

**USE OF SHEEP IN THE CONTROL OF AN INVASIVE BUNCHGRASS**

BY:

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Baccalaureate of Science in Bioresource Research,

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Baccalaureate of Science in Bioresource Research,  
Animal Reproduction and Development

Thesis of Ryan Scholz

Presented on June 1, 2007

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**AN ABSTRACT OF THE THESIS OF**

Ryan Scholz for the degree of Baccalaureate of Science in Bioresource Research, Animal Reproduction and Development Option, presented on June 1, 2007.

Title: Use of Sheep in the Control of an Invasive Bunchgrass

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Studies were conducted to assess influencing the effectiveness of sheep for grazing control of false brome (*Brachypodium sylvaticum*) an invasive grass species. Orphan lambs were exposed to false brome via grass extract in their milk and naturally reared lambs were exposed by grazing dense stands of false brome with their mothers pre-weaning. When orphan lambs were subsequently offered fresh false brome under pen conditions, prior exposure had no effect on intake. When naturally reared lambs were tested under the same conditions, lambs previously exposed to false brome consumed 50% more than controls. The effect was also observed under grazing conditions when naturally reared lambs were placed on dense plots of false brome. Concerns about seed remaining viable after passing

through the sheep gut were examined by a variety of digestion trials. When suspended in the rumen for varying times, seed was partially digested and viability correspondingly dropped until no viable seed was found after 48 hrs. *In vitro* digestion resulted in no viable seeds after 24 hrs. When loose seed was placed directly into the rumen for *in vivo* digestion, no intact seed could be found in feces and no seed germination occurred in fecal samples.

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## CHAPTER 1

### INTRODUCTION

Sheep are often used in the biological control of weed species. While the usefulness of sheep in these settings has been well documented (Walker et al., 1992), several problems have been shown to accompany the use of sheep in controlling weeds (Walker et al., 1992; Olson et al., 1990). One of the primary concerns is the potential to spread weed seed from infested to clean fields via sheep feces (Olson et al., 1990).

It may also prove difficult to convince sheep to consume plants to which they have had no prior exposure (Thorhallsdottir et al., 1990). This can often become an issue if the sheep show an initial aversion to the plant such as is the case with slender false brome (*Brachypodium sylvaticum*).

These studies were designed to examine the effect of pre-weaning exposure of lambs to eating false brome on their affinity for eating the plant later in life, as well as the effect of ruminal and post-ruminal digestion on the germination of false brome and seeds.

## CHAPTER 2

### REVIEW OF LITERATURE

#### 2.1. BACKGROUND

False brome (*Brachypodium sylvaticum*) is an invasive, perennial bunchgrass that appears to be rapidly spreading throughout western Oregon (Dexter et al., 2001). The species is native to Europe, Asia, and Northern Africa (Tu, 2002). Occasionally cultivated for ornamental purposes, false brome was first discovered as an escaped invader in North America near Eugene, Oregon in 1939 (Chambers, 1966). Since the 1960's, false brome has spread rapidly through Polk, Benton, and Lane counties and is spreading beyond, becoming common in woodlands and forest understories (Kaye, 2001). It thrives under a wide range of conditions including closed-canopy coniferous forests, riparian forests, forest edges, and sunny upland prairies (Tu, 2002).

At early stages of invasion, false brome may appear as small tufts sparsely dispersed throughout native grasses and herbaceous species (Dexter et al., 2001, Tu 2002). As the invasion progresses, original plants reproduce by seed and populations quickly expand to form a dense cover of grass capable of excluding almost all other plants (Dexter et al., 2001). False brome has been shown to be capable of suppressing

threatened and endangered species such as Kincaid's Lupine (*Lupinus sulphureus* subsp. *kincaidii*), the host plant for the endangered Fender's Blue Butterfly (*Icaricia icariodes fenderi* Lycaenidae (Kaye, 2001). On managed forests, it has also been shown to inhibit seedling establishment (Kaye, 2003).

Until now, the only successful methods of controlling false brome which have been proven effective have been chemicals such as glyphosate and super-heated foam applied with a Waipuna machine (Kaye, 2003). Because of the nature of many of the ecosystems that have been invaded by false brome, non chemical methods are urgently needed. Mowing and burning have both been explored as possible non-chemical means for controlling the spread of false brome, but they have been ineffective unless combined with chemicals (Kaye, 2003).

## **2.2. SHEEP CONTROL OF FALSE BROME**

For several years, operations staff at Oregon State University have been aware of false brome colonies spreading through oak-grasslands at the OSU Sheep and Horse Centers (Meyer, 2004). For the past three years, Sheep Center staff have attempted to control the invasion by enclosing sheep onto areas infested with false brome to encourage heavy grazing of the grass. Observations suggested that grazing appears to be

reducing the vigor of the false brome plants and studies are currently underway to validate these observations.

The palatability of false brome for wildlife and livestock is reported to be low (Kaye, 2003); however, this assumption is based on observations and research conducted in Europe. In its native geographic range, one strain of false brome is host to a fungus, *Epichloë sylvatica*, which offers the plant partial resistance to herbivory by producing biologically active alkaloids (Brem 2001). While Oregon populations of false brome have not been tested for endophyte content, the particular variety which hosts this endophyte has not been found in Oregon (Dexter et al. 2001). Even without the presence of this endophyte, grazing control could be difficult because false brome is a relatively coarse grass which sheep seem to find unpalatable.

### **2.3. TRAINING SHEEP TO EAT FALSE BROME**

Studies have shown that lambs which are exposed to novel foods in social settings (such as with their mothers) have an increased affinity for such food when retested later in life (Thorhallsdottir et al., 1990, Nolte et al., 1990). Studies have also shown training to be most effective in conditioning lambs to prefer certain foods when exposed at an early age (Squibb et al., 1990, Nolte et al., 1992).

Previous research on leafy spurge (*Euphorbia esula* L.) indicates that exposing lambs at a young age with their mothers can be trained to consume plants which they would otherwise avoid (Walker et al., 1992).

#### **2.4. SEED VIABILITY**

One of the principle criteria in weed control practice is to insure that short-term gains do not cause long-term losses. One of the concerns which have been raised regarding the use of sheep as control tools in false brome is that if sheep being grazed on false brome plants consumed viable seed, the later expulsion of any undigested seed from the body in the feces could potentially germinate and actually cause further spread of the plant.

Several laboratory digestion techniques can be used to examine the effect of ruminal digestion on the viability of seeds (Ocumpaugh and Swakon, 1993). Post-digestion seeds are tested for viability.

The most common technique used to observe the effect of ruminal digestion on the viability of seeds is *in sacco* digestion. Seeds are sealed in a small fine-mesh bag and suspended into the rumen of a cannulated animal for varying periods of time. Seeds are then weighed to determine seed mass

lost due to digestion and tested for viability (Ocumpaugh and Swakon 1993).

*In vitro* digestion techniques may also be used to determine the effects of both ruminal and post-ruminal digestion on the viability of seeds (Ocumpaugh and Swakon 1993).

Using these techniques, researchers have shown that while some weed seeds do pass through the digestive track unharmed (Fredrickson et al. 1997), many suffer damage which significantly decreases subsequent germination rates (Olson et al. 1997).

**CHAPTER 3****EFFECT OF PRE-WEANING EXPOSURE OF LAMBS TO FALSE BROME ON  
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### 3.1. ABSTRACT

In mid-June, 26 Polypay ewes rearing ewe/wether sets of Suffolk-sired twin lambs were assigned to graze stands of either 75-90% false brome (treatment group) or normal pasture (control) with their lambs for two weeks prior to weaning. Following weaning, all lambs remained on their existing grazing regimen for an additional seven days before being moved to a central barn for weighing. Ewe lambs were then moved to good pasture for grazing as a single group for a 30 day interim.

Immediately following removal from training pastures wether lambs underwent a confinement intake trial. The wethers then joined the ewe lambs on pasture for 28 days at which time the intake trial was repeated.

Following a 30 day interim period, ten ewe lambs from each treatment group were grazed for 15 hr on 100 m<sup>2</sup> plots of high density stands of false brome. Ewe lambs were removed to normal pasture for two days then the grazing exercise was repeated on fresh false brome stands. The entire trial was repeated the following year with new lambs of the same genetic makeup.

At the first testing of the confinement feeding trial, treatment lambs consumed 116g of false brome compared to 78g for control lambs; on the following day, intakes were 152g vs. 119g. At retesting 28 days later, intake on the first day was 176g vs.

145g for controls and on the second day the intakes were 232g vs. 140g.

During the first testing the second year, treatment lambs consumed 72g compared to 64g for control lambs; on the next day, intakes were 64g vs. 75g, respectively. At retesting 28 days later, intake on the first day was 87g vs. 56g for controls and on the second day the intakes were 87g vs. 58g.

At first testing during the grazing trial, biomass reduction in the false brome grazing plots was 71% for the treatment lambs vs. 22% for the controls. When retested on fresh plots two days later, biomass reduction was 71% for treatment lambs vs. 15% for the controls.

Biomass reduction during the initial grazing in the second year of the trial was 82% for the treatment lambs vs. 31% for the controls. When retested two days later, biomass reduction was 78% for the treatment lambs vs. 12% for the controls.

Pre-weaning exposure of lambs to false brome greatly increased their short term predisposition to eat the plant in a pasture setting.

## **3.2. INTRODUCTION**

### **3.2.1. Research Question**

Will sheep exposed to false brome as lambs with their mothers have an increased affinity for *B. sylvaticum* compared to unexposed sheep in a confinement and pasture setting?

### **3.2.2. Hypothesis**

Sheep exposed to *B. sylvaticum* as lambs with their mothers will have an increased affinity for false brome compared to unexposed sheep when exposed in a confinement and pasture setting.

### **3.2.3. Experimental Design**

Two groups of 12 ewe/wether twin sets of F-1 Suffolk x Polypay cross lambs (naïve, exposure), were assigned to graze either good pasture (naïve) or stands of dense false brome (exposure) for two weeks with their mothers followed by and a third week after weaning from their mothers. At the end of the three week training, ewe lambs from each group were combined on good pasture free of false brome for four weeks while awaiting pasture testing. Wether lambs were removed from training pastures to the first confinement testing.

After the first confinement testing, wether lambs joined the ewe lambs on good pasture for four weeks. At the end of the

four week interim, the wether lambs were removed to the second confinement trial, while the ewe lambs were moved to pasture test plots.

### **3.3. MATERIALS AND METHODS**

#### **3.3.1. Animals for the Experiment**

Preliminary groups were selected before ewes and lambs were turned out to pasture from the lambing barn to ensure that all lambs used in the study were naïve to false brome prior to scheduled treatment. Twin pairs were selected to reduce the amount of grazing pressure exerted by the ewes on the training plots. Prior to the study, ewes and lambs were managed as one group on good pasture completely free of false brome.

Twelve sets of twin lambs of uniform weight and their mothers were selected for naïve (control) and exposure (treatment) groups. Following assignment to experimental groups, the treatment lambs were moved with their mothers to pastures containing 75-90% false brome by weight, while the control sheep grazed good pasture for the duration of the training period. After two weeks of training, lambs were weaned by removal of ewes, and lambs remained on their respective plots for an additional seven days to minimize any behavioral effect from weaning on subsequent trial results.

Treatment group ewe lambs were then moved to good pasture to join the control lambs for a 30 day interim period, while all wether lambs were moved to the barn for confinement testing.

### 3.3.2. **Confinement Feeding**

#### 3.3.2.1. *Sample Preparation*

On the morning of each confinement feeding trial, 12kg of false brome was collected using a hand scythe. The forage was separated into 24 equal allotments for individual feeding.

#### 3.3.2.2. *Confinement Feeding*

Following removal from their training pastures, wether lambs were fasted for 18 hours prior to the beginning of the first confinement feeding. All lambs were allowed *ad libitum* access to fresh water during this time.

Lambs were individually penned and offered 250g of fresh cut false brome. After 30 minutes, lambs were returned to the holding pen and fasted for an additional 24 hours before a second confinement feeding. Uneaten false brome was collected and weighed to determine the amount consumed.

Forage desiccation during each feeding and forage dry matter content was also determined for dry matter calculations.

Confinement feeding was repeated in a second testing four weeks later.

### 3.3.3. Pasture Grazing

#### 3.3.3.1. Plot Selection and Preparation

Prior to the grazing test, four adjacent 100m<sup>2</sup> plots containing 75-90% false brome by weight were selected and fenced using portable electric fencing. Two plots were assigned to each group of lambs, and four 1m<sup>2</sup> clippings were taken from each plot to estimate beginning standing biomass.

Residual forage was estimated following lamb removal by clipping four additional 1m<sup>2</sup> clippings within each plot. Consumption was estimated for each plot as the difference between pre- and post-grazing forage estimates

#### 3.3.3.2. Plot Grazing

Early on the day of initial testing, 10 ewe lambs from each group were hauled by tractor and trailer to each group's respective plot to arrive 30 minutes before sunrise. The sheep remained on the plots until 30 minutes after sundown. All lambs had *ad libitum* access to fresh water while on the plots.

The grazing procedure was repeated two days later on the remaining two plots. The entire trial was repeated with new lambs the following year.

#### 3.3.4. **Data Analysis**

Data from the confinement trial were analyzed by analysis of variance with the amount consumed as the dependent variable. Treatment, year and testing were fitted as independent variables.

### **3.4. RESULTS**

#### **3.4.1. Confinement Feeding**

As seen in Table 3-1, at the first testing treatment lambs consumed 116g compared to 78g for control lambs; on the following day, intakes were 152g vs. 119g, respectively. At retesting 28 days later, intake was higher overall with 176g vs. 145g on the first day and 232g vs. 140g on the second day.

When new lambs were tested a year later, the treatment lambs consumed 72g compared to 64g for the control lambs at first testing; on the next day, intakes were 64g vs. 75g respectively. At retesting 28 days later, intake on the first day was 87g vs. 56g for controls and on the second day the intakes were 87g vs. 58g.

Across both years, the treatment lambs consumed 122g while the control lambs averaged 93g ( $p < 0.001$ ).

### 3.4.2. **Pasture Grazing**

At first testing in 2004, treatment lambs consumed nearly three times as much as the control lambs with an average of 62g per m<sup>2</sup>, while control lambs consumed an average of 22g per m<sup>2</sup> (Table 3-3). When retested two days later, treatment lambs consumed nearly six times as much as the controls at 83g per m<sup>2</sup>, with control lambs consuming an average of 14g per m<sup>2</sup>.

When new lambs were retested in 2005 similar results were attained with treatment lambs removing an average of 106g per m<sup>2</sup>, while control lambs consumed an average of 42g per m<sup>2</sup>. When retested two days later, treatment lambs consumed 70g per m<sup>2</sup>, with control lambs consuming an average of 11g per m<sup>2</sup>.

Table 3-1: Mean consumption of false brome in a confinement setting among lambs naïve and pre-exposed to false brome in a pasture setting.

|      |        |       | Treatment | Control |
|------|--------|-------|-----------|---------|
| 2004 | Test 1 | Day 1 | 116g      | 78g     |
|      |        | Day 2 | 152g      | 119g    |
|      | Test 2 | Day 1 | 176g      | 145g    |
|      |        | Day 2 | 232g      | 140g    |
| 2005 | Test 1 | Day 1 | 72g       | 64g     |
|      |        | Day 2 | 64g       | 75g     |
|      | Test 2 | Day 1 | 87g       | 56g     |
|      |        | Day 2 | 87g       | 58g     |

Table 3-2: Analysis of Variance for amount of false brome consumed in confinement by lambs naïve and previously exposed in a pasture setting to false brome.

| Source            | Sum of Squares | Df  | Mean Square | F-Ratio | P-Value |
|-------------------|----------------|-----|-------------|---------|---------|
| Main Effects      |                |     |             |         |         |
| A: Treatment      | 41301.3        | 1   | 41301.3     | 23.82   | 0.0000  |
| B: Testing        | 43681.3        | 1   | 43681.3     | 31.49   | 0.0000  |
| C: Year           | 264924.0       | 1   | 264924.0    | 152.80  | 0.0000  |
| Interactions      |                |     |             |         |         |
| AB                | 12352.1        | 1   | 12352.1     | 8.90    | 0.0032  |
| AC                | 17633.3        | 1   | 17633.3     | 12.71   | 0.0005  |
| BC                | 34668.8        | 1   | 34668.8     | 24.99   | 0.0000  |
| Residual          | 320759.0       | 185 | 1733.83     |         |         |
| Total (Corrected) | 671213.0       | 191 |             |         |         |

Table 3-3: Mean biomass per square meter of false brome plots before and after grazing by lambs naïve and previously exposed to false brome in a pasture setting, and estimated forage removal by each group.

|      |          |            | Treatment | Control |
|------|----------|------------|-----------|---------|
| 2004 | Day<br>1 | Beg Weight | 88g       | 101g    |
|      |          | End Weight | 26g       | 80g     |
|      |          | Consumed   | 62g       | 22g     |
|      | Day<br>2 | Beg Weight | 118g      | 100g    |
|      |          | End Weight | 35g       | 86g     |
|      |          | Consumed   | 83g       | 14g     |
| 2005 | Day<br>1 | Beg Weight | 129g      | 138g    |
|      |          | End Weight | 23g       | 96g     |
|      |          | Consumed   | 106g      | 42g     |
|      | Day<br>2 | Beg Weight | 91g       | 95g     |
|      |          | End Weight | 21g       | 84g     |
|      |          | Consumed   | 70g       | 11g     |

### 3.5. CONCLUSIONS

This study shows that lambs which are exposed with their mothers to false brome prior to weaning had a significantly increased propensity to consume false brome in a confinement setting ( $p < 0.001$ ). Lambs also show a increased propensity for the plant in a pasture setting.

The pasture training method explored in this study appears to be a viable method for training sheep to consume false brome.

CHAPTER 4

EFFECT OF EARLY EXPOSURE OF ORPHAN LAMBS TO FALSE BROME IN  
MILK ON SUBSEQUENT CONFINEMENT INTAKE OF FALSE BROME

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#### 4.1. ABSTRACT

Twenty-four lambs of uniform age were raised on milk replacer as orphan lambs. Twelve lambs received an extract of false brome in their milk for 14 days (28-42 days of age). At six months of age, the lambs were fasted for 18 hours, individually penned and offered 500g fresh cut false brome for 30 minutes. They were then held off feed a further 24 hours and the false brome testing repeated. Lambs were then returned to pasture for 28 days after which the intake trial was repeated.

At the first testing, treatment lambs consumed 28g compared to 16g for control lambs; on the following day, intakes were 50g vs. 35g, respectively. At retesting 28 days later, false brome intake on the first day was 78g vs. 53g and on the second day the intakes were 59g vs. 69g.

Testing of very young lambs to false brome juice in their milk pre-weaning did not have a significant effect on their intake of false brome forage when tested at six months of age ( $p=0.7933$ ; consumption by both groups was very low).

## **4.2. INTRODUCTION**

### **4.2.1. Research Question**

Will sheep exposed to *B. sylvaticum* as lambs in their milk have an increased affinity for *B. sylvaticum* compared to unexposed sheep in?

### **4.2.2. Hypothesis**

Sheep exposed to *B. sylvaticum* as lambs in their milk will have an increased affinity for *B. sylvaticum* compared to unexposed sheep when exposed in a confinement setting.

### **4.2.3. Experimental Design**

Two groups of 12 lambs born via caesarian section on a common day were weaned from their mothers at birth and raised as orphans. One group of lambs (exposure) received a juice pressure extracted from false brome using water in their milk from day 24 to 35 of age. Following training, lambs were weaned from milk onto grain and hay. Beginning at two months of age, lambs were allowed to graze perennial ryegrass (good) pasture.

At six months of age lambs were fasted overnight and exposed to fresh cut false brome in a confinement setting for 30 minutes. Following testing, lambs were fasted an additional 24 hours before the testing was repeated. Lambs were then returned

to pasture for 30 days before the confinement testing was repeated.

### **4.3. MATERIALS AND METHODS**

#### **4.3.1. Animals for the Experiment**

Ewes were synchronized using PGF<sub>2</sub> $\alpha$  and bred over a 24 hour period by natural service to ensure that lambs used in this study were uniform age. All lambs were born by caesarian section performed by students at the Oregon State University College of Veterinary Medicine.

Each lamb was fed colostrum via stomach tube every 3 hours for the first 24 hours after birth. Lambs were then trained to nurse reconstituted milk replacer (Land O Lakes- Saint Paul, MN) from a hanging calf bottle fitted with a lamb nipple (Nasco- Fort Atkinson, WI). When lambs were able to nurse independently, they were penned individually and allowed *ad libitum* access to fresh milk, creep feed, soybean meal and water.

#### **4.3.2. Orphan Lamb Training**

When the lambs were 24 days old, 12 began receiving milk in which a portion of the water was replaced with juice pressure-extracted from fresh false brome forage and frozen the previous summer. The juice initially constituted 5% of the liquid fraction of the milk; this was increased by 5% daily until

reaching 25% on day 28. The 25% concentration was fed *ad libitum* morning and evening through day 35 and continued as the lambs were gradually weaned to solid food over the next 5 days.

#### 4.3.3. **Sample Preparation**

On the morning of each confinement feeding trial, 12kg of false brome was collected using a hand scythe. The forage was separated into 24 equal allotments for individual feeding.

#### 4.3.4. **Confinement Feeding**

Lambs were placed in a holding pen and fasted for 18 hours prior to the beginning of the first confinement feeding. All lambs were allowed *ad libitum* access to fresh water during this time.

Lambs were individually penned and offered 250g of fresh cut false brome. After 30 minutes, lambs were returned to the holding pen and fasted for an additional 24 hours before a second confinement feeding. Uneaten false brome was collected and weighed to determine the amount consumed.

Forage desiccation during each feeding and forage dry matter content was also determined for dry matter calculations.

Confinement feeding was repeated in a second testing four weeks later.

#### 4.3.5. **Data Analysis**

Data from the confinement trial were analyzed by analysis of variance with the amount consumed as the dependent variable. Treatment and testing were fitted as independent variables.

#### 4.4. RESULTS AND DISCUSSION

At the first testing, treatment lambs consumed 28g compared to 16g for control lambs; on the following day, intakes were 50g vs. 35g, respectively (Table 4-1). At retesting 28 days later, false brome intake on the first day was 78g vs. 53g for controls and on the second day the intakes were 59g vs. 69g.

Orphan lambs exposed to false brome juice in their milk did not have an increased propensity to consume the plant later in life ( $p=0.7933$ ).

Table 4-1: Mean consumption of false brome in a confinement setting among lambs naïve and pre-exposed to false brome in milk.

|      |        |       | Treatment | Control |
|------|--------|-------|-----------|---------|
| 2005 | Test 1 | Day 1 | 28g       | 16g     |
|      |        | Day 2 | 50g       | 35g     |
|      | Test 2 | Day 1 | 78g       | 53g     |
|      |        | Day 2 | 59g       | 69g     |

Table 4-2: Analysis of Variance for amount of false brome consumed in confinement by lambs naïve and previously exposed to false brome in milk.

| Source            | Sum of Squares | Df | Mean Square | F-Ratio | P-Value |
|-------------------|----------------|----|-------------|---------|---------|
| Main Effects      |                |    |             |         |         |
| A: Treatment      | 67.5919        | 1  | 67.5919     | 0.07    | 0.7933  |
| B: Testing        | 24163.1        | 1  | 24163.1     | 24.70   | 0.0000  |
| Interactions      |                |    |             |         |         |
| AB                | 5378.68        | 1  | 5378.68     | 5.50    | 0.0213  |
| Residual          | 86082.2        | 88 | 978.207     |         |         |
| Total (Corrected) | 116741.0       | 91 |             |         |         |

#### **4.5. CONCLUSIONS**

While there was no significant effect of exposure of very young lambs to false brome juice in their milk pre-weaning on their intake of false brome forage when tested at six months of age (consumption by both groups was very low).

CHAPTER 5

EFFECT OF IN SACCO DIGESTION ON THE GERMINATION OF SLENDER  
FALSE BROME SEEDS

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## 5.1. ABSTRACT

To determine the effect of digestion by rumen micro flora on the viability of seeds (*in sacco* digestion technique), false brome seeds were digested *in sacco* for 3, 6, 12, 24, 48, or 72 hours in four ruminally cannulated wethers. Following digestion, seed samples were weighed to determine the amount of dry matter removed by ruminal digestion and germinated to determine viability.

Following a 3 week cold, wet stratification, and two weeks germination, 81% of false brome seeds which underwent a 3 hour digestion were viable. Subsequent digestions yielded viabilities of 83% after 6 hours, 85% after 12 hours, and 55% after 24 hours. After 48 hours of digestion, no viability was observed in false brome seeds.

## 5.2. INTRODUCTION

### 5.2.1. Research Question

Will false brome seeds which are digested *in sacco* (in the rumen) by sheep have decreased germination rates when compared to undigested seed?

### 5.2.2. Hypothesis

False brome seeds which are digested *in sacco* (in the rumen) by sheep will have decreased germination rates when compared to undigested seed.

### 5.2.3. Experimental Design

False brome seed was digested suspended in fine mesh bags in the rumens of four ruminally-cannulated sheep for 3, 6, 12, 24, 48, and 72 hours. Following digestion, seeds were given a three week cold, wet stratification period followed by two weeks in a growth chamber with a 14 hour, 25°C light period, followed by a 10 hour, 15°C dark period.

## 5.3. MATERIALS AND METHODS

### 5.3.1. Animals for the Experiment

In June 2005, (several months prior to the start of this study) four 15 month old Polypay X Suffolk wether lambs each weighing 52 kg were surgically fitted with 3 inch ruminal cannulas (Model #8C, Bar-Diamond, Inc).

Following surgery, the sheep were housed together in a 6m X 12m pen until the beginning of the trial. During this time, sheep received 1 kilogram alfalfa pellets per head daily and Timothy hay (good hay) *ad libitum*. Each sheep's cannula was

washed thoroughly and sprayed with insecticide twice weekly to prevent infection and fly strike.

Three weeks prior to the beginning of this trial, each sheep was individually penned in 1.5m X 3m pens for the duration of the trial to ensure equal consumption by each sheep and facilitate safe sample insertion and collection. Each sheep was also gradually switched from alfalfa pellets and good hay to 1.5 kg of Perennial Ryegrass/Tall Fescue hay (poor hay) daily.

#### 5.3.2. **Seed Collection**

Prior to the beginning of this study, 2 kg of false brome seeds were collected by manually stripping seeds from ripe seed heads. Seeds were stored in a cool, dry area until use in the trial.

#### 5.3.3. **Sample Preparation**

False brome seeds were dried in a 35°C drying oven for 48 hours and allowed to air-equilibrate. Ten 100 seed samples were weighed using an analytical balance accurate to 1/10000g to determine the average weight of one-hundred seeds (CWT).

Twenty-eight samples of seed weighing approximately 2.5g each were prepared and weighed using an analytical balance accurate to 0.0001g. The number of seeds in each sample was then calculated using the CWT and beginning weight.

Each sample was heat sealed into individual 5cm x 10cm Dacron digestion bags. Cotton string was secured to each digestion bag.

Seed bags were randomly allotted to sheep and digestion time, so that each sheep had one false brome sample for each of the six digestion times.

#### 5.3.4. ***In Sacco Seed Digestion***

*In sacco* digestion analysis was utilized to estimate the ruminal digestion of false brome seeds by sheep grazing on a low quality forage diet (similar in nutrient value to false brome in seed). Six Dacron bags were prepared using the aforementioned technique and were sequentially inserted into the rumen on a time schedule to allow 72, 48, 24, 12, 6, and 3 hour of digestion prior to removal at a common time.

Prior to inserting samples into the rumen, bags were soaked in warm water while the cannula plug was being cleaned and removed. Bags were inserted into the rumen cranial to the dorsal pillar and ventral to the ruminal cardia and secured to the cannula using the cotton string.

Following digestion for the prescribed time period, the bags were carefully removed from the rumen and immediately placed in ice water to arrest any microbial activity. Following a short immersion in ice water, each bag was rinsed under a

steady stream of cool water until the water draining from the bag was clear.

Bags were dried in a 35°C drying oven for 48 hours then allowed to air-equilibrate. Seeds were then removed from the bag and weighed to determine the amount of dry matter removed by ruminal digestion.

#### 5.3.5. **Seed Viability**

Seeds were germinated at the OSU Seed lab following standard germination procedures. Fifty seeds from each sample were subjected to a three week cold, wet stratification period followed by a two week grow-out temperature regiment of 25°C for 14 hours followed by 15°C for 10 hours.

#### 5.3.6. **Data Analysis**

Data from the *in sacco* trial were analyzed by a one-way analysis of variance with the seed germination as the dependent variable. Digestion time was fitted as an independent variables.

### **5.4. RESULTS AND DISCUSSION**

Viability of false brome seeds remained steady at approximately 80% for the first 12 hours of digestion, after which the viability dropped to 55% at 24 hours. No viability was observed in false brome seeds after 48 hours of digestion.

Table 5-1: Percent germination of false brome seeds following ruminal digestion by sheep for prescribed amount of time\*.

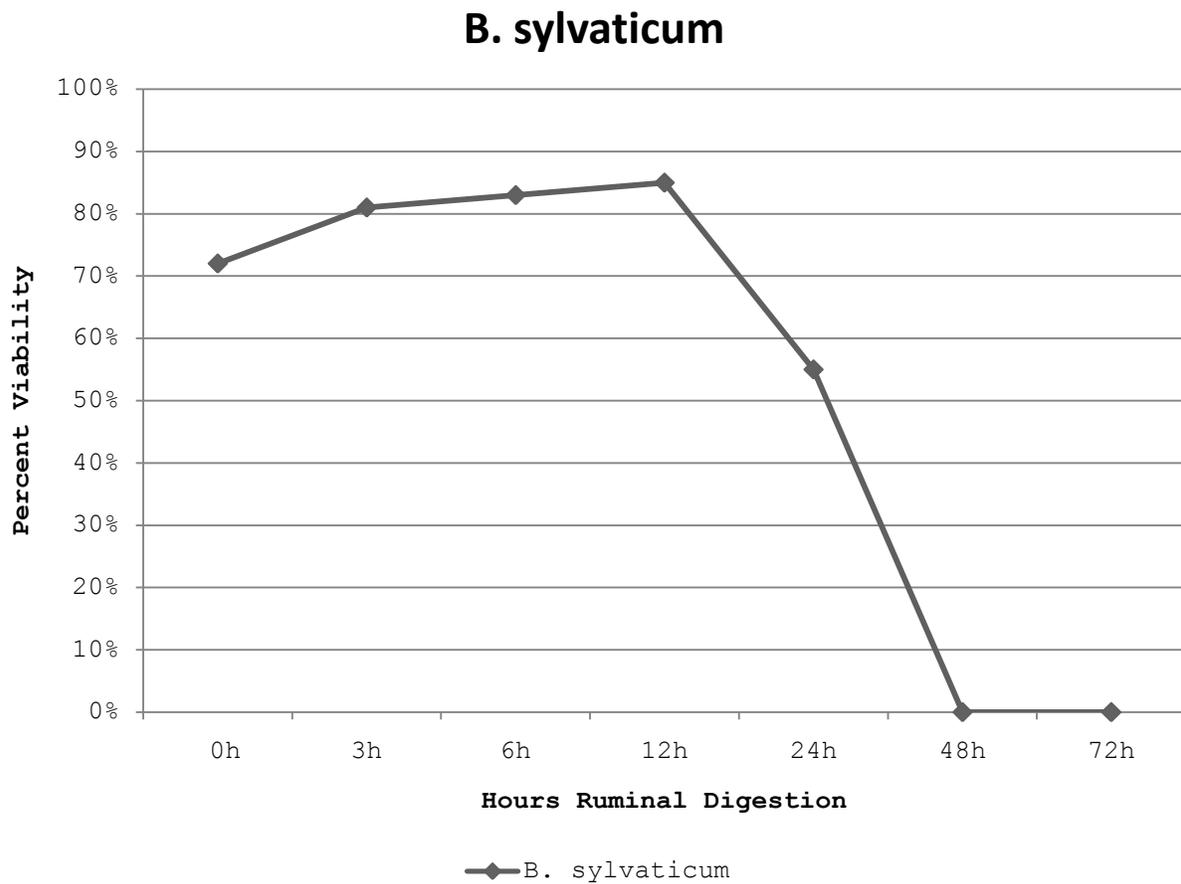
| Digestion Time | Germination Percent |
|----------------|---------------------|
| 0 hours        | 72% <sup>A,B</sup>  |
| 3 hours        | 81% <sup>A</sup>    |
| 6 hours        | 83% <sup>A</sup>    |
| 12 hours       | 85% <sup>A</sup>    |
| 24 hours       | 55% <sup>B</sup>    |
| 48 hours       | 0% <sup>C</sup>     |
| 72 hours       | 0% <sup>C</sup>     |

\* Samples not sharing a common superscript are statistically different at  $p < .05$ .

Table 5-2: Analysis of Variance for percent germination of false brome following ruminal digestion by sheep.

| Source            | Sum of Squares | Df | Mean Square | F-Ratio | P-Value |
|-------------------|----------------|----|-------------|---------|---------|
| Between Groups    | 8631.21        | 6  | 1438.54     | 39.26   | 0.0000  |
| Within Groups     | 769.5          | 21 | 26.6429     |         |         |
| Total (Corrected) | 9400.71        | 27 |             |         |         |

Figure 5-1: Percent germination of false brome seeds following ruminal digestion by sheep for prescribed amount of time\*.



### **5.5. CONCLUSIONS**

Viability of false brome seeds was significantly affected by digestion within 24 hours. No viability was observed in false brome seeds after 48 hours of ruminal digestion.

*CHAPTER 6*

EFFECT OF *IN VIVO* DIGESTION ON THE GERMINATION OF SLENDER  
FALSE BROME SEEDS

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### 6.1. ABSTRACT

Samples of false brome and Perennial Ryegrass seeds were digested *in vivo* to determine the effect of ruminal and post ruminal digestion on seed viability.

Fecal samples were collected daily for seven days from sheep which had been ruminally dosed false brome and Perennial Ryegrass seeds. Fecal samples were sieved to recover any whole seeds present before the feces was germinated following standard germination protocol.

No seeds were found in the feces, no seeds germinated from the fecal samples, and no seeds were identified from the fecal samples exposed to germination.

## **6.2. INTRODUCTION**

### **6.2.1. Research Question**

Will false brome seeds which are digested *in vivo* by sheep have decreased germination rates when compared to undigested seed?

### **6.2.2. Hypothesis**

False brome seeds which are digested *in sacco* by sheep will have decreased germination rates when compared to undigested seed.

### **6.2.3. Experimental Design**

Seed samples weighting 5g were introduced directly into the rumens of four ruminally cannulated sheep. Fecal samples were collected every 24 hours for 7 days following introduction of the seed. One aliquot of each fecal sample was sieved to find any undigested seeds, while a second was germinated in a growth chamber. Following germination, the sample was sieved to find any intact seeds which had failed to germinate.

## **6.3. MATERIALS AND METHODS**

### **6.3.1. Animals for the Experiment**

In June 2005 (several months prior to the start of this study) four 15 month old Polypay X Suffolk wether lambs each

weighing 52kg were surgically fitted with 3 inch ruminal cannulas (Model #8C, Bar-Diamond, Inc) were surgically installed into.

Following surgery, the sheep were housed together in a 6m X 12m pen until the beginning of the trial. During this time, sheep received 1kg alfalfa pellets per head daily and Timothy hay (good hay) *ad libitum*. Each sheep's cannula was washed thoroughly and sprayed with insecticide twice weekly to prevent infection and fly strike.

Three weeks prior to the beginning of this trial, each sheep was individually penned in 1.5m X 3m pens for the duration of the trial to ensure equal consumption by each sheep and facilitate safe sample insertion and collection. Each sheep was also gradually switched from alfalfa pellets and good hay to 1.5 kg of Perennial Ryegrass/Tall Fescue hay (poor hay) daily.

#### 6.3.2. **Seed Collection**

Prior to the beginning of this study, 2kg of false brome seeds were collected by manually stripping seeds from ripe seed heads. Seeds were stored in a cool, dry area until use in the trial.

#### 6.3.3. **Sample Preparation**

False brome seeds were dried in a 35°C drying oven for 48 hours, and allowed to air-equilibrate. Ten 100 seed samples were

weighed using an analytical balance accurate to 1/10000g to determine the average weight of one-hundred seeds (CWT).

Four approximately five gram samples of false brome seeds were prepared and weighed using an analytical balance accurate to 0.0001g. The number of seeds in each sample was calculated using the CWT and beginning weight.

#### 6.3.4. ***In vivo* Seed Digestion**

*In vivo* digestion analysis was utilized to determine the effects of ruminal and post-ruminal digestion of false brome seeds on subsequent seed recovery and viability. Samples were prepared using the aforementioned procedures (Section 6.3.3).

Seeds were introduced directly into the rumen of each sheep and feces were collected daily for seven days.

#### 6.3.5. **Seed Recovery**

Following daily fecal collection, 10% of each sample was sieved using water to recover any whole seeds.

#### 6.3.6. **Seed Viability**

Fecal samples were germinated at the OSU Seed lab following standard germination procedures. 20g from each sample was subjected to a three week cold, wet stratification period followed by a two week grow-out temperature regiment of 25°C for 14 hours followed by 15°C for 10 hours.

#### **6.4. RESULTS**

No seeds were found in the feces, no seeds germinated from the fecal samples, and no seeds were identified from the fecal samples exposed to germination.

*CHAPTER 7*

**EFFECT OF *IN VITRO* DIGESTION ON THE GERMINATION OF SLENDER  
FALSE BROME SEEDS**

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### 7.1. ABSTRACT

Samples of false brome seeds were digested *in vitro* to quantify the germination results of *in sacco* and *in vivo* digestion techniques.

No germination was observed for false brome seeds following either 24 or 48 hours of *in vitro* ruminal digestion.

## **7.2. INTRODUCTION**

### **7.2.1. Research Question**

Will false brome seeds which are digested *in vitro* using sheep rumen fluid have decreased germination rates when compared to undigested seed?

### **7.2.2. Hypothesis**

False brome seeds which are digested *in vitro* using sheep rumen fluid will have decreased germination rates when compared to undigested seed.

### **7.2.3. Experimental Design**

False brome seeds were digested using standard *in vitro* digestion techniques for either 24 or 48 hours. Following digestion, seeds were given a three week cold, wet stratification period followed by two weeks in a growth chamber with a 14 hour, 25°C light period, followed by a 10 hour, 15°C dark period.

## **7.3. MATERIALS AND METHODS**

### **7.3.1. Animals for the Experiment**

In June 2005 (several months prior to the start of this study) four 15 month old Polypay X Suffolk wether lambs each weighing 52 kg were surgically fitted with 3 inch ruminal

cannulas (Model #8C, Bar-Diamond, Inc) were surgically installed into.

Following surgery, the sheep were housed together in a 6m X 12m pen until the beginning of the trial. During this time, sheep received 1 kilogram alfalfa pellets per head, daily and Timothy hay (good hay) *ad libitum*. Each sheep's cannula was washed thoroughly and sprayed with insecticide twice weekly to prevent infection and fly strike.

Three weeks prior to the beginning of this trial, each sheep was individually in 1.5m X 3m pens for the duration of the trial to ensure equal consumption for each sheep and facilitate safe sample insertion and collection. Each sheep was also gradually switched from alfalfa pellets and good hay to 1.5 kg of Perennial Ryegrass/Tall Fescue hay (poor hay) daily.

#### 7.3.2. **Rumen Fluid Collection**

On the day of the trial, one 500ml aliquot of rumen fluid was collected from each sheep. Aliquots were then filtered through cheesecloth and mixed to form a uniform inoculum.

#### 7.3.3. **Sample Preparation**

False brome seeds were dried in a 35°C drying oven for 48 hours, and allowed to air-equilibrate. Ten 100 seed samples were weighed using an analytical balance accurate to 1/10000g to determine the average weight of one-hundred seeds (CWT).

Six 0.25g false brome samples were prepared and weighed. The number of seeds in each sample was calculated using the CWT and beginning weight. Three samples of each seed type were assigned to 24 hour digestion while the other three samples were assigned to 48 hour digestion.

#### 7.3.4. ***In vitro* Seed Digestion**

*In vitro* digestion analysis was utilized to quantify viability results obtained from the ruminal and post-ruminal digestion of false brome. Samples were prepared using the aforementioned procedures (section 7.3.3) and run in the OSU forage lab using the following procedures.

Three blank samples containing only ruminal fluid were run with each time block to account for solids in the ruminal fluid which were not removed by filtering through cheesecloth.

Each 0.25g seed sample was placed in an individual 250ml Erlenmeyer flask with 0.25g of false brome substrate which had been previously ground to pass a 0.5mm screen. 90ml of rumen fluid and 10ml of McDougal's buffer ( $\text{NaCO}_2$ ) were then added to each sample after which the flasks were capped with a rubber stopper fitted with a one-way valve to allow gasses to escape the flask.

Samples were incubated at  $38.5^\circ\text{C}$  for the prescribed times, agitating them every 8 hours.

After 24 or 48 hours (depending on treatment) the samples were removed from the incubator, filtered, and rinsed under suction to remove all rumen fluid. Samples were then dried at 35°C for 24 hours, weighed, sieved under DI water to remove all substrate, dried, and germinated

#### 7.3.5. **Seed Viability**

Seeds were germinated at the OSU Seed lab following standard germination procedures. Fifty seeds from each sample were subjected to a three week cold, wet stratification period followed by a two week grow-out temperature regiment of 25°C for 14 hours followed by 15°C for 10 hours.

#### **7.4. RESULTS**

No viability was observed for seeds following either the 24 or 48 hour digestion.

## CHAPTER 8

### CONCLUSIONS

Results indicate that enclosing sheep on false brome with their mothers as lambs may be an effective means for pre-conditioning them to graze the grass. Additionally, false brome seeds are not likely to retain viability after passing through the sheep digestive system; therefore, use of sheep as grazing tools to control this invasive weed species is not likely to pose a threat of spreading viable seed. Since the seed has a prominent awn, spread via transport in wool remains a concern so it is recommended that sheep used for grazing control be shorn.

## CHAPTER 9

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CHAPTER 10

APENDIX 1: EFFECT OF IN SACCO DIGESTION ON THE WEIGHT OF  
SLENDER FALSE BROME SEEDS

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## **10.1. MATERIALS AND METHODS**

### **10.1.1. Animals for the Experiment**

In June 2005, (several months prior to the start of this study) four 15 month old Polypay X Suffolk wether lambs each weighing 52 kg were surgically fitted with 3 inch ruminal cannulas (Model #8C, Bar-Diamond, Inc).

Following surgery, the sheep were housed together in a 6m X 12m pen until the beginning of the trial. During this time, sheep received 1 kilogram alfalfa pellets per head daily and Timothy hay (good hay) *ad libitum*. Each sheep's cannula was washed thoroughly and sprayed with insecticide twice weekly to prevent infection and fly strike.

Three weeks prior to the beginning of this trial, each sheep was individually penned in 1.5m X 3m pens for the duration of the trial to ensure equal consumption by each sheep and facilitate safe sample insertion and collection. Each sheep was also gradually switched from alfalfa pellets and good hay to 1.5 kg of Perennial Ryegrass/tall fescue hay (poor hay) daily.

### **10.1.2. Seed Collection**

Prior to the beginning of this study, 2 kg of false brome seeds were collected by manually stripping seeds from ripe seed

heads. Seeds were stored in a cool, dry area until use in the trial.

#### 10.1.3. **Sample Preparation**

False brome seeds were dried in a 35°C drying oven for 48 hours and allowed to air-equilibrate. Ten 100 seed samples were weighed using an analytical balance accurate to 1/10000g to determine the average weight of one-hundred seeds (CWT).

Twenty-eight samples of seed weighing approximately 2.5g each were prepared and weighed using an analytical balance accurate to 0.0001g. The number of seeds in each sample was then calculated using the CWT and beginning weight.

Each sample was heat sealed into individual 5cm x 10cm Dacron digestion bags. Cotton string was secured to each digestion bag.

Seed bags were randomly allotted to sheep and digestion time, so that each sheep had one false brome sample for each of the six digestion times.

#### 10.1.4. ***In Sacco* Seed Digestion**

*In sacco* digestion analysis was utilized to estimate the ruminal digestion of false brome and Perennial Ryegrass seeds by sheep grazing on a low quality forage diet. Twelve Dacron bags

(six of each species of seeds) were prepared using the aforementioned technique and were sequentially inserted into the rumen on a time schedule to allow 72, 48, 24, 12, 6, and 3 hour of digestion prior to removal at a common time.

Prior to inserting samples into the rumen, bags were soaked in warm water while the cannula plug was being cleaned and removed. Bags were inserted into the rumen cranial to the dorsal pillar and ventral to the ruminal cardia and secured to the cannula using the cotton string.

Following digestion for the prescribed time period, the bags were carefully removed from the rumen and immediately placed in ice water to arrest any microbial activity. Following a short immersion in ice water, each bag was rinsed under a steady stream of cool water until the water draining from the bag was clear.

Bags were dried in a 35°C drying oven for 48 hours then allowed to air-equilibrate. Seeds were then removed from the bag and weighed to determine the amount of dry matter removed by ruminal digestion.

#### 10.1.5. **Calculations**

To determine the amount of dry matter removed from the seeds by ruminal digestion, it was necessary to first determine the dry matter content for undigested seeds. To do this, three

1.0g samples of undigested seed from each species was weighed, dried at 100 °C the re-weighed to determine percent dry matter of the undigested seeds (DMU).

Likewise, nine 1/3g samples of the digested seed within each digestion time were randomly selected from each species of grass and combined to make three 1g samples. Each sample was weighed, dried at 100 °C and weighed again to determine the percent dry matter of the digested seeds (DMD).

After final weights were collected, the final weight was calculated as a percent of the initial sample weight, representing the percent of dry matter removed by digestion. This percent was then used in all subsequent calculations and analysis.

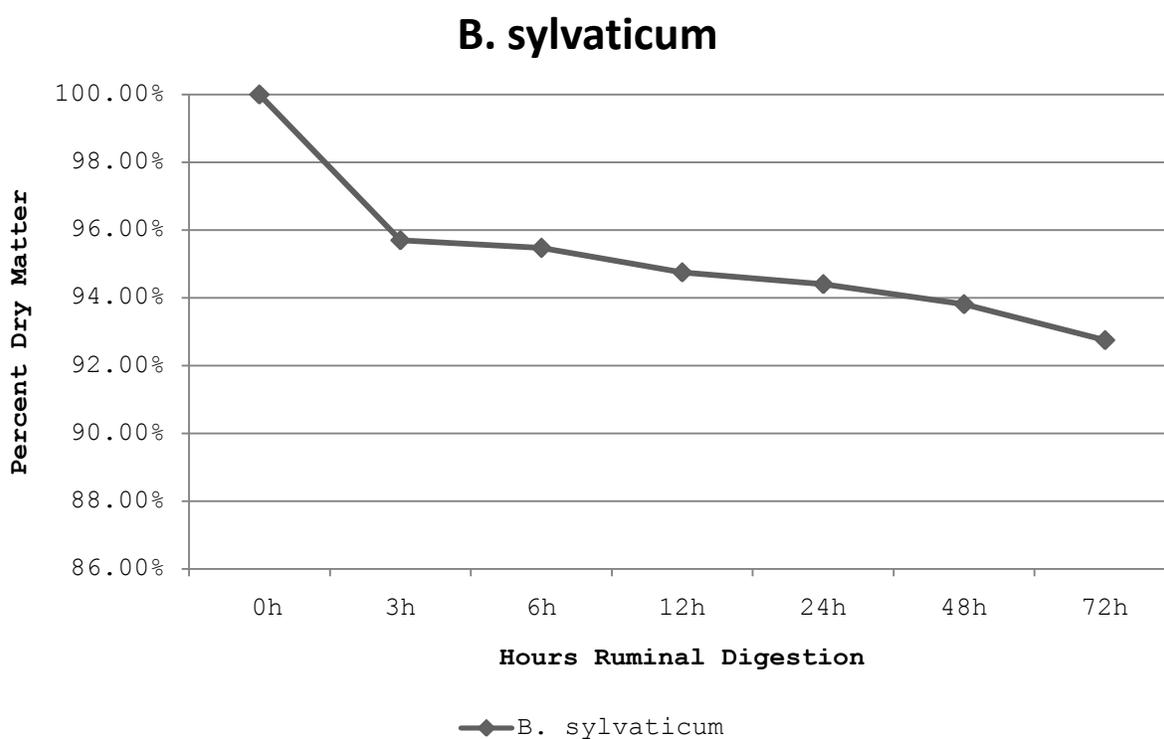
## 10.2. RESULTS AND DISCUSSION

Mean seed digestion percentages for *in sacco* digestion of false brome and Perennial Ryegrass for the various digestion times are shown in **Error! Reference source not found.** Mean digestion rates for each sheep are summarized in **Error! Reference source not found.** No significant difference in digestion rates was observed between sheep used in this trial.

Table 10-1: Percent of original dry matter for false brome seeds following ruminal digestion by sheep for prescribed amount of time.

| Digestion Time | Germination Percent |
|----------------|---------------------|
| 3 hours        | 96%                 |
| 6 hours        | 95                  |
| 12 hours       | 95%                 |
| 24 hours       | 94%                 |
| 48 hours       | 94%                 |
| 72 hours       | 93%                 |

Figure 10-1: Percent of dry matter remaining after *in sacco* ruminal digestion of false brome by sheep for prescribed times.



CHAPTER 11

APENDIX 2: EFFECT OF IN SACCO DIGESTION ON THE GERMINATION  
OF SLENDER FALSE BROME AND PERENNIAL RYEGRASS SEEDS

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### **11.1. MATERIALS AND METHODS**

Perennial ryegrass seeds were prepared using methods detailed in Chapter 6.

### **11.2. RESULTS AND DISCUSSION**

Viability of Perennial Ryegrass seeds remained steady at approximately 90% through 12 hours of digestion. Viability was reduced to 73% at 24 hours, 43% after 48 hours, and 26% after 72 hours.

Table 11-1: Percent germination of perennial ryegrass seeds following ruminal digestion by sheep for prescribed amount of time\*.

| Digestion Time | Germination Percent |
|----------------|---------------------|
| 3 hours        | 90%                 |
| 6 hours        | 90%                 |
| 12 hours       | 92%                 |
| 24 hours       | 86%                 |
| 48 hours       | 64%                 |
| 72 hours       | 22%                 |

Figure 11-1: Percent germination of perennial ryegrass seeds following ruminal digestion by sheep for prescribed amount of time.

