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Effects of seeds ingestion by Lagune cattle and other pre-planting treatments on the germinability of *Centrosema pubescens* Benth seeds in Soudanian region of Benin (West Africa)

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In Benin under extensive management of grassland, there is little information about legumes regeneration and management in pastures. This trial evaluated the germination of *Centrosema pubescens* seeds after passage through the digestive tract of three young bulls and three heifers of *Lagune* breed cattle. Following seeds ingestion by cattle, total faeces were collected at 24-h intervals for 6 days after which time the faeces were sieved and the surviving intact seeds were then collected, counted and germination tests undertaken. Moreover, the effect of soaking seeds with hot water and mechanical scarification on breaking of dormancy in seeds of *C. pubescens* were studied through seven treatments: control (1); scarification using sandpaper (2); and seeds were immersed in hot water (80°C) for 2 (3), 4 (4), 6 (5), 8 (6), and 10 min (7). The total number of seeds recovered represented 7.65% of the number fed. The number of seeds recovered after 72 h represented more than 91.00% of total seeds recovery. Overall germination percentage of seeds recovered (45.09%) was greater than that of untreated seeds (31.00%). Seedling emergence was significantly higher when dung was broken-down than when left intact. Generally, it was observed that mechanical scarification was the method that had the highest percentage of germination (96.00%), followed by seeds immersed in hot water at 80°C for 2 to 4 min and seeds ingested by cattle. Therefore, endozoochory and other pre-planting seeds treatments can potentially favour seed germination of *C. pubescens* and contribute to the improvement of degraded grassland.

Key words: *Centrosema pubescens*, sandpapering, endozoochorous, hot water, germination, Benin.

INTRODUCTION

In tropical regions, grassland is secondary habitats formed due to human activities resulting in degradation of

deciduous forests (Mandar, 2016; Houndjo et al., 2018a). Spread throughout south and north of Benin, natural savannahs and fallows are important from economic and ecological points of view and are the prime source of fodder for the large population of livestock (Sinsin, 1993; Houinato, 2001; Adjolahoun, 2008; Koura, 2015; Lesse, 2016). In these grasslands, legumes species are scarce and overexploited thus leading to their low abundance whereas native grasses and weeds are largely dominant especially in the dry season (Michiels et al., 2000; Lesse, 2016). Uncontrolled use of forage legumes species leads to degradation and reduction of their habitat and population in grassland (Sinsin, 1993; Mandar, 2016). Several studies have been conducted on legume forage species adaptation to the environmental conditions in Benin considering their yield and nutritive value (Michiels et al., 2000; Adjolahoun, 2008; Babatoundé et al., 2010; Musco et al., 2016). However, under extensive management of these pastures, there is little information about legumes regeneration and management in pastures.

Centrosema pubescens (Centro) belongs to the Fabaceae family and is a perennial, training-climbing herb with strong tendency to root at nodes of trailing stems. It is native to Central America and can be grown in many tropical regions. Centro is widely used as forage and source of calcium and phosphorus to livestock (Cook et al. 2005). Centro has been identified by several authors as potential forage legume for the tropical regions (Cook et al., 2005; Adjolahoun, 2008; Houndjo et al., 2018a). Green matter yield of *C. pubescens* varies from 13.5 to 40.0 tons ha⁻¹ year⁻¹ (Ajayi et al., 2008; Houndjo et al., 2018b). *C. pubescens* forage is very rich in protein (19.6%) and can be used as green manure crop in rubber, coconut and oil palm plantation and its forage can be grown for stall feeding, grazing or preserved as hay or silage for use during the dry season when there is a scarcity of grazeable materials (Ajayi et al., 2008; Houndjo et al., 2018b). It can be established by oversowing in natural or artificial pastures by enrichment planting or direct seeding (Adjolahoun, 2008). However, without seeds treatment, establishment of Centro is difficult mainly due to high proportion of hard seeds (Win et al., 1975; Muhammad, 2015; Houndjo et al., 2018b). Seed is the basic agricultural input and its quality is extremely important.

Some leguminous species with hard seeds are known to survive digestion and be dispersed by ruminants including cattle and in some cases have gone on to become established and develop into being environmental weeds (Berner et al., 1995; Paynter et al., 2003). Temporal patterns in the defecation of seeds after

ingestion indicate that germination increased as the length of retention in the digestive tract increased (Jolaosho et al., 2006). Mastication and/or action of acid and enzymes present in the digestive tract of cattle could separate the seed from the shell, soften seed and/or scarify the seed coat (Traveset and Verdu, 2002).

The retention time of seeds in the digestive tract varies, depending on the type of animal (Gökbulak, 2003). Some authors suggest that the larger body size and longer intestinal tract in cattle was responsible for the lower recovery rates of *Albizia saman* seeds (Jolaosho et al., 2006). Other factors such as seed characteristics, diet quality, health, age, sex, and stress level may also influence seed retention time (Raymundo et al., 2018). Lagune cattle is the main cattle breed in south of Benin. It is of the smallest cattle breeds of Benin (Gbangboche et al., 2011; Houndjo et al. 2018a) and little is known about the recovery and germination of seeds after ingestion by Lagune cattle in Coastal region of West Africa.

Cattle ingestion and later excretion of seeds (endozoochory) of *C. pubescens* as a method of seeds dispersal would have the potential to act as a low cost alternative for transport large numbers of seeds and deposit them in a germinable form into an environment suitable for establishment (Doucette et al., 2001). Dung depositions generate gaps and provide nutrients and organic matter that facilitate seedling emergence and growth (Osvaldo et al., 2010). However, other studies indicate that seed inclusion in dung can suppress seedling emergence (Uytvanck et al., 2010; Milotić and Hoffman, 2016).

As it is desirable to contribute to the rehabilitation of degraded grassland in Benin, endozoochory, mechanical scarification, acid treatment, or hot water treatment, may be of value. Sulfuric acid was reported as having the highest positive effect in breaking seed dormancy (Muhammad, 2015). However, sand papering and hot water treatments could be considered for substitution because sulfuric acid application by most farmers is not easy (Muhammad, 2015).

This study was conducted to determine:

- (1) the survival and germination of *C. pubescens* seeds fed to penned Lagune cattle;
- (2) the influence of sex and seed retention time in the digestive tract on seeds germination;
- (3) the effect of being contained in dung for seedling emergence;
- (4) the effect of treatment with hot water and mechanical scarification on the germination of untreated seeds in order to compare different methods of breaking dormancy of *C. pubescens* seeds.

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MATERIALS AND METHODS

Plant

Seeds of *C. pubescens* were selected to examine the rate of recovery and the subsequent germination after passing through the digestive tract of bovines. Seeds were obtained from the experimental study on the influence of cattle manure rates in combination with plant row spacing conducted by Houndjo et al. (2018b). Seeds were harvested in November 2016 and stored in plastic jars in ambient temperature to prevent seed quality deterioration (Razanamandranto et al., 2004). Morphological characteristics of seeds are shown in Table 1. *C. pubescens* seed mean number per pod and thousand-seed weights were 18.09 and 34.62 g, respectively. There were 27809 to 33761 seeds/kg, with an average of 28885 seeds/kg (Table 1). The investigation involved three complementary activities which included (a) retrieval of seeds from dung and their germination testing, (b) seedling emergence directly from dung, and (c) seed treatment with mechanical scarification or hot water.

Seeds retrieved and germination after the digestive process

The first part of the investigation examined the survival and germination of *C. pubescens* seeds fed to penned cattle.

Seeds ingestion procedure

six cattle (*Bos taurus*) namely 3 young bulls (90.20 ± 5.30 kg) and 3 heifers (70.50 ± 3.50 kg) of Lagune cattle breed were used to examine seeds recovery and germinability after passage through the digestive tract of cattle. Mean age of cattle were 18 ± 2 months. Lagune cattle were used as it is the main cattle breed in the region (Gbangboche et al., 2011; Assogba et al., 2016). The feeding experiment was conducted during 2 months (from December 2017 to January 2018) in the farm of the Faculty of Agricultural Sciences of the University of Abomey-Calavi at Sékou located between 6° 21' - 6° 42' N and 2° 13' - 2° 25' E. The area has a sub-equatorial climate with two rainy seasons alternated with two dry seasons of unequal duration (Houndjo et al., 2018a).

Before seeds ingestion began, each cattle was housed in an individual pen for an adaptation period of two weeks, they were treated to control ectoparasites and endoparasites. This procedure was done to avoid any stress behavior due to the captive environment that could influence or perturb any feeding or digestion aptitude. During this period, the animals were regularly fed *ad libitum* with *Panicum maximum-Stylosanthes hamata* forage mixture (70:30, dry matter basis) (Doucette et al., 2001). Animals received also fresh water and mineral blocks salt *ad libitum*. This was done to be sure that they did not accidentally ingest other seeds than the ones from the study species. The same food was supplied in the same way until the end of the experiment.

The ingestion procedure required that seeds were directly placed into the oesophagus of the animals. To keep track of recovered seeds from the dung and to obtain a sufficient number of replicates, seeds of *C. pubescens* were supplied to 3 young bulls and 3 heifers, kept individually in pens. Quantities of seeds containing exactly 1000 seeds were put into a 65 cl glass bottle mixed with 50 cl of water. The neck of the bottle was introduced into the mouth and shaken to be sure that all seeds were ingested by each of the animals individually in pens. No seed was observed to be spat out during the experiment period. Seeds therefore suffered no mechanical damage during ingestion but could be ruminated later (Gardener et al., 1993a).

Table 1. Pod length and weight of seeds of *Centrosema pubescens* used in the study.

Parameter	Average	Range
Pod length (cm)	15.66	6.00 - 17.30
Number of seeds/pod	18.09	8 - 23
1000 seeds weight (g)	34.62	29.62 - 35.96
Number of seeds/kg	28885	27809 - 33761

Source: Houndjo et al. (2018b).

Dung collection and extraction of seeds

The dung of each animal was separately and daily collected in plastic trays at 24-h intervals, during 6 days after seed ingestion. The collection ended after 6 days because previous trial showed that no more seeds were found in the dung after 5 days (Razanamandranto et al., 2004; Gardener et al., 1993a). All dung was collected from each pen at the end of each 24-h period, that is, at 24, 48, 72, 96, 120 and 144 h after consuming the seeds. Total fresh dung in each tray was weighed and mixed individually for 15 min prior to sampling. Three, 200 g sub-samples were sampled for each cattle by collection time. One sub-sample was retained for dry matter (DM) determination, one for germination assays, and another for seed recovery estimates. *C. pubescens* seed recovery was conducted using a method similar to Jones and Bunch (1977). According to this, the 200 g faecal sub-sample was placed in a 2-L container. Then 1 L of water was added and gently mixed to create a slurry, which was flowed down through a series of 2 stacked sieves with decreasing apertures of 1 and 0.42 mm. Sieves were gently washed with water, then seeds and the larger particles of digesta were sifted from the fine particles. The seeds and the larger particles that remained from each screen were transferred to trays lined with paper towel and dried for approximately 24 h at 28°C in a forced air oven. Each sample of dried faeces was carefully inspected, and all identified seeds were retrieved and separated according to seed type (Doucette et al., 2001). Whole undigested seeds were separated from broken seeds, counted and retained for germination testing in the laboratory (Jolaosho et al., 2006). Seed recovery for each day was calculated on the basis of the overall faecal collection for that time and seed densities from the 200 g sub-sample, as follows:

$$TNRS = \left[\frac{NRS_{(200g)}}{200} \right] \times TFO$$

where *TNSR* = Total Number of Recovered Seed of the period, *NRS*_(200g) = Number of Recovered Seeds from the 200 g sub-sample of the period, and *TFO* = total faecal output of the period.

The percentages of seeds recovered were determined as follows:

$$SR(\%) = \left(\frac{NRS}{1000} \right) \times 100$$

where *SR* (%) is seed recovered in percentage, *NRS* refer to the cumulative number of recovered seeds up to 96 h after ingestion and 1000 the number of seed ingested.

Germination testing

All intact seeds recovered in the cattle trial were submitted to germination. Three replicates of a split-plot experiment were used

with retention time (24, 48, 72 and 96 h) as the main plots in a randomized complete block design (RCBD), sex (male and female) as the subplots combined to give a total of eight treatments with 24 plots. Prior to germination test, seeds of all replicates were disinfected and rinsed with sterile distilled water (Grande et al., 2013). Soil of the experimental site was collected and sieved to remove stones, leaves, stems and other materials. Due to small number of seeds recovered, for each treatment and for each replicate, all intact seeds recovered per day and per cattle were placed on the surface of 150 mm diameter pots filled with sieved, sterilized sand and kept moist. The pots were kept in a shade house at ambient temperature (approximately 29/20°C). The number of seed that germinated was recorded daily for 20 days. Seeds were considered germinated when the radicle had emerged through the integument (ISTA, 1996). Germinated seeds were removed after each count. At the end of the test, seeds that had not germinated were categorized into hard and dead components by touching and piercing with a needle. Hard seeds could not be pierced with the needle (Hassen et al., 2004).

Three parameters were calculated:

(1) Germination percentage (GP) was calculated with the formula:

$$GP(\%) = \frac{\sum_{i=1}^{20} ni}{20} \times 100$$

where n_i = number of seeds germinated and removed on day di and $i = 1 \dots i = 20$ the duration in days of the test. 20 = number of seeds of each repetition placed on each pot at the beginning of germination test.

(2) Germination speed (GS) was calculated following the formula given by Czabator (1962) as follows:

$$GS(\text{seed/day}) = \sum_{20}^1 \frac{ni}{di}$$

where n_i = number of seeds germinated and removed on day di .

(3) Mean germination time (MGT), was calculated as formula given by Ellis and Roberts (1981) as follow:

$$MGT(\text{day}) = \left[\frac{\sum_{i=1}^{20} nidi}{\sum_{i=1}^{20} ni} \right]$$

where n_i = number of seeds germinated and removed on day di , and 20 the duration in days of the test.

Seedling emergence from dung

The second part of the investigation observed the effect of being contained in dung for seedling emergence. Three replicates of a split-plot experiment were used with retention time (24, 48, 72 and 96 h) as the main plots in a randomized complete block design (RCBD), sex (male and female) as the subplot, dung form (broken down and intact) as the sub subplots combined to give a total of 16 treatments with 48 plots. A sampling of 100 g fresh dung from each

day and from each animal (generated in feeding activity) were placed in soil on a tray (12 cm width × 20 cm long × 8 cm depth) and was placed outdoors and left for 2 months. The faeces were either broken down the soil surface (simulating crumbling of dung by rainfall or by animals) or left intact on the soil surface (Mancilla-Leytón et al., 2012). The soil was kept moist. After two months, the number of plants emerging from each faeces was counted (Ghassali et al., 1998).

Other pre-planting seeds treatment of *C. pubescens*

For the third part of the investigation the effect of soaking seeds with hot water and mechanical scarification were studied in a split-plot design replicated 5 times. There were seven treatments: control (1); Sand paper: mechanical scarification using sand paper (2); and seeds were immersed in hot water (80°C) for 2 (3), 4 (4), 6 (5), 8 (6), and 10 min (7). For each treatment and for each replicate, 20 seeds were placed on the surface of 150 mm diameter pots filled with sieved, sterilized sand and kept moist and the germination test were conducted using the standard procedure mentioned above (ISTA, 1996).

Statistical analysis

The General Linear Model procedure of SAS (SAS Institute Inc. 1989) was used for analysis of variance of the germination parameters in the first and third part of the investigation. For the first part eight treatments [4 retention times (24, 48, 72 and 96 h) × 2 sex (male and female)] were considered. In both activities, the total percentages of germinated, hard and rotten seeds were subjected, after arcsine transformation, to analysis of variance using Proc GLM of SAS (1989). When Fisher's F values were significant at $P < 0.05$, the analysis was continued by comparing the means using Tukey's test at the threshold of $P < 0.05$. Arcsine-transformed means were back transformed for presentation. In the second part of the investigation the number of *C. pubescens* seedlings emerged from intact and crumbled faeces was compared using t-test.

RESULTS

Faeces recovery and number of seeds recovered from cattle

The data of number of seeds fed to the cattle that were recovered intact from the faeces was presented in Figure 1. A mean of 1.13 kg DM of faeces per animal male and 1.09 per animal female was produced over the 120 h period. The total number of seeds recovered from young bull (71.37 seeds) and heifer (81.63 seeds) accounting for 7.14 and 8.16% of the seeds ingested by the cattle, respectively were not significantly different ($p > 0.05$) (Figure 1). The average number of seeds recovered from the cattle at the end of 96 h represented 7.65% of the number fed. There was a definite pattern of excretion of seeds by the cattle, with a distinct peak during the 48 to 72 h after ingestion (Figure 1). The number of seeds recovered after 72 h represented more than 91.00% of total seeds recovery. Seeds recovered during the 48 to 72 h after ingestion from young bull (63.91 seeds) and heifer (70.34 seeds) was significantly ($p < 0.05$) higher than the numbers recovered at other times for all cattle

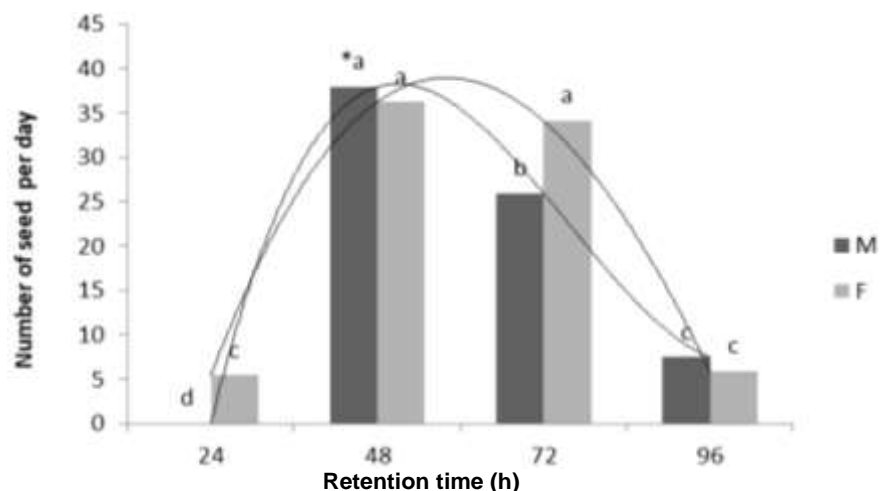


Figure 1. Number of seeds per head recovered from cattle faeces. *Different letters indicate significant differences among number of seeds per head recovered from cattle faeces (Tukey test; $p < 0.05$).

(Figure 1). While the numbers of seeds recovered from the heifer at 24 h (5.43 seeds), and 96 h (5.86 seeds) was similar and significantly lower than those recorded at 48 or 72 h (Figure 1).

Seed germination after ingestion by cattle

Mean effects of ingestion by cattle on germination are shown in Table 2 and Figure 2. All three germination indices (percentage of germinated seeds, mean germination time and germination speed) were significantly influenced by the retention time in digestive tract of cattle ($p < 0.05$). Overall, germination percentage of seeds recovered from faeces (45.09%) was significantly higher ($p < 0.05$) than that of untreated seeds (31.00%) (Figure 2). Percentage of germinated seeds of *C. pubescens* did not increase, as the retention time increased from 48 h (47.83%) to 72 h (43.50%) (Table 2). The analyses also revealed a significant retention time \times animal sex interaction for percentage of germinated seeds of seeds recovered from faeces 48 h after ingestion. Germination speed significantly decreased ($p < 0.05$) as the retention time increased from 48 h (4.24 seeds/day) to 72 h (0.95 seed/day) (Table 2).

Hard and germinable levels of seeds were also determined before and after passage through the cattle (Tables 3 and 4). Before passage through the cattle tract, the amount of hard seeds (53.00%) was significantly ($p < 0.05$) higher than the amount of germinable seeds (31.00%) (Table 3). After seed passage through cattle tract, the proportion of hard seed 38.99% (61.14 divided by 156.80; Table 4) was significantly lower than germinable seed 46.73% (73.27 divided by 156.80; Table 4). However, as increasing times of excreted seeds, the fraction of germinable seeds in the recovered fraction

was lower and the fraction of hard seeds higher (Table 4). Germinable seeds (164.00 seeds), hard seeds (117.50 seeds) and rotten seeds (60.28 seeds) recovered at 48 h after ingestion was significantly ($p < 0.05$) higher than those recovered at other times for all cattle (Table 4). Germinable seeds (8.57 seeds), hard seeds (11.17 seeds) and rotten seeds (1.77 seeds) recovered at 96 h after ingestion was significantly ($p < 0.05$) lower than those recovered at other times for all cattle (Table 4). The total number of germinable seeds from young bull (253.00 seeds) and heifer (259.88 seeds) at the end of 96 h were not significantly different ($p > 0.05$) (Table 4).

Establishment in trays of *C. pubescens* seedlings from faeces

Number of *C. pubescens* seedlings emerged from intact and crumbled faeces is shown in Figure 3. The number of seedlings emerging out of crumbled faeces (7 plants per cattle) was significantly higher ($p < 0.05$) compared to seedling emergence out of intact faeces (2 plants per cattle) (Figure 3). Additionally, the day of faeces collection had influence on seedling number. Number of seedling emergence from crumbled faeces at 48 h (4.35 plants per cattle) was significantly higher ($p < 0.05$) than the numbers of seedlings recovered at other times for all cattle (Figure 3). There was no seedling emergence in intact faeces at 24 h after ingestion.

Effects of pre-planting seed treatment on germination parameters

The results of the germination test for other methods (soaking in hot water or sand paper) of breaking

Table 2. Characteristics of seeds germination after passage through digestive tract of cattle and faeces dry matter per cattle per day.

Retention time (h)	Sex	Percentage of germinated seeds (%)	Mean germination time (day)	Germination speed (seed/day)	Faeces dry matter (g)
24	Young bull	0 ^{Bd1}	0 ^{Bd}	0 ^{Be}	214.39 ^{Ad}
	Heifer	50.00 ^{Aa}	7.00 ^{Aa}	1.08 ^{Ac}	55.96 ^{Be}
	Mean	25.00	3.50	0.54	135.17
48	Young bull	55.00 ^{Aa}	6.64 ^{Aa}	4.74 ^{Aa}	108.66 ^{Ac}
	Heifer	40.67 ^{Bb}	7.22 ^{Aa}	3.74 ^{Bb}	108.36 ^{Ac}
	Mean	47.83	6.93	4.24	108.51
72	Young bull	44.33 ^{Ab}	5.75 ^{Ab}	0.87 ^{AcD}	221.35 ^{Ad}
	Heifer	42.67 ^{Ab}	6.16 ^{Ab}	1.03 ^{Ac}	216.73 ^{Ad}
	Mean	43.50	5.95	0.95	219.04
96	Young bull	33.00 ^{Bc}	7.00 ^{Aa}	0.08 ^{Be}	284.87 ^{Bb}
	Heifer	50.00 ^{Aa}	2.00 ^{Bc}	0.50 ^{Ad}	348.00 ^{Aa}
	Mean	41.50	4.50	0.29	316.43
Overall mean		45.09	5.96	1.72	194.79

¹For the same column, means followed by the same lower letter are not significantly different ($p < 0.05$). Within the same column and for the same retention time, means followed by the same upper letter are not significantly different ($p < 0.05$).

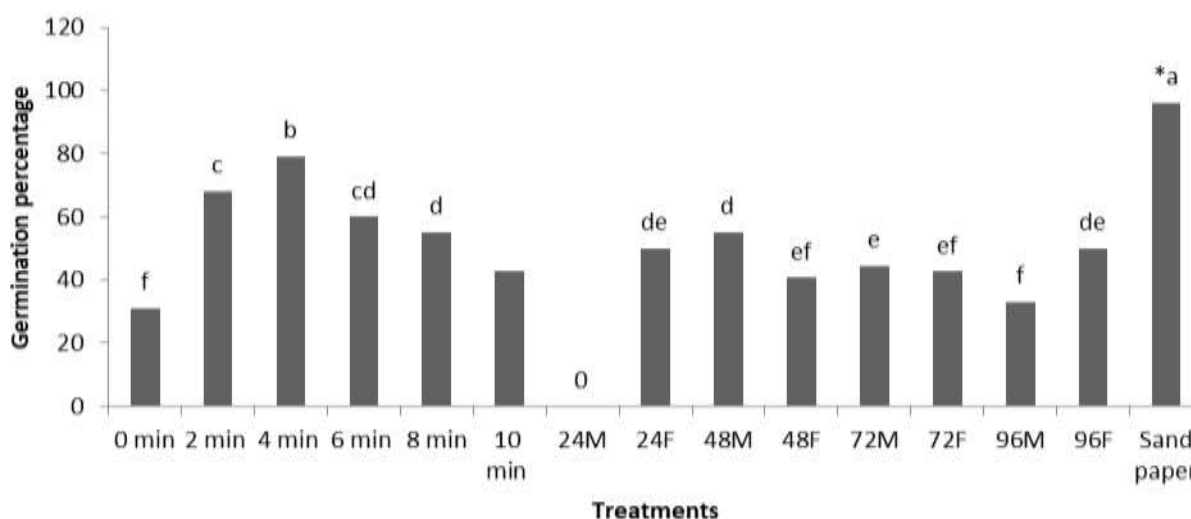


Figure 2. Germination percentage of treated *C. pubescens* seeds. (Sand paper: mechanical scarification using sand paper; and seeds were immersed in hot water (80°C) for 2, 4, 6, 8, and 10 min. Also seeds recovered in the cattle trial with retention time (24, 48, 72 and 96 h) and sex (male (M) and female (F)) were combined to give a total of eight treatments (24 M, 24 F, 48 M, 48 F, 72 F, 72 M, 96 M, 96 F). *Different letters indicate significant differences among Germination percentage of treated *C. pubescens* seeds (Tukey test; $p < 0.05$). 0: In these time periods no seeds were recovered.

dormancy of *C. pubescens* seeds are shown in Figure 2 and Table 3. There was significant difference ($p < 0.05$) in parameters measured between treatments. In the present study, as increasing soaking time, percentage of germination and germination speed values were increased and peaked at 4 min soaking. However, with the longer time of exposure to hot water, the values of the

two germination indices were decreased (Figure 2 and Table 3). Mechanical scarification with sand paper significantly increased ($p < 0.05$) seeds germination compared to control, hot water scarification or passage through cattle tracts (Figure 2). Mechanical scarification was the method which revealed to be more efficient to remove seed dormancy as it had the highest percentage

Table 2. Characteristics of seeds germination after passage through digestive tract of cattle and faeces dry matter per cattle per day.

Retention time (h)	Sex	Percentage of germinated seeds (%)	Mean germination time (day)	Germination speed (seed/day)	Faeces dry matter (g)
24	Young bull	0 ^{Bd1}	0 ^{Bd}	0 ^{Be}	214.39 ^{Ad}
	Heifer	50.00 ^{Aa}	7.00 ^{Aa}	1.08 ^{Ac}	55.96 ^{Be}
	Mean	25.00	3.50	0.54	135.17
48	Young bull	55.00 ^{Aa}	6.64 ^{Aa}	4.74 ^{Aa}	108.66 ^{Ac}
	Heifer	40.67 ^{Bb}	7.22 ^{Aa}	3.74 ^{Bb}	108.36 ^{Ac}
	Mean	47.83	6.93	4.24	108.51
72	Young bull	44.33 ^{Ab}	5.75 ^{Ab}	0.87 ^{AcD}	221.35 ^{Ad}
	Heifer	42.67 ^{Ab}	6.16 ^{Ab}	1.03 ^{Ac}	216.73 ^{Ad}
	Mean	43.50	5.95	0.95	219.04
96	Young bull	33.00 ^{Bc}	7.00 ^{Aa}	0.08 ^{Be}	284.87 ^{Bb}
	Heifer	50.00 ^{Aa}	2.00 ^{Bc}	0.50 ^{Ad}	348.00 ^{Aa}
	Mean	41.50	4.50	0.29	316.43
Overall mean		45.09	5.96	1.72	194.79

¹For the same column, means followed by the same lower letter are not significantly different ($p < 0.05$). Within the same column and for the same retention time, means followed by the same upper letter are not significantly different ($p < 0.05$).

Table 3. Characteristics of seeds germination for the various other pre-planting seeds treatments.

Treatment	Germinable seeds (%)	Hard seeds (%)	Rotten seeds (%)	Mean germination time (day)	Germination speed (seed/day)
0 min	31.00 ^{f1}	53.00 ^a	16.00 ^c	3.59 ^a	2.44 ^c
Sand paper	96.00 ^a	0.00 ^d	4.00 ^d	1.75 ^b	13.26 ^a
2 min	68.00 ^c	17.00 ^b	15.00 ^c	4.87 ^a	5.06 ^b
4 min	79.00 ^b	9.00 ^c	12.00 ^{cd}	4.11 ^a	6.53 ^b
6 min	60.00 ^{cd}	10.00 ^c	30.00 ^b	3.82 ^a	5.31 ^b
8 min	55.00 ^d	12.00 ^{bc}	33.00 ^b	4.67 ^a	2.84 ^c
10 min	43.00 ^e	8.00 ^{c2}	49.00 ^a	4.64 ^a	2.01 ^c
Overall mean	61.71	15.57	22.71	3.92	5.35

¹For the same column, means followed by the same lower letter are not significantly different ($p < 0.05$).

of germination (96.00%) and germination speed (13.26 seeds/day). In contrast, it had the lowest mean germination time values (1.75 day). This method was followed by seeds immersed in hot water for 2 to 4 min and seeds ingested by cattle (Table 2 and Table 3). The lowest percentage of germination (31.00%), were recorded in control (T0), the highest mean germination time (5.96 days) and the lowest germination speed (1.72 seed/day) values were recorded in seeds ingested by cattle (Table 2 and Table 3).

DISCUSSION

The investigation involving three complementary activities were conducted to facilitate the improvement of degraded grasslands in Benin. The first part of the investigation

examined the survival and germination of *C. pubescens* seeds fed to penned cattle.

Since the seeds were supplied directly into the esophagus of cattle, they were not exposed to chewing during ingestion. Seeds therefore suffered no mechanical damage during ingestion but could be ruminated later (Gardener et al., 1993a). Seeds are damaged by masticating (Ozer, 1979). Also, the chewing time during rumination is much greater compared to chewing time during ingestion (Minson, 1990; Babatoundé, 2005). So, the percentage of seeds recovered from faeces is likely to be greater than when seeds are fed to animals (Gardener et al., 1993a). However, the influence should be small because much of the damage to ingested seeds occurs in the rumen and some in the abomasum (Simao et al., 1987). The average number of seeds recovered from the cattle at the end of 96 h represented 7.65% of

Table 4. Distribution of germinating, hard and rotten seeds after excreted seeds in 1000g DM faeces per day.

Retention time (h)	Sex	Excreted seeds in 1000 g DM	Germinable seeds	Hard seeds	Rotten seeds
24	Young bull	0.00 ^{Be}	0.00 ^{Bd}	0.00 ^{Bh}	0.00 ^{Bb}
	Heifer	97.00 ^{Ac1}	48.52 ^{Ac}	27.33 ^{Ae}	21.16 ^{Ab}
	Mean	48.50	24.26	13.66	10.58
48	Young bull	349.20 ^{Aa}	192.20 ^{Aa}	94.33 ^{Bb}	62.65 ^{Aa}
	Heifer	334.30 ^{Aa}	135.75 ^{Bb}	140.67 ^{Aa}	57.90 ^{Aa}
	Mean	341.80	164.00	117.50	60.28
72	Young bull	117.20 ^{Bc}	52.08 ^{Ac}	59.67 ^{Bd}	5.49 ^{Ab}
	Heifer	157.30 ^{Ab}	67.18 ^{Ac}	83.68 ^{Ac}	6.43 ^{Ab}
	Mean	137.30	59.63	71.67	5.95
96	Young bull	26.20 ^{Ad}	8.72 ^{Ad}	14.67 ^{Af}	2.81 ^{Ab}
	Heifer	16.80 ^{Ade}	8.43 ^{Ad}	7.68 ^{Bg}	0.73 ^{Ab}
	Mean	21.52	8.57	11.17	1.77
Overall mean		156.80	73.27	61.14	22.45

¹For the same column, means followed by the same lower letter are not significantly different ($p < 0.05$). Within the same column and for the same retention time, means followed by the same upper letter are not significantly different at ($p < 0.05$) 0: In these time periods no seeds were recovered.

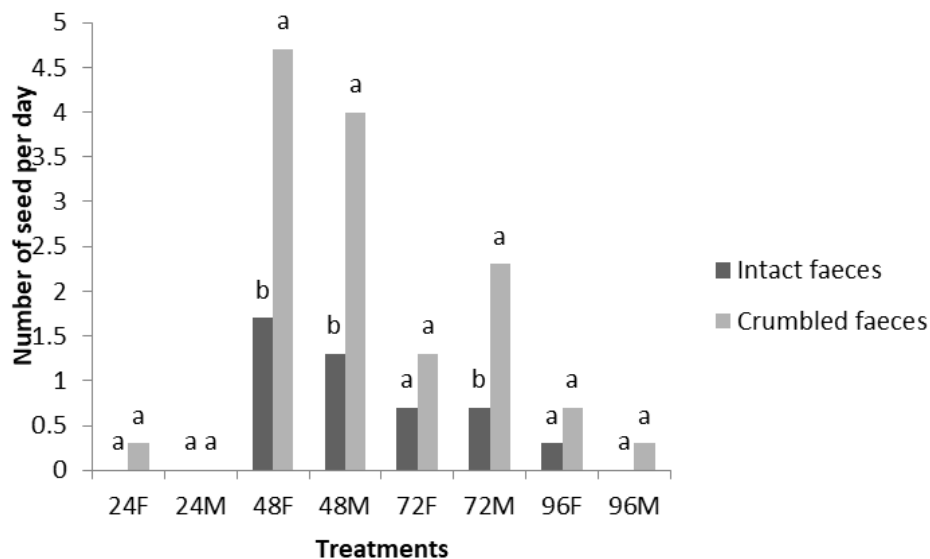


Figure 3. Number of *C. pubescens* seedlings per cattle (male (M) or female (F)) emerged from intact and crumbled faeces on the soil surface collected after 24, 48, 72 and 96 h after ingestion. *Different letters indicate significant differences among number of *C. pubescens* seedlings per cattle emerged from intact and crumbled faeces on the soil surface collected after 24, 48, 72 and 96 h after ingestion (T-test; $p = 0.001$).

the number fed. This confirms the result of Gardener et al. (1993b) that the fraction of rotten *C. pubescens* seeds is 89% after digestion by cattle. Cattle digested

considerably higher amounts of *C. pubescens* seeds.

Overall germination percentage of seeds recovered from faeces (45.09%) was significantly higher ($P < 0.05$)

compared to untreated seeds (31.00%) (Table 2 and Figure 2). This is in agreement with previous studies on frugivores gut treatment (Traveset, 1998; Razanamandranto et al., 2004). Before passage through the cattle the proportion of hard seeds (53.00%) in seed lot was significantly ($P < 0.05$) higher than the amount (38.99%) after passage through cattle tract (Tables 3 and 4). Conversely, the amount of germinable seeds before passage through cattle tract was lower than that after passage (Tables 3 and 4). The mechanisms by which the digestive system stimulate germination could be through the separation of the seed from the shell, softening and scarification of the seed coat through mastication or action of acids and enzymes in saliva and stomach, action of faecal material present in the dung (moistening and fertilizer) (Traveset and Verdu, 2002). However anthelmintic applied may impact negatively seeds germination not only indirectly (reduced breakdown of faeces by dung beetles) but also directly through toxic effects (Eichberg et al., 2016). Also, the quantities of germinable and hard seeds recovered depended on the hard seeds content of the untreated seeds and the fraction of the hard seeds breaking down in the digestive system (Gardener et al., 1993a). The majority of the hard seeds of *C. pubescens* softened in the digestive tract. The breakdown of hard seeds followed by digestion explains the poor percentage of seeds recovered from faeces at the end of 96 hours (Gardener et al., 1993a). However, the results indicate that significant quantities of *C. pubescens* seeds can pass through the digestive tract of grazing cattle intact and almost 46.73% of these seeds will germinate within a month of wetting. These confirm the results of Lamphrey (1967), Souza and Júlio (2001) and Simão Neto and Jones (1987) who reported that, hard seeds often pass through the digestive tract of ruminants without being damaged. The survival of *C. pubescens* seeds after ingestion would serve two purposes: first, provide a cheap method of dispersing seeds and, second provide access to otherwise inaccessible land (Barrow and Havstad, 1992).

Seed retention time in the digestive tract is another important factor that affects the germination of seeds of some plant species (Traveset, 1998; Souza and Júlio, 2001). The number of seeds recovered after 72 h represented more than 91.00% of total seeds recovery. Barrow and Havstad (1992) found that about 95% of the recovered seeds of gelatin-encapsulated seeds fed to cattle passed through the steers within 72 h. It is generally believed that the longer the retention time, the more the seed coat will be exposed to mechanical abrasions, action of acids and enzymes and the better will be the germination of egested seeds (Razanamandranto et al., 2004). By contrast our result shows that the percentage of germinated seeds of *C. pubescens* did not increase, as the retention time increased from 48 h (47.83%) to 72 h (43.50%) (Table 2)

which agrees with previous studies (Gardener et al., 1993a). This result is probably due to the fact that an increasing time of seed retention in the digestive tract had lowered fraction of germinable seeds and increased fraction of hard seeds (Table 4). The possible explanation could be that mechanical or chemical scarification of seeds with a long residence time in digestive tract may have allowed acids and enzymes to diffuse through the seed coat into the inner tissue, which eventually resulted in death of the embryo (Doucette et al., 2001). Some *C. pubescens* seedlings were observed in dung collected 48 to 72 h after injection. Seeds retention in the digestive tract of cattle for longer time may also induce germination during the digestive tract, followed by death of the seedlings (Stiles, 2000).

The total number of germinable seeds from young bull (253.00 seeds) and heifer (259.88 seeds) at the end of 96 h were not significantly different ($p > 0.05$) (Table 4). The hypothesis of a difference in retention time for males and females due to their difference in body size which may affect the germination of seeds was not confirmed (Raymundo et al., 2018; Razanamandranto et al., 2004). According to the present results, both Lagune cattle sexes can potentially favor seeds dispersal of *C. pubescens* as sex did not significantly influence seeds recovery and seeds germination ($p > 0.05$) (Figures 1 and 2).

The second part of the investigation observed the effect of being contained in dung for seedling emergence. Dung collected 2 and 3 days after ingestion of *C. pubescens* seeds had the highest concentration of viable (that is, hard and germinable) seeds. This dung was placed outdoors in a tray and left for 2 months. 100 g of fresh faeces collect each day during trial and broken-down on the soil surface produced an average of 7 plants per cattle, while those left intact on the soil surface produced 2 plants ($p < 0.05$). So coprophageous insects that break-down the dung, trampling by grazing animals or another processes that disperse fresh manure, such as, heavy rain, may allow successful germination and emergence of *C. pubescens* seeds (Eichberg et al., 2016; Mancilla-Leytón et al., 2012). However, when dungs are left intact for some months, the tight structure of dung act as a mechanical barrier and seedling emergence is thought to be unlikely (Grande et al., 2013; Andrews, 1995). Additionally, seedling emergence in *C. pubescens* seeds retrieved from crumbled faeces at 48 h after ingestion was significantly higher ($p < 0.05$) than the numbers of seedlings recovered at other times for all cattle (Figure 3).

The result demonstrates that break-down the dung and day of faeces collection had influence on seedling number. *C. pubescens* seeds are retained in cattle for 3 days and cattle can walk up to 14 km day⁻¹ (Ghassali et al., 1998; Squires, 1981). Seeds can be disseminated over a large area, management of livestock to control

spread of the plant is important. If the aim is to introduce *C. pubescens* to an area by passing seeds through cattle, the animals should be confined on that area for a minimum of 72 h after ingestion of seeds and preferably for at least 96 h to obtain a maximum recovery of seeds (Jolaosho et al., 2006). However, the endozoochorous dispersal involves cost and this cost is a sacrifice in the number of seeds surviving the passage through the digestive tract of cattle (Cosyns et al., 2005). This involves that a large number of seeds must have been consumed to compensate for the generally low seeds recovered and almost 46.73% of these seeds will germinate within a month of wetting.

For the third part of the investigation, the effect of soaking seeds with hot water and mechanical scarification were studied. Generally, it was observed that mechanical scarification was the method that had the highest percentage of germination (96.00%), germination speed (13.26 seeds/day), and the lowest mean germinated times values (1.75 days), followed by seeds immersed in hot water at 80°C for 2 to 4 min and seeds ingested by cattle. Some authors observed that mechanical scarification was the most effective way of breaking dormancy in seeds of *Leucaena leucocephala* and *Chrysophyllum abidum*, respectively (Aduradola et al., 2005; Duguma et al., 1998). Mechanical scarification has a positive effect on breaking dormancy because the damaging of lignified palisade cells after sandpapering permits water and oxygen to enter the cells (Yildiztugay et al., 2012). Mechanical scarification and duration of immersion in hot water affected the amount of germinable and hard seeds ($p < 0.05$), compared with the germinable and hard seeds content of the untreated seeds. In the present study, increasing soaking time, increased the germination percentage and germination speed values and peaked at 4 min, while with longer time of exposure, the values of the two germination indices decreased. The highest of some germination indices of seeds immersed in hot water for 4 min might be attributed to the increased penetration of water and oxygen into the seeds. Gisachew and Scarisbrick (1999) and NFTA (1995) reported that treatment with hot water proved a very useful alternative for increasing the rate of germinable seeds. The highest rotten seeds were recorded in seeds immersed in hot water for 10 min (49.00%). The poor germination rates after immersing the seeds for 6, 8 and 10 min are probably a result of the death of embryo as caused by long time of exposure to hot water. So, a long time of exposure to hot water increased the amount of rotten seeds and decreased the amount of germinable seeds. Rincon et al. (2003) reported that soaking the seeds in hot water induced seeds germination; however, increasing the contact time of the seeds with hot water decreased seeds germination.

Endozoochory by Lagune cattle, mechanical scarification, hot water treatment at 80°C for 2 to 4 min

can potentially favor seeds germination of *C. pubescens* and contribute to the rehabilitation of degraded grassland through enrichment planting. However, little is known about the relative costs and benefits of endozoochorous, mechanical scarification, and hot water treatment at 80°C for 2 to 4 minutes for enrichment planting. To understand from a plant's-eye-view, the role and relative importance of endozoochory compared with mechanical scarification and hot water treatment at 80°C for 2 to 4 min in rehabilitation of degraded grassland in Benin, we need to quantify the relative contribution of different techniques to later generation of the plants (Cosyns et al., 2005). This will require an integrate approach combining information from field observations with data concerning the region where seeds are deposited, in relation to cost of seeds lost from digestion for endozoochorous dispersal and consequences of seeds arriving on native species (Doucette et al., 2001; Gökbülak, 1998).

Conclusion

The findings suggest that both Lagune cattle sexes can potentially favor seeds dispersal of *C. pubescens* as sex did not significantly influence seeds recovery and seeds germination. The average number of seeds recovered from the cattle at the end of 96 h represented 7.65% of the number fed. Overall germination percentage of seeds recovered from faeces significantly increased compared to untreated seeds. The percentage of germinated *C. pubescens* seeds was not positively affected, as the retention time increased from 48 to 72 h. The result also demonstrates that break-down of the dung increased seedling number. It was observed that mechanical scarification was the method that had the highest percentage of germination (96.00%), followed by seeds immersed in hot water for 2 to 4 min at 80°C and seeds ingested by cattle. However, further studies are needed to understand the role, the relative cost and benefits of endozoochorous compared with the use of mechanical scarification using sand paper and hot water treatment at 80°C for 2 to 4 min in rehabilitation of degraded grassland in Benin.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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