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Experimental assessment of endozoochorous dispersal of *Prosopis flexuosa* seeds by domestic ungulates.

Running title: Endozoochory by domestic ungulates

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Abstract

Question: Spatial and temporal dynamics of plant communities in various ecosystems are shaped by the movements of seeds expressed as different dispersal modes. In desert rangeland, many plants produce relatively large fruits that are limited in their long-distance dispersal abilities and, therefore, depend on transport inside animals, a process termed endozoochory. Evaluating the effectiveness of this process from experimental data is crucial for assessing the potential of domestic ungulates as effective long-distance seed dispersers and, consequently, as management and restoration tools in degraded rangelands.

Methods: In this study we jointly estimated recovery, gut retention time (RT) and germination probability of *Prosopis flexuosa* seeds (a tree species of Monte desert in Argentina) transported by goats, horses and cattle (N=4) through a seed feeding experiment. Also, in horses and cattle, we used plastic particles to distinguish between seed loss due to mastication and due to degradation in the gut.

Results: Results showed higher seed recovery in horses (26%) than in cattle (3%) and goats (5%), and different values of RT and germination of seeds among species (in decreasing order, RT was: cattle>goats>horses; and germination was: goat>horses>cattle=control seeds).

Conclusions: We concluded that the quality of seed treatment by horses is better than by the other species, because of the high seed recovery and high germination compared to control seeds. To get a complete picture of this mutualistic interaction, future studies could inquire about the physical and chemical properties of faeces as substrate and the environmental conditions of sites where seeds are deposited.

Keywords: seed feeding experiment, endozoochory, germination, gut retention time, seed recovery, ingestive and rumination chewing, *Prosopis flexuosa*, desert rangeland

Introduction

Plant regeneration comprises all plant life stages from seeds to seedlings and adult plants (Wang et al., 2002). The transition between these stages is facilitated by multiple ecological processes including pollination, seed dispersal and recruitment (Wang et al., 2002). Among these processes, seed dispersal is one of the most sensitive to human disturbances and has become an important issue in plant ecology and anthropogenic landscape management (Neuschulz et al., 2016).

In particular, endozoochory by large herbivores may allow seeds to travel several kilometres away from the parent plant, favouring connectivity among distant populations within metapopulations or colonization of unoccupied suitable patches (Cousens et al., 2010; Baltzinger et al., 2019). Domestic and wild ungulates with large home ranges, high travel speed, large gut capacity and long seed retention time are good examples of this (Pakeman, 2001; Cousens et al., 2010). In fact, some livestock species have been considered dynamic “ecological corridors” and potential tools for restoration of habitats degraded by fragmentation processes (Cosyns et al., 2005). However, it is also widely recognized, in this plant-animal mutualism that animal species vary in the benefits they provide to plants, such that livestock species can be positioned on an effectiveness gradient ranging from very effective long-distance seed dispersers to seed predators (Schupp et al., 2017).

The seed dispersal effectiveness (SDE) concept defined by Schupp (1993) and Schupp et al. (2010) affords a conceptual framework for comparing different livestock species as potential long-distance plant dispersers in extensive livestock farming systems. According to this framework, fluctuations in SDE can be explained in relation to multiple factors grouped into two components, quantitative and qualitative ones. The former relates to how many seeds are loaded by the vector (emigration phase), the latter to what is the probability of a loaded seed becoming an adult plant (transfer and immigration phases). In the case of endozoochory, the qualitative component combines quality of the treatment exerted by the vector (mouth and gut), quality of the seed deposition substrate (dung) and quality of the deposition site.

Seed survival in the digestive system is one of the main determinants of successful endozoochorous dispersal. Both the gut environment which seeds are exposed to and its consequences for seed survival and germination are products of the interaction among diet composition, seed traits and animal traits (Traveset, 1998). The quali-quantitative characteristics

of diet in free-ranging domestic ungulates result from the interplay between their feeding strategies, and the quality and quantity of available food (Egea et al., 2014). For seeds, this means variations in scarification level and time spent in the digestive tract (retention time), and hence variation in their germination success (Baltzinger et al., 2019). Scarification level and retention time of seeds also depend on seed traits such as size, shape, specific gravity, permeability or thickness of the seed coat (Pakeman et al., 2002; Smýkal et al., 2014; Albert et al., 2015), and on animal traits such as body size or morphophysiological specializations of the digestive system that are expressed as different digestion modes (ruminant or not) and feeding preferences (browser and grazers) (Hofmann, 1989).

Among digestive system specializations, selective particle size retention, and ingestive and rumination chewing are key factors for understanding how the flow and pooling of ingesta in the digestive tract affect the fate and survival of seeds. Considering these digestive physiological features, seeds are faced with two very contrasting scenarios. On the one hand, Clauss et al. (2009) showed that, in ruminant species, small food particles (including small seeds) are passed to the lower digestive tract, whereas larger particles (including large seeds) are selectively retained and then regurgitated to be re-chewed (rumination mechanism). On the other hand, in non-ruminant species, digestive tracts do not contain filtering mechanisms that promote retention of coarse particles, in fact selective expulsion of larger particles has been interpreted as a strategy to rid the digestive tract of difficult-to-digest material in order to maximize the animal's ingestive capacity (i.e., food intake) (Hummel et al., 2018). Furthermore, among ruminants, the ability for selective particle retention is expressed as a decreasing gradient among from grazer to intermediate and browser feeders (Hofmann, 1989).

From a methodological point of view, using an experimental approach allows monitoring digestive kinetic parameters (e.g. retention time) in livestock species and their consequences for seed germination success since many processes influencing the endozoochorous process are controlled (e.g. density-dependent germination, alternating environmental conditions, diet composition). Moreover, using inert particles that do not interact with microbial fermentation allows assessing the influence of physical characteristics, such as size and specific gravity, on mean seed retention time (King & Moore, 1957; Campling & Freer, 1962), and also permits distinguishing between seed loss due to mastication and due to degradation in the gut (Dufreneix et al., 2019). From this perspective, in the present study we experimentally assessed quality of the seed treatment in the digestive systems of three species of domestic ungulates considered potential endozoochorous

dispersers of *Prosopis flexuosa*, a keystone tree species of the Argentine dry woodlands. The three ungulate species included two foregut fermenters (i.e. ruminants, cattle and goats) and one hindgut or colon fermenters (i.e. non-ruminant, horse). The main objective was to determine the combined impact of seed intake and gut passage, testing the following hypotheses:

(1) Because in ruminant species the combination of a density-dependent sorting mechanism with the rumination cycle facilitates a very efficient size reduction of ingested particles (Van Soest, 1994), recovery of whole seeds will be higher in horses than in cattle or goats.

(2) For goats and cattle (ruminant species), mean seed retention time will be longer compared to the horse (non-ruminant species). In order to face the problem of an inverse relationship between feed intake and digestive efficiency, ungulate species have different digestive specializations. These involve retention of particles in the forestomach and subsequent reduction of its size to enhance food digestibility in ruminants, and selective expulsion of particles in non-ruminants to rid the digestive tract of undigested material in order to enhance food intake (Hummel et al., 2018).

(3) Because the effect of chewing is similar for seeds and plastic pellets, and the effect of long chemical digestion on seeds can reduce seed recovery, we expect a similar mean retention time between seeds and plastic pellets in cattle and horse, a similar recovery between seeds and plastic pellets in horse, and a lower recovery of seeds than of plastic pellets in cattle.

(4) *Prosopis flexuosa* seeds have physical dormancy enforced by impermeable seed coats. Dormancy can be broken when seeds pass through animal digestive tracts (Campos et al., 2008). As the prolonged exposure of seeds to digestive fluids may result in embryo damage (Traveset & Verdú, 2002), we expect that germination success of recovered seeds will be lower for seeds ingested by ruminant species (cattle and goats) compared to the non-ruminant one (horse). For all cases, germination of ingested seeds will be higher compared to seeds collected from trees.

(5) The passage through the gastrointestinal tract and its effects on seeds is a time-dependent and compartmentalized process (Bernard et al., 1998). As the time elapsed from seed ingestion increases, we expect a low germination success of recovered seeds. In other words, germination will be lower in seeds recovered towards the end of the experiment (period 2) than in seeds recovered during the first days of the experiment (period 1)

Materials and methods

Plant species

Prosopis flexuosa fruit is a modified indehiscent pod with a thin epicarp, a mesocarp that can be fleshy, sugary or fibrous, and several endocarp segments (Burkart, 1976). Given its nutritional composition (40% carbohydrates, 32 to 36% protein, 17% fat, 5% ash and 6% fibre) and high digestibility (Burkart, 1952, 1976), the *P. flexuosa* fruit is used as raw material for elaboration of food for human consumption (Capparelli, 2008; Moreno et al., 2018) and represents a valuable forage resource for domestic and wild animals. Seed germination is hindered by physical dormancy, and seed scarification by different agents increases germination (Catalán & Balzarini, 1992; Peláez et al., 1992; Peinetti et al., 1993; Campos & Ojeda, 1997; Baes et al., 2002; Campos et al., 2008).

The role of many wild animals as seed predators or dispersers of *P. flexuosa* seed is well known (Campos & Ojeda, 1997; Campos et al., 2007, 2008, 2017, 2018, 2020; Campos & Velez, 2015; Velez et al., 2016, 2018), as well as the quantitative aspects related to number of seeds dispersed under different land uses (Campos *et al.*, 2016; Bessega et al., 2017; Tabeni et al., 2017; Miguel et al., 2017, 2018a, 2018b). For domestic animals like cattle, previous studies analysed the number and germinability of *P. flexuosa* seeds recovered from faeces collected in the field (Campos & Ojeda, 1997) and established the quality of sites where faeces and seeds are deposited (Campos et al., 2011).

Experiments of ingestion and recovery of seeds and plastic pellets

The feeding experiments were conducted from November 2016 to April 2017 at the experimental campus of the Argentinean Institute of Arid Zone Research (IADIZA), and at the School of Agricultural Science (National University of Cuyo). All experimental procedures and animal care practices were in agreement with the provisions of the Guide for Care and Use of Agricultural Animals in Research and Teaching (FASS, 2010). For seed ingestion and recovery experiments, we monitored twelve individual animals of all three species: goat (*Capra hircus*, N = 4), cattle (*Bos taurus*, N = 4) and horse (*Equus ferus caballus*, N = 4). The animal species were assumed to have a different mean retention time of digestive products (as seeds) according to their variation in body mass and digestive system (Illius & Gordon, 1992). In order to evaluate the mechanical effect of digestion on seed recovery in animal species of similar size and with different morphophysiological types of digestive systems, cattle (N = 2) and horses (N = 4) were also fed with plastic pellets similar to *Prosopis* seeds in shape and specific gravity.

All animals were kept in individual pens with *ad libitum* access to water, shade and trace mineral salt block, and fed daily with an alfalfa hay-based diet until the end of the experiment. Each

animal was offered controlled amounts of *P. flexuosa* fruits and, some of them, also plastic pellets. Both fruits and pellets were offered mixed with alfalfa hay, ground corn and molasses. We began the experiments in the morning, ensuring that each animal ingested as many seeds and plastic pellets as possible (Appendix S1). We interrupted the first day feeding session when it appeared that all seeds and plastic pellets had been ingested or when the animal refused to eat, and the remaining seeds and plastic pellets were removed and counted.

The fruits of *P. flexuosa* used in feeding experiments were collected from 20 adult trees randomly selected at the Ischigualasto Provincial Park (San Juan province) during the fruiting seasons (January to March) of 2015 to 2016; they were stored in paper bags in a refrigerator at 5 °C until the start of experiments, following the methodology proposed by Cony (1993) for preserving seeds. The offered fruits were selected, discarding by external observation the ones with holes in the epicarp caused by emergence of adult insects (Velez et al., 2018). We quantified the number of seeds provided, assuming a single seed in each pod segment.

The days following ingestion of seeds and plastic pellets, the faeces of each animal were collected daily and stored in labelled paper bags until processing. To ensure that we covered retention time until reaching a plateau in the cumulative proportion of retrieved seeds, we collected all fresh faeces for ten days. To recover both seeds and plastic pellets, each faecal sample was thoroughly washed under running water in a sieve (71 µm). Seeds were dried at room temperature, and stored in the laboratory until the germination experiment. Seed and plastic pellets recovery was calculated as the proportion of viable seeds daily removed from faeces to the viable seeds consumed by individuals. Because animals were fed on whole fruits containing viable and non-viable seeds, pre-ingestion loss was estimated using seeds collected from trees.

Germination and viability of seeds

Germination and viability were measured for seeds ingested by animals. In July 2018 and May 2019, germination tests were performed in incubators (Precision GCA Corporation, Scientific Model 818, Chicago, Illinois, USA) with constant light and temperature (30 °C). Seeds were placed in sterile, plastic Petri dishes (9 cm diameter) containing filter paper disks resting on a thin layer of cotton, all materials having previously been sterilised. Ingested seeds were previously immersed in a solution of sodium hypochlorite (2%) for 10 minutes to remove fungi and superficial bacteria (Sauer & Burroughs, 1986). Dishes were initially moistened with a suspension of Captan fungicide at 2g/L, being thereafter moistened as needed with sterile water. Dishes were randomly repositioned every week to avoid a chamber position effect. Germination, identified as

visible radicle protrusion, was recorded every day for 30 days. Apparently healthy seeds (i.e. seeds without visible damage) recovered from animal faeces were cultivated, with sample sizes determined by seed availability. Seeds recovered from each individual per deposition day were kept in separate Petri dishes containing 25 seeds at maximum. We used visually healthy seeds with endocarps collected from trees and made 25 replicates of 20 seeds to better represent the condition and provenance of seeds ingested by animals. It should be noted that there is a 32 percent pre-dispersal loss of *P. flexuosa* seeds due to abortion and insect predation. In many cases, insects die during development and do not emerge, then endocarps remain intact and seed loss cannot be externally determined (Velez *et al.*, 2018).

To establish whether seeds that had failed to germinate in the previous experiment were viable, seeds from trees and ingested by animals that had not germinated were subjected to the standard treatment with a 2,3,5-triphenyltetrazolium chloride test (Pili-Sevilla, 1987), which detects seed viability by staining the embryo tissue pink/red. Then, those seeds that germinated and those that were positive in the viability test were considered viable seeds.

Data analysis

In order to analyse differences among animal species in the recovery of ingested seeds (prediction 1), we used a generalized linear mixed model (GLMM) with a binomial error distribution and a logit link function because there was no overdispersion. The total number of seeds recovered from faeces was used as numerator in the response variable, and the number of seeds ingested by individuals as denominator. Species were included as explanatory variables and individuals were considered a random effect nested within species.

Mean retention time is the time that the average digested particle remains in the digestive tract (Warner, 1981) or the time taken for a 50 percent seed recovery. Expressing the results in terms of mean retention time is a convenient method for comparing the curves of cumulative seed recovery over time along their entire lengths (Castle, 1956). The mean retention time of seeds and plastic pellets ingested by each individual was computed as the weighted sum of particles recovered per day related to the total number of particles recovered (Gardener *et al.*, 1993; Razanamandranto *et al.*, 2004). Differences among species in mean seed retention time (prediction 2) were analysed using a Kruskal-Wallis rank sum test with a posteriori pairwise Wilcoxon test.

Comparisons of mean retention time and number of seeds and plastic pellets recovered after gut passage for horses and cattle (prediction 3) were made using a Two Sample t-test in the case of cattle and the non-parametric Wilcoxon signed-rank test for horses.

Differences in germination (prediction 4) were analysed between seeds recovered from faeces and seeds collected from trees using a GLMM with a binomial error distribution and a logit link function. The total number of germinated seeds was used as numerator in the response variable. The number of seeds recovered from faeces of individuals or the number of viable seeds in the replicates of seed collected from trees were used as denominator. Species were included as explanatory variables and individuals were considered a random effect nested within species.

In order to assess the effect of retention time in the digestive tract on seed germination (prediction 5), we estimated for every species the time needed for an 80 percent seed recovery; using this information, we grouped the data into two periods, the former considering the days required to reach 80 percent seed recovery (period 1: P1), and the latter including the days until the end of the experiments (period 2: P2). We chose this percentage of recovery because values showed low variations among individuals of each species. We used a GLMM with a binomial error distribution and a logit link function to analyse the effect of periods (P1 and P2) on the percentages of germinated seeds. For this model, the number of germinated seeds during every period was used as numerator in the response variable and the total number of germinated seeds as denominator. Period was included as explanatory variable and individuals nested within species were considered random effects.

All analyses and graphs were performed in R version 3.6.1 (Team RC, 2016).

Results

A mean of 65 percent (SE = 4.5) of the seeds obtained from trees were viable. Viability was established from seeds with endocarps and represents the viability of seeds ingested by animals in our experiment. Similar results were found by Velez et al. (2018).

Total seed recovery after passage through digestive tracts was 5, 3 and 26 percent of the viable ingested seeds for cattle, goats and horses respectively (Table 1). The fitted model showed that seeds ingested by cattle and goats were recovered in a lower percentage than those ingested by horses (Table 2).

The mean retention time of ingested seeds varied among the studied species. Cattle showed the longest mean retention time, followed by goats and horses in decreasing order. Only between cattle and horses was there a statistically significant difference (Kruskal-Wallis chi-squared = 8.11, df = 2, p-value = 0.02; Table 1). The mean retention times of plastic pellets and seeds were similar for cattle ($t = -1.48$, df = 2.35, p-value = 0.32) and horses ($W = 3$, p-value = 0.20; Table 1).

The recovery of plastic pellets ingested by cattle was significantly higher in comparison with seed recovery ($X^2 = 15.75$, $df = 1$, $p < 0.00001$; Table 1), whereas there were similar recoveries of plastic pellets and seeds when ingested by horses ($W = 5$, $p = 0.48$; Table 1).

The model fitted to test whether percentages of germinated seeds differ among sources (recovered from faeces and collected from trees) showed that germination was significantly low for seeds collected from trees and seeds ingested by cattle (Table 2). Total seed germination was intermediate for seeds consumed by horses and high for those consumed by goats (Table 1).

The germination analysis of seeds recovered after two periods of retention in the digestive tract showed lower germination for seeds recovered during P2 (Table 2), that is, seeds that remained in the digestive tracts after 80 percent of ingested seeds had been recovered (Table 1).

Discussion

Recovery of seeds and plastics pellets

Although a fairly large number of seed feeding trials with different ungulate species have been carried out (Baltzinger et al., 2019), no clear-cut conclusions can be drawn with respect to mechanical and chemical effects of the gastrointestinal tract on seed recovery. In our study, the use of plastic pellets allowed us to assess the physical effects of digestion on seed recovery without interactions with chemical digestion. Recovery of both seeds and plastic pellets was higher in horses than in cattle. However, in cattle, the proportion of recovered pellets doubled that of recovered seeds (10 and 5%, respectively), while there were no significant differences between recovery of seeds and plastic pellets in horses or between retention time of seeds and plastic pellets in both animal species. In light of these findings, we reached two main conclusions. Firstly, seed recovery in horses is determined almost entirely by the physical aspect of digestion, as was evidenced by the similarity between recovery of seeds and plastic pellets in this animal species. This supports the result obtained by Janzen et al. (1985) who found that horses destroy a higher proportion of seeds during ingestive mastication than do cattle. Secondly, the higher disappearance rate of seeds consumed by cattle was attributed to the synergistic effects of mechanical and chemical digestion; evidence of this was the conjunction of higher recovery of plastic pellets (twice that of seeds) and similar retention times between seeds and plastic pellets in this animal species. Like other species of domestic ruminants, goats and cattle must reduce ingested particle sizes (like seeds) via regurgitation and rechewing before material can flow from the rumen-reticulum to the lower gut, which prevents bulky material filling the gut and limits dry matter intake. Conversely, non-ruminant species, such as horses, are not limited by the particle-size

restrictions that control the flow of material from the foregut, and do not exhibit the regurgitation/rechewing behaviours that typify ruminant feeding (Munn et al., 2008). These specializations in chewing, sorting and filtration mechanisms help explain strong differences in seed recovery between ruminant (<5%) and non-ruminant species (26%). Indeed, Gardener et al. (1993) found that, in ruminant species, most of the chewing damage to seeds may be caused during rumination rather than during ingestion. On the other hand, it is generally believed that the smaller the size of the oral cavity and gut, the more will the seeds be in contact with teeth and gut wall, and hence the higher the risks of mastication and chemical and mechanical abrasion of the gut wall (Gardener et al., 1993; Edward et al., 1998). However, contrary to results obtained by Razanamandranto et al. (2004) in a seed feeding experiment with two grazer species of different body size (cattle and sheep), in our study differences in body mass did not translate into lower seed recovery in goats than in cattle. A possible explanation could be that small selector ruminants, such as goats, have less-developed omasum and less-selective retention than grazer species, and as a result goats can pass coarser matter and even bypass the rumen (Hofmann, 1989). This supports results obtained by Udén and Van Soest (1982) who fed sheep, goats, cattle and horses with grass hay and found larger faecal particles in goats compared to sheep; authors considered this result as partial evidence of adaptation in browsing ruminant species.

Seed germination and retention time of seeds and plastic pellets

As aforementioned, we showed that retention time of seeds and plastic pellets varied among the animal species studied. Seeds and plastic pellets passed through the gastrointestinal tract of horses more quickly than through the gastrointestinal tract of cattle, whereas goats showed an intermediate value for seed retention time between cattle and horses. These results were explained in relation to differences in body size and adaptive feeding strategies developed by domestic ungulates, which allow them to reduce the dietary load of unavailable lignified residue and meet their nutritional requirements. As a general rule of thumb, digestive adaptations of domestic ungulates are limited to their capacity to retain ingested food for a sufficient time to extract nutrients (Van Soest, 1994). Among bulk and roughage eaters (grazers), ruminant species like cattle are functionally restricted by the sieving and sorting process in the reticulorumen and omasum, which promotes maximal retention time and fibre digestion at the expense of net intake, whereas in non-ruminant species, such as horses, filtering mechanisms are not a limitation and their feeding strategy consists of lower extraction and higher consumption (Van Soest, 1994; Foose, 1982). This explains, at least in part, the higher retention time of seeds and plastic pellets in

cattle than in horses, which supports observations by Van Soest (1994) and Pearson et al. (2006). In the case of goats, a small selector ruminant, the feeding strategy consists of feeding selectively on more nutritious plant parts, reducing retention time and bypassing indigestible load (Hofmann, 1989). Functionally, bypass would place goats somewhere between a non-ruminant grazer (horse) and a ruminant grazer (cattle), which explains the intermediate length of time spent by seeds in the digestive tract of goats. On the other hand, in our study, body mass appeared unrelated to seed retention time, but instead related to differences in retention time of plastic pellets and seed between horses and cattle. This supports results of previous studies (Schwarm et al., 2008; Picard et al., 2015) which suggest that the effects of digestion strategy may dominate those of body mass on seed retention time.

Regarding seed germination, our results showed that seeds excreted by goats and horses doubled the germination success (38 and 31% respectively) of seeds with no treatment (i.e., recovered directly from mother plants). Similar effects of goat's digestion on hard-coated seed germination were found by Shiferaw et al. (2004) who reported germination rates of 37 percent for *P. juliflora* seeds after passage through goats, and 21 percent for seeds with no treatment, and by Baraza and Valiente-Banuet (2008) who found that passage through the gut of domestic goats greatly increased germination of leguminous seeds but reduced that of gramineous seeds. Among hindgut fermenters, unlike our results, intake of *P. flexuosa* (Campos et al., 2008) and *P. ferox* (Baes et al., 2002) seeds by donkey does not appear to directly improve seed germination success in comparison with healthy seeds collected from trees. Moreover, in our study, the catalyst effect of the digestive process on germination of *P. flexuosa* seed was not evidenced in cattle for there were no significant differences in germination between seeds excreted by cattle and seeds collected from trees (20 and 15 %, respectively), which does not support observations by Campos and Ojeda (1997), who found a greater germination capacity in seeds recovered from cattle dung. It should be noted that, unlike in our study, previous results were obtained from field experiments without knowing the initial number of ingested seeds, using hard-coated seeds collected from dung of free-ranging livestock. The germination success of seeds collected in this way depends not only on animal species acting as dispersers, but also on other factors such as qualitative-quantitative characteristics of the diet or quality of faecal depositions (Baltzinger et al., 2019).

Relationship between germination and retention time of seeds

How retention time affects the germination success of gut-passed seeds has been the trigger of interesting hypotheses and numerous studies over the last decades. In general terms, the

relationship among these variables has been conceptualized as a trade-off between herbivores and seeds. From the animal's perspective, seed retention time is a trade-off between retaining fruits for a sufficient period of time to assimilate nutrients and discarding indigestible seeds as rapidly as possible (Van Soest, 1994). From the plant's perspective, seed retention time is a trade-off between dispersal distance and seed viability (Murray et al., 1994). While strong evidence suggests that germination of soft-coated seeds decreases as retention time in the gastrointestinal tract increases (Gardener et al., 1993); results for hard-coated seeds are less conclusive. Our prediction about an inverse relationship between both variables was partially fulfilled since results showed an increase in seed germination as a consequence of low to intermediate retention times (in goats and horses respectively), whereas a longer retention time had no consequences on total germination success of seed excreted by cattle. For many plant species with hard-coated seeds, the time that seeds remain in a disperser's gastrointestinal tract can have negative, positive or even null consequences for the germination success of gut-passed seeds (Traveset et al., 2007), depending on other factors such as digestive capacity of dispersers (Colucci et al., 1990) or intra and interspecific seed coat variations (Smýkal et al., 2014). In line with these statements and with our results, Campos et al. (2020) found no differences in germination of *P. flexuosa* seeds after their passage through the wild herbivores *Chelonoidis chilensis* (Argentine tortoise) and *Dolichotis patagonum* (Patagonian hare), despite the marked difference in seed retention time between both animal species. This suggests that differences in germination of seeds after passing through the digestive tract of dispersers can be better explained by considering the set of characteristics that define their feeding strategies instead of considering retention time as an isolated explanatory variable. Considering that (i) studied animal species differ in their feeding strategies and hence in their capacity to digest forage, or more specifically to digest plant structural carbohydrates like cellulose and hemicelluloses (in decreasing order: cattle > goats > horses) (Foose, 1982; Van Soest, 1994; Pearson et al., 2006), and that (ii) the softening of hard seed coats during the digestive process can break down dormancy and stimulate seed germination (Baskin et al., 2000; Traveset & Verdú, 2002; Smýkal et al., 2014), the high to intermediate values of germination of seed ingested by goats and horses were attributed to their low digestive capacity and hence gentle treatment of seeds during their passage through the gastrointestinal tract. The low ability of goats and horses to digest forage has been mainly related to a fast passage of ingested particles through their guts, a characteristic common to the feeding strategies of both animal species, which is compensated for by eating for bulk and volume (horse) or by selectively

eating more nutritious plant parts (goats) (Hoffman, 1989). Evidence of fast passage in our study is given by the low to intermediate values of seed retention time in goats and horses, which means less exposure to chemical digestion, in particular to enzymatic digestion of hard-coated seeds by ruminal and cecal microflora in goats and horses respectively (Howard & Elliot, 1988; Defonbelle et al., 2003). Unlike observed in goats and horses, our results showed similar germination values between *P. flexuosa* seeds recovered from cattle and collected from trees, even when these grazing ruminants have a higher digestive capacity than goats and horses (Van Soest, 1994).

As mentioned above, the increased ability of cattle to digest forage has been mainly related to more efficient selective particle retention and particle size reduction via rumination, which means retaining food for maximal digestion at the expense of net intake (Clauss et al., 2008, 2009). Evidence of this feeding strategy in our study was the lower recovery and higher retention time of seeds and plastic pellets estimated in cattle, which was attributed to buoyancy and specific gravity of both particles (1.8 and 1.6 respectively). Pell et al. (1988) demonstrated that particles with a specific density greater than 1.3 have a low probability of being ruminated or of quickly passing through the gastrointestinal tract of cattle, and attributed the long period elapsing before particles are recovered from the faeces both to retention time in rumen and to the slow passage through the lower gastrointestinal tract. From the seed perspective, an increase in time of seed exposure to cattle ruminal fluid allows ruminal bacteria to produce more cellulase, which can degrade the cellulose polymers of seed coats and promote seed imbibition and germination (Howard & Elliott, 1988).

It is known that once a coat is disrupted and becomes permeable to water, additional time in the gastrointestinal tract may be detrimental to its viability and germinability, and even seed destruction may occur (Traveset, 1998). These observations were supported by our results since we found a higher germination success of seed recovered during Period 1 than of seed recovered during Period 2. These results suggest that both seed passage through the gastrointestinal-tract and its consequences on seed recovery and germination are time-dependent processes; knowing this is valuable for better interpretations of the total germination value of gut-passed seeds since this value is usually estimated as a proportion of total seeds recovered.

Conclusion and implications

This study starts from the premise that contribution of endozoochorous dispersers to the future reproduction of a plant species is product of two components, and that consequences of the qualitative component for seed dispersal effectiveness depend largely on the conditions seeds are

exposed in the digestive tract. As these conditions vary depending on herbivore feeding strategies, domestic ungulates on extensive systems can be positioned on an effective seed dispersal gradient. Long-distance seed dispersal by large ungulates could be an important management and restoration mechanism in native forests since it may favour connectivity among distant populations within metapopulations or colonization of unoccupied suitable patches. In light of evidence related to the treatment seeds receive in the digestive tract, we identify horses as more effective dispersers than cattle or goats. Future studies could inquire about the quality of depositions for seed germination and seedling establishment, considering the physical and chemical properties of faeces as substrate and the environmental conditions of sites where seeds are deposited.

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Author contributions: AVE and CMC conceived and designed the research; AVE, MSC, MIC, CMC did the feeding experiments; AVE, MSC, MIC, CS and CMC did the germination and viability trials; CMC analyzed the data; AVE and CMC led the writing of the manuscript. All authors contributed critically to the final draft and gave final approval for publication.

Data availability statement

The data that support the findings of this study are available in Appendix S2.

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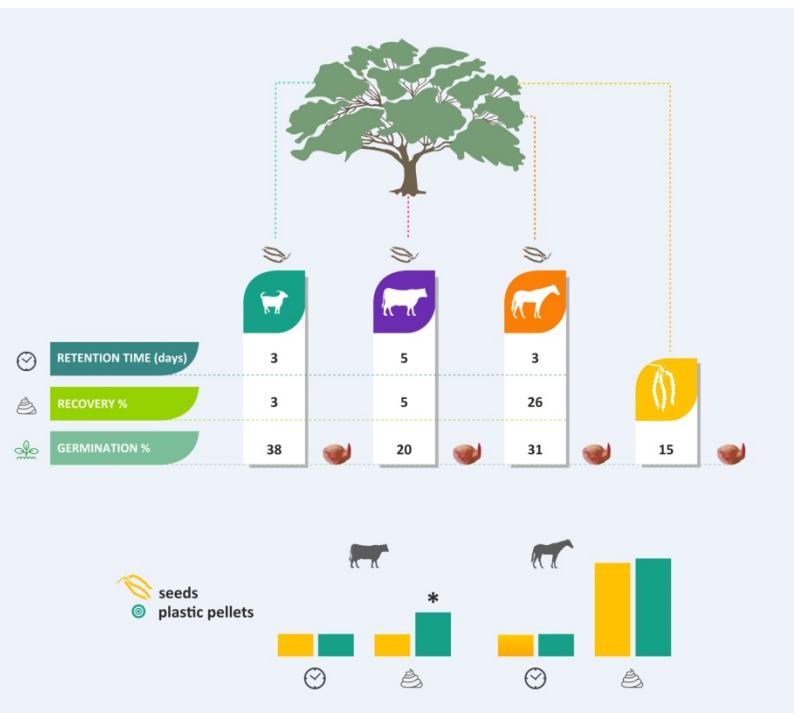
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Support information

Appendix S1: Additional details of experimental design.

Appendix S2: Data that support the findings of this study.

Graphical abstract



Domestic animals are endozoochorous dispersers of *Prosopis flexuosa* seeds. We experimentally assessed the effects of gut passage through ruminants (goats and cattle) and non-ruminant (horse) species on seed recovery, retention time and germination success. Quality of the seed treatment by horses is better than by the other species, because of the high seed recovery and germination compared to control seeds.

Table 1. Percentages of seeds and plastic pellets recovered from faeces, mean retention time (days) of seeds and plastic pellets, percentages of total germinated seeds and germination after the two periods of retention in the digestive tracts of animals. Mean values (SE) are shown. Values of recovered seeds were recalculated considering the viability obtained for ingested seeds (65%). Differences among rows are indicated with capital letters; differences between periods and between recovery and retention time of seeds and plastic pellets are indicated with lowercase letters.

| | Recovery | | Retention time | | Germination | | |
|--------|----------------|-----------------|----------------|-----------------|---------------|---------------|---------------|
| | Seeds | Plastic pellets | Seeds | Plastic pellets | Total | Period 1 | Period 2 |
| Cattle | 4.8 (0.9) B b | 10.2 (2.1) B a | 5.5 (0.6) A a | 6.1 (0.1) A a | 19.6 (3.9) BC | 91.3 (5.4) a | 8.7 (5.4) b |
| Goat | 3.3 (1.9) B | | 3.4 (0.5) AB | | 38.0 (9.7) A | 47.8 (21.2) a | 32.2 (19.3) a |
| Horse | 26.2 (2.1) A a | 29.3 (6.8) A a | 2.6 (0.2) B a | 3.8 (0.9) B a | 31.3 (4.0) AB | 83.2 (5.5) a | 16.8 (5.5) a |
| Trees | | | | | 14.8 (3.4) C | | |

Differences among rows are indicated with capital letters. Differences between recovery and retention time of seeds and plastic pellets are indicated with lowercase letters. Differences between seed germination in the two periods are indicated with lowercase letters. Different letters indicate significant differences ($P < 0.05$).

Table 2. Results of generalized linear mixed models with a binomial error structure testing whether the proportion of *P. flexuosa* seeds recovered from faeces differs among species, the proportion of germinated seeds differs among sources (faeces and trees), and between retention periods (P1 and P2) in the digestive tracts of animals.

| Adjusted model | Effects | Estimate | Std. error | z value | p value |
|---|------------------|----------|------------|---------|--------------|
| Proportion of recovered seeds ~ species + (1 species/individual) | Intercept (Goat) | -3.77 | 0.33 | -11.26 | < 0.0001 *** |
| | Cattle | 0.69 | 0.45 | 1.54 | 0.122 |
| | Horse | 2.42 | 0.44 | 5.53 | < 0.0001 *** |
| Total proportion of germinated seeds ~ sources + (1 species/individual) | Intercept (Goat) | -0.77 | 0.27 | -2.82 | 0.005 * |
| | Cattle | -0.79 | 0.38 | -2.09 | 0.036 * |
| | Horse | -0.39 | 0.29 | -1.33 | 0.182 |
| | Trees | -1.19 | 0.33 | -3.58 | < 0.001 ** |
| Proportion of germinated seeds ~ period + (1 species/individual) | Intercept (P1) | -0.17 | 0.10 | -1.67 | 0.095 |