



Effect of yak rumen content treatments on seed germination of 11 alpine meadow species on the Qinghai-Tibetan Plateau



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ABSTRACT

The Qinghai-Tibetan Plateau is located in the 'Third Pole' of the world, characterized by a harsh environment. Despite this, the alpine meadow ecosystem have developed over a wide area but serious grassland degradation is threatening the ecological environment on the Plateau. Recruitment of new plants to the population, via germination and establishment, is vital to plant community survival. Previous work on the seeds in this area has mainly focused on community-wide germination strategies, seed germination characteristics and their correlations with seed size and seed mass. However, there have been no studies on the effects of soaking in rumen contents on the plant seed germination characteristics of alpine meadow species. The present study had two main objectives: (i) to determine the effect of fresh rumen content from yaks on seed germination characteristics and seedling growth of species common to the eastern Tibetan Plateau alpine meadow, and (ii) to develop an effective method to enhance seed germination. Seeds of 11 common species were collected together with fresh rumen content from three yaks that grazed there. Seed germination tests were conducted after they had been soaked in rumen content for one of six soaking periods (12, 24, 36, 48, 60 or 72 h). The seeds were incubated under natural light conditions of 8 h light at 25 °C and 16 h darkness at 15 °C, for the germination period of 34 days. The results showed that seed germination and seedling growth were affected by soaking time, seed coat completeness and seed type. After soaking in rumen content, the germination percentages of scarified (peeled or with the seed coat cut through) seeds of some species (true seeds *Oxytropis ochrocephala* and *Medicago ruthenia* var *inschanica*, nutlet *Carex enervis*, achenes *Anemone rivularis* and *Polygonum sibiricum*) and complete seeds of *C. enervis*, and *A. rivularis* were improved but the duration of soaking was also important. Seed germination of caryopsis *Achnatherum inebrians* (a toxic grass) was significantly inhibited by any exposure to rumen fluids. Scarified seeds generally had higher germination percentages than complete ones after treatment, but with the increase in soaking time, germination percentages declined and scarified seeds were more sensitive to the treatment than the complete seeds. After soaking in yak rumen content, the germination indices of scarified *M. ruthenia* at 12 h treatment, *O. ochrocephala* and achene *Rumex acetosa* at 12–24 h treatment, nutlet *Kobresia humilis* at 24 h treatment, *P. sibiricum* at 24–48 h treatment, *C. enervis* at 12–48 h treatment and *A. rivularis* at 12–60 h treatment were significantly higher than the control ($P < 0.05$), while the germination indices of complete *C. enervis* seeds at 12 h and 36 h treatment, and *A. rivularis* at 12–60 h treatment were significantly higher compared with the control. The germination indices of other species gradually decreased with the increase in soaking time. We concluded that yak rumen digestion could enhance, inhibit or not affect seed germination and seedling growth of the alpine meadow species, which might influence seedling recruitment, interspecific competition, and the plant community structure of the eastern Tibetan Plateau alpine meadow. Overall, yak digestion has a positive effect on alpine meadow seed germination and seed dispersal.

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0. Introduction

The Qinghai-Tibetan Plateau is located in the 'Third Pole' of the world, with special conditions of challenging natural geography, a harsh environment [1]. Despite this alpine meadow ecosystems

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have developed over a wide area but is extremely fragile [2], and serious grassland degradation is threatening the ecological environment of the Plateau. The Alpine meadow community succession, vegetation restoration has caused widespread concern of many scientists at present [3,4]. Plant regeneration is vital in the processes of community succession and vegetation restoration [5], and successful germination and establishment of the seeds are the keys and grazing animals have role. Seed germination directly relates to the species breeding, populations of maintaining and expanding ecological processes, and directly affects the distribution of the vegetation, community dynamics and biodiversity [6]. The seed digestive dispersal (Endozoochory) is that the seeds in the feces are away from the mother plants after frugivores (birds and primates) swallowing fleshy fruit and the herbivores foraging herbs and shrubs with seeds [7,8]. It is of great theoretical and practical significance to clarify the endozoochorous dispersal of seeds and other disseminules by herbivores plays an important role in understanding plant regeneration, species diversity, community structure and succession. The actual mechanisms deserve further study.

Partial seeds with germinability are passed out after grazed by animals, the seed germination percentage and germination speed are all improved [9]. In grazing ecosystems, herbivores feed, digest and pass out plant seeds, thereby affecting both seed dispersal and germination capacity [10]. Zhang et al. reported that after eaten and digested by cattle and goats, *Zoysia japonica* seeds lost little vitality but increased seed germination percentage [11]. Robles and Castro [12] found that after incubation in the rumen of herbivores (sheep and goat), seed germination of *Helianthemum apenninum* was boosted, while Li et al. [13] showed that yak and Tibetan sheep juices significantly inhibited seeds germination of *Saussurea japonica* and *Saussurea iodostegia*. Research has showed that yak digestive tract effects seed germination of species of alpine meadow in Qinghai-Tibetan Plateau, and then impacts the population regeneration, species diversity, community structure and succession of this region [14]. Researches have been reported on seed germination of alpine meadow species [15–19], however, there is no published work on the effect of livestock's rumen content on seed germination. In this study, we conducted the effects of fresh yak rumen contents on seed germination and seedling growth of 11 plant species from alpine meadow to provide a basis for seed dispersing and management of grassland ecosystem in Qinghai-Tibetan Plateau.

1. Materials and methods

1.1. Study area

Tianzhu Alpine Grassland Experimental Station is located to the southwest foot of Wushao Ridge of Gansu Province with humid climate, thin air and strong solar radiation. The annual average temperature is -0.1°C , the lowest average temperature is -18.3°C in January while the highest is 12.7°C in July. Greater than 0°C accumulated temperature is 1380°C , and there are still low temperatures below 0°C in July. Annual rainfall is 416 mm concentrated in July, August and September and annual evaporation is 1592 mm. The grazing seasons are classified either as cold or warm.

1.2. Study species

In August to September 2011, fully mature seeds of 11 common alpine meadow species (Table 1) were manually collected. These species are perennial and adapted to alpine meadow conditions. Most provide forage for livestock and a variety of wildlife species.

Table 1

Eleven common plants in alpine meadow and a guide to their disseminules.

Plant names	Plant family	Seed types
<i>Achnatherum inebrians</i>	Gramineae	Caryopsis
<i>Anemone rivularis</i>	Ranunculaceae	Achenes
<i>Carex enervis</i>	Cyperaceae	Nutlets
<i>Elymus nutans</i>	Gramineae	Caryopsis
<i>Kobresia humilis</i>	Cyperaceae	Nutlets
<i>Medicago ruthenia</i> var <i>inschanica</i>	Leguminosae	True seed
<i>Oxytropis ochrocephala</i>	Leguminosae	True seed
<i>Pedicularis kansuensis</i>	Scrophulariaceae	True seed
<i>Poa crymophila</i>	Gramineae	Caryopsis
<i>Polygonum sibiricum</i>	Polygonaceae	Achenes
<i>Rumex acetosa</i>	Polygonaceae	Achenes

The selected species included dominant species such as *Elymus nutans*, *Kobresia humilis*, *Kobresia capillifolia*, as well as toxic species *Achnatherum inebrians*, *Oxytropis ochrocephala* and *Pedicularis kansuensis* which are poisonous to livestock either all the time or only when green [14]. The seeds were taken to the laboratory after collection, dried and then stored in manila envelopes at room temperature. These seeds were then used in the experiment.

1.3. Methods

1.3.1. Yak rumen content collection and measurement

Early in December 2011, fresh rumen contents were collected at a slaughterhouse in Tianzhu County, Gansu Province, from 3 healthy yaks raised under the same growth conditions. Rumen contents were stored in a dark place at 25°C , and quickly transported to the lab and placed in a dark incubator at 39°C prepared for the test see (Table 2).

Seeds of 11 species of intact and scarified disseminules (seeds of *P. kansuensis*, *O. ochrocephala* and *M. ruthenia* were cut through the seed coat, while others had the seed coat peeled). Then all were encased in small cotton bags ($6\text{ cm} \times 4\text{ cm}$), which were distributed evenly throughout the yak rumen content for periods of 12 h, 24 h, 36 h, 48 h, 60 h and 72 h. Of these exposure times, 24 h, 48 h and 72 h are widely accepted as standards for determination of the effects on seed germination [20,21]. Rumen contents were incubated under dark condition at 39°C to simulate the temperature condition inside the animal rumen. After the soaking tests, seeds in the cotton bags were rinsed with tap water and put into 75% alcohol disinfection for 1 min, then washed with distilled water 4 times for the germination test.

1.3.2. Seed germination test

Three replicates of seeds from each plant species were placed on moistened filter paper in Petri dishes for the germination tests. Petri dishes were incubated under natural light conditions of 8 h light at 25°C and 16 h darkness at 15°C , for the germination period of 34 days. The complete seeds untreated were as a control, each Petri dish received 90 seeds per replicate. Every day, the percentage of seeds that had germinated was recorded. Seeds were regularly watered with distilled water, a seed was considered as germinated when the radicle was visible. Seedling length was measured at Day 13 [22,23].

1.3.3. Data analysis

Germination percentage (GP) for each treatment was calculated after 34 days. The germination index (GI) is based on number of seeds that germinated and the germination percentage. These parameters were also calculated from the following formulas.

$$GP = 100 \times GN/SN$$

where GN is the total number of germinated seed and SN is the total number of seeds tested.

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