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Research Article

Initial growth of fabaceae seedlings in goat and sheep feces

Crescimento inicial de plântulas de fabáceas nas fezes de caprinos e ovinos

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ABSTRACT

The objective of this study was to evaluate the emergence of Fabaceae seedlings: butterfly-pea, puero and Campo Grande stylosanthes in goats and sheep feces in order to check if the stools allow the initial development of these plants; and the effects of the passage through digestive tract. For this, 50 g of each Fabaceae seed were offered mixed with concentrated to nine sheep and nine goats, both with mean body weight of 40 kg. Animal feces were collected at six hours intervals up to 72 hours after the ingestion of the seeds, and taken immediately to the greenhouse after each collection. It was evaluated the total number of seedlings emerged in the feces and the emergence rate index (GSI) for thirty days after ingestion of the seeds. It was used a completely randomized design in a factorial scheme: 3 Fabaceae x 12 collection times, with three replicates (animals). The best performance for total number of seedlings germinated in goats and sheep feces was obtained by puero, followed by stylosanthes. Butterfly-pea presented poor results due to high degradation of seeds by mastication. Goats and sheep feces do not prevent seedling emergence for the species assessed, so these animals can be considered true seed dispersers of these plants in pastures.
KEYWORDS: Dispersion. Emergency. Fecal plate.

RESUMO

Objetivou-se com este estudo avaliar a emergência de plântulas de Fabaceae: cunhã, kudzu tropical e estilosantes Campo Grande nas fezes de caprinos e ovinos, a fim de verificar se as fezes permitem o desenvolvimento inicial dessas plantas; e os efeitos da passagem pelo trato digestório. Para isso foram oferecidos 50 g de sementes de cada fabácea misturados ao concentrado a nove cabritos e nove cordeiros, ambos com peso corporal médio de 40 kg. As fezes dos animais foram coletadas em intervalos de seis horas após a ingestão das sementes, até completar 72 horas, sendo as mesmas levadas para a casa de vegetação imediatamente após cada coleta. Foram avaliados o número total de plântulas emergidas nas fezes e o índice de velocidade de emergência (IVE) durante trinta dias, após a ingestão das sementes. Foi utilizado delineamento inteiramente ao acaso, em esquema fatorial: 3 fabáceas x 12 períodos de coleta, com três repetições (animais). O melhor desempenho quanto ao número total de plântulas germinadas nas fezes de caprinos e ovinos foi obtido pelo kudzu, seguido pelo estilosantes. A cunhã apresentou baixos resultados devido à alta degradação das sementes pela mastigação. As fezes de caprinos e ovinos não impedem a emergência das plântulas das espécies avaliadas, logo estes animais podem ser considerados legítimos dispersores destas sementes nas pastagens.
PALAVRAS-CHAVE: Dispersão. Emergência. Placa fecal.

INTRODUCTION

The inclusion of fabaceae in exclusive poaceae pastures has often increased the system's productivity. Forage fabaceae can be used in conjunction with poaceae, representing an interesting source of food from a nutritional point of view (as they have a high protein content and good digestibility) and strategic point of view (to reserve green food in the dry season of the year, due to their root system be deeper). Other advantages of using fabaceae are: nitrogen fixation for poaceae in intercropped systems, recycling of nutrients, and recovery of pastures or degraded areas (SEIFFERT & THIAGO 1983, FREITAS et al. 2003, SPÓSITO et al. 2018, OLIVEIRA ABRANCHES et al. 2021).

One of the main obstacles to the adoption of intercropped pastures between poaceae and fabaceae, where the former is already established, is the cost of implementing these into the exploitation system (DEMINICIS 2009a). However, herbivorous animals can alter pasture heterogeneity naturally during grazing, through seed dispersal. For this dispersion to occur, the seeds pass through the animal's digestive tract (DT) and are eliminated in the feces (endozoochory); this being the main form of seed dispersal (BRUUN & POSCHLOD 2006, PETERSEN & BRUUN 2019).

After animals ingest the seeds, damage occurs through chewing and digestion. The percentage of damaged seeds is related to their hardness and shape, the animal species, the quality of the diet consumed and the time spent in the DT (TJELELE et al. 2014). In addition to factors related to passage through DT, seed germination in feces can be influenced by fecal fermentation, high contamination by fungi and bacteria, positioning of seeds in the fecal cake and dehydration of the fecal cake (DEMINICIS et al. 2009). Likewise, seed germination and initial seedling establishment can be inhibited due to the toxicity (nitrogenous compounds) of fecal plaque (TRAVESET et al. 2001, BRAZ et al. 2002, DÍAZ-CHUQUIZUTA et al. 2017). On the other hand, feces can provide nutrients to the seedlings that emerge from them, promoting plant growth and establishment (STILES 1992).

The genus Stylosanthes is native to Central and South America, and its cultivation has expanded to subtropical America and Australia. Most species are perennial, with deep roots, drought-tolerant, and adapted to poor soils, forming symbiotic relationships with nitrogen-fixing bacteria, making them ideal for tropical conditions. Stylosanthes species are important forage legumes in tropical and subtropical regions due to their economic significance (MEDEIROS & FLORES 2014). *Clitoria ternatea* is a tropical perennial forage legume with deep roots, cultivated as a protein-rich forage crop well-suited to hot, semi-arid climates. It propagates via seeds, is drought-tolerant, and can thrive in areas with as little as 380 mm of annual rainfall (DE ALMEIDA et al. 2024). *Pueraria phaseoloides* is a species native to Malaysia and Indonesia, and is now widely distributed throughout the humid tropics, particularly in the Amazon. In this region, *P. phaseoloides* stands out as a valuable forage crop due to its high nutritional value, drought resistance, and nitrogen-fixing capability (CABRAL & SOUZA 2017).

Considering that the dispersal of forage seeds by ruminants can be used for the dynamics of fabaceae populations in consortium with poaceae, the aim of this work was to evaluate the initial growth of seedlings of butterfly pea, Campo Grande

stylosanthes and tropical kudzu in goat feces and sheep.

MATERIALS AND METHODS

The experiment was conducted in the goat farming sector of the Animal Science Research Support of State Agricultural Technical School Antonio Sarlo - ETEAAS/FAETEC, and in a greenhouse at the Research Support Unit, both belonging to the North Fluminense State University – Darcy Ribeiro, Campos dos Goytacazes County, Rio de Janeiro State, Brazil. To carry out the experiment, was used seeds of butterfly pea (*Clitorea ternatea*), Campo Grande Stylosanthes (*Stylosanthes capitata* and *Stylosanthes macrocephala*) and tropical kudzu (*Pueraria phaseoloides*), whose batches were previously analyzed regarding germination, according to the recommendations of the Rules for Seed Analysis (RAS) (BRASIL 2009).

For the study, nine kids of the Saanen and alpine brown breeds and nine crossbred lambs (Santa Inês x Dorper) were used, all castrated and with an average weight of 40 kg. The goats were housed in individual stalls, and to allow the collection of feces, they used collection bags attached to the body by “collars”. There was an adaptation period of seven days for both individual accommodation and the use of collection bags. The sheep were placed individually in metabolic cages, which have trays at the bottom, allowing the accumulation of feces. They also went through a seven-day adaptation period. Feeding was carried out individually, following a forage:concentrate ratio of 60:40, with 700g.day^{-1} of concentrate based on corn and soybean bran and 4kg.day^{-1} of chopped elephant grass daily (quantities divided into two treatments and water ad libitum. The grass was managed at a height of 1.20 m. The diet was balanced according to NRC (2007) for a gain of 150g per day.

Fifty grams of seeds from each fabaceae were mixed with 350g of concentrate and fed to the animals, once, in the morning (6am) on the first day of the experiment. This quantity corresponds to approximately 1,000 seeds of butterfly pea, 17,500 of stylosanthes and 4,850 of tropical kudzu (BRASIL 2009), all fresh (without scarification).

Feces were collected up to 72 hours after seed ingestion, at intervals of 6 hours (6, 12, 18, 24, 30, 36, 42, 48, 54, 60, 66 and 72 hours). The 6-hour waste was discarded, as preliminary tests did not reveal the presence of seeds during this period. For the control test, four repetitions were carried out with 100 seeds of each species placed directly in the sand, inside trays, without passing through the animals' DT.

After being collected, the feces were placed in identified plastic bags and taken to the greenhouse, where they were placed in rectangular plastic trays (36 cm long, 23 cm wide and 6 cm high), identified and perforated to allow drainage of water. These trays were filled with approximately 3 kg of washed and sieved sand. The feces were deposited in the trays according to the collection times, where they remained for 30 days. During the entire experimental period, feces were watered at least twice a day. The average temperature and relative humidity in the greenhouse during the experimental period were, respectively, 22.75 ºC and 71.95%.

The emerged seedlings were counted daily, and at the end of 30 days they were removed and weighed whole (aerial part + root), obtaining the natural and dry matter values. After being removed from the feces, the seedlings were washed in running

water and dried on sheets of absorbent paper to obtain the weight of natural matter. For dry matter weight, the seedlings were placed in a ventilated greenhouse at 70 ºC for 72 hours. The emergency speed index (GSI) was also evaluated through daily counting, with the calculation being made using the adaptation of Maguire's formula (MAGUIRE 1962).

The experimental design used was completely randomized, in a 3 x 12 factorial scheme (fabaceae x passage times), with 3 replications (animals). The results were subjected to analysis of variance by SAS (2009). The Scott-Knott test was used to group the means, at 5% significance, using the GENES program (CRUZ 2006). An individual analysis of variance was performed on the data to verify the homogeneity of variance (Cochran's test), determining whether or not the transformation of the residual degrees of freedom was required for the combined analysis. An analysis of variance (5%) was performed for the results of the total number of seedlings emerged in goat feces as a function of passage time through the digestive tract (h).

RESULTS

The analysis of variance of the total number of seedlings emerged in goat and sheep feces indicated significance for fabaceae, collection times and F*T interaction (P<0.01) (Tables 1 and 2).

Table 1. Analysis of variance of the total number of seedlings (TNS), Germination speed index (GSI), Natural matter weights (NMW) and dry matter weights (DMW) of seedlings emerged in goat feces.

Sources of Variation	DF	MS			
		TNS	GSI	NMW (mg)	DMW (mg)
Fabaceae(F)	2	43168,03**	359,86**	484815,59^{ns}	9764,18^{ns}
Time(T)	11	13013,12**	166,94**	241355,10^{ns}	5942,00^{ns}
F*T	22	5823,69**	52,20**	169042,50^{ns}	4740,36^{ns}
Residue	72	511,52	7,46	75835,72	1733,02
Average		34,61	3,98	159,48	24,89
CV(%)		65,35	68,67	172,67	167,25

*Significant at 5% by F test; **Significant at 1% by F test; ns = not significant.

Table 2. Analysis of variance of the total number of seedlings (TNS), Germination speed index (GSI), Natural matter weights (NMW) and dry matter weights (DMW) of seedlings emerged in sheep feces.

Sources of Variation	DF	MS			
		TNS	GSI	NMW (mg)	DMW (mg)
Fabaceae(F)	2	43168,03**	359,86**	528998,31**	14074,17*
Time(T)	11	13013,12**	166,94**	193232,90**	6144,32*
F*T	22	5823,69**	52,20**	118393,93*	4508,17*
Residue	72	511,53	7,46	41083,05	1563,66
Average		34,61	3,98	157,55	26,35
CV(%)		65,35	68,67	128,65	150,15

*Significant at 5% by F test; **Significant at 1% by F test; ns = not significant.

When compared to other species, kudzu showed a greater number of seedlings in goat feces in the periods of 24, 36 and 72 hours after ingesting the seeds. Adding up the seedlings emerged between 12 and 72 hours of passage, kudzu showed the best result (493 seedlings), followed by stylosanthes (255 seedlings) (Figure 1). The analysis of variance did not show significance for the study of the total number of seedlings emerged in goat feces as a function of passage time through the digestive tract (h).

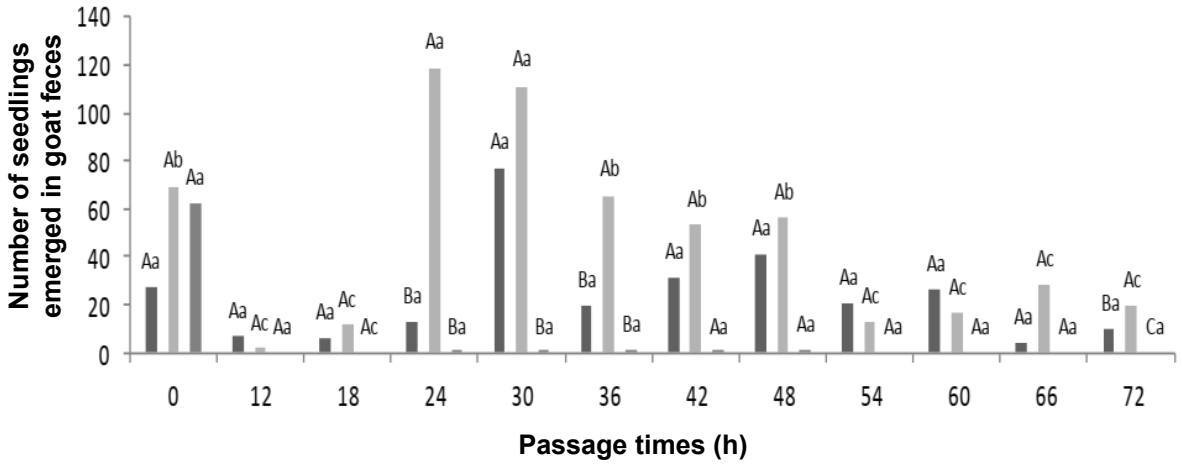

Figure 1. Total number of stylosanthes, kudzu and butterfly pea seedlings emerged in goat feces, as a function of passage time through the digestive tract (h). *At each time point, the columns correspond respectively to Stylosanthes, kudzu, and butterfly pea.

Among the fabaceae plants, there was a significant difference in the total number of seedlings in sheep feces between periods 0, 18 and 36 hours of dispersal, with the number of kudzu seedlings being significantly higher compared to the other species at times 18 and 36 hours (Figure 2). The analysis of variance did not show significance for the study of the total number of seedlings emerged in seep feces as a function of passage time through the digestive tract (h).

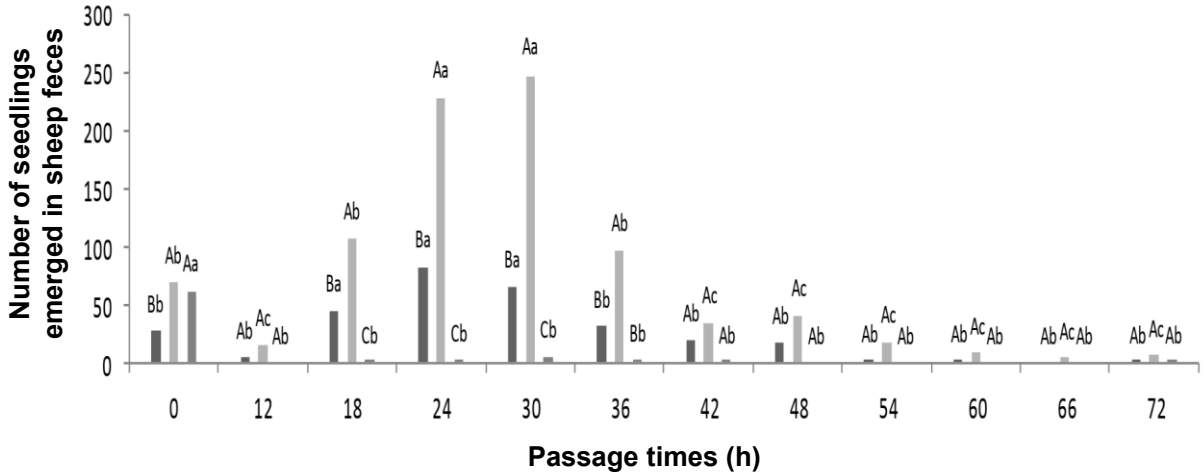

Figure 2. Total number of stylosanthes, kudzu and butterfly pea seedlings emerged in sheep feces, as a function of passage time through the digestive tract (h).
*At each time point, the columns correspond respectively to Stylosanthes, kudzu, and butterfly pea.

DISCUSSION

In the test with goats, there was an effect of passage times on the number of seedlings emerged in feces only for kudzu seeds, with the highest results being obtained in periods of 24 and 30 hours after ingestion of the seeds. Homogeneity of variance was confirmed using Cochran's method, allowing for the use of analysis of variance. The ratio between the largest and smallest residual mean square was 6.95, which did not require adjusting the residual degrees of freedom for conducting the combined analysis of the experiments (PIMENTEL-GOMES 2009).

In a study of the dispersion of fabaceae seeds in the feces of cattle in the field, SILVA (2008) also found a greater number of kudzu seedlings (146) when compared to macrotyloma, calopogonium and Stylosanthes. However, the results with cattle were lower than those observed with goats. These results may be related to the efficiency of breaking dormancy and degradation caused by passage through the DT of cattle.

For the emergence of stylosanthes seedlings, there was no significant effect between dispersal times. However, there was a higher average number of stylosanthes seedlings within 30 hours after ingestion of the seeds (Figure 1). The results found in the present study, regarding the number of seedlings germinated in goat feces, were higher than those found by VALENTE et al. (2016) and by DEMINICIS (2009b) found an average of 23 stylosanthes plantlets in the feces in bovine fecal plaques and in the present study an average of 3 plants were observed in goat feces. This author attributed the low percentage of germination of stylosanthes seedlings to the high degradability that occurred during the passage through the cattle DT.

The number of wedge seedlings did not differ statistically between passage times (Figure 1). These results suggest that these seeds were degraded by chewing and/or by the digestion processes that occurred during their passage through the DT of goats. In data not yet published (SILVA et al. 2019), there was a 50% emergence of wedge seedlings when the seeds were incubated “in situ” between 6 and 24 hours, eliminating damage from chewing. Based on these results, it is possible to infer that chewing is mainly responsible for the degradation of butterfly pea seeds, possibly due to their larger size.

DEMINICIS (2009b), when studying the germination of fabaceae seeds in bovine fecal plaques, observed an average of 48 butterfly pea seedlings between 12 and 30 hours after ingesting the seeds. These results are superior to those found in this work, where in the same period, between 12 and 30 hours after ingestion of butterfly pea seeds, there was an average emergence of 0.2 seedlings in goat feces. These results highlight the differences between goats and cattle in terms of the degradation of the food consumed. In a study on the chewing efficiency of herbivores, FRITZ et al. (2009) and CLAUSS et al. (2023) reported that the size of the particle to be ingested must be proportional to the animal's body mass, thus, goats have a greater chewing intensity when compared to cattle. In this case, it is assumed that the greater intensity of chewing observed in goats is the preponderant factor in reducing the number of wedge seedlings emerging in the feces of these animals.

In the test with sheep, there was an effect of passage time on the number of seedlings emerged in feces for the three species evaluated. For Stylosanthes, the highest number of seedlings in feces occurred at passage times of 18, 24 and 30 hours.

For kudzu, the highest number of seedlings occurred in the periods of 24 and 30 hours after ingestion of the seeds. For butterfly pea, the control treatment was significantly greater than the passage times in terms of the number of seedlings in sheep feces.

In general, kudzu presented the highest number of seedlings in sheep feces, totaling 809 seedlings between 6 and 72 hours after ingestion of the seeds, followed by stylosanthes and butterfly pea, with 268 and 11 seedlings, respectively (Figure 2). These results suggest that kudzu seeds are less susceptible to damage caused by chewing and passing through the DT of sheep than stylosanthes and butterfly pea seeds. This greater emergence of kudzu seedlings in feces may be related to the shape and texture of the seed coat. According to SIMÃO NETO et al. (1987) shorter seeds are less damaged when passing through the DT, just as spherical seeds with a smooth seed coat make damage during chewing more difficult.

The ingestion and passage through the DT of sheep reduced the vigor of the butterfly pea seeds, presenting less developed seedlings, that is, of lower weight, when compared to the seedlings originating from seeds that did not pass through the DT (control). The weights of natural matter in the passage times were always lower than in the control for the butterfly pea seedlings, while the weight of dry matter in the 36-hour period was statistically equal to the control. These seeds are probably more degraded due to their larger size, which induces greater chewing intensity (FRITZ et al. 2009). Furthermore, large seeds are more prone to damage caused by chewing, as a small crack in the seed coat is enough to expose them to attack by ruminal microorganisms and other effects of digestion (SIMÃO NETO et al. 1987).

The results obtained with goats and sheep indicate that the animals' passage through the DT did not reduce the vigor of the seeds, as the seedlings in the passage periods showed the same development as the seedlings originated from seeds that did not pass through the DT (control).

From the results of number of seedlings and weight of natural matter, it was possible to verify that goat and sheep feces did not prevent the emergence of the fabaceae studied. These results are in line with those found by RAMOS et al. (2006) who stated that the growth of seedlings was not blocked by being surrounded by sheep feces, on the contrary, feces can give greater vigor to plants due to the release of nutrients (TRAVESET et al. 2001).

Contradictorily, in a study on the dispersal of calopogonium, pigeon pea and leucaena seeds, NAKAO & CARDOSO (2010) stated that bovine feces should not constitute a favorable medium for the initial growth of seedlings, given the low or no germination in this medium. , which may have proved toxic to seedlings, mainly due to variations in pH and nitrogenous compounds caused by microbial activity. These authors believe that the length of time the seeds remain in the feces is directly related to their mortality (JANZEN 1984, SRIDHARA et al. 2016), in addition to the total dehydration of the fecal plate in two days, under the conditions of the experiment (BRAZ et al. 2002).

Despite this, in natural field conditions, trampling, rain, and especially the action of coprophagous beetles can modify the substrate and create a microenvironment favorable to the germination of seeds and growth of seedlings deposited in feces, so

that they do not come to act, as a limiting factor for dispersion (NAKAO & CARDOSO 2010).

CONCLUSION

Kudzu showed a greater number of seedlings emerged in the feces of goats and sheep, and a higher GSI. The period from 24 to 30 hours after ingestion of kudzu seeds showed greater seedling emergence. The highest GSI of kudzu seedlings occurred at 24, 30 and 36 hours in goat feces and at 24 and 30 hours in sheep feces. Passage through the Digestive Tract of goats and sheep did not reduce the vigor of the seeds of the fabaceae evaluated, except for Clitorea. Goat and sheep feces do not impede the initial growth of seedlings.

AUTHOR CONTRIBUTIONS

All authors participated in every stage of the study, and all authors have read and agreed to the published version of the manuscript.

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DATA AVAILABILITY STATEMENT

The data can be made available upon request.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analysis or interpretation of data; in writing the manuscript and deciding to publish the results.

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