



Potential for endozoochorous seed dispersal by sheep and goats: Risk of weed seed transport via animal faeces

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Subject Editor: Diego Batlla

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Abstract

Endozoochory is known as an important mechanism for the spread of weeds. We carried out experiments to assess the fate of seeds of several weed species (*Convolvulus arvensis*, *Cuscuta campestris*, *Rumex crispus*, *Hordeum spontaneum* and *Sorghum halepense*) after passing through the gut of sheep and goat. Eighteen animals of both sheep and goat received diet mixed with seeds of the weed species or control with only wheat bran (five weed species + control × three replications). Results showed that a higher proportion of seeds were missing after passage through the sheep gut than in goats. In goats, a greater proportion of seeds were dead after passage, but the number of seeds collected from dung was also greater. Weed species differed, with the highest seed recovery and viability in *Cuscuta campestris*. Based on time of seed passages through the animal gut estimated for the different weed species, we recommend that sheep should be kept in a corral for 96 hr to minimise seed transportation via their faeces. For goats, if *R. crispus* and *C. arvensis* seeds could be excluded from the diet, then maintaining them for 96 hr in an animal stall would ensure little seed transportation via dung, but we found *R. crispus* and *C. arvensis* seeds to be present and viable in goat dung even 120 hr after feeding. Very large numbers of viable seeds can be found in goat and sheep dung, so the use of rotted manure is highly recommended to avoid transportation of viable seeds via manure fertilisers.

KEY WORDS

gut passage, seed recovery, seed transmission, seed viability, weed species

1 | INTRODUCTION

Seed dispersal plays a crucial role in the 'success' of many weed species. An important effective way of seed dispersal is by animals through both ectozoochorous and endozoochorous modes (Wang et al., 2017). In regions with regular livestock movement, for example in central Asia, zoochory is probably one of the most important mechanisms of seed dispersal (Bläß et al., 2010). Seed dispersal by animals is not only important in rangelands and natural ecosystems, but also in non-grazed ones. Weed seeds can be transported after ingestion by animals and

shed directly in pastures or spread via fresh manure to arable fields (Petit et al., 2013). Manures from sheep, goats, cows and pigs are used as fertilising materials in organic agricultural fields (Issaka et al., 2012). In sampling of 12 milking-cow and heifer barns on six New York farms, 133 thousand weed seeds per kg fresh manure were observed (Pleasant & Schlather, 1994). Sheep can disperse 40 thousand seeds per animal every year (Welch, 1985). Thirty-seven per cent of the local plant species were found as viable seeds in sheep dung (Pakeman, 2001). Seeds of weed species infesting forage production farms are reported to be frequent in daily diet of livestock (Shaheen et al., 2014).

After passage through the animal gut, some seeds are killed, while others may become less dormant and hence more germinable (Baskin & Baskin, 2014). Seed germination response to animal gut can be highly species-specific. In cattle, the estimated time to 50% viability loss after seed intake ranged from 65 hr for *Rumex crispus* L. (curly duck) to 76 hr for *Polygonum aviculare* L. (common knotweed) (Rahimi et al., 2016). In a study by Wang et al. (2017), percentage seed recovery ranged between 12.6% and 17.6% for leguminous species and between 0.8% and 3.2% for gramineous species.

There are great differences among animal species, but also among individuals of the same species, in seed fate through their guts (Cirujeda et al., 2019). One animal may have numerous viable seeds in the pellets, while, another has none (Heady, 1954). Rahimi et al. (2016) showed different cattle types were different in seed recovery and viability.

The size of animal is important in seed dispersal. It was found that larger primates (i.e. those with longer digestive tracts) may be more effective seed dispersers as seeds germinated earlier and seedlings performed better the longer the time they spent in the acid (i.e. stimulated gut; Chac, 2014). Retention time is also positively correlated with animal size; therefore, it is lowest in rabbit gut which is 31 hr while in cattle, sheep and horse it is 71, 41 and 66 hr, respectively (Illius & Gordon, 1992). For goat, the passage time of seeds through the gut ranged from 12 to 96 hr depending on the weed species (Harrington et al., 2011; Wang et al., 2017).

Apart from strong effect of animals and weed species, the interactions between weed species and animals are also important (Bilal, 2015). Fazelian et al. (2014) tested the seed germination of 39 plant species excreted from faeces of cattle, sheep and goat, showing that while seed traits highly determined their fate through animal guts, this could be very interactive with physical and chemical properties of animal digestive tracts.

Convolvulus arvensis L. (field bindweed) (Orloff et al., 2018), *Cuscuta campestris* Yuncker (dodder) (Saric-Krsmanovic et al., 2018), *R. crispus* (Iqbal et al., 2019), *Sorghum halepense* L. Pers. (johnson grass) (Peerzada et al., 2017) and *Hordeum spontaneum* L. (wild barley) (Hosseini et al., 2019) are common grassland weeds and they vary in seed characteristics, such as size, shape and hardness (Hogan

& Phillips, 2011). As their seeds remain viable in dairy manure (Demirhan and Ozyazici, 2019; Liu et al., 2020; Rahimi et al., 2016), restrictions are often imposed when using organic fertiliser containing weed seeds (Mt & Schlather, 1994).

Worldwide, stock numbers have increased in the last 20 years from 1 billion to 1.1 billion for sheep and from 0.4 billion to 0.5 billion for goats (Terrili, <http://www.fao.org/3/ah221e/AH221E01.htm>). These two species are commonly grazed and raised in the same flocks (Yakhchali & Ranjbargarmabolia, 2008). Because of their different body mass and gut traits, they also differ in their potential for endozoochory (Degen, 2007) and, therefore, must be treated differently to control movement of weed seeds through their grazing and in their fresh manure.

In this study, we ask four main questions: (a) how different do the seeds of the common weeds *C. arvensis*, *C. campestris*, *R. crispus*, *S. halepense* and *H. spontaneum* pass through the gut of sheep and goat? (b) what is the duration of the species-specific passage and excretion through the digestive tract? (c) for how long will the seeds of these weeds remain viable in the fresh manure of sheep and goats? (d) based on our results, what can be recommended with regard to the safe movement of sheep and goats to avoid transportation of viable and germinable seeds to new areas?

2 | MATERIALS AND METHODS

2.1 | Seed collection

Intact seeds of *C. arvensis*, *C. campestris*, *S. halepense*, *H. spontaneum* and dry fruits of *R. crispus* were collected from maize (*Zea mays* L.) and alfalfa (*Medicago sativa* L.) fields in Karaj, Iran (35.8400°N, 50.9391°E) in October 2013 and 2015. The collected seeds were air-dried, and waste materials and immature seeds were removed. Undamaged seeds were stored in paper bags at room temperature until start of the experiment (one month at the temperature of 20–23°C). Three replicates of 1,000 seeds from each weed species were weighted to obtain 1,000-Seed weight. The length and width of seeds were also measured using Vernier calliper (Table 1).

TABLE 1 Seed characteristics of the weed species used in the experiment (\pm SE)

Weed species	2013			2015		
	Length (mm)	Width (mm)	1,000-seed weight (g)	Length (mm)	Width (mm)	1,000-seed weight (g)
<i>Convolvulus arvensis</i> L.	3.00 (0.001)	2.00 (0.002)	4.00 (0.280)	3.00 (0.002)	2.00 (0.01)	4.50 (0.560)
<i>Cuscuta campestris</i> Yuncker.	1.50 (0.02)	1.50 (0.001)	0.76 (0.110)	1.80 (0.01)	1.80 (0.02)	0.81 (0.002)
<i>Hordeum spontaneum</i> C. Koch.	13.20 (0.03)	0.60 (0.01)	46.40 (1.360)	12.10 (0.003)	0.45 (0.002)	42.00 (1.050)
<i>Rumex crispus</i> L. ^a	2.50 (0.002)	2.00 (0.001)	2.00 (0.280)	2.50 (0.002)	1.90 (0.002)	1.50 (0.043)
<i>Sorghum halepense</i> L.	4.00 (0.001)	1.50 (0.001)	4.61 (0.050)	4.50 (0.001)	2.00 (0.01)	5.10 (0.310)

^aFor *Rumex crispus*, the characteristics of fruits were measured.

2.2 | Experimental design

A repeated measure experiment was conducted in a factorial arrangement of treatments with three replications. The experiment was repeated twice during 2013 and 2015. Experimental factors were animals, comprised of indigenous sheep (*Ovis aries* L.) and goats (*Capra aegagrus hircus*) and weed species as described above. Female sheep and goats with average body weight of, respectively, 44.5 (± 0.39) and 36.9 (± 0.31) kg in 2013 and 45.0 (± 0.25) and 38.5 (± 0.44) kg in 2015 were chosen for the experiments. Eighteen sheep and 18 goats were placed individually in wooden boxes (1.5 m \times 0.7 m) with 1.25 m distance and fed with common diet (Table 2).

Animals were put inside the boxes 24 hr before the experiment to allow them to acclimate to the experimental conditions. Daily amount of sheep and goat food was considered 3 and 1.5 kg (Lacey et al., 1992). Seeds of each weed species were added to the diet in a ratio of 1,500 seeds kg^{-1} diet. Hence, the diet fed to every sheep and goat on the first day only consisted of 4,500 and 2,250 seeds, respectively. Weed species seeds were mixed homogeneously with 200 g wheat bran and then added to each animal's daily diet. Fresh water and salt blocks were also provided ad libitum.

Each animal received diet mixed with one of the weed species seeds or control diet with only wheat bran (seed-free diet) (five weed species + control \times three replicates = 18 animals). The diet was hourly monitored to make sure that all of seeds were eaten. Faeces collection commenced 24 hr after feeding and was continued for 5 days. Daily faecal materials were carefully collected off the floor and the boxes became completely clean (Lacey et al., 1992). At each faeces collection, five subsamples were taken from each animal faeces using a cream scoop. Each subsample represented about 4% to 7% of the total daily output of an individual animal, as the bulk density of the dung was not the same over time (Lacey et al., 1992). Faeces were washed with faucet water through a wire sieve (80 mesh) until the water was clear. Hard faeces were immersed in cold water until soft (Tjelele et al., 2012). A sifter (60 mesh) was used to separate seeds from faecal remains. Undamaged seeds derived from

daily faeces were counted to determine seed recovery for each day. Seeds were then stored in plastic bags in a cool dry place for further testing (Tjelele et al., 2012). On average, more than 60 seeds were recovered per sample for sheep and more than 38 seeds for goats. In order to standardise the tests, we used 30 seeds for each germination test that were placed in a Petri dish.

2.3 | Viability and germination testing

Seeds before feeding to animals and after recovery from each animal were subjected to germination and viability tests. Germination test was conducted in a germination chamber. Seeds of *C. arvensis* and *S. halepense* were maintained at 30°C in darkness; *C. campestris* seeds were maintained at the same temperature, but exposed to 8 hr daily light (Benvenuti et al., 2005) and *H. spontaneum* and *R. crispus* seeds were exposed to 25°C and darkness for germination testing (Taylorson & Hendricks, 1972). From each replicate, 30 seeds of each weed species were put in 8 cm glass dishes that had one disc of germination paper and 10 ml of distilled water. Germination was counted daily based on radicle protrusion (2 mm) for 14 days. The tetrazolium test was used to determine viable seeds. For this, non-germinated seeds were first pricked or scratched to break any hard seed coats and then sectioned to expose the embryo and stained with 2,3,5-triphenyl tetrazolium chloride solution in distilled water with pH 7 at 20°C in darkness. After 48 hr, seeds whose embryos had stained red and had firm flesh were classified as viable. Non-germinated seeds with complete embryo colouring were thus considered to be dormant seeds. At any time after feeding (TAF), the total number of viable seeds comprised the number of recovered seeds that germinated and the number that were determined as dormant.

2.4 | Statistical analyses

A combined analysis was performed to data; as the effect of year was significant, therefore the data of each experiment (year) were analysed and shown separately. The effects of animal type, weed species and TAF on recovery, viability and germinability of ingested seeds were subjected to mixed effects analysis that was performed in R-studio using package nlme (Pinheiro et al., 2020). Percentage data were arcsine transformed before analysis to meet the normality assumption for the analysis of variance (Zar, 2014). For statistical comparisons, we used protected least significant difference (LSD). The interactions were analysed and presented using non-linear regression. SigmaPlot (12.5) was used for non-linear regression analysis. Seed recovery (%) in sheep and viability (%) in both sheep and goat over TAF were described using a logistic model as follows:

$$y = \frac{a}{1 + \left(\frac{\text{TAF}}{T_{50}}\right)^b} \quad (1)$$

TABLE 2 Ingredients of the diet of sheep and goats

Diet materials	Diet amount (g)	
	Sheep	Goat
Wheat straw	470	240
Alfalfa	360	190
Barley	450	215
Beet pulp	110	50
Wheat bran ^a	120	62
Mineral and vitamin	20	11
Salt	10	6
Corn silage	1,500	780
Total	3,040	1,554

^aIn addition to these amounts, 200 g wheat bran was mixed with the seeds before adding seeds to the daily diet of each animal.

where y represents seed recovery or viability (%), a is the maximum seed recovery or viability (%) that occurs with initial extraction time, T_{50} denotes the time for 50% reduction in a , and b is the slope at the linear part of the curve (rate of seed recovery or viability decrease over TAF).

The percentage of germination over TAF for both sheep and goat and recovery for goat were described with a Gaussian model as follows:

$$y = y_0 + c * \exp\left(-0.5 \left(\frac{TAF - x_0}{d}\right)^2\right) \quad (2)$$

where y is seed recovery or germination (%), c is the maximum seed recovery or germination (%), x_0 is the time at which the c value occurs, y_0 is seed germination or recovery at first assessment (24 hr after feeding) and d is the standard deviation of y .

Model fit was assessed using the lack of fit test, root mean square of error (RMSE), adjusted R -squared (R_{adj}^2) and the standard error of parameters.

3 | RESULTS

The seed viability and germination of each weed species at the beginning of the experiment are shown in Table 3. Seeds of *H. spontaneum* were completely lost through the digestive tract of both sheep and goats and no seeds were recovered from the faeces samples over TAF. For the remaining weed species, mixed effect analysis indicated significant interactions between animal type, weed species and time (TAF) on seed recovery, viability and germination percentage ($p \leq 0.05$). Therefore, interactions were further described using non-linear regression.

3.1 | Comparison between sheep and goat

Figure 1 shows an overall comparison of recovery, viability and germinability of weed seeds in sheep and goat dung after 120 hr. Averaged over both years, a significantly higher number of seeds were missed in sheep than goat guts (Figure 1). However, per cent

of dead and dormant seeds were higher in goat dung. Also, seeds extracted from goat faeces were more germinable than those obtained from sheep dung. The per cent dead, viable and germinable seeds differed between weed species, as in sheep, a higher per cent of the recovered seeds of *C. campestris* and *R. crispus* were dormant, while for other weed species the ratios were approximately the same. In goat, a high per cent of *C. campestris* seeds were dormant.

Averaged over years and weed species, sheep dung contained 19% of the fed seeds, of which 4.25% (standard error = 1.32) were dead, 11% (4.3) were dormant and 3.62% (1.38) were germinable. Goat dung contained 33% of the fed seeds of which 14% (6.35) were dead, 22% (5.19) were dormant and 7% (2.3) were germinable. Therefore, a significantly higher number of seeds were recovered from goat dung ($p < 0.05$), with a higher percentage of dead, dormant and germinable seeds ($p < 0.05$, with LSD values for dead, dormant and germinable seeds of 4.2, 4.5 and 1.4, respectively).

Averaged over all weed species, seed recovery was significantly lower in 2013 than in 2015 ($p < 0.01$, $LSD = 4.7$), with the recovery of 14.8% (2) and 23% (3.4) of seeds for sheep and 32.3% (3) and 54% (4.1) for goats in 2013 and 2015, respectively.

3.2 | Seed fate through the sheep gut

3.2.1 | Seed recovery over TAF

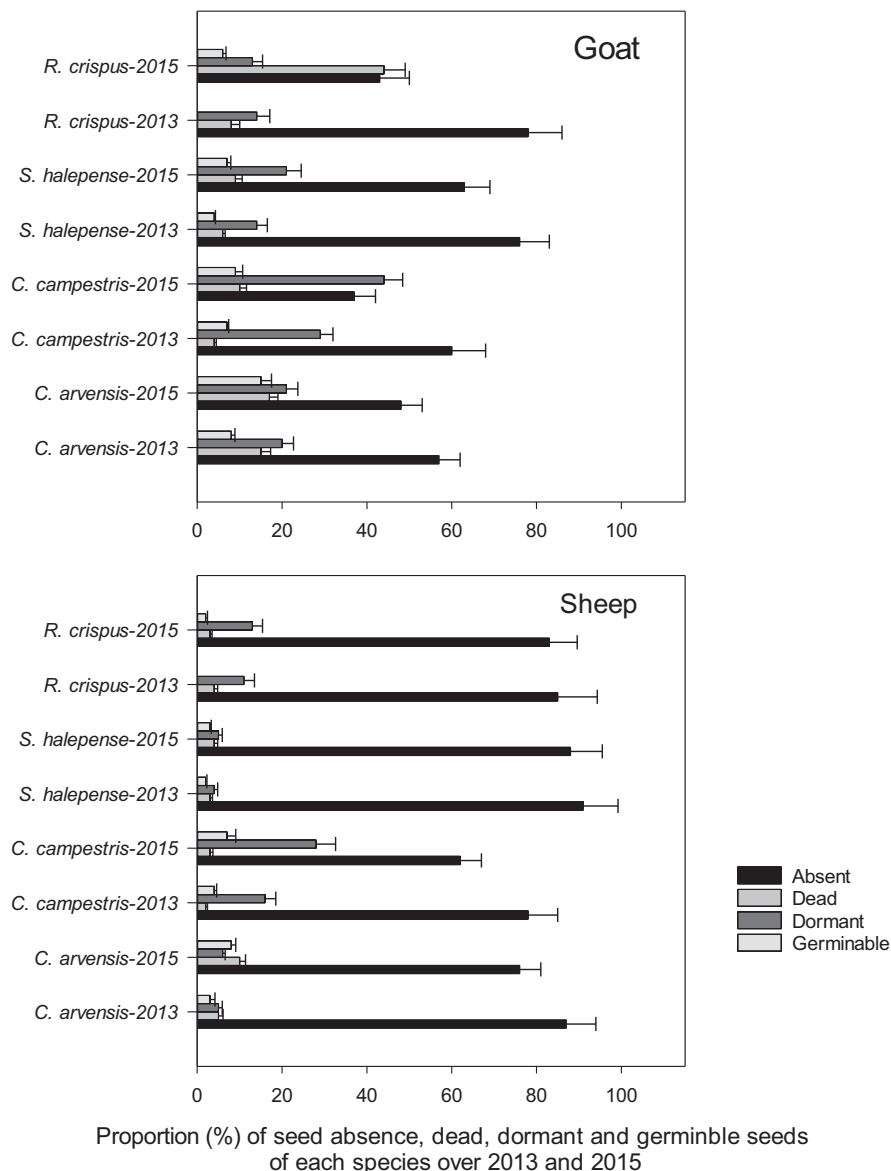
For both years, *C. campestris* showed the highest seed recovery (Table 4). In general, seed recovery was higher at the beginning and decreased with TAF. For example, in *C. campestris* with increasing TAF from 24 to 120 hr, seed recovery decreased from 10% (± 2.0) to 0.67% (± 0.2) in 2013, and from 16.50% (± 3.4) to 1% (± 0.1) in 2015 (Figure 2). The decreasing trend of seed recovery (%) over TAF was described using Equation 1.

The seed recovery of *C. arvensis* was initially (TAF = 24 hr) higher than *S. halepense* and *R. crispus* (Table 4). In 2013, 48 hr after feeding, the three weed species had the same amount of seed recovery, while in 2015, *C. arvensis* showed higher seed recovery than *S. halepense* and *R. crispus* with TAF (Figure 2).

Weed species	2013		2015	
	Viability (%)	Germination (%)	Viability (%)	Germination (%)
<i>Convolvulus arvensis</i> L.	100.0 (0.00)	7.0 (1.70)	98.8 (0.00)	6.0 (2.30)
<i>Cuscuta campestris</i> Yuncker.	98.6 (0.01)	13.0 (2.00)	99.8 (0.01)	10.0 (3.50)
<i>Hordeum spontaneum</i> C. Koch.	100.0 (0.00)	19.0 (5.30)	100.0 (0.00)	17.0 (4.30)
<i>Rumex crispus</i> L.	100.0 (0.00)	8.0 (1.40)	100.0 (0.00)	12.0 (4.00)
<i>Sorghum halepense</i> L.	99.8 (0.00)	4.5 (0.80)	100.0 (0.00)	3.0 (0.50)

TABLE 3 Initial weed seed viability and germination at the beginning of the experiments ($\pm SE$)

FIGURE 1 Proportion (%) of seed absence, dead, dormant and germinable seeds in sheep and goat faeces after 120 hr. Different weed species for 2 years (2013 and 2015) are included. Bars show the standard error



3.2.2 | Seed viability over TAF

Seeds viability over TAF was well described with logistic model equations (Equation 1). In both years, *C. campestris* seeds showed high levels of viability through the experiment. Viability of seeds collected 24 hr after feeding was between 96 (± 4.4) and 100%, but decreased to 83% after 120 hr (Figure 2). *Rumex crispus* seeds were also highly viable, while *C. arvensis* seeds showed on average the least viability of all weed species studied. *Sorghum halepense* seeds showed also a low viability, as in 2015, the seeds extracted 120 hr after feeding had viability less than 20%.

3.2.3 | Seed germination over TAF

Germination (%) over TAF significantly differed among weed species. *Rumex crispus* seeds showed no germination in 2013 and less than 7% in 2015, with no visible trend over time. With 24 hr after feeding, the highest germinable seeds belonged to *S. halepense* with 23% (± 3.6)

and 16.5% (± 2.0) germination in 2013 and 2015, respectively; however, those severely decreased with TAF (Figure 2). Germination of *C. arvensis* and *C. campestris* seeds were low at the beginning and latest samplings, while showed a significant rise in middle time, therefore, a Gaussian model was used to describe changes in seed germination over TAF (Figure 2). However, the peak time was different between *C. arvensis* and *C. campestris* (parameter x_0 in Table 4). According to model estimates, for *C. campestris*, the faeces samples collected 85–90 hr TAF contained highest germinable seeds, while for *C. arvensis* the peak times were between 51 and 62 hr after feeding (Table 4).

3.3 | Seed fate through the goat gut

3.3.1 | Seed recovery over TAF

Seed recovery from goat faeces showed different trends from sheep. As for all weed species, the seed recovery over TAF was peak type and well

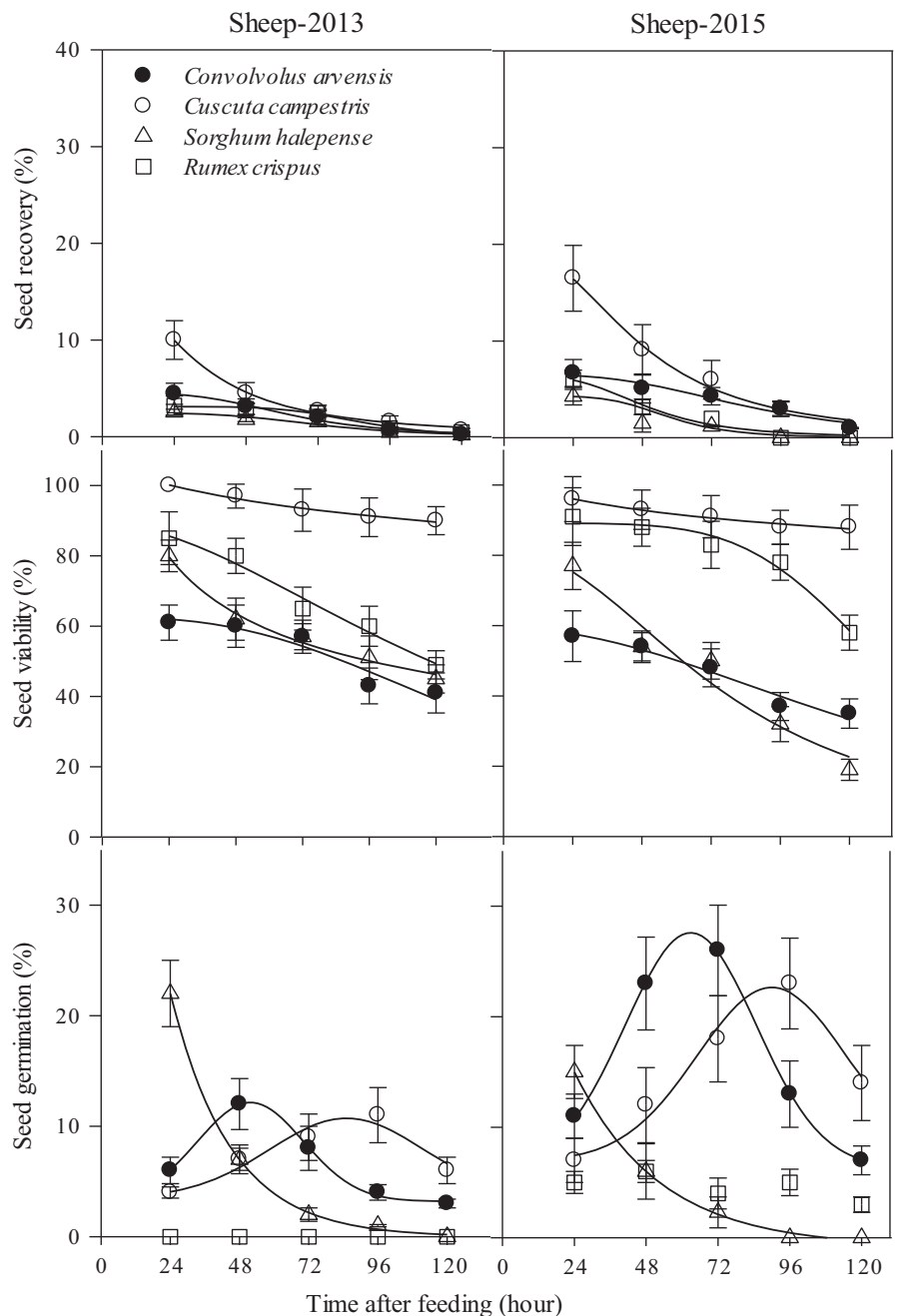
TABLE 4 Parameter estimates of fitting non-linear models (Equations 1 and 2) to proportion (%) of seed recovery, viability and germination over TAF for seeds extracted from sheep faeces (\pm SE)

Weed Species		Parameter estimates																
		Equation 1					Equation 2											
		b	a	T ₅₀	y ₀	c	x ₀	d	R ² _{adj}	RMSE								
2013	2015	2013	2015	2013	2015	2013	2015	2013	2015	2013	2015	2013	2015					
Seed recovery	<i>Convolvulus arvensis</i>	3.65 (0.8)	3.20 (1.2)	4.50 (0.4)	6.50 (0.8)	63.02 (5.1)	84.00 (10.0)	-	-	-	-	-	-	0.97	0.90	0.28	0.67	
	<i>Cuscuta campestris</i>	2.00 (0.3)	2.40 (0.5)	16.32 (4.3)	19.61 (3.2)	30.12 (8.8)	46.80 (8.9)	-	-	-	-	-	-	0.99	0.98	0.30	0.90	
	<i>Rumex crispus</i>	6.30 (1.4)	5.11 (1.0)	3.12 (0.2)	4.35 (0.3)	85.60 (3.7)	57.22 (3.0)	-	-	-	-	-	-	0.97	0.98	0.20	0.25	
	<i>Sorghum halepense</i>	4.10 (1.9)	3.50 (1.5)	2.42 (0.4)	6.31 (1.1)	73.10 (10.4)	49.80 (0.8)	-	-	-	-	-	-	0.89	0.93	0.33	0.65	
	<i>Convolvulus arvensis</i>	2.68 (1.3)	2.10 (0.7)	62.45 (4.2)	59.00 (4.0)	145.55 (20.3)	136.00 (12.0)	-	-	-	-	-	-	0.84	0.93	3.78	2.60	
Seed viability	<i>Cuscuta campestris</i>	2.71 (1.3)	0.40 (0.1)	100.00 (0.0)	96.00 (12.0)	-	-	-	-	-	-	-	-	0.85	0.94	3.70	0.81	
	<i>Rumex crispus</i>	1.80 (0.5)	4.90 (1.2)	89.00 (5.7)	89.00 (2.0)	134.00 (10.0)	136.00 (6.0)	-	-	-	-	-	-	0.95	0.95	2.30	2.73	
	<i>Sorghum halepense</i>	0.40 (0.2)	2.10 (0.9)	100.00 (0.0)	81.00 (13.0)	11.00 (1.7)	77.00 (16.0)	-	-	-	-	-	-	0.97	0.90	1.70	6.70	
	<i>Convolvulus arvensis</i>	-	-	-	-	-	-	3.22 (0.3)	6.40 (0.5)	8.90 (0.5)	21.21 (0.6)	51.83 (1.0)	62.91 (0.4)	18.38 (1.2)	0.98	0.99	0.39	0.40
	<i>Cuscuta campestris</i>	-	-	-	-	-	-	3.50 (1.6)	7.00 (2.5)	7.16 (2.7)	15.65 (2.7)	85.13 (6.0)	90.00 (3.3)	26.73 (3.0)	0.80	0.89	1.50	1.92
Seed germination	<i>Rumex crispus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	<i>Sorghum halepense</i>	-	-	-	-	-	-	-	23.12 (3.6)	16.50 (2.0)	47.31 (7.1)	51.00 (3.1)	4.00 (0.2)	0.99	0.99	0.30	0.60	

Note: Adjusted R-squared and RMSE of model fit are also included.

b, the slope at the linear part of the curve (rate of seed recovery or viability decrease over TAF); a, the maximum seed recovery (%) or viability (%); T₅₀, the time for 50% reduction in a; y₀, initial seed germination (%) that occurs with initial extraction time; c, the maximum seed germination (%); x₀, the time at which the c value occurs; d, the standard deviation of seed germination (%) over TAF for sheep; RMSE, root mean square of error; R²_{adj}: adjusted R-squared.

FIGURE 2 Change in seed recovery (%), seed viability (%) and seed germination (%) with TAF for sheep and different weed species. The fitted lines are model predictions. Bars show the standard error of means



described by Gaussian model (Figure 3). *Cuscuta campestris* seeds were the most abundant seeds found in goat dung (Table 5). For both years, the peak seed recovery of *C. arvensis*, *C. campestris*, and *S. halepense* occurred between $40 (\pm 1.4)$ and $49 (\pm 0.6)$ h after feeding. For *R. crispus*, the peak of seed recovery was estimated to occur $81 (\pm 2.9)$ h after feeding (Table 5).

3.3.2 | Seed viability over TAF

The viability of seeds found in goat dung decreased with TAF. The change in seed viability (%) was well described using a logistic model (Equation 1; Figure 3), however, parameter estimates suggested significant difference between weed species. *Cuscuta campestris* showed highest viability (100%) with early and middle TAF, however, with

96 hr after feeding decreased down to $70\% (\pm 5.0)$ and $56\% (\pm 3.6)$ in 2013 and 2015, respectively (Figure 3). *Rumex crispus* seeds also maintained their viability until 70 hr after feeding then decreased. For *S. halepense*, maximum viability was $84\% (\pm 0.6)$ observed in 2013 (Table 5). A rapid decline in the viability of *S. halepense* seeds occurred from 40 and 70 hr after feeding, respectively for 2013 and 2015 (Table 5; Figure 3). *Convolvulus arvensis* seeds found 24 hr after feeding had a viability of 60%, suffering a 10% decrease over TAF.

3.3.3 | Seed germination over TAF

Germination of *R. crispus* seeds was lower than 2% and 7%, respectively for 2013 and 2015. Germination of the other weeds followed

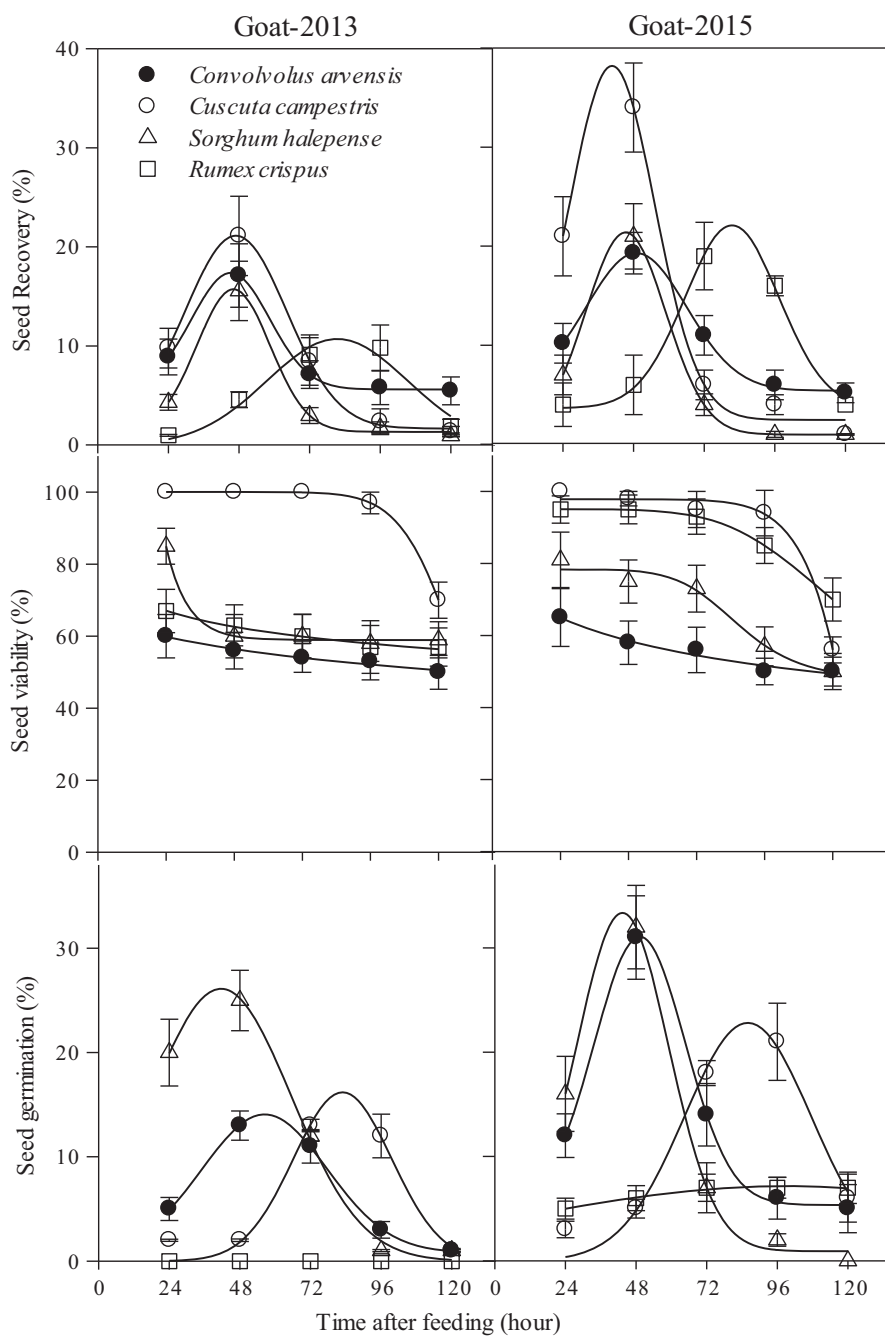


FIGURE 3 Change in seed recovery (%), seed viability (%) and seed germination (%) with TAF for goat and different weed species. The fitted lines are model predictions. Bars show the standard error of means

a peak trend over TAF (Figure 3). Goat dung collected between 38 and 85 hr after feeding contained the highest number of germinable seeds (Figure 3). *Sorghum halepense* seeds were the most frequent germinable seeds in goat dung and its peak time was estimated to be 38 (± 0.6) hr after feeding. The peak time for *C. arvensis* was estimated 82 (± 1.8) hr after feeding and occurred shortly after that for *S. halepense* with 56 (± 0.3) hr after feeding (Table 5).

4 | DISCUSSION

The fate of plant seeds following consumption by animals differs among weed species. Even congeneric plants often show little

consistency in their response because of variations in seed morphology or age (Kuiters & Huiskes, 2010). The current study shows the differential fate of seed recovery, viability and germination after passing through the animal gut. Seed recovery from digestive tracts depends on features such as size, shape, mass, hardness and age of ingested seeds (Gardener et al., 1993; Kuiters & Huiskes, 2010; Wang et al., 2017).

Animal species also play an important role in seed dispersal by endozoochory. Sheep and goats are closely related and both belong to the subfamily Caprinae. However, there are differences in their anatomy, physiology and feeding behaviour. Sheep are grazing animals while goats are essentially browsing (Devendra, 1990). Grazers and browsers have measurable differences in the morphology of the

TABLE 5 Parameter estimates of fitting non-linear models (Equations 1 and 2) to proportion (%) of seed recovery, viability and germination over TAF for seeds extracted from goat faeces (\pm SE)

Parameter estimates		Equation 1												Equation 2											
		Equation 1						Equation 2						Equation 1						Equation 2					
		<i>b</i>	<i>a</i>	<i>T</i> ₅₀	<i>Y</i> ₀	<i>c</i>	<i>x</i> ₀	<i>D</i>	<i>R</i> ² _{adj}	RMSE	<i>b</i>	<i>a</i>	<i>T</i> ₅₀	<i>Y</i> ₀	<i>c</i>	<i>x</i> ₀	<i>D</i>	<i>R</i> ² _{adj}	RMSE						
Weed Species	2013	2015	2013	2015	2013	2015	2013	2015	2013	2015	2013	2015	2013	2015	2013	2015	2013	2015	2013	2015					
Seed recovery																									
<i>Convolvulus arvensis</i>	–	–	–	–	–	–	5.64 (0.2)	5.42 (0.3)	11.73 (0.3)	13.88 (0.4)	45.10 (0.5)	49.00 (0.6)	13.22 (0.4)	17.13 (0.5)	0.99	0.98	0.2	0.34							
<i>Cuscuta campestris</i>	–	–	–	–	–	–	1.63 (0.3)	2.47 (1.5)	19.52 (0.5)	35.73 (3.2)	46.91 (0.5)	40.73 (1.4)	17.33 (0.5)	14.57 (1.9)	0.99	0.96	0.4	2.10							
<i>Rumex crispus</i>	–	–	–	–	–	–	–	3.60 (0.8)	10.73 (1.1)	18.45 (1.2)	81.42 (2.9)	81.41 (0.9)	23.90 (3.0)	15.93 (2.0)	0.91	0.98	1.2	0.81							
<i>Sorghum halepense</i>	–	–	–	–	–	–	1.32 (0.4)	0.98 (0.0)	14.41 (0.7)	20.40 (0.0)	46.12 (1.2)	45.30 (0.0)	12.45 (0.8)	13.63 (0.0)	0.99	0.98	0.56	0.11							
Seed viability																									
<i>Convolvulus arvensis</i>	0.53 (0.4)	0.83 (0.3)	69.20 (14.5)	50.10 (6.0)	140.00 (14.0)	72.10 (7.1)	–	–	–	–	–	–	–	–	0.92	0.87	0.79	1.65							
<i>Cuscuta campestris</i>	11.44 (0.1)	12.92 (3.3)	100.00 (0.0)	97.74 (1.4)	128.90 (2.0)	122.76 (1.3)	–	–	–	–	–	–	–	–	0.98	0.90	0.09	4.10							
<i>Rumex crispus</i>	0.30 (0.0)	5.02 (0.2)	100.00 (0.0)	95.25 (0.2)	271.00 (17.3)	146.88 (1.3)	–	–	–	–	–	–	–	–	0.96	0.99	0.80	0.32							
<i>Sorghum halepense</i>	0.13 (0.0)	2.71 (0.9)	84.00 (0.6)	81.15 (3.8)	25.00 (2.1)	140.17 (11.9)	–	–	–	–	–	–	–	–	0.98	0.98	0.90	0.12							
Seed germination																									
<i>Convolvulus arvensis</i>	–	–	–	–	–	–	0.80 (0.2)	5.30 (0.4)	13.20 (0.2)	25.72 (0.6)	56.39 (0.3)	49.33 (0.5)	21.20 (0.5)	15.39 (0.4)	0.99	0.99	0.18	0.60							
<i>Cuscuta campestris</i>	–	–	–	–	–	–	–	0.90 (0.3)	16.21 (1.9)	32.44 (1.7)	82.86 (1.8)	43.45 (0.9)	16.70 (2.5)	15.67 (1.0)	0.94	0.98	1.40	1.32							
<i>Rumex crispus</i>	–	–	–	–	–	–	–	–	–	7.15 (0.1)	–	98.62 (5.5)	–	88.06 (8.6)	–	0.96	–	0.16							
<i>Sorghum halepense</i>	–	–	–	–	–	–	–	–	31.72 (2.2)	22.81 (1.9)	37.95 (0.6)	86.01 (2.0)	14.40 (1.3)	21.50 (2.2)	0.99	0.94	1.00	2.00							

Note: Adjusted R-squared and RMSE of model fit are also included.

b, the slope at the linear part of the curve (rate of seed viability decrease over TAF); *a*, the maximum seed viability (%) that occurs with initial extraction time; *T*₅₀, the time for 50% reduction in *a*; *Y*₀, initial seed recovery (%) or germination (%) that occurs with initial extraction time; *c*, the maximum seed recovery (%) or germination (%); *x*₀, the time at which the *c* value occurs; *d*, the standard deviation of seed recovery (%) or germination (%) over TAF for goat; RMSE, root mean square of error; *R*²_{adj}: adjusted R-squared.

foregut (rumen, reticulum, abomasums, and omasum), the hindgut, salivary glands, mouth, teeth, liver, and body mass that may influence their ability to consume grasses and browses and its digestion (Odashima et al., 1991). Sheep have significantly longer rumen retention and mean retention times. In contrast, goats have faster passage rate through the digestive tract (Tsiplakou et al., 2011). Shipley (1999) also showed that the gut length of sheep is longer than that of goats. In line with this, we found that a larger proportion of seeds did not pass through the sheep gut, while more seeds were recovered from goat faeces. Goats have a higher fermentation rate in the rumen and also a higher absorption rate (Zoidis et al., 2018). This leads to more damage to seeds and thus, higher dead seeds were found in goat faeces. Moreover, the number of germinable seeds was higher in goat than sheep, but this could also be due to the chewing system in the goat that produces sever scarification to hard seed coats during the passage (Neto et al., 1987). The acids within the tract may have further promoted scarification of some of these hard seed coats (Harrington et al., 2011).

In sheep, the time of recovery was similar for seeds of the different weed species in both years. However, the magnitude of recovery (%) was higher for *C. campestris*, which could be due to the size and shape of *C. campestris* seeds. The average size of the *C. campestris* seeds is about 1.5 mm (the smallest among the seeds studied) with spherical shape. According to Jensen et al. (1995), there might be an inverse relationship between seed size and recovery, as the small seeds are in general less digestible compared to the big seeds and more likely to escape mastication (Gökbulak, 2006). In line with this, we also found an overall trend of decrease of per cent seed recovery from animal dung with increasing seed weight (Figure 4). Seeds of *H. spontaneum* were completely lost through the digestive tract of both sheep and goat over TAF. This could be due to the larger seed size of this species compared to the other seeds. Larger seeds are more likely to get damaged than smaller seeds by chewing

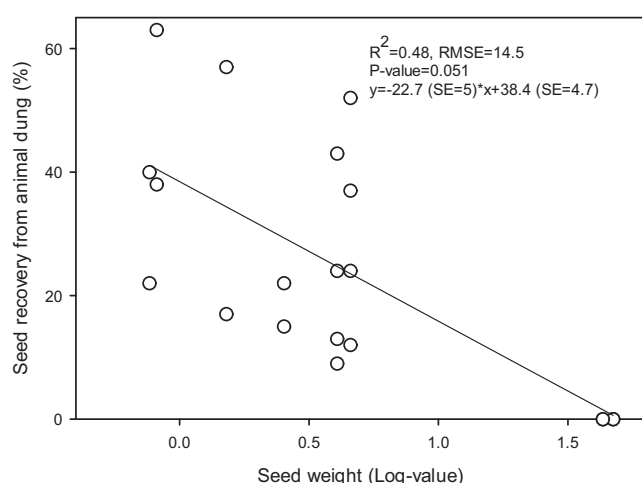


FIGURE 4 Relationship between overall per cent seed recovery from animal dung and seed weight (log-value) (pooled data of sheep and goats). R-square, RMSE value, *p*-value and line equation are shown

during rumination. Harrington et al. (2011) found a negative linear relationship between seed size and the percentage of seeds recovered following passage through goats. In their trial, the two longest seeds had the lowest recovery. However, other studies showed that the percentage of recovery was positively correlated with seed size (Gardener et al., 1993; Wang et al., 2017) and that the shape was the main factor in seeds remaining undamaged. Furthermore, spherical seeds are reported to escape more easily from mastication and also pass the digestive tract more rapidly (Peco et al., 2006), while elongated seeds are more often lost through the animal gut. In line with this, we found that *H. spontaneum*, the species with the most elongated seeds among the weeds studied, was absent in animal dung and showing zero seed recovery, and the recovery was also low for *S. halepense* seeds that are relatively elongated. In contrast, seeds of *C. campestris* and *C. arvensis* are rounder and smaller and were more often recovered as viable seeds in animal dung (Table 1; Figure 1).

Traveset et al. (2008) suggested that in addition to seed size, the hardness of the seed coat is also an important factor in the success of seeds in passing through the digestive tract. *Convolvulus arvensis* seeds are of medium size (Traveset and Verdu, 2002), but classified as having a hard seed coat (Weaver & Riley, 1982). *Cuscuta campestris* seeds also have a hard seed coat (Baskin & Baskin, 2014) and a small size (Traveset and Verdu, 2002). The hard seed coat of these species may thus explain their higher recovery than the other weeds in sheep (Figure 1). In goats, *C. campestris*, *C. arvensis* and *S. halepense* were similar in recovery time, while the peak time for *R. crispus* seeds occurred with more delay (Figure 3). This could be because of the low specific gravity in *R. crispus* seeds (Rahimi et al., 2016). Gardener et al. (1993) found a negative correlation between retention time and specific gravity of seeds. They further reported that less dense seeds had the slowest passage and longest retention time through the digestive tract of cattle.

As shown, *R. crispus* seeds had a low seed recovery (Figures 2 and 3). The *R. crispus* seeds are not classified as hard seeds (Baskin & Baskin, 2014) and they are relatively large, so a large number of *R. crispus* seeds may be damaged or lost during passage through the digestive tract.

Seed dormancy of *C. arvensis* and *C. campestris* is classified as physical dormancy, while *R. crispus* and *S. halepense* are classified to have physiological dormancy (Baskin & Baskin, 2014). We found *Sorghum halepense* seeds to be more germinable, but only in goat faeces. Chemical treatment with H_2SO_4 proved to be an effective method to break seed dormancy in *S. halepense* (Podrug et al., 2014), thus, germination of *S. halepense* seeds is expected to increase after passage through the animal gut.

Impermeable seed coats in *C. campestris* have two palisade layers and only the inner one is impermeable (Lyshede, 1992). This impermeable seed coat in *C. campestris* prevents germination. It has been shown that break of dormancy of *C. campestris* seeds can be reached after 20 min soaking in H_2SO_4 (Lados, 1998). Partial scarification in passing through the digestive tract could further breakdown seed dormancy of *C. arvensis* and *C. campestris*. To remove the innate dormancy of seeds of *R. crispus*, an initial period of low-temperature

stratification is needed (Pye & Andersson, 2009). Therefore, breaking the dormancy of *R. crispus* seeds is not expected to occur through the animal guts (Figure 3).

5 | RECOMMENDATIONS TO AVOID TRANSPORTATION OF VIABLE AND GERMINABLE SEEDS TO NEW AREAS

A higher per cent of seeds is missed through sheep than the goat gut. In goat, a larger number of seeds are recoverable from animal faeces; however, the number of dead seeds is also high. To compare weed species, the *C. campestris* seeds showed the highest seed recovery while no *H. spontaneum* seed was observed in animal dung. The recovered *C. campestris* seeds were mostly viable. In general, seeds viability decreased over TAF.

In sheep, seed recovery decreased in both years to less than 5% after 72 hr, and especially for *R. crispus* and *S. halepense*, very few seeds were recovered after this time point. Viability of *C. campestris* seeds remained above 80% until 120 hr. The viability of the other weeds was also above 40% over 120 hr, except for *S. halepense* that fell to about 20% in the 2015 data. However, after 96 hr, very few seeds remained in sheep dung. Thus, we recommend that sheep should be kept in a corral for 96 hr to minimise seed transportation via their faeces.

For goats, at least 50% of *C. arvensis* and *R. crispus* seeds were recovered during 120 hr in 2015. In 2013, *C. arvensis* seeds showed about 50% recovery. In addition, at least 50% of seeds remained viable after 120 hr. If *R. crispus* and *C. arvensis* seeds could be excluded from the diet of goats, then maintaining them for 96 hr in an animal stall would ensure little seed transportation via goat dung. However, we found *R. crispus* and *C. arvensis* seeds to be present and viable in goat dung even 120 hr after feeding. The total number of viable seeds in animal faeces after 120 hr (at which point more than 10% of seeds remained viable, often a much higher percentage) can be enormous if large numbers of seeds are fed to goat or sheep, especially for some weed species such as *C. campestris* that were still largely viable. Therefore, using rotted manure is highly recommended to avoid transportation of viable seeds in dung via manure fertilisers.

ACKNOWLEDGEMENTS

We wish to thank the Fadak Agro Industry Company for the financial support during the fieldwork.

PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1111/wre.12461>.

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