through the digestive system of goats and sheep, consequently improving physiological parameters compared to non-ingested seeds.

The recovery rate of *L. ferrea* and *S. spectabilis* seeds was higher in sheep than in goats, in the first hours after ingestion.

In goats, the recovery occurred slowly and over longer periods, which can potentially favor the perpetuation of the species within the habitat.

Sheep and goats grazing in the Caatinga may contribute to the dispersal of plant species such as *L. ferrea* and *S. spectabilis* when ingested and excreted in the animal feces.

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