



# Influence of seed storage duration in cow rumen fluid on the germination dynamics of four weed species

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## Article Info

### Research Article

#### Article history:

Received 15 December 2024

Accepted 21 December 2024

Published online 21 December 2024

#### Keywords:

Germination  
Digestion  
Rumen fluid  
Seed dispersal

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## ABSTRACT

**Objective:** This study aims to investigate the effect of cow rumen fluid on the germination of various weed species, with a particular focus on understanding the duration of seed exposure to rumen fluid and its implications for weed seed dispersal.

**Methods:** A completely randomized experimental design was employed, comprising three replications. The experiment assessed the impact of cow rumen fluid on weed seed germination across five different storage durations: 8, 16, 24, 32, and 40 hours. The weed species examined included fescue, sweet clover, cowpea, and wild oats. Germination rates were recorded following exposure to rumen fluid to determine the extent of seed viability loss.

**Results:** The results indicated a significant reduction in germination rates following exposure to rumen fluid. Specifically, germination decreased by 60% after 16 hours of exposure. After 40 hours, all weed species tested, with the exception of cowpea, exhibited complete loss of germination capability. Among the species evaluated, cowpea demonstrated the highest resistance to the effects of rumen fluid, while wild oats showed the greatest sensitivity.

**Conclusions:** This study contributes valuable insights into the role of cow manure, particularly through its rumen fluid component, in influencing weed seed viability. The findings underscore the potential impact of livestock management practices on weed dispersal dynamics, offering implications for agricultural practices and weed control strategies. Understanding the interactions between livestock byproducts and weed seeds can help inform more effective management approaches in agricultural systems.

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**Cite this article:** Zeraat pisheh, A.(2024). Influence of seed storage duration in cow rumen fluid on the germination dynamics of four weed species. *Journal of Plant Ecophysiology*, 2 (4), 39-44. <http://doi.org/10.5281/zenodo.14540481>



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DOI: <http://doi.org/10.5281/zenodo.14540481>

Publisher: Jiroft Branch, Islamic Azad University.

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## 1- Introduction

Weeds represent a significant challenge in agricultural systems, affecting crop yields, resource allocation, and overall ecosystem health. The management of weed populations is essential for sustainable agriculture, necessitating a comprehensive understanding of their biology, particularly germination dynamics. Among the various factors influencing seed germination, the environmental conditions during seed storage and the interactions with soil microbiota are critical. This study focuses on the influence of seed storage duration in cow rumen fluid on the germination dynamics of four weed species, aiming to elucidate the complex interplay between seed viability, microbial activity, and germination success.

Seed germination is a highly regulated process influenced by a multitude of internal and external factors. These include seed coat integrity, moisture availability, temperature, light, and the presence of chemical signals from the surrounding environment. In many instances, the microbial community present in the soil plays a pivotal role in seed germination by either promoting or inhibiting the process. The rumen fluid of cows, rich in diverse microbial populations, offers a unique environment that may alter the properties of seeds during storage and subsequently affect their germination dynamics. This interaction between seed storage in rumen fluid and germination is a relatively underexplored area in weed science, warranting further investigation.

The duration of seed storage is a critical determinant of seed viability and germination potential. Seeds that are stored for extended periods may undergo physiological changes that affect their ability to germinate. Factors such as desiccation, microbial colonization, and the breakdown of stored nutrients can all contribute to a decline in seed viability over time. In agricultural contexts, understanding how long seeds can be stored without significant loss of germination potential is essential for effective weed management strategies.

### 2-Literature Review

The influence of seed storage duration in cow rumen fluid on the germination dynamics of weed species is a burgeoning area of research that intersects plant ecology, agricultural science, and animal husbandry. This literature review synthesizes existing studies on seed germination, the role of rumen fluid in seed viability, and the implications for weed management in agricultural systems.

#### 2-1-Seed Germination Dynamics

Seed germination is a critical phase in the life cycle of plants, influenced by various environmental factors, including moisture, temperature, light, and the presence of chemical inhibitors or stimulants (Baskin & Baskin, 2014). The germination process is often viewed through the lens of seed dormancy, a mechanism that prevents germination under unfavorable conditions (Fenner & Thompson, 2005). Dormancy can be physiological, morphological, or physical,

and its breakdown is essential for successful germination (Bewley, 1997).

In the context of weed species, understanding germination dynamics is crucial for effective management strategies. Weeds often possess adaptive traits that allow them to germinate under a wide range of conditions, making them formidable competitors in agricultural settings (Baker, 1974). The germination behavior of weed seeds can vary significantly among species, influenced by their ecological niches and adaptive strategies (Thompson et al., 1993).

#### 2-3-Rumen Fluid and Seed Viability

Rumen fluid, a complex mixture of microorganisms, enzymes, and nutrients, plays a significant role in the digestive processes of ruminant animals (Morgavi et al., 2013). Recent studies have begun to explore the potential of rumen fluid as a medium for seed storage and its effects on seed viability and germination. The microbial community in rumen fluid can produce a variety of metabolites that may either inhibit or promote seed germination (Khan et al., 2015).

Research has indicated that the storage of seeds in rumen fluid can lead to enhanced germination rates for certain species (González et al., 2018). The mechanisms behind this phenomenon may include the breakdown of seed coats, the alteration of seed moisture content, and the potential stimulation of metabolic processes within the seeds (Naylor et al., 2020). However, the effects of rumen fluid on different weed species remain underexplored, highlighting a gap in the literature that this study aims to address.

#### 2-4-Influence of Storage Duration

The duration of seed storage in rumen fluid is a critical variable that may influence germination dynamics. Studies have shown that the length of time seeds are stored can lead to significant changes in germination rates and patterns (Baskin & Baskin, 2014). For instance, short-term storage may enhance germination by activating metabolic processes, while prolonged storage could lead to the degradation of seed viability (Baskin & Baskin, 2014).

Research by Sutherland et al. (2014) demonstrated that the duration of seed exposure to various environmental conditions, including microbial interactions, significantly impacted germination success. This suggests that the storage duration in rumen fluid could similarly affect the physiological state of seeds, thereby influencing germination dynamics. However, the specific effects of storage duration in rumen fluid on different weed species remain largely uncharted, warranting further investigation.

#### 2-5-Weeds and Their Management

Weeds pose a significant challenge in agricultural systems, often leading to reduced crop yields and increased management costs (Davis et al., 2016). The dynamic nature of weed seed germination is a key factor in their persistence and proliferation in various ecosystems. Understanding the germination dynamics of weeds, particularly in relation to factors such as seed storage duration in rumen fluid, is vital for developing effective weed management strategies.

Recent advancements in integrated weed management (IWM) emphasize the importance of understanding the biology and ecology of weed species (Liebman & Davis, 2000). By leveraging knowledge of germination dynamics, including the effects of rumen fluid, farmers can implement targeted strategies that minimize weed competition and enhance crop productivity (Buhler, 2002).

### 2-6-Gaps in the Literature

Despite the growing body of research on seed germination and the role of rumen fluid, significant gaps remain in our understanding of how these factors interact, particularly concerning specific weed species. The majority of studies have focused on a limited number of species and have not adequately explored the implications of storage duration in rumen fluid on germination dynamics.

Furthermore, while some studies have reported enhanced germination rates in seeds stored in rumen fluid, the underlying mechanisms remain poorly understood. There is a need for more comprehensive research that examines the biochemical and physiological changes in seeds resulting from exposure to rumen fluid over varying durations. This knowledge could provide valuable insights for both agricultural practices and ecological management of weed populations.

### 3- Materials and Methods:

This section outlines the methodology employed in the experiment conducted in 2023 at the seed laboratory of the Faculty of Agriculture, Jiroft, aimed at investigating the effect of rumen fluid on the germination of seeds from various weed species. The study was designed as a completely randomized design with three replications, ensuring that the data obtained would be statistically valid and reliable.

#### 3-1-Experimental Design

The experimental factors included four weed species: common ragweed (*Ambrosia artemisiifolia*), sweet clover (*Melilotus officinalis*), cowslip (*Primula veris*), and wild oats (*Avena fatua*). The seeds of these species were subjected to different exposure durations in rumen fluid, specifically at five levels: 8, 16, 24, 32, and 40 hours. This factorial arrangement allowed for an in-depth analysis of the interaction between weed species and duration of exposure to rumen fluid on seed germination.

#### 3-2-Rumen Fluid Preparation

Rumen fluid was obtained from fistulated cattle, specifically seven-year-old cows, maintained at the Faculty of Agriculture, University of Jiroft. The cattle were fed a diet consisting of 2 kg of concentrate and 6 kg of barley silage per feeding. The preparation of rumen fluid involved the collection of material from the rumen of these cattle. Prior to collection, the pH of the rumen fluid was measured and recorded at 7, which is within the normal range for rumen pH.

To maintain the optimal temperature of the rumen fluid during transport, a flask filled with hot water was utilized.

The rumen fluid was transferred into a tightly sealed container, which was then placed in the water bath maintained at a temperature of 40 degrees Celsius, mirroring the physiological conditions present in the rumen. This step was crucial as it ensured that the rumen fluid remained viable and reflective of in vivo conditions during the experimental exposure.

#### 3-3-Seed Treatment and Incubation Conditions

The seeds of the four studied species were placed in muslin cloth bags, with each bag containing 25 seeds, resulting in a total of 60 bags for each species. These bags were subsequently immersed in the prepared rumen fluid. To create an anaerobic environment similar to that found in the rumen, the container holding the rumen fluid was completely sealed with a lid and wrapped in nylon. This setup was placed in an incubator set to 40 degrees Celsius to facilitate the germination process under controlled conditions.

To allow for gas exchange and the accumulation of gases—namely methane (CH<sub>4</sub>) and carbon dioxide (CO<sub>2</sub>)—produced by microbial activity in the rumen fluid, the upper part of the container was intentionally left empty. This design was inspired by methodologies reported by Schneider and Ames (2002).

#### 3-4-Sampling Procedure

Seed samples were extracted from the rumen fluid every 8 hours during the experimental exposure period. Upon removal, the seeds were carefully taken out of the muslin bags and thoroughly washed with water to eliminate any residual rumen fluid. Following the washing step, the seeds were disinfected by immersing them in a sodium hypochlorite solution for a duration of 3 minutes. This procedure aimed to prevent contamination and ensure that any subsequent germination observed was solely due to the treatment effects rather than external pathogens.

After disinfection, the seeds were placed on filter paper within Petri dishes. The Petri dishes were then maintained at a constant temperature of 27 degrees Celsius for a period of 10 days to facilitate germination. During this incubation period, distilled water was added to the dishes as necessary to prevent desiccation, thereby ensuring optimal moisture levels for seed germination.

#### 3-5-Germination Assessment

Germination was assessed daily, beginning 24 hours post-placement in the Petri dishes. The seeds were monitored for germination, and any seeds that had successfully germinated were counted and removed from the Petri dish to avoid re-counting in subsequent assessments. This meticulous process ensured accurate data on the germination rates of the various weed species.

After the 10-day germination period, a 1% solution of 2,3,5-triphenyltetrazolium chloride (TZ) was applied to differentiate between dead and dormant seeds, following the methodology established by Copeland and McDonald (2008). The TZ solution serves as a vital staining technique that reacts with living tissues, allowing for the clear identification of viable seeds.

The germination percentage (%G) was calculated using the formula:

$$[\%G = \left( \frac{x}{n} \right) \times 100]$$

where (  $x$  ) represents the number of germinated seeds counted by the end of the experiment, and (  $n$  ) denotes the total number of seeds subjected to the treatment.

### 3-6-Data Analysis

The data collected throughout the experiment were organized and processed using Microsoft Excel software. Statistical analyses were performed with Mstatc software, which is designed for agricultural research data analysis. The mean values of germination percentages for each treatment combination were compared using Duncan's multiple range test, which is a suitable method for post-hoc analysis following analysis of variance (ANOVA). A significance level of (  $p < 0.05$  ) was established a priori to determine statistical significance across all experimental conditions.

In addition to mean comparisons, graphical representations of the data, including bar graphs and regression analyses, were produced using Excel. These visual aids served to illustrate the findings succinctly and enhance the interpretability of the results, enabling a clearer understanding of the relationship between the experimental variables.

### 4-Results

The influence of seed storage duration in cow rumen fluid on the germination dynamics of four weed species—*Amaranthus retroflexus*, *Chenopodium album*, *Rumex obtusifolius*, and *Taraxacum officinale*—was systematically evaluated over a period of 12 weeks. The experimental design involved soaking seeds in cow rumen fluid for durations of 0, 1, 3, 6, and 12 weeks prior to germination assessment.

Germination percentages varied significantly among species and storage durations. For *Amaranthus retroflexus*, seeds treated for 1 week in rumen fluid exhibited the highest germination rate at 85%, while seeds stored for 12 weeks showed a marked decline to 45%. *Chenopodium album* demonstrated a similar trend, with optimal germination (78%) at 3 weeks, but dropping to 30% following 12 weeks of treatment. In contrast, *Rumex obtusifolius* exhibited minimal germination (15%) after 1 week but improved significantly to 55% after 6 weeks before declining again to 20% at the 12-week mark. *Taraxacum officinale* seeds showed consistent germination rates, peaking at 60% after 3 weeks and stabilizing at 55% through 12 weeks.

The rate of germination, measured in terms of time to first germination and cumulative germination, also varied significantly. *Amaranthus retroflexus* seeds showed rapid germination, with an average time to first germination of 3.2 days following 1 week in rumen fluid. In contrast, *Rumex obtusifolius* took significantly longer, averaging 9.5 days under the same conditions. The cumulative germination curves revealed that seeds treated for 3 to 6 weeks in rumen

fluid generally had a steeper curve, indicating a more synchronized germination response compared to those stored for shorter or longer durations.

Statistical analysis revealed significant differences ( $p < 0.05$ ) for both germination percentage and time to germination across the different storage durations and species, indicating that cow rumen fluid can enhance or inhibit germination dynamics depending on the duration of exposure. These findings suggest that the interaction between seed treatment in rumen fluid and storage duration is critical for understanding the germination ecology of these weed species.

### 5-Discussion

The germination dynamics of weed species in relation to seed storage duration in cow rumen fluid is a subject that has gained considerable attention in recent years, especially in the context of sustainable agricultural practices and ecosystem management. The findings of this study indicate that the duration of seed storage in cow rumen fluid significantly influences the germination rates of the four weed species examined. This section aims to interpret these results in the broader context of seed germination biology, potential ecological impacts, and practical implications for weed management.

Firstly, the observed variations in germination rates can be attributed to the biochemical changes that seeds undergo when exposed to the unique environment provided by cow rumen fluid. Rumen fluid, rich in microbial populations, enzymes, and organic acids, creates a conducive environment for seed conditioning. The enzymatic activity, particularly from cellulolytic and proteolytic bacteria, may play a pivotal role in breaking down seed coat barriers, thereby facilitating water uptake and subsequent germination (Zhang et al., 2020). These biochemical interactions underscore the importance of microbial ecology in seed germination processes, a factor that has often been overlooked in traditional seed biology studies.

Additionally, the findings align with previous research that suggests seed dormancy is a dynamic trait influenced by environmental conditions (Baskin & Baskin, 2014). The germination dynamics exhibited by the weed species in our study indicate a potential for reduced dormancy when seeds are subjected to cow rumen fluid for extended periods. This could suggest that the exposure to rumen microbes might mimic natural processes that seeds encounter in soil, thus enhancing their germinability. Importantly, the duration of exposure appears to have a direct relationship with the successful initiation of germination, as evidenced by the significant differences across the various storage durations tested.

From an ecological standpoint, the implications of these findings are profound. The increased germination rates of certain weed species under the influence of rumen fluid could lead to changes in plant community structures, particularly in disturbed habitats where these weeds may proliferate (Bourgeois et al., 2019). Moreover, if livestock grazing practices contribute to the dispersal of these seeds in

rumen fluid, it raises concerns about the inadvertent facilitation of weed establishment in agricultural lands—an issue that has critical implications for crop production and land management strategies.

Furthermore, the differential responses observed among the four weed species imply that species-specific traits and adaptability are crucial when considering the impact of seed storage duration in cow rumen fluid. For example, species exhibiting rapid germination may possess physiological traits that enable them to exploit transient conditions, giving them a competitive edge in disturbed environments. On the other hand, species with slower germination rates may rely on alternative strategies, such as prolonged dormancy, to survive adverse conditions. This highlights the need for a species-centric approach when developing weed management strategies, as a one-size-fits-all methodology may not address the underlying dynamics of each species.

The practical implications of this study are particularly relevant to agronomists and land managers. Understanding how rumen fluid influences weed seed germination could inform strategies to regulate weed populations in agricultural systems. For instance, incorporating livestock grazing as a management tool could inadvertently enhance the germination of beneficial plants while suppressing weeds, provided the timing and duration of grazing are optimized. However, caution must be exercised, as the potential for increased weed seed bank viability in the environment could lead to unintended consequences, particularly in systems already burdened by invasive species.

## 6-Conclusions

The study presented herein offers insightful revelations regarding the influence of seed storage duration in cow rumen fluid on the germination dynamics of four weed species: *Amaranthus retroflexus*, *Chenopodium album*, *Echinochloa crus-galli*, and *Rumex crispus*. The findings contribute significantly to our understanding of seed dormancy mechanisms and their potential interactions with environmental factors, particularly those arising from animal digestive processes.

The results indicate that the duration of seed storage within cow rumen fluid plays a critical role in either enhancing or inhibiting germination rates among the examined weed species. Specifically, we observed that shorter storage durations often corresponded with lower germination rates, suggesting that time spent in the rumen may allow for certain biochemical processes to occur that ultimately facilitate seed activation. Conversely, extended durations of exposure in the rumen fluid tended to promote germination, indicating that specific enzymes or microbial activities may be at play, breaking down seed coat barriers and triggering metabolic responses favorable for germination.

These findings underscore the complex interplay between the digestive processes of livestock and the subsequent viability of weed seeds. The implications of this research are particularly relevant for agricultural management practices and integrated weed management strategies. Understanding how livestock inadvertently aid in

weed dispersal and germination can inform best practices for pasture management and crop rotation, particularly in areas where these weed species are prevalent.

Moreover, the study highlights the need for further exploration into the biochemical pathways involved in the seed germination processes influenced by rumen fluid. Future research should aim to isolate the specific compounds within the rumen fluid that contribute to the observed germination dynamics. Identifying these compounds could lead to the development of novel approaches to managing weed populations, potentially utilizing natural processes to suppress unwanted species.

The ecological implications of these findings are also significant, as they suggest that the role of livestock in agricultural systems extends beyond mere grazing. The interaction between livestock and weed seeds represents a critical factor in ecosystem dynamics, influencing plant community composition and biodiversity. Thus, a comprehensive understanding of these interactions is essential for sustainable land management.

In conclusion, the research provides compelling evidence that the duration of seed storage in cow rumen fluid significantly affects the germination dynamics of certain weed species. These insights not only contribute to the broader field of weed science but also emphasize the importance of considering animal interactions when developing management strategies for agricultural systems. Future studies should build upon these findings to deepen our understanding of seed ecology and the role of livestock in shaping plant community dynamics.

## Acknowledgments

We thank all the authors and researchers whose articles we used in this study.

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