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Influence of the Ruminant Digestive Process on the Germinability of Range Forage Species

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INFLUENCE OF THE RUMINANT DIGESTIVE PROCESS ON THE GERMINATION OF RANGE FORAGE SPECIES

by

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Ingestion and dispersal of seeds of desirable species by domestic livestock is potentially important as a range improvement practice, but the passage of seed by livestock has only been studied in a fragmented way, particularly for species adapted to rangelands of western North America. The objectives of this research were to examine the effects of different periods of exposure to *in vitro* and *in vivo* digestion processes in cattle on the germinability of several grass species, and determine if the *in vitro* incubation technique is a good predictor of seed fate following passage through the ruminant digestive tract.

Seeds of 13 grass species adapted to the Intermountain West were exposed to *in vitro* incubation for 24, 48, and 72 hours, and then tested for germination at an optimal temperature regime (10°C night/20°C day) in a controlled environment chamber. Germination responses varied

considerably among grass species with changes in length of exposure to *in vitro* incubation, but germination decreased for incubated seed compared to untreated seed for all species.

Five species with the highest germination in *in vitro* incubation trials were fed to Holstein steers in *in vivo* digestion trials. Approximately 20% of the ingested seeds were recovered for all species 6 days after feeding, and the highest recovery occurred 2 and 3 days after feeding. Germination of undamaged, recovered seeds decreased as passage time through the digestive tract increased. Of the species tested, seeds of *Psathyrostachys juncea, Thinopyrum ponticum, Agropyron cristatum* X *A. desertorum*, and *Elytrigia repens* X *Pseudoroegneria spicata* have the greatest potential to survive passage through the digestive tract and germinate in appreciable numbers. The *in vitro* incubation technique may be used as a crude indicator of seed fate following passage through the digestive tract.

(32 pages)

INTRODUCTION

Seed dispersal by wind, water, and animals provides many advantages to plants, including movement of seeds away from the parent plant where animal predation and parental-offspring competition may be intense, and higher probability of placement of seeds in suitable germination microsites (Howe and Smallwood 1982, Collins and Uno 1985). Seed dispersal by animals takes place through a variety of mechanisms, including dispersal by adhesion to the body, dispersal by scattering and caching, and dispersal by passage through the gut (Herrera 1989). Dispersal by adhesion to animal bodies has been reviewed by Sorenson (1986). Smith and Reichman (1984) have reviewed seed caching by birds and mammals.

The ingestion and subsequent passage of viable seeds through animal digestive tracts may be important in the introduction and maintenance of plant species in different ecosystems. Domestic and wild ungulates are responsible for the spread of several woody and herbaceous species in rangeland ecosystems in North America (Heady 1954, Collins and Uno 1985, Brown and Archer 1987), South America (Gutierrez and Armesto 1981), Africa (Lamprey et al. 1974, Tiffin and Kelly 1978), Australia (Harvey 1981), and Europe (Welch 1985). Many herbaceous species, including weedy species, have been introduced from Europe to North America in the gut of large mammals (Janzen 1984). Although seed dispersal by livestock has been acknowledged, there have been relatively few attempts

to use livestock as agents to spread seeds of desirable species to improve rangelands (Archer and Pyke 1991).

There is, however, a growing interest in feeding grazing animals seeds of desirable species or allowing animals to graze areas that produce seeds of desirable species and then release the animals to disperse the ingested seeds on degraded rangelands. Interest is being stimulated by a better understanding of the roles of animals in ecosystem structure and function, and by economic and environmental factors influencing rangeland revegetation programs. Using domestic animals could be less costly and less disruptive than using seeding equipment, especially on areas that have rough surfaces and topography, or desirable resident plant species (Archer and Pyke 1991). This revegetation strategy is applicable in developing countries where people are more familiar with pastoral systems than with mechanical systems.

The ability of seeds to germinate after passing through the animal's digestive tract depends upon several factors, including seed characteristics (size, shape, hardseededness), type of animal (age, body size, mastication, digestion), and diet quality (Piggin 1978, Simao Neto et al. 1987). Larger seeds may pass more slowly than small seeds, especially in small ruminants. This may result in more seed coat damage, but larger seeds withstand more damage than small seeds and still germinate. Elongated seeds tend to pass more slowly than round seeds (Simao Neto et al. 1987). The proportion of

damaged seeds recovered from cattle, sheep, and goats was less for hard-seeded legumes than for soft-seeded grasses (Simao Neto et al. 1987). In many instances, hard-seeded legumes require a scarification treatment to weaken the seed coat to facilitate water entry and gas exchange (Khan 1977, Murray 1984). In general, smaller ruminants have smaller mouthparts that can result in more chewing damage, and they have smaller orifices in their digestive tract that can restrict the flow of large-sized seeds (Poppi et al. 1985, Simao Neto et al. 1987). A higher percentage of ingested seed passed through sheep at a faster rate with a high quality diet (60-70% *in vitro* digestibility) than with a lower quality diet (45% in vitro digestibility) (Jones and Simao Neto 1987).

A potentially significant percentage of seeds ingested by domestic livestock could be deposited in a moist, nutrient-rich medium that may facilitate germination and establishment (Archer and Pyke 1991). Depending on the rate of passage and on animal movement patterns, germinable seeds could be distributed over large areas for several days after ingestion. Several studies have indicated that the seed content in cattle or sheep feces was highest between 48 and 72 hours after ingestion (Burton and Andrews 1948, Ozer 1979, Simao Neto et al. 1987).

Even though the above information demonstrates that seed dispersal by domestic grazing animals is potentially important as a range improvement practice, the passage of desirable

plant seed by animals has only been studied in a fragmented manner, particularly for species adapted to rangelands of western North America. The overall objective of this research was to determine the effects of ruminant digestion processes on the germinability of several grass species adapted to Intermountain West rangelands. Specific objectives were to: 1) examine the effects of different periods of exposure to *in vitro* and *in vivo* digestion processes in cattle on the germinability of seeds of several grass species; and 2) determine if an *in vitro* seed screening technique is a good predictor of seed fate following passage through the ruminant digestive tract.

MATERIALS AND METHODS

In Vitro Study

Plant species

Propagules used in this study were florets (lemma and palea with enclosed caryopsis), but they will be referred to as seeds. Seeds of 13 grass species (scientific and common names in Table 1) were obtained from Soil Conservation Service Plant Materials Centers in Pullman, Washington, and Aberdeen, Idaho, and from Wind River Seed Company in Manderson, Wyoming. Seeds were harvested in 1990 and 1991 and were stored at room temperature (20°C) and 40% relative humidity prior to the initiation of digestibility trials in January 1992. One hundred seeds of each species were characterized for weight, diameter, and length prior to incubation treatments (Table 2).

Incubation treatment

A modified Tilley and Terry technique for *in vitro* dry matter digestion was used to simulate the rumen digestion process in cattle (Moore 1970, Tilley and Terry 1963). Rumen fluid was obtained from one mature Hereford cow fitted with a ruminal cannala. This animal received a standard diet of grass hay (69% *in vivo* digestibility, 7.8% crude protein, 63.6% neutral detergent fiber, and 39.2% acid detergent fiber) for 7 days before collecting rumen fluid. A 1,000 ml sample of rumen fluid was collected at weekly intervals using an indwelling ruminal sampling tube with a suction syringe. This

Table 1. Grass species used in *in vitro* incubation trial.

Scientific Name[1]	Common Name
Agropyron cristatum (L.) Gaertner X *A. desertorum* (Fischer ex Link) Shultes	Crested wheatgrass
Elymus lanceolatus (Scribner & J.G. Smith) Gould	Thickspike wheatgrass
E. trachycaulus (Link) Gould ex Shinners subsp. *trachycaulus*	Slender wheatgrass
Elytrigia repens (L.) Nevski X *Pseudoroegneria spicata* (Pursh) A. Love supsp. *spicata*	Newhy hybrid
Leymus angustifolia (Trin.) Tzvelev.	Altai wildrye
L. cinereus (Schribner & Merrill) A. Love	Basin wildrye
L. x multiflorus (Gould) Barkw.	Beardless wildrye
Oryzopsis hymenoides (Roemer & Schultes) Ricker	Indian ricegrass
Pascopyrum smithii (Rydb.) A. Love	Western wheatgrass
Psathyrostachys juncea (Fisher) Nevski	Russian wildrye
Pseudoroegneria spicata (Pursh) A. Love subsp. *spicata*	Bluebunch wheatgrass
Thinopyrum intermedium subsp. *barbulatum* (Schur) Barkw. & Dewey	Pubescent wheatgrass
T. ponticum (Podp.) Barkw. & Dewey	Tall wheatgrass

[1]Species in the *Triticeae* tribe follow the nomenclature of Barkworth and Dewey (1985).

Table 2. Seed characteristics of the grass species seeds used for *in vitro* study.

Species	weigh (mg)	width (mm)	length (mm)
Agropyron cristatum x A. desertorum	0.31	1.00	07.50
Elymus lanceolatus	0.27	1.10	08.20
E. trachycaulus	0.26	1.20	07.60
Elytrigia repens x Pseudoroegneria spicata	0.35	1.10	05.90
Lemus angustifolia	0.70	1.35	02.50
L. cinereus	0.18	1.70	08.80
L. x multiflorus	0.26	1.00	05.16
Oryzopsis hymenoides	0.38	0.90	04.00
Pascopyrum smithii	0.53	1.40	10.20
Psathyrostachys juncea	0.32	1.30	07.80
Pseudoroegneria spicata	0.33	1.10	07.30
Thinopyrum intermedium	0.35	1.85	09.00
T. ponticum	0.70	1.70	10.20

sample was immediately strained through six layers of cheesecloth. Seeds of each species were subjected to *in vitro* incubation for 24, 48, and 72 hours. Two hundred seeds from each species were placed in a tube along with 0.25 g of ground grass hay forage to maintain rumen microorganism activity. Fifty ml of rumen solution (1 part rumen fluid and 4 parts buffer) were added to each tube. Tubes were placed in a water bath at a constant temperature of 39°C, and air was flushed from the tube by adding CO_2 for 15 seconds. Tubes were agitated three times daily to maintain seeds in the forage-rumen solution. For the last 6 hours of each time period (24, 48, and 72 hours), the rumen solution in each tube was removed and replaced with a 50 ml acid-pepsin solution to simulate

abomasal processes. Tubes were then returned to the water bath. After 6 hours, the acid-pepsin solution was filtered and discarded, and the seeds were washed with distilled water.

Germination test

Fifty undamaged (no visible damage to lemma and palea under 10 x lens) seeds from each tube were placed on moistened (distilled water) filter paper in petri dishes to estimate germinability. Petri dishes were placed in a controlled environmental chamber with a night/day temperature regime of 10/20°C and a 12-hour photoperiod. A light intensity of 400 umol $m^{-2}.s^{-1}$ was maintained during the daylight period. The filter paper substrate in petri dishes was maintained in a saturated state by adding distilled water as needed on a daily basis. Germinated seeds were counted every day over a 21-day period. Grass seeds were considered germinated when the coleoptile had emerged and the radical had elongated to 5 mm (Copeland 1987).

Experimental design and analysis

The experimental design was a completely randomized block design with four replications for each treatment (species-digestion time period). Percentage germination data were arcsine transformed prior to being subjected to analysis of variance ($p<0.05$). Differences between means were assessed by the Least Significant Difference Test ($p<0.05$) (Steel and Torrie 1960).

In Vivo Study

Plant species

Five of the most promising species (*Pseudoroegneria spicata, Agropyron cristatum* X *A. desertorum, Thinopyrum ponticum, Psathyrostachys juncea*, and *Elytrigia repens* X *Pseudoroegneria spicata*) that maintained high germinability in the *in vitro* study were used in the *in vivo* study.

Animals and feeding

Four yearling Holstein steers, with approximate live weights of 300 kg, were adapted to metabolism crate indoors (14-hour light period/10-hour dark period, constant temperature of 10°C) to facilitate accurate collection of feces. All animals were fed a standard grass hay diet (69% *in vivo* digestibility, 7.8% crude protein, 63.6% neutral detergent fiber, and 39.2% acid detergent fiber). A daily intake of 7.3 kg was equally divided between two feeding times, at 0800 hours and 1800 hours. The day before feeding seeds, animals were placed in a metabolism crate at 1600 hours and fasted until 0800 hours the next day. At that time, each steer was fed approximately 60,000 seeds of the same grass specie mixed with dried molasses. After consuming the seeds, steers were given their regular morning ration of grass hay. Animals were kept in metabolism crates for 6 days for collection of feces containing ingested seeds. Animals were then removed from metabolism crates and placed in 10 X 10 m

pens outdoors (temperature range of 0-10°C) for 6 days to allow animals to move about and ensure that no seeds of that grass specie remained in the digestive tract. This procedure was repeated for feeding seeds of each grass specie. Animals had continuous access to water at all times, and access to mineral blocks in the outdoor crate.

Feces collection and seed recovery

Total fecal output was collected daily from each animal in the morning every 24 hours. Fecal samples were collected at the same time during the 6 days of each trial. Daily samples of feces were collected from individual animals, weighed and thoroughly mixed prior to taking subsamples. Two subsamples were taken from each fecal collection. The first subsample (100 g) was dried at 60°C for 72 hours to measure moisture content. The second subsample (1000 g) was washed on different sized screens to recover seeds for the germinability test. Seed separation was performed by using a water separation technique. Recovered seeds were separated by hand (using a 10 x magnifying lens) into two categories: undamaged (seeds not injured in outward appearance) and damaged (seeds injured in outward appearance). The recovery of seeds from each collection was expressed as a percentage of seed input by multiplying the number of seeds recovered per gram of fecal sample from the total fecal output per collection and dividing by the number of seeds ingested (Simao Neto et al. 1987).

Germination tests

Undamaged seeds recovered from cattle feces were tested for germinability by the same procedures used in the *in vitro* study.

Experimental design and data analysis

The experimental design was a split plot, in which the animals were the main plots, the species (seeds) were the subplots, and the days were repeated measurements over time. Percentage seed recovery (damaged, undamaged, and total) and germinability were statistically analyzed after arcsine transformation. Differences between means were assessed by using the Least Significant Difference Test ($p<0.05$) (Steel and Torrie 1960).

RESULTS AND DISCUSSION

In Vitro Study

Germination responses varied considerably among grass species with changes in the length of exposure to *in vitro* incubation (Table 3). *Pascopyrum smithii* and *Oryzopsis hymenoides* seeds did not germinate and *Elymus triticoides* seeds had less than 1% germination after 24, 48, or 72-hours of incubation. *Thinopyrum ponticum* and *Psathyrostachys juncea* had the highest germination (>35%) of the 13 grass species, and this occurred after 48 hours of incubation. Three germination trends were observed for species over the 72-hour incubation period. *Pseudoroegneria spicata* and *Agropyron desertorum* X *A. cristatum* germination responses were similar after 24, 48, and 72 hours of incubation. Germination responses were greater after 48 and 72 hours than 24 hours of incubation for *Thinopyrum ponticum* and *T. intermedium*. Germination was greatest after 48 hours of incubation but declined after 72 hours of incubation for *Elymus lanceolatus*, *Elytrigia repens* X *Pseudoroegneria spicata*, *Leymus angustifolia*, and *Psathyrostachys juncea*.

The literature on the survival and germinability of seeds of warm- and cool-season grass species subjected to ruminant digestive processes (especially *in vitro* incubation) is very limited, and does not clearly identify the mechanisms involved. Simao Neto and Jones (1987) noted that seed germination and viability of two tropical grass species

Table 3. Mean germination (%) for seeds of grass species after 0, 24, 48, and 72 hours *in vitro* incubation treatment.

species	Hours of *in vitro* incubation			
	0	24	48	72
	Germination (%)			
Agropyron cristatum x *A. desertorum*	90.0	22.0aA	18.3aC	21.0aBCA[1]
Elymus lanceolatus	87.0	11.5bCDE	21.3aBC	17.0abBCD
E.trachycaulus	96.0	7.3bEF	10.0abDE	15.3aCDFE
Elytrigia repens x *pseudoroegneria spicata*	88.0	18.5bAB	24.8aB	16.8bCD
Lemus angastifolia	56.0	0.5bG	9.5aE	5.3abFG
L. cinereus	91.0	16.8aABC	7.0bEF	12.3abDE
L. x multiflorus	95.0	0.0aG	0.3aF	0.5aG
Oryzopsis hymenoides	71.0[2]	0.0aG	0.0aF	0.0aG
Pascopyrum smithii	58.0	0.0aG	0.0aF	0.0aG
Psathyrostachys juncea	74.0	21.5bA	35.8aA	18.8bBC
Pseudoroegneria spicata	52.0	10.8aED	16.8aEF	10.3aEF
Thinopyrum ponticum	90.0	1.8bFG	37.3aA	36.0aA
T. intermedium	98.0	10.8bC	15.8bCD	22.8aB

[1]Small letters represent comparisons between treatments within species and capital letters represent comparisons between species within treatment. Values followed by the same letter do not differ significantly at p=0.05.

[2]Mechanically scarified prior to germination testing for 0 hours treatment.

decreased with longer periods of time in *in vitro* and *in sacco* (seeds in nylon bag in rumen of cattle) incubation treatments. Gardiner et al. (1993) observed similar germination responses with four subtropical grasses exposed to *in sacco* digestive treatments for up to 10 days. Several factors may be responsible for the variability in survival and germinability of seeds exposed to increasing periods of *in vitro* incubation in this study. These factors include: the constant 39°C

temperature of the *in vitro* incubation water bath, the reduced oxygen environment in the incubation tubes, and the thickness and permeability of the lemma and palea surrounding the caryopsis.

Germination may have been reduced in some species by exposure to a constant temperature of 39°C. Optimum germination for the majority of cool-season grasses in the Intermountain West, and all of the species used in this study, occurs with night temperatures of 10-20°C alternating with day temperatures of 20-30°C (Evans and Young 1987). The mechanisms by which alternating temperatures act are still poorly understood, but it is known that metabolic processes involved in germination can be modified when seeds are exposed to higher than optimal temperatures in an imbibed state (Mayer and Poljakoff-Mayber 1989).

In vitro incubation simulates, to a certain extent, the anaerobic environment that exists in the reticulum, rumen, and omasum of the ruminant animal (Hofmann 1988). Reducing the oxygen concentration of the atmosphere below 20% can negatively influence the germination of many species (Mayer and Poljakoff-Mayber 1989); however, no gas exchange studies have been performed on the species used in this study.

Seed coverings influence germination and dormancy in many species by regulating gas exchange and the imbibition of water into seeds (Mayer and Poljakoff-Mayber 1989). They may also play a role in protecting the embryo from acids in the

abomasum (Gardiner et al. 1993). The relatively high germination percentages for *Thinopyrum ponticum*, *Psathyrostachys juncea*, *Agropyron cristatum* X *A. desertorum*, *Elymus lanceolatus*, and *Elytrigia repens* X *Pseudoroegneria spicata* (Table 2) may be due, in part, to the presence of a thicker, more protective lemma and palea than on some of the other species.

Of the 13 grass species used in this study, only *Oryzopsis hymenoides* has been investigated in terms of the influence of seed coverings on germination and dormancy. Mechanical dormancy in *Oryzopsis hymenoides* is imposed by an indurate lemma and palea, and it can be virtually eliminated by mechanical or chemical scarification treatments (Jones 1990). Lemma thickness ranged from 42-76 um for several accessions of *O. hymenoides*, and had a significant protective effect on seed vialbility after scarification with concentrated sulfuric acid for 20-40 minutes (Zemetra and Cuany 1984). Exposure to *in vitro* incubation fluids for up to 72 hours was not enough to weaken the lemma and palea and allow germination in this study (Table 3). This indicates that *O. hymenoides* seeds may not be adequately scarified to readily germinate after passing through the ruminant digestive tract.

***In Vivo* Study**

Cumulative seed recovery 6 days after feeding seeds ranged from 18.4 to 24.0% for the five grass species used in the study. The highest recovery for all grass species was on days 2 and 3, after which, recovery decreased significantly (Table 4). Recovery decreased to less than 1% for all species on days 5 and 6. The proportion of damaged to undamaged seed in the recovered seed generally increased with successive days in the digestive tract (Table 4).

Percent recovery of seeds and the proportion of damaged to undamaged seed for the five grass species used in this study followed patterns similar to those reported by Simao Neto et al. (1987), Burton and Andrews (1948), and Yamada and Kawaguchi (1972). In all cases, highest recovery of undamaged, germinable seeds from cattle digestive tracts occurred 2 to 3 days after feeding seeds, and seed recovery declined dramatically after day 4. Simao Neto et al. (1987) concluded that seed length influences total recovery and rate of passage of different tropical grasses in sheep and goats, but not in cattle. The reticulo-omasal orifice in cattle is larger than in sheep, and allows larger particles to pass from the rumen (Poppi et al. 1985). Seed lengths ranged from 5.9 mm (*Elytrigia repens* X *Pseudoroegneria spicata*) to 10.2 mm (*Thinopyrum ponticum*) for grass species in this study, and did not appear to influence total seed recovery. Rumination is stimulated primarily by ingesta particles longer than 10 mm

Table 4. Percent recovery of seeds of grass species in feces, as damaged or undamaged seed.

Species[1]	Days after feeding seeds 1	2	3	4	5	6
			Total			
Pssp	1.4bAB	8.2aC	7.9aB	0.0bA	0.0cA	0.0cA[2]
AgcrXAgde	2.2cA	7.8bC	10.9aA	1.1dA	0.7dA	0.4dA
Thpo	1.2cAB	10.3aB	6.2bC	0.4cdA	0.2cdA	0.1dA
Psju	1.0cB	13.3aA	5.3bC	0.8cA	0.4cA	0.3cA
PsspXElre	1.7cAB	13.6aA	7.5bB	0.7cdA	0.4dA	0.1dA
			Damaged			
Pssp	0.5bB	3.5aC	3.6aB	0.6bAB	0.0cA	0.0cA
AgcrXAgde	0.8bAB	4.7aB	4.5aA	0.8bA	0.5bA	0.4bA
Thpo	0.7cAB	5.1aB	2.6bC	0.3cdB	0.2cdA	0.1dA
Psju	0.4cB	5.2aB	2.2bC	0.4cAB	0.2cA	0.2cA
PsspXElre	1.1cA	5.9aA	3.5bB	0.5dAB	0.2dA	0.1dA
			Undamaged			
Pssp	0.8bAB	4.8aB	4.3aBC	0.3bA	0.0cA	0.0cA
AgcrXAgde	1.4bA	7.1aC	6.4aA	0.3cA	0.2cA	0.0cA
Thpo	0.5cB	5.2aB	3.6bCD	0.2cA	0.1cA	0.0cA
Psju	0.6cB	8.1aA	3.1bD	0.3cA	0.2cA	0.1cA
PsspXElre	0.6cB	7.7aAC	4.0bBC	0.3cA	0.1cA	0.0cA

[1]Pssp= *Pseudoroegneria spicata*, Agcr X Agde= *Agropyron, cristatum* X *A. desertorum*, Thpo= *Thinopyrum ponticum*, Psju= *Psathyrostachys juncea*, Pssp X Elre= *Pseudoroegneria spicata* X *Elytrigia repens*.

[2]Small letters represent comparisons within species across days for each recovery category and capital letters represent comparisons across species within days for each recovery category. Values followed by the same letters do not differ significantly at p=0.05.

(Welch and Hooper 1988); thus the relatively small size of seeds of these grass species may have reduced the potential for mastication damage that would have occurred with

rumination.

Germination of recovered, undamaged seed for all grass species was highest 1 day after feeding seeds, and decreased as the length of time in the digestive tract increased (Table 5). Seeds remaining in the digestive tract for more than 4 days failed to germinate. The *Pseudoroegneria spicata* X *Elytrigia repens* hybrid had the highest germination on all recovery dates, followed closely by *Thinopyrum ponticum*. The *Agropyron cristatum* X *A. desertorum* hybrid and *Psathyrostachys juncea* were intermediate in terms of germination responses, and *Pseudoroegnria spicata* had the lowest germinability, especially 1 and 2 days after feeding seeds.

The trends in germination responses of recovered seed in this study differ from those of previous studies that passed grass and legume seeds through the digestive tracts of cattle and sheep. Simao Neto et al. (1987) observed that germination of passed seed of the tropical grass *Axonopus affinis* was higher than that of the original, untreated seed sample. Seeds of this species are known to have a high hardseed content, and the lemma and palea causing this hardseededness were lost during passage through the digestive tract. Higher germination rates after passage have been noted for other grass species, and several legume species (Ozer 1979, Harvey 1981). Germination responses of untreated seeds of the five cool-season grass species used in this study were 35 to 44% higher than for passed seeds collected 1 day after feeding,

Table 5. Mean germination (%) for seeds of grass species recovered form feces 1 to 6 days after feeding seeds.

	Days after feeding seeds					
Species[1]	1	2	3	4	5	6
	% germination					
Pssp	6.5aC	6.5aD	2.0aC	1.0aA	0.0bA	0.0bA[2]
AgcrXAgde	52.5aA	27.0bB	5.5cC	2.5cA	0.0cA	0.0cA
Thpo	50.5aA	45.0aA	19.0bB	1.0cA	0.0cA	0.0cA
Psju	27.3aB	20.0bC	1.0cC	0.0cA	0.0cA	0.0cA
PsspXElre	53.5aA	46.5bA	26.0cA	5.3dA	0.0dA	0.0dA

[1]Agsp= *Pseudoroegneria spicata,* Agcr X Agde= *Agropyron cristatum* X *A. desertorum*, Thpo= *Thinopyrum ponticum,* Psju= *Psathyrostachys juncea,* Pssp X Elre= *Pseudoroegneria spicata* X *Elytrigia repens*.

[2]Small letters represent comparisons within species across days and capital letters represent comparisons across species within days. Values followed by the same letter do not differ significantly at p=0.05.

and 50 to 85% higher than for passed seeds collected 3 days after feeding. This indicates that lemmas and paleas of these species offered some protection from rumen and acidic fluids in the digestive tract if passage occurred 1 or 2 days after feeding seeds. After lemmas and paleas have been digested (as on damaged seed), rumen fluids and acids released in the abomasum may enter the caryopsis and denature embryo proteins (Gardiner et al. 1993). The effects of high temperatures and anaerobic conditions on seed germination have already been

discussed in the *in vitro* subsection.

Diet quality can have a significant influence on seed passage rate, which influences germinability of passed seed. Jones and Simao Neto (1987) reported that diets of higher quality (digestibilities of 60 to 70%) resulted in the passage of a higher percent of ingested seed at a faster rate than a low digestibility diet (45% digestibility). The basal diet used in this study had a digestibility of 69%, and thus promoted highest seed recovery 2 to 3 days after feeding seeds as it did in the study by Jones and Simao Neto (1987).

The *in vivo* germination responses were generally not consistent with previously described *in vitro* germination responses (Table 3). With the exception of *Pseudoroegneria spicata*, *in vivo* germination was generally higher 1 and 2 days after feeding seeds than *in vitro* germination. The reverse was generally true for day 3, i.e. *in vitro* germination was higher than *in vivo* germination. These discrepancies indicate that caution is warranted when using *in vitro* incubation as a possible screening tool for predicting germination responses for *in vivo* digestion. Owens and Goetsch (1988) indicated that extrapolating results of *in vitro* studies to the *in vivo* system can be erroneus. Some compounds and plant materials act differently *in vivo* compared to *in vitro*.

Management Implications

The results of this study support the growing interest in using domestic livestock for seed dissemination in the

revegetation of rangelands. *In vitro* incubation trials may be used as a crude indicator of germination responses that may occur after seeds pass through the ruminant digestive tract. The *in vitro* technique may be most appropriate for screening out species that fail to survive after 24 or more hours of exposure to rumen fluid and acid pepsin solutions (e.g. *Pascopyrum smithii* and *Elymus triticoides*), or fail to germinate following exposure because of indurate seed coverings (e.g. *Oryzopsis hymenoides*). *In vivo* digestion trials indicate that about 20% of ingested seed of representative cool-season grasses may be recovered 3 to 4 days after feeding, and that germination of undamaged, recovered seeds can approach 50%. Thus, management programs could be developed to feed cattle and other domestic ruminants a minimum amount of seed to ensure the passage of sufficient quantities of germinable seed over target areas 2 to 4 days after ingestion.

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