

PROVIDING SUPPLEMENT, WITH OR WITHOUT PEG, TO REDUCE THE
EFFECTS OF CNICIN AND ENHANCE GRAZING OF SPOTTED KNAPWEED BY
SHEEP AND CATTLE

by

Melany Cheeseman

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of the requirements for the degree

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Dr. Bret E. Olson

Approved for the Department of Animal and Range Sciences

Dr. Wayne F. Gipp

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ABSTRACT

Spotted knapweed (*Centaurea maculosa* Lam.), an invasive forb from Eurasia, infests over 1.7 million hectares in Montana and costs millions of dollars in forage losses each year. Some wildlife and livestock graze this species, however, most large herbivores, including cattle and horses, avoid spotted knapweed and prefer native plants. Some herbivores may avoid or reduce their intake of spotted knapweed because the plant contains cnicin. Cnicin is a bitter-tasting sesquiterpene lactone that may cause negative post-ingestive consequences in the rumen. If an appropriate supplement and/or anti-toxicant can be identified that will reduce the negative post-ingestive effects of cnicin to rumen microbes, sheep and cattle may consume more spotted knapweed and grazing may help control this invasive species.

I determined the effects of cnicin, rates of an anti-toxicant (polyethylene glycol), and a nutrient/energy supplement, with or without polyethylene glycol, on sheep and cattle rumen microbial activity, efficiency, and mass using a modified *in vitro* system. Sheep and cattle rumen microbes were negatively affected by cnicin. Polyethylene glycol, at the rates provided, had limited effects on sheep and cattle rumen microbial response to spotted knapweed plant parts. The nutrient/energy supplement, with or without polyethylene glycol, enhanced sheep and cattle rumen microbial efficiency. Because the nutrient/energy supplement may reduce some of the negative effects of cnicin on rumen microbes, sheep and cattle may consume more spotted knapweed in the field when provided with the supplement.

I hypothesized that providing a nutrient/energy supplement, with or without polyethylene glycol, would increase sheep and cattle intake and time spent consuming spotted knapweed in a drylot, and in the field in July and August. Animal behaviors were recorded by focal animal sampling. Providing a nutrient/energy supplement, with or without polyethylene glycol, did not enhance sheep and cattle intake or time spent consuming spotted knapweed in a drylot, or in the field in July and August. Therefore, supplementing sheep and cattle with the nutrient/energy supplement, with or without polyethylene glycol, provided in these trials, may not be an appropriate method for managing the spread of spotted knapweed.

CHAPTER 1

INTRODUCTION

In the northwestern United States, invasive plant species displace native vegetation and decrease forage (Watson and Renney, 1974; Tyser and Key, 1988). Many alternatives have been considered to decrease the number and spread of invasive species. Sheep grazing can control some undesirable species (Sharrow and Mosher, 1982; Ralphs et al., 1991; Bell et al., 1996), and grazing can be a cost-effective method of weed control (Williams et al., 1996).

Spotted knapweed (*Centaurea maculosa* Lam.), an invasive species from Eurasia, infests over 1.7 million ha in Montana (Montana Weed Management Task Force, 2005) and costs millions of dollars in forage losses each year (Bucher, 1984; Hirsch and Leitch, 1996). Some wildlife and livestock species graze spotted knapweed (Wright and Kelsey, 1997; Olson and Wallander, 2001); however, some livestock, especially cattle and horses, avoid spotted knapweed and select native plants.

Morphophysiological adaptations, such as mouth size and mobility of mouthparts affect sheep and cattle grazing preferences. Cattle and sheep are roughage eaters (Hofmann, 1989), however, cattle prefer grasses to forbs and sheep will consume grasses and forbs (Grant et al., 1885; Ruyle and Bowns, 1985). Cattle have large mouths and cannot be very selective while grazing (Hofmann, 1989; Meyer et al., 1957). In contrast, sheep have small, mobile lips and can selectively consume individual plant parts.

Some ruminants may consume less spotted knapweed because of the secondary compound cnicin. Cnicin is a bitter-tasting (Suchy and Herout, 1962) sesquiterpene lactone that is present in all aboveground spotted knapweed plant parts (Olson and Kelsey, 1997). Sheep rumen microbial activity is lower when cnicin concentrations in spotted knapweed plant parts are high (Olson and Kelsey, 1997). A ruminant experiences negative post-ingestive feedback (PIF) when an ingested plant negatively affects the ruminant or rumen microbes (Freeland and Janzen, 1974). Ruminants will avoid or reduce their intake of plant species associated with negative PIF (Provenza, 1990). Sheep and cattle may consume less spotted knapweed if it causes negative PIF. However, if an appropriate supplement and/or anti-toxicant can be identified that will reduce the negative effects of cnicin, sheep and cattle may consume more spotted knapweed.

Microbial activity in the rumen may detoxify some plant secondary compounds (Freeland and Janzen, 1974; Smith, 1992), such as cnicin. Nutrient and energy supplements can increase rumen microbial activity and mass (Garg and Gupta, 1992; Srinivas and Gupta, 1997; Moujahed et al., 2000; Hess et al., 2004). If increased rumen microbial activity reduces the negative effects of cnicin, ruminants may consume more spotted knapweed. Providing supplemental nutrients and energy may increase intake and digestibility of plants containing secondary compounds (Banner et al., 2000; Villalba et al., 2002a, b).

Anti-toxicants may reduce the negative effects of secondary compounds on ruminants and rumen microbes (Smith, 1992). The anti-toxicant polyethylene glycol (PEG) binds with tannins (Jones and Mangan, 1977), and may also bind with other

secondary compounds. Rumen microbes exposed to tannin-containing forages have greater activity, efficiency, and mass *in vitro* when PEG is included in the flask (Saarisalo et al., 1999; Getachew et al., 2000, 2001; Garcia et al., 2004). If PEG binds to cnicin and reduces its negative effects on rumen microbes, ruminants may consume more spotted knapweed when provided with the anti-toxicant. Herbivores consume more tannin-rich forages when PEG is provided (Titus et al., 2001; Bhatta et al., 2002; Landau et al., 2002).

Supplement blocks are an effective way to deliver PEG to ruminants (Ben Salem et al., 1999, 2002). If ruminants consume the block several times throughout the day, it may increase the effectiveness of the anti-toxicant. Polyethylene glycol is more effective *in vitro* when provided in several small doses instead of one large dose (Getachew et al., 2001). Supplemental nutrients and energy can also benefit ruminants. If providing a supplement block with PEG decreases the negative effects of spotted knapweed on rumen microbes, herbivores may consume more spotted knapweed. Herbivores consume more tannin-rich forages when supplemental feed blocks containing PEG are provided (Moujahed et al., 2000; Ben Salem et al., 2000, 2002).

I hypothesized that *in vitro* microbial activity (gas production), efficiency (*in vitro* dry matter disappearance), and mass (purines) would be: 1) decreased by the secondary compound cnicin, 2) greater at greater rates of the anti-toxicant PEG, and 3) enhanced by a nutrient/energy supplement with or without PEG. I determined sheep and cattle rumen microbial response to cnicin, PEG, and a nutrient/energy supplement (Chapter 2). I used a modified *in vitro* system to measure rumen microbial activity, efficiency, and mass.

The effects of pure cnicin may support earlier research that indicates this secondary compound is responsible for the negative effects of spotted knapweed on rumen microbes. Different rates of PEG were added to flasks containing different plant parts of spotted knapweed to determine an appropriate rate to bind with cnicin in spotted knapweed. The nutrient/energy supplement was added to flasks containing spotted knapweed leaves, stems, and buds to determine if supplement would increase rumen microbial function of sheep and cattle that consumed these plant parts of spotted knapweed.

I hypothesized that sheep provided with a nutrient/energy supplement, with or without PEG, would consume more spotted knapweed: 1) in a drylot, 2) in the field during July, and 3) in the field during August. The effects of providing sheep with a nutrient/energy supplement, with or without PEG, were determined in a drylot, and in the field in July and August (Chapter 3). Ewes were provided with no supplement, a nutrient/energy supplement, or a nutrient/energy supplement with PEG. Intake and time spent consuming fresh-cut spotted knapweed and fresh-cut grass were measured in a drylot. Bite rates and percent time grazing spotted knapweed, perennial grasses, and other forbs were determined for ewes in the field.

I hypothesized that cattle provided with a nutrient/energy supplement, with or without PEG, would consume more spotted knapweed: 1) in a drylot, 2) in the field during July, and 3) in the field during August. The effects of providing cattle with a nutrient/energy supplement, with or without PEG, were determined in a drylot and in the field in July and August (Chapter 4). Heifers were provided with no supplement, a

nutrient/energy supplement, or a nutrient/energy supplement with PEG. Intake and time spent consuming fresh-cut spotted knapweed and fresh-cut grass were measured in a drylot. Bite rates and percent time grazing spotted knapweed, perennial grasses, and other forbs were measured for heifers in the field. Results and implications of these three studies are summarized in Chapter 5.

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CHAPTER 2

RUMEN MICROBIAL RESPONSE

Introduction

In the northwestern United States, invasive plants displace native vegetation and decrease forage (Watson and Renney, 1974; Tyser and Key, 1988). Spotted knapweed (*Centaurea maculosa* Lam.), an invasive species from Eurasia, invades disturbed sites (Watson and Renney, 1974) and spreads rapidly by seeds (Schirman, 1981). Spotted knapweed currently infests over 1.7 million ha in Montana (Montana Weed Management Task Force, 2005) and costs millions of dollars in forage losses each year (Bucher, 1984; Hirsch and Leitsch, 1996).

Spotted knapweed contains secondary compounds that may reduce grazing by livestock (Locken and Kelsey, 1987; Olson and Kelsey, 1997). Plant secondary compounds are a defense mechanism that can cause negative post-ingestive feedback (PIF) in some herbivores (Freeland and Janzen, 1974). Negative PIF occurs when an ingested plant negatively affects the ruminant or the rumen microbes, and can lead herbivores to avoid or reduce their intake of certain plant species (Provenza et al., 1990). Ruminants may continue to consume a plant if they can detoxify the secondary compounds and thereby reduce or eliminate negative PIF.

Cnicin, the major aboveground secondary compound in spotted knapweed, is a bitter-tasting (Suchy and Herout, 1962) sesquiterpene lactone with anti-microbial and anti-bacterial properties (Cavallito and Bailey, 1949; Karioti et al., 2002; Saragou et al.,

2005). Cnicin is present in all aerial spotted knapweed plant parts; however, concentrations are highest in leaves and lowest in stems (Olson and Kelsey, 1997). A diet of greater than 70% spotted knapweed (leaves, 0.86-3.86% cnicin; buds, 1.95% cnicin) negatively affects sheep rumen microbes. Microbial activity and mass are greater when cnicin concentrations of spotted knapweed plant parts are reduced via extraction (Olson and Kelsey, 1997). These results indicate that rumen microbes respond negatively to spotted knapweed because of cnicin; however, the effects of the compound cnicin on rumen microbes have not been assessed.

Microbial activity in the rumen can detoxify plant secondary compounds (Freeland and Janzen, 1974; Smith, 1992), such as cnicin. Introducing supplemental nutrients and energy into the rumen can increase microbial populations and activity (Freeland and Janzen, 1974; Garg and Gupta, 1992; Srinivas and Gupta, 1997; Moujahed et al., 2000; Hess et al., 2004). A supplement block, such as Sweet 14% (Ridley Block Operations, Table 12), contains nutrients and energy that may reduce the negative effects of spotted knapweed on rumen microbes, and increase intake of the plant. Providing a nutrient, energy, or protein supplement can enhance intake of forages containing secondary compounds (Banner et al., 2000; Villalba et al., 2002a, b).

Anti-toxicants may bind with secondary compounds and possibly reduce their negative effects on ruminants (Smith, 1992). For example, the anti-toxicant polyethylene glycol (PEG) increases microbial activity and mass, and *in vitro* dry matter disappearance (IVDMD) when rumen microbes are exposed to tannin-rich forages (Saarisalo et al., 1999; Getachew et al., 2000, 2001; Garcia et al., 2004). Polyethylene glycol binds to

hydroxyl groups of tannin molecules through hydrogen bonding (Jones and Mangan, 1977). Similarly, PEG may bond to hydroxyl groups of cnicin molecules and reduce the negative effects of this compound. If PEG reduces the negative effects of cnicin, sheep and cattle may consume more spotted knapweed when provided with the anti-toxicant. Grazing herbivores increase their intake of tannin-containing forages (*Coleogyne ranosissima* Torr., *Prosopis cineraria* L., *Pistacia lentiscus* L.) when provided with 5 – 50 g of PEG per day (Titus et al., 2001; Bhatta et al., 2002; Landau et al., 2002).

Adding PEG to a nutrient/energy supplement may further reduce the negative effects of cnicin in spotted knapweed. Digestibility and intake of tannin-containing forages is greater when PEG is delivered in a supplement block than when it is delivered in drinking water, sprayed on forage, or provided in concentrate (Ben Salem et al., 1999, 2002). Polyethylene glycol is more effective *in vitro* when offered in a slow release form rather than when added all at once (Getachew et al., 2001). If ruminants consume the supplement block several times during the day, they will receive several small doses of PEG and the anti-toxicant should be more effective. Providing a supplemental feed block with PEG increases rumen microbial activity and mass, and enhances intake when ewes are fed a tannin-rich diet (Moujahed et al., 2000; Ben Salem et al., 2000, 2002). Grazing animals may consume spotted knapweed more readily if Sweet 14% with or without PEG enhances rumen microbial function.

I hypothesized that *in vitro* microbial activity (gas production), efficiency (IVDMD), and mass (purines) would be: 1) decreased by the secondary compound cnicin, 2) greater at greater rates of the anti-toxicant PEG, and 3) enhanced by a

nutrient/energy supplement with or without PEG. My objectives were to determine the effects of: 1) the secondary compound cnicin (Trial 1), 2) rates of an anti-toxicant (PEG; Trial 2) and 3) supplemental nutrients/energy with or without an anti-toxicant (Sweet 14% with or without PEG; Trial 3) on sheep and cattle rumen microbial activity, efficiency, and mass *in vitro*.

Methods and Materials

Plant Materials and Nutritive Value

Spotted knapweed was collected at an infested site near Belgrade, Montana (45°45'N, 111°10'W) in June 2004. The elevation is about 1376 m and the soil is a loamy-skeletal over sandy or sandy skeletal, mixed, superactive, frigid Aridic Argiustoll (USDA, 2002). Spotted knapweed was air-dried under shade for 10 d, and then separated into leaves, stems, and buds. These plant parts and a low-quality grass hay, as a standard, were ground to 1 mm and used for all *in vitro* trials.

Ground spotted knapweed leaves, stems, and buds and the grass hay were dried at 60° C for 48 h and analyzed for DM, CP, and ash using standard methods (AOAC, 2002). Acid detergent fiber (ADF) and neutral detergent fiber (NDF) were analyzed using methods for an ANKOM fiber analyzer (Ankom Technology Corp., Fairport, NY; Van Soest et al., 1991).

Neutral detergent fiber of spotted knapweed leaves was much lower than spotted knapweed stems and buds, and grass hay (Table 1). Acid detergent fiber was lowest for spotted knapweed leaves, highest for spotted knapweed stems, and intermediate for grass hay and spotted knapweed buds. Crude protein of grass hay and spotted knapweed stems

was lower than that of spotted knapweed leaves and buds. Organic matter was similar for all forages.

Table 1. Neutral detergent fiber (NDF), acid detergent fiber (ADF), crude protein (CP), and organic matter (OM) of *Centaurea maculosa* plant parts, and of grass hay

Plant part	NDF (%)	ADF (%)	CP (%)	OM (%)
<i>C. maculosa</i>				
stems	46.3	37.2	6.2	88.1
leaves	20.4	14.3	12.2	84.5
buds 1 ^a	41.3	30.8	11.5	89.0
buds 2 ^b	38.5	28.5	11.4	89.0
Grass Hay	49.1	31.5	6.9	85.1

a – *C. maculosa* buds 1 were used in Trials 2a and 3

b – *C. maculosa* buds 2, from a different year, were used in Trial 2b

Trial 1 - Cnicin

I measured sheep and cattle rumen microbial response to the secondary compound cnicin. Flasks were randomly assigned to 1 of 3 treatments: 1) grass hay, 2) grass hay with cnicin, or 3) spotted knapweed leaves. Dried spotted knapweed leaves may contain up to 3.86% cnicin (Olson and Kelsey, 1997). Fresh and dried spotted knapweed have similar cnicin concentrations (Kelsey and Mihalovich, 1987). Cnicin (0.06g, 95% pure, R. Kelsey, pers. comm.) was added to grass hay (1.94g) to mimic a 3% concentration in spotted knapweed leaves.

Trial 2 – Anti-toxicant

I measured sheep and cattle rumen microbial response to 2 sets of 3 rates of PEG (MW = 3350). Polyethylene glycol rates were based on predicted average and predicted maximum Sweet 14% (Ridley Block Operations) supplement intake relative to total feed intake. Predicted average (Trial 2a) and maximum (Trial 2b) PEG intake rates were

12.5% of supplement intake rates, to correspond with the dose of anti-toxicant in the supplement with PEG. Average PEG rates per flask were 9 mg, 18 mg, and 36 mg for sheep, and 2.5 mg, 5 mg, and 10 mg for cattle. Maximum PEG rates per flask were 15 mg, 30 mg, and 60 mg for sheep, and 5 mg, 10 mg, and 20 mg for cattle. Each dose of PEG was mixed with 3 ml reverse osmosis (RO) water. Spotted knapweed leaves and stems were used in Trial 2a, whereas buds and stems were used in Trial 2b. I replaced buds with leaves in Trial 2b because Trial 3 (described below) indicated that rumen microbial response to the supplement containing PEG was somewhat greater for buds than leaves. Flasks were randomly assigned to 1 of the 3 treatment rates and 2 forage types.

Trial 3 – Nutrient/energy Supplement

I measured sheep and cattle rumen microbial response to a nutrient/energy supplement (Sweet 14%, Ridley Block Operations), with or without PEG, using spotted knapweed leaves, stems, and buds, and grass hay (Table 1). Flasks were randomly assigned to each forage type and 1 of 3 treatments: 1) control, 2) nutrient/energy supplement, and 3) nutrient/energy supplement with PEG (12.5% PEG). The supplement was freeze-dried and crushed into a fine powder using a mortar and pestle. Doses of supplement per flask were based on the predicted average supplement intake relative to total feed intake for sheep (143 mg) and cattle (40 mg) (M. Robbins, pers. comm., Ridley Block Operations). Five ml RO water was mixed with each supplement dose and also added to each control flask.

Rumen Microbial Response

I measured microbial gas production (activity), dry matter disappearance (efficiency), and purines (mass) using a modified *in vitro* system. Rumen fluid from 2 cannulated white-faced ewes (mean BW = 54 kg) and 2 cannulated beef cows (mean BW = 771 kg) fed a diet of grass hay was used for all trials. Rumen fluid was collected and strained through 8 layers of cheesecloth. For each treatment, duplicate 250 ml flasks were prepared containing 50 ml strained rumen fluid, 2 g forage, and 100 ml McDougall's buffer. Flasks were placed in a shaking water bath at 39°C, and gas production was measured by water displacement in inverted burettes at 2, 3, 4, 6, 12, 18, and 24 hr (Roberts and Olson, 1999). Flask contents were filtered after gas trials were completed. Residues were dried at 60°C for 48 hr and weighed to determine IVDMD. Residues were also analyzed for purine concentrations of attached bacteria (Zinn and Owens, 1986).

Statistical Analyses

Cumulative gas production, IVDMD, and purine concentrations were analyzed using ANOVA (PROC Mixed Procedure, SAS, 2004). The model included random error due to bath ($n = 2$) and run ($n = 3$ replicate runs for each trial: 1, 2a, 2b, 3). The experimental unit was the flask and the sample size for each trial was $n = 6$. Sheep and cow data were analyzed separately for all trials because the animal species x forage type interaction was significant in Trial 1 ($P < 0.0001$), and different doses of PEG and supplement were added to sheep and cattle flasks in Trials 2 and 3.

Effects of forage type ($n = 3$; hay, hay with cnicin, spotted knapweed leaves) were analyzed for Trial 1. Forage types were compared using the Tukey method for pairwise comparisons (Tukey, 1953).

Effects of rate of PEG ($n = 3$; 0.5x, 1x, 2x) were analyzed by forage type ($n = 2$) for Trial 2a and 2b. Treatment means were compared using the Tukey method for pairwise comparisons (Tukey, 1953).

Effects of treatment ($n = 3$; control, supplement, supplement with PEG) were analyzed by forage type ($n = 4$; hay, spotted knapweed leaves, stems, and buds) for Trial 3. Planned contrasts were used to compare treatment means of: 1) flasks with supplement vs. flasks without supplement, and 2) supplemented flasks with PEG vs. supplemented flasks without PEG.

I present P -values less than 0.15 (Gill, 1981). Data that did not meet the assumption of normality were transformed using appropriate transformations. Actual means and standard errors are presented in text, tables, and figures.

Results

Trial 1 - Cnicin

Sheep and cattle rumen microbes produced more gas in 24 h when exposed to grass hay than when exposed to grass hay with cnicin ($P < 0.001$; Figure 1A). Sheep rumen microbes produced more gas when exposed to spotted knapweed leaves than when exposed to hay with cnicin ($P < 0.001$). In contrast, cattle rumen microbes produced similar amounts of gas from the 2 forages.

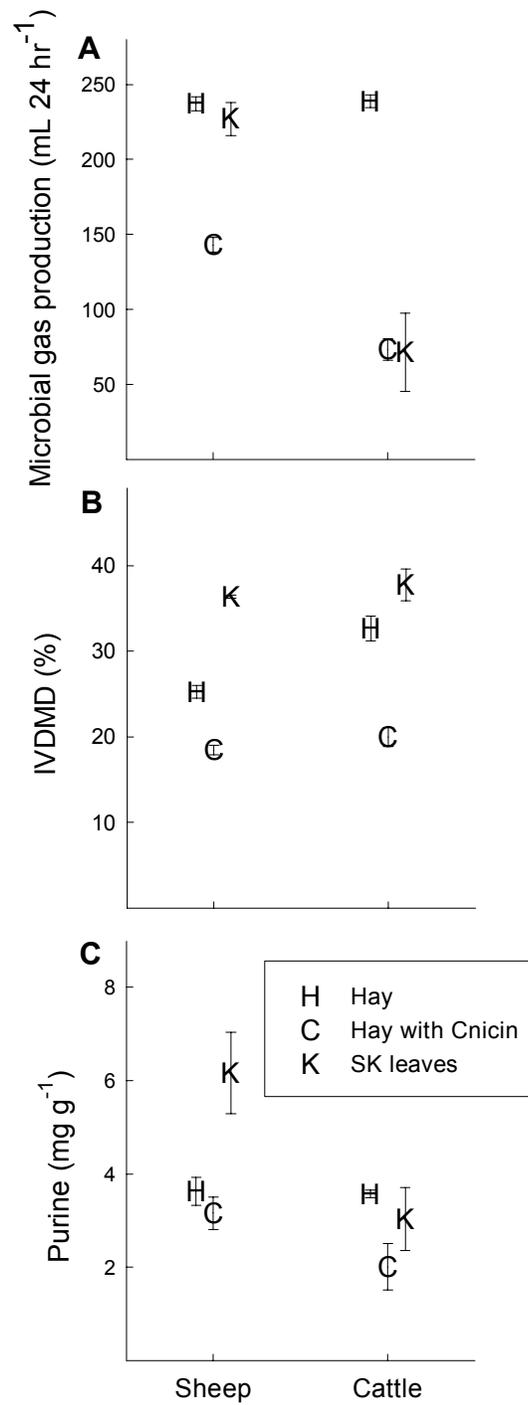


FIG. 1. Cumulative microbial gas production (A), *in vitro* dry matter disappearance (B), and microbial mass (C) of sheep and cattle rumen microbes exposed to hay (H), hay with cnicin (C), and spotted knapweed leaves (K). Means \pm 1 S.E.

In vitro dry matter disappearance trends were similar for sheep and cattle rumen fluid (Figure 1B). *In vitro* dry matter disappearance was greater for grass hay than for grass hay with cnicin ($P < 0.001$). Spotted knapweed leaves had the greatest IVDMD ($P < 0.001$).

In vitro microbial mass was greater for sheep rumen microbes exposed to spotted knapweed leaves than those exposed to grass hay or grass hay with cnicin ($P = 0.001$, $P = 0.001$, respectively; Figure 1C). Cattle rumen microbes exposed to grass hay had greater microbial mass than those exposed to grass hay with cnicin ($P = 0.02$).

Trial 2 – Anti-toxicant

When predicted average rates of PEG were used, gas produced by sheep rumen microbes exposed to spotted knapweed stems varied with PEG rate ($P = 0.08$; Figure 2A). More gas was produced at the 0.5x and 2x rate than the 1x rate ($P = 0.11$, $P = 0.13$, respectively). When maximum rates of PEG were used, gas produced by sheep rumen microbes exposed to spotted knapweed buds varied with the rate of PEG ($P = 0.07$). Less microbial gas was produced at the 0.5x rate than the 1x rate ($P = 0.06$). Gas produced by cattle rumen microbes exposed to spotted knapweed plant parts was not affected by rates of PEG. When average and maximum PEG rates were used, IVDMD did not vary among rates of PEG (Figure 2B).

When maximum rates of PEG were used, microbial mass produced by sheep rumen microbes exposed to spotted knapweed stems varied depending on the rate of PEG ($P = 0.01$, Figure 2C). Microbial mass was greater at the 2x PEG rate than the 0.5x and

1x rates ($P = 0.01$, $P = 0.11$, respectively). Microbial mass produced by cattle rumen microbes was not affected by rate of PEG.

Trial 3 – Nutrient/energy supplement

Sheep rumen microbes exposed to grass hay, and spotted knapweed stems and buds produced more gas when supplement, with or without PEG, was added ($P < 0.001$, $P = 0.05$, $P = 0.01$, respectively; Figure 3A). Cattle rumen microbes exposed to grass hay produced more gas when supplement with or without PEG was added ($P = 0.02$). In control flasks, sheep rumen microbes produced the most gas when exposed to spotted knapweed stems compared with the other forages tested. Cattle rumen microbes produced more gas when exposed to spotted knapweed stems and buds than when exposed to grass hay and spotted knapweed leaves.

In vitro dry matter disappearance of grass hay and spotted knapweed buds was greater when supplement, with or without PEG, was added to flasks containing sheep rumen fluid ($P = 0.01$, $P = 0.07$, respectively; Figure 3B). Supplement, with or without PEG, increased IVDMD of grass hay and spotted knapweed leaves, stems, and buds when added to flasks containing cattle rumen fluid ($P = 0.02$, $P = 0.05$, $P = 0.10$, $P = 0.03$, respectively). In control flasks, IVDMD of grass hay was lowest, and IVDMD of spotted knapweed leaves was greatest when sheep and cattle rumen microbes were exposed to these forages.

Sheep rumen microbes exposed to spotted knapweed leaves had greater microbial mass when supplement without PEG was added instead of supplement with PEG ($P = 0.06$; Figure 3C). Adding supplement with or without PEG did not affect microbial mass

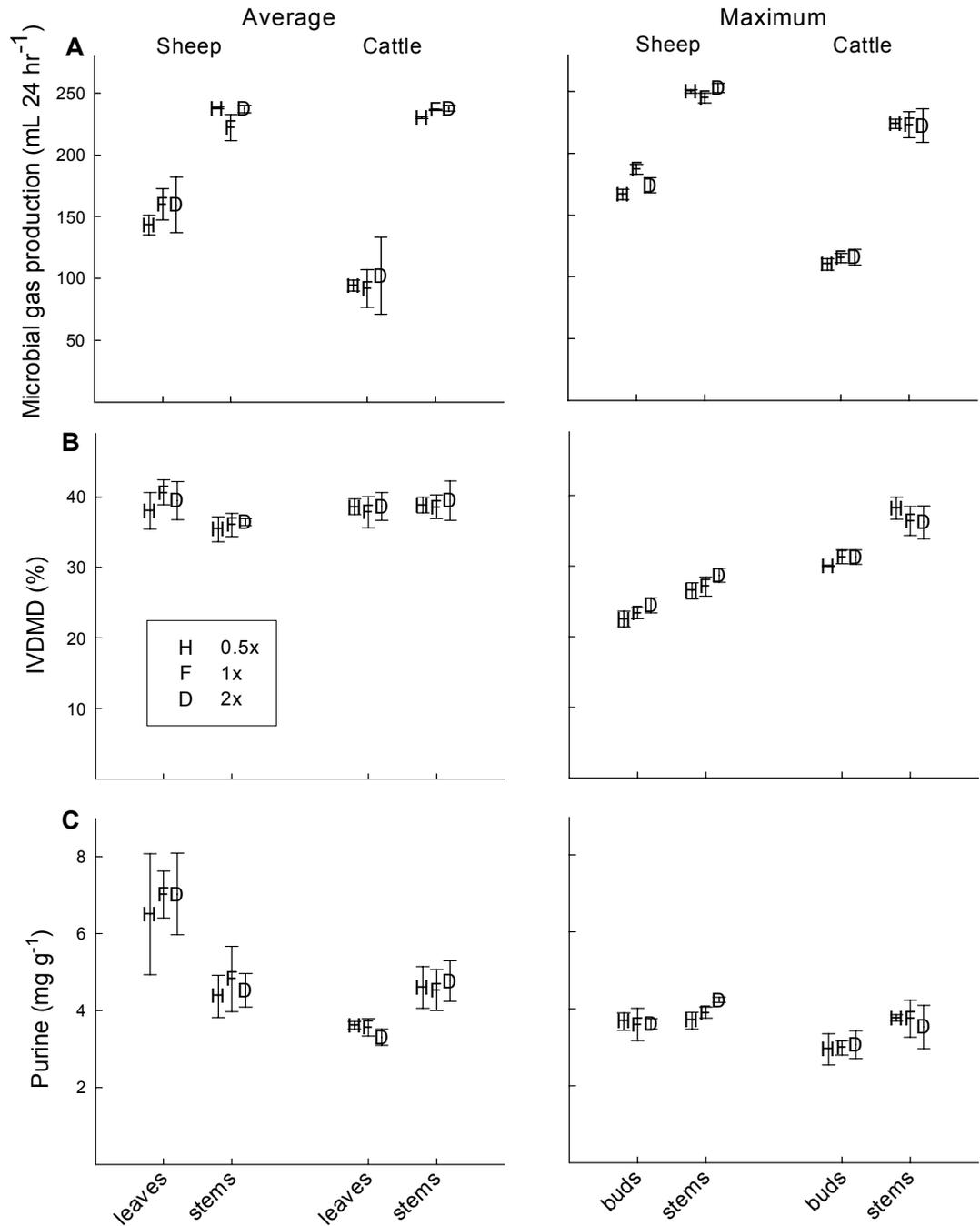


FIG. 2. Cumulative microbial gas production (A), *in vitro* dry matter disappearance (B), and microbial mass (C) of sheep and cattle rumen microbes exposed to spotted knapweed leaves, stems, or buds and a half, 0.5x (H), full, 1x (F), or double, 2x (D) dose of PEG at average and maximum rates. Means \pm 1 S.E.

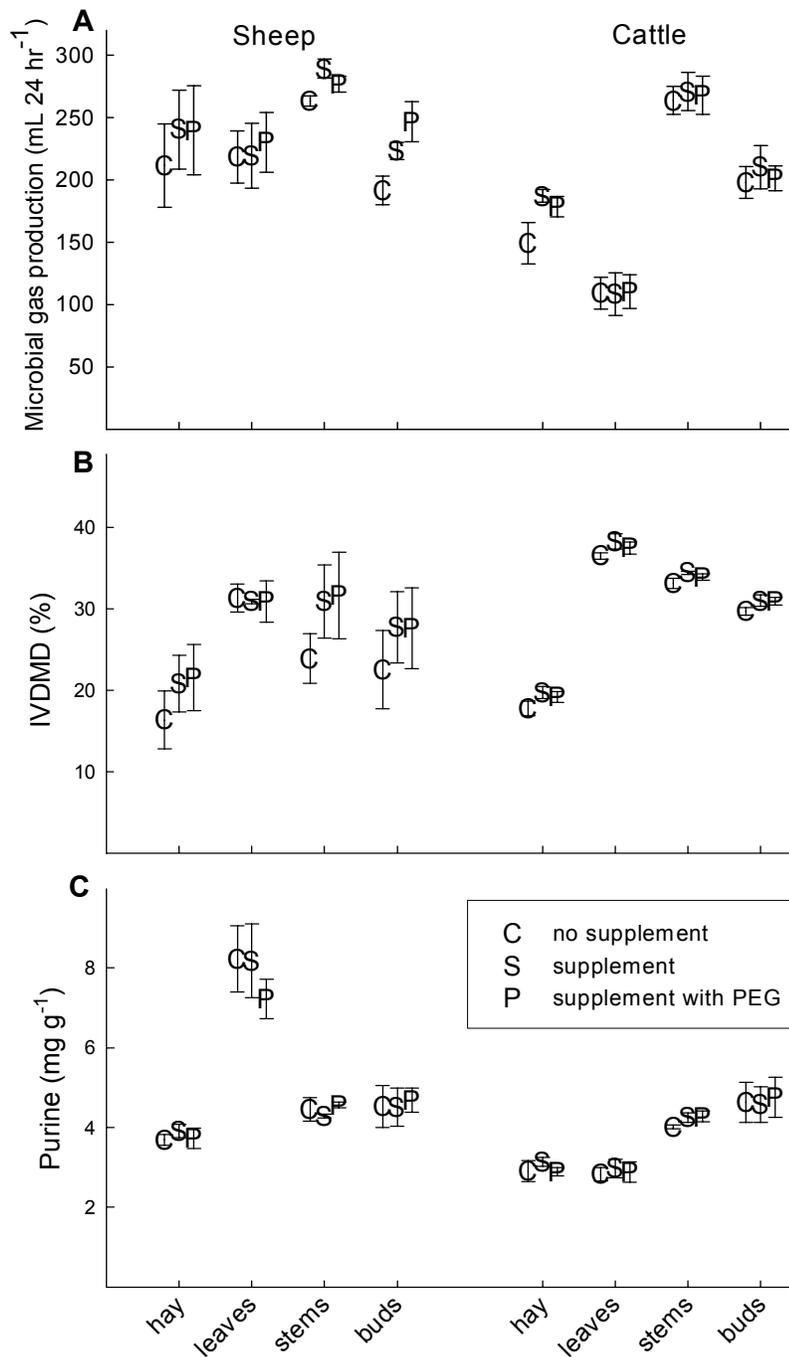


FIG. 3. Cumulative microbial gas production (A), *in vitro* dry matter disappearance (B), and microbial mass (C) of sheep and cattle rumen microbes exposed to grass hay, or spotted knapweed leaves, stems, or buds and no supplement (C), a nutrient/energy supplement (S), or a nutrient/energy supplement with 12.5% PEG (P). Means \pm 1 S.E.

when cattle rumen fluid was used. In control flasks, sheep rumen microbes exposed to spotted knapweed leaves had the greatest microbial mass compared with the other forages tested. In contrast, cattle rumen microbes exposed to spotted knapweed buds had the greatest microbial mass compared to the other forages tested.

Discussion

Based on traditional measures, spotted knapweed is a nutritious forage (Kelsey and Mihalovich, 1987; Olson and Kelsey, 1997). Crude protein, NDF, and ADF of spotted knapweed plant parts were similar to those previously reported, with leaves and buds having greater nutritive value than stems (Olson and Kelsey, 1997).

In Trial 1, cattle rumen microbes produced less microbial mass than sheep rumen microbes when exposed to spotted knapweed leaves. Because IVDMD of spotted knapweed leaves was similar with rumen microbes from both animal species, this indicates that small populations of cattle rumen microbes were more efficient than large populations of sheep rumen microbes when exposed to spotted knapweed. However, the procedure used to determine microbial mass does not distinguish between live and dead microbes and many of the sheep microbes may have been dead.

Microbial activity and efficiency were lower when sheep and cattle rumen microbes were exposed to hay with cnicin. Microbial mass was also reduced when cattle rumen microbes were exposed to hay with cnicin. These results indicate that cnicin negatively affects rumen microbial function. Similarly, microbial activity and mass of sheep rumen microbes exposed to spotted knapweed leaves were greater when most cnicin was extracted (3.86 to 0.59%, dry wt.; Olson and Kelsey, 1997). The negative

effects of cnicin on rumen microbial function may cause negative PIF, which would cause herbivores to avoid or consume less spotted knapweed.

Unexpectedly, hay with cnicin had different effects on sheep and cattle rumen microbial response than spotted knapweed leaves, which may reflect different cnicin concentrations in these forages. Cnicin concentrations of spotted knapweed leaves range from 0.6% – 3.9% dry weight (Locken and Kelsey, 1987; Olson and Kelsey, 1997); however, leaves used in this experiment were not assayed for cnicin concentration. Rumen microbial function may have been inhibited more if the cnicin concentration of hay with cnicin (3% cnicin) was greater than the cnicin concentration of spotted knapweed leaves used in this study. However, differences in microbial response probably reflect the nutritional content of the spotted knapweed leaves and grass hay. In general, forages with higher CP and lower fiber contents are more digestible (Van Soest, 1994). Spotted knapweed leaves had twice the CP, and much lower NDF and ADF, than the grass hay.

In Trial 2, the response of sheep and cattle rumen microbes exposed to spotted knapweed and PEG was specific to plant part and rate. For example, among the 0.5x, 1x, and 2x maximum intake rates, sheep rumen microbes produced the most gas at the 1x rate when exposed to stems and the least gas at the 1x rate when exposed to buds. Polyethylene glycol increases microbial activity, mass, and dry matter disappearance when rumen microbes are exposed to tannin-rich forages (Saarisalo et al., 1999; Getachew et al., 2000; Garcia et al., 2004). However, providing PEG at the rates that I tested did not seem to enhance microbial activity, mass, or dry matter disappearance

when sheep and cattle rumen microbes were exposed to spotted knapweed plant parts. Sheep and cattle rumen microbial response to spotted knapweed plant parts and rates of PEG was similar to the microbial activity, efficiency, and dry matter disappearance in control flasks in Trials 1 and 3. The anti-toxicant, at the rates tested, probably would not reduce negative PIF or enhance spotted knapweed intake by sheep and cattle.

In Trial 3, control flasks reflect differences in sheep and cattle rumen microbial response to different plant parts. Sheep and cattle rumen microbes produced the most gas when exposed to spotted knapweed stems, which have low cnicin concentrations (Olson and Kelsey, 1997), and the least gas when exposed to leaves and buds, which have greater cnicin concentrations (Olson and Kelsey, 1997). In a previous study, sheep rumen microbes produced more gas when exposed to spotted knapweed stems than leaves and produced more gas when cnicin concentrations of spotted knapweed leaves were reduced via extraction (Olson and Kelsey, 1997). This indicates that cnicin inhibits microbial activity when sheep and cattle rumen fluid are used, which is consistent with the results from Trial 1 where rumen microbes exposed to hay with cnicin had lower microbial activity than those exposed to hay alone.

In vitro dry matter disappearance was greatest when sheep and cattle rumen microbes were exposed to spotted knapweed leaves. This probably reflects the CP content of the forage; grass hay had the lowest CP and also the lowest IVDMD. Spotted knapweed leaves also had greater IVDMD than stems or buds when sheep rumen fluid was used in a previous study (Olson and Kelsey, 1997). This indicates that CP content affects sheep and cattle rumen microbial IVDMD more than cnicin content of forages.

Microbial mass differed by animal species and plant part. Sheep rumen microbes produced the most microbial mass when exposed to spotted knapweed leaves. In a previous study, sheep rumen microbes exposed to spotted knapweed leaves produced more microbial mass than spotted knapweed stems or flower buds, although the cnicin concentration in leaves was greater (Olson and Kelsey, 1997). Sheep rumen microbes exposed to spotted knapweed leaves may have died quickly. Microbial mass was analyzed by a procedure that does not distinguish between live and dead microbes. Cattle rumen microbes produced slightly more microbial mass when exposed to spotted knapweed stems and buds than when exposed to spotted knapweed leaves and grass hay. This indicates that greater cnicin concentrations in spotted knapweed leaves (Olson and Kelsey, 1997) may inhibit microbial growth of cattle rumen microbes, which supports the results of Trial 1.

The supplement, with or without PEG, tended to increase IVDMD of spotted knapweed stems and buds, and grass hay when sheep and cattle rumen fluid were used. Supplemental nutrients, energy and protein can increase microbial activity, intake and digestibility when provided with low quality forages (Garg and Gupta, 1992; Liu and McMeniman, 2001; Hess et al., 2004; Leupp et al., 2005). Polyethylene glycol did not seem to enhance the effects of the supplement, which was not surprising because PEG had limited effects in Trial 2. The nutrient/energy supplement without PEG may reduce negative PIF by enhancing the efficiency of rumen microbes exposed to spotted knapweed.

Spotted knapweed was provided as 100% of the forage in all flasks. Ruminants select mixed diets to mitigate the effects of secondary compounds (Villalba et al., 2002c, 2004), and will not likely consume a 100% spotted knapweed diet when grazing. Rumen microbes become negatively affected when spotted knapweed is between 30% and 70% of the diet, though the exact threshold is unknown (Olson and Kelsey, 1997). Potentially, the supplement, with or without the anti-toxicant, may have had a greater effect if spotted knapweed had constituted a lower percentage of the diet.

Conclusions

If large herbivores would enhance their intake of spotted knapweed, the spread of this invasive species in forests and rangelands could be reduced. The secondary compound cnicin appears to cause negative PIF when spotted knapweed is presented as a large portion of the diet. If an anti-toxicant or supplement can be identified that will reduce the negative effects of cnicin, it should increase herbivore intake of spotted knapweed. Polyethylene glycol, in the form and rate provided, may not effectively bind with the secondary compound cnicin. My nutrient/energy supplement enhanced rumen microbial efficiency and may increase intake of spotted knapweed by grazing sheep and cattle.

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CHAPTER 3

SHEEP TRIALS

Introduction

In the northwestern United States, invasive plant species displace native vegetation and decrease forage (Watson and Renney, 1974; Tyser and Key, 1988). Spotted knapweed (*Centaurea maculosa* Lam.), an invasive species from Eurasia, infests over 1.7 million ha in Montana (Montana Weed Management Task Force, 2005) and costs millions of dollars in forage losses each year (Bucher, 1984; Hirsch and Leitch, 1996). Many alternatives are being considered to reduce the number and spread of invasive plant species. Chemical herbicides are the most common method of control (DiTomaso, 2000). Livestock grazing may offer a cost-effective method of control for some rangeland weeds (Williams et al., 1996).

Increasing sheep intake of an undesirable species to a higher percentage of their diet may help control some species. Sheep grazing has been used to control some undesirable species (Bowes and Thomas, 1978; Sharrow and Mosher, 1982; Ralphs et al., 1991; Bell et al., 1996).

Sheep will graze spotted knapweed (Olson and Wallander, 2001) and repeated sheep grazing can reduce the number of spotted knapweed plants and recruitment of young plants into the community (Olson et al., 1997). However, spotted knapweed negatively affects sheep rumen microbes *in vitro* when it constitutes between 30% and 70% of the diet, although the exact threshold is unknown (Olson and Kelsey, 1997).

Sheep may reduce their intake of spotted knapweed if the plant negatively affects rumen microbes and causes negative post-ingestive feedback (PIF). If the negative effects of this plant to sheep rumen microbes could be reduced, sheep may consume more spotted knapweed, and further reduce the spread of this invasive species.

Based on traditional measures, spotted knapweed is a nutritious forage for ruminants (Kelsey and Mihalovich, 1987; Olson and Kelsey, 1997; Wright and Kelsey, 1997). However, spotted knapweed contains the bitter-tasting (Suchy and Herout, 1962) sesquiterpene lactone, cnicin (Locken and Kelsey, 1987; Olson and Kelsey, 1997; Wright and Kelsey, 1997). Cnicin is present in all aboveground plant parts, although concentrations are higher in leaves and buds than stems (Olson and Kelsey, 1997). Cnicin negatively affects sheep rumen microbial activity, efficiency, and mass (Olson and Kelsey, 1997; Chapter 2), and thereby may cause negative post-ingestive feedback (PIF) (Freeland and Janzen, 1974). Ruminants avoid or reduce their intake of plant species associated with negative PIF (Provenza et al., 1990).

Introducing supplemental nutrients and energy into the rumen system increases microbial populations and activity (Freeland and Janzen, 1974; Garg and Gupta, 1992; Srinivas and Gupta, 1997; Moujahed et al., 2000), and may reduce the negative effects of some secondary compounds (Hess et al., 2004). Ruminant intake of plants containing secondary compounds is greater when supplemental nutrients, energy, and protein are provided (Banner et al., 2000; Villalba et al., 2002a, b). Supplements can also enhance weight gain or reduce weight loss when herbivores consume low quality forages (Cohen, 1974; Mulholland and Coombe, 1979; Titgemeyer et al., 2004). Supplement blocks, like

Sweet 14% (Ridley Block Operations; Table 12), contain nutrients and energy that may increase rumen microbial populations and activity, and thereby increase digestion and intake of spotted knapweed.

Anti-toxicants can bind with secondary compounds and reduce their negative effects on rumen microbes and ruminants (Smith, 1992). The anti-toxicant polyethylene glycol (PEG) binds to the hydroxyl groups of tannins (Jones and Mangan, 1977), and reduces the negative effects of this secondary compound. The effects of PEG have not been assessed with other secondary compounds but cnicin has several hydroxyl groups and may also bind with PEG. Ruminants provided with 5 - 300g PEG consume more tannin-rich forage in feeding (Silanikove et al., 1996,1997; Villalba and Provenza, 2001, Villalba et al., 2002a) and grazing trials (Titus et al., 2001; Bhatta et al., 2002; Landau et al., 2002). In some cases, providing PEG does not increase intake, but increases crude protein and fiber digestibility of plants containing secondary compounds (Ben Salem et al., 1999; Moujahed et al., 2005). Ewes provided with PEG may consume more spotted knapweed if PEG binds to cnicin and reduces the negative effects of this compound on rumen microbes.

Polyethylene glycol is more effective when delivered in a supplement block than when delivered in drinking water, sprayed on forage, or provided in concentrate (Ben Salem et al., 1999, 2002). A self-fed, free-access supplement may provide small doses of nutrients, energy, and PEG, if animals consumed the block several times throughout the day. Polyethylene glycol is more effective *in vitro* when delivered in several small doses rather than when added all at once (Getachew et al., 2001). Herbivores provided with a

supplement containing PEG consumed more tannin-rich forage in feeding (Moujahed et al., 2000; Ben Salem et al., 2000, 2002; Bhatta et al., 2005) and grazing trials (Bhatta et al., 2004). Providing a nutrient/energy supplement with PEG may enhance sheep intake of spotted knapweed if the dose of PEG provided binds with cnicin.

My objectives were to determine if sheep provided with a nutrient/energy supplement, with or without PEG, would consume more spotted knapweed: 1) in a drylot (Drylot Trial), 2) in the field during July (Field Trial 1), and 3) in the field during August (Field Trial 2).

Materials and Methods

Drylot Trial (June 10-18)

On June 1, 2005, 36 white-faced yearling ewes (mean BW = 50.3 kg \pm 4.17 S.E.) were transferred from the Montana State University Red Bluff Research Ranch to the Fort Ellis research facility near Bozeman, Montana. Ewes were randomly assigned to 3 treatment groups (n = 12 per group): 1) no supplement (C), 2) nutrient/energy supplement (S), and 3) nutrient/energy supplement with PEG (P).

Ewes (n = 4) were penned in their assigned treatments (n = 3 blocks; 174.2 m² per treatment in each block) in an area with minimal grass cover. Sheep consumed grass growing in pens and dry grass hay for 2 d, and had free access to their assigned supplement at this time. For the next 8 d, ewes in the S and P treatments were provided supplement tubs at 0800 h. Between 1100 and 1200 h each ewe was offered a choice of 700 g (130.1 g DM basis) chopped spotted knapweed (*C. maculosa*), and 1000 g (244.9 g

DM basis) mixed perennial grass, which was primarily smooth brome (*Bromus inermis* L.). Spotted knapweed and grass were fresh-cut each day before the trial. Four pairs of piles (spotted knapweed, fresh-cut grass) were placed in each pen to reduce agonistic behavior among the 4 ewes.

Within each block, 3 observers were randomly assigned to a treatment (C, S, P). Observers recorded animal feeding behavior with Polycorder Dataloggers (Omnidata, Logan, Utah) for 30 min. Behaviors were classified as: consuming spotted knapweed or fresh-cut grass, using supplement, and other (traveling, watering, etc.). After 30 min, intake was determined by collecting and weighing orts from each pen. Around 1400 h, supplement tubs were removed and weighed to determine supplement intake. Ewes were then fed a low quality grass hay at 1.75% BW. Animals had access to fresh water and salt at all times. Ewes were weighed after the trial to determine weight change.

Field Trial 1 (July 7-15)

Grazing behavior was observed for 7 d in mid-July at the Perkins Ranch near Deerlodge, Montana (46°19'N, 112°52'W). The mean annual precipitation is 38.1 – 48.26 cm year⁻¹ and the elevation is about 1403 m. The soil is a fine-loamy, mixed, superactive, frigid Typic Argiustoll with 0 – 4% slopes (USDA, 2003). Ewes (n = 36, mean BW = 57.3 kg ± 0.8 S.E.) that had previous experience with the supplement and fresh-cut, chopped spotted knapweed in the drylot at Fort Ellis (Drylot Trial) were used in this trial. Ewes remained in their same treatments (C, S, P), but were reassigned to blocks. Using portable electric fence, three replicate blocks (2090 m²) were created and sub-divided into three lanes. Treatments were randomly assigned to lanes.

Ewes were transported to the field site and adjusted to the site and fence conditions for 1 d. For the next 7 d, grazing behavior was observed for approximately 90 min during morning (0530 h) and evening (1800 h) grazing periods using focal animal sampling (Altmann, 1974). Three trained observers were randomly assigned to blocks for the entire trial. Observers recorded each animal's bite rate and feeding behavior for three, 3 min periods using a Polycorder Datalogger (Omnidata, Logan, Utah). Observers rotated through animals within their block so that observation periods were non-consecutive for each ewe. Behaviors were classified as: grazing spotted knapweed, forbs, or perennial grasses, ruminating, using supplement, and other. Ewes had access to fresh water, salt, and their assigned supplement tub (except controls) at all times. Supplement tubs were weighed daily to determine group intake for those assigned supplement. Ewes were weighed after the trial to determine weight change. Percent use of spotted knapweed and perennial grasses were determined by ocular estimates after the trial.

Field Trial 2 (August 16-22)

Grazing trials were repeated for 6 d in mid-August. Average pre-trial weight of the 36 ewes was $60.1 \text{ kg} \pm 2.6 \text{ S.E.}$ Pens were relocated to new sites within the same area as the July trial. Methods remained the same except only 2 observers recorded animal behavior. Observers started in a randomly assigned block each morning and evening grazing period and rotated through blocks, observing each ewe's activity for one, 3 min observation period each. Ewes were weighed after the trial to determine weight change. Percent use of spotted knapweed and perennial grasses were determined by ocular estimates after the trial.

Forage Analysis and Vegetation Measurements

Spotted knapweed and grass samples were collected 3 times during the Drylot Trial, whereas only 1 sample of basal diet grass hay was collected. During each field trial (Field Trial 1, Field Trial 2), species composition was determined using the Daubenmire canopy cover method (USDA-USDI, 1996) in each lane along three, 30 m transects using a 0.1 m² Daubenmire frame at 1 m intervals. To determine biomass, the standing crop of current years growth was clipped in 3 randomly located 0.1 m² plots adjacent to each block, and separated into forage type (spotted knapweed, other forbs, perennial grasses). All collected samples were oven-dried at 60° C for 48 h, weighed, and ground in a Wiley mill through a 1 mm screen. Forages were analyzed for DM, CP, and ash using standard methods (AOAC, 2002). Acid detergent fiber (ADF) and neutral detergent fiber (NDF) were analyzed using an ANKOM fiber analyzer (Ankom Technology Corp., Fairport, NY; Van Soest et al., 1991). *In vitro* dry matter disappearance (IVDMD) was analyzed using a Daisy Incubator (Ankom Technology Corp., Fairport, NY; Harris, 1970).

Nutritive value of forages provided during the Drylot Trial did not vary by day (Table 2). The grass hay fed as a basal diet was of low quality. Spotted knapweed had lower NDF and ADF concentrations than fresh-cut grass. Fresh-cut grass and spotted knapweed had high CP levels during this trial.

Species composition and biomass were similar for all blocks in the July (Table 3) and August (Table 4) grazing trials. Spotted knapweed averaged about 45% species composition and 15% canopy cover in July and August. Total biomass was 798.3 kg ha⁻¹ ± 7.5 S.E. in July and 503.8 kg ha⁻¹ ± 7.5 S.E. in August.

Table 2. Dry matter (DM), neutral detergent fiber (NDF), acid detergent fiber (ADF), crude protein (CP), organic matter (OM), and *in vitro* dry matter disappearance (IVDMD) of grass hay, fresh-cut grass, and spotted knapweed sampled during the sheep Drylot Trial

Forage and date collected	DM (%)	NDF (%)	ADF (%)	CP (%)	OM (%)	IVDMD (%)
Grass Hay	95.9	54.45	30.84	7.0	89.5	66.6
Fresh-cut grass						
6/15/05	96.1	60.0	31.0	12.5	90.8	70.1
6/17/05	96.5	59.3	30.1	*	88.7	72.9
6/18/05	96.5	62.1	31.0	19.1	90.4	75.0
Spotted Knapweed						
6/15/05	94.2	34.9	23.6	14.7	90.8	72.5
6/17/05	94.5	35.4	22.9	16.2	89.4	70.7
6/18/05	94.6	37.8	21.0	16.5	90.2	71.3

* Data not available

Table 3. Canopy cover and species composition (\pm S.E.) of forage types by block during sheep Field Trial 1

Forage	Block	Canopy cover (%)	Species composition (%)
Spotted knapweed	1	11.7 \pm 1.2	39.3 \pm 3.3
	2	14.9 \pm 1.9	43.8 \pm 2.8
	3	18.0 \pm 2.3	59.1 \pm 1.7
Grasses	1	10.7 \pm 1.0	37.7 \pm 3.2
	2	9.6 \pm 0.2	30.0 \pm 3.1
	3	7.4 \pm 0.6	25.6 \pm 0.5
Other forbs	1	6.9 \pm 1.0	23.0 \pm 2.0
	2	9.0 \pm 1.9	26.2 \pm 3.8
	3	4.5 \pm 0.1	15.6 \pm 1.5

Forage nutritive values were similar among blocks in the July (Table 5) and August (Table 6) field trials. In July, spotted knapweed had lower NDF and higher CP than grasses. Neutral detergent fiber and ADF of other forbs was similar to spotted knapweed, however, CP of other forbs was higher and IVDMD was lower. In August, spotted knapweed had higher ADF and CP than grasses; however, grasses had higher

IVDMD than spotted knapweed. Other forbs had the highest CP and lowest ADF and NDF, however IVDMD was intermediate. Grasses had the highest IVDMD and spotted knapweed had the lowest.

Table 4. Canopy cover and species composition (\pm S.E.) of forage types by block during sheep Field Trial 2

Forage	Block	Species composition	
		Canopy cover (%)	(%)
Spotted knapweed	1	17.9 \pm 2.3	50.4 \pm 5.1
	2	15.0 \pm 3.1	43.0 \pm 8.9
	3	11.2 \pm 1.8	39.7 \pm 2.7
Grasses	1	13.0 \pm 1.4	37.9 \pm 4.1
	2	15.4 \pm 2.5	44.3 \pm 6.9
	3	11.6 \pm 0.4	42.5 \pm 3.6
Other forbs	1	4.0 \pm 0.5	11.7 \pm 1.4
	2	4.4 \pm 0.8	12.7 \pm 2.1
	3	4.9 \pm 0.8	17.8 \pm 1.1

Table 5. Neutral detergent fiber (NDF), acid detergent fiber (ADF), crude protein (CP), organic matter (OM), and *in vitro* dry matter disappearance (IVDMD) (\pm S.E.) of spotted knapweed, grasses, and other forbs sampled during sheep Field Trial 1

Forage	NDF (%)	ADF (%)	CP (%)	OM (%)	IVDMD (%)
Spotted knapweed	46.4 \pm 0.7	34.0 \pm 1.4	7.5 \pm 0.2	92.0 \pm 0.8	55.9 \pm 1.7
Grasses	63.2 \pm 0.3	33.3 \pm 1.2	5.6 \pm 0.2	92.5 \pm 0.2	59.5 \pm 1.7
Other forbs	48.6	36.3	8.1	89.4	42.9

Table 6. Neutral detergent fiber (NDF), acid detergent fiber (ADF), crude protein (CP), organic matter (OM), and *in vitro* dry matter disappearance (IVDMD) (\pm S.E.) of spotted knapweed, grasses, and other forbs sampled during sheep Field Trial 2

Forage	NDF (%)	ADF (%)	CP (%)	OM (%)	IVDMD (%)
Spotted knapweed	62.7 \pm 2.7	49.3 \pm 2.8	5.7 \pm 1.2	94.7 \pm 0.3	37.8 \pm 2.1
Grasses	69.2 \pm 1.7	36.6 \pm 1.6	4.9 \pm 0.3	93.8 \pm 0.5	51.2 \pm 1.3
Other forbs	49.0	37.0	7.4	92.7	43.2

Statistical Analyses

Drylot trial data were analyzed with repeated measures ANOVA (PROC Mixed, SAS, 2004). Time spent consuming (%) and intake (% consumed) were analyzed for fresh-cut grass and spotted knapweed. Field trial data were also analyzed by repeated measures ANOVA. Time spent grazing (%) and bite rate (bites min⁻¹) were analyzed for grasses, forbs, and spotted knapweed. The model included effects of treatment as the between factor, and day as the within factor, and their interaction. Random error due to block (n = 3) was also included in the model. Treatment groups within blocks were the experimental unit.

Covariance structures (autoregressive order-1 and compound symmetry) were compared to determine which was appropriate for each analysis. Planned contrasts were used to compare treatment means: 1) non-supplemented ewes versus supplemented ewes (C vs. S, P), and 2) ewes provided with supplement versus ewes provided with supplement with PEG (S vs. P).

A t-test was used to compare supplement intake between S and P treatment groups in each trial. A one-way ANOVA was used to compare supplement intake among blocks.

Differences of $P < 0.15$ are presented (Gill, 1981). Data were transformed when the assumption of normality was not met. Actual means are presented in the text, tables, and figures.

Results

Drylot Trial (June 10-18)

Percent time consuming spotted knapweed and grass varied by day and by treatment (day, $P = 0.002$, treatment, $P = 0.005$; Fig. 4A). C ewes spent more time consuming spotted knapweed and less time consuming grass than S and P ewes ($P = 0.02$, $P = 0.03$, respectively). S ewes spent more time consuming spotted knapweed and less time consuming grass than P ewes ($P = 0.003$, $P = 0.006$, respectively).

Spotted knapweed and grass intake varied by day during the 8 d trials (day, $P = 0.0001$; Fig. 4B). Spotted knapweed intake varied by treatment (treatment, $P = 0.005$). C ewes consumed more spotted knapweed than S and P ewes ($P = 0.006$). S ewes consumed more spotted knapweed than P ewes ($P = 0.03$).

Supplement intake did not vary between S and P treatments or among blocks. On average, each ewe consumed $0.15 \text{ kg} \pm 0.04 \text{ S.E.}$ of supplement daily during the 8-d trial. Ewe gains over the 8-d trial were similar among treatments, averaging $7.0 \text{ kg} \pm 0.5 \text{ S.E.}$

Field Trial 1 (July 7-15)

Bite rate of ewes grazing spotted knapweed and grasses varied during the 7-d trial (day, $P < 0.0001$, day, $P < 0.0001$, respectively; Fig. 5). Percent time grazing spotted knapweed and percent time grazing grass varied during the trial (day, $P < 0.0001$, $P < 0.0001$, respectively; Fig. 6) but not in a consistent manner.

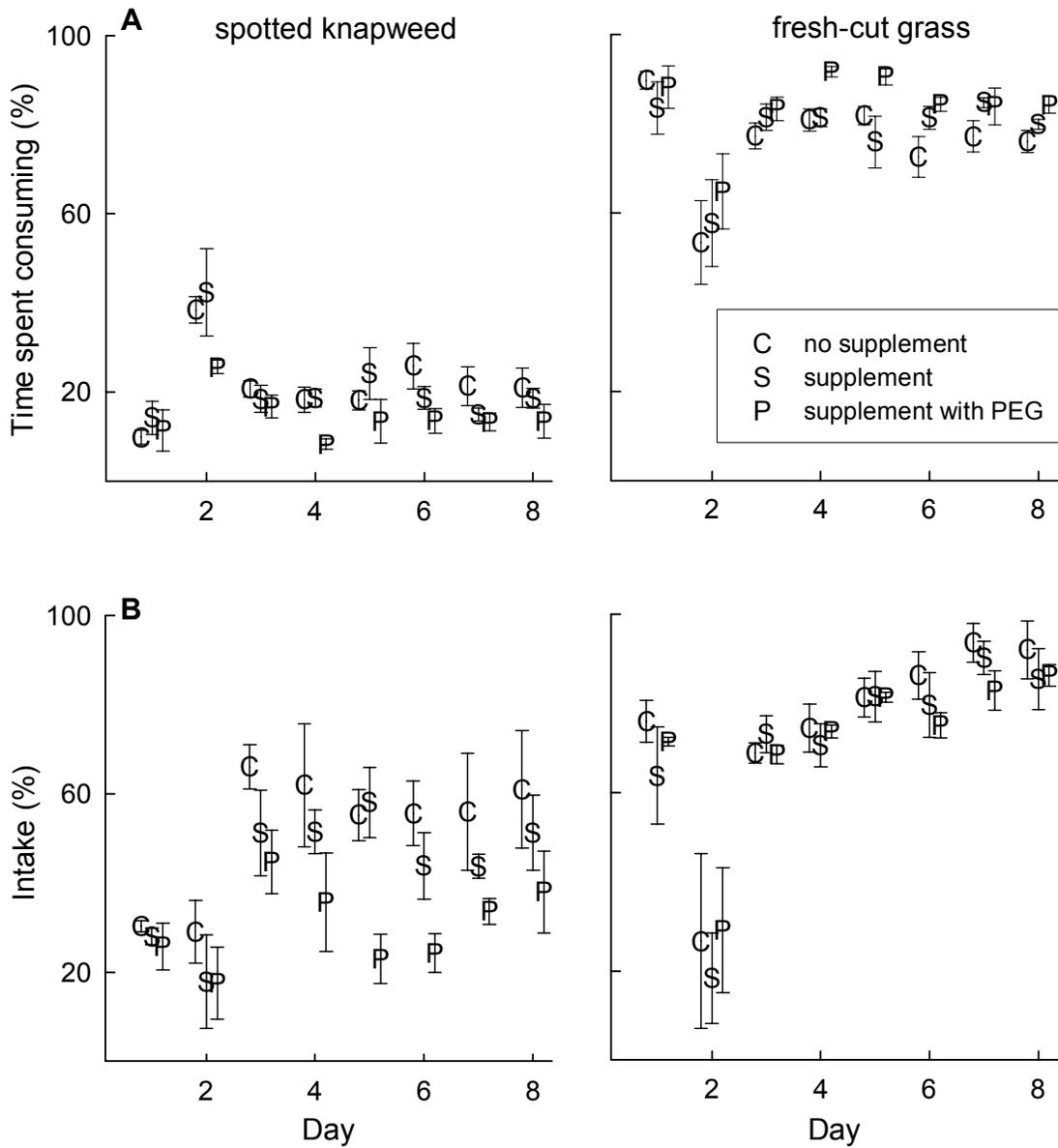


FIG. 4. In the drylot, time spent consuming (A) and intake (B) of fresh-cut spotted knapweed, and fresh-cut grass for yearling ewes provided with no supplement (C), a nutrient/energy supplement (S), or a nutrient/energy supplement with PEG (P) for 8 days beginning June 10, 2005. Means \pm 1 S.E.

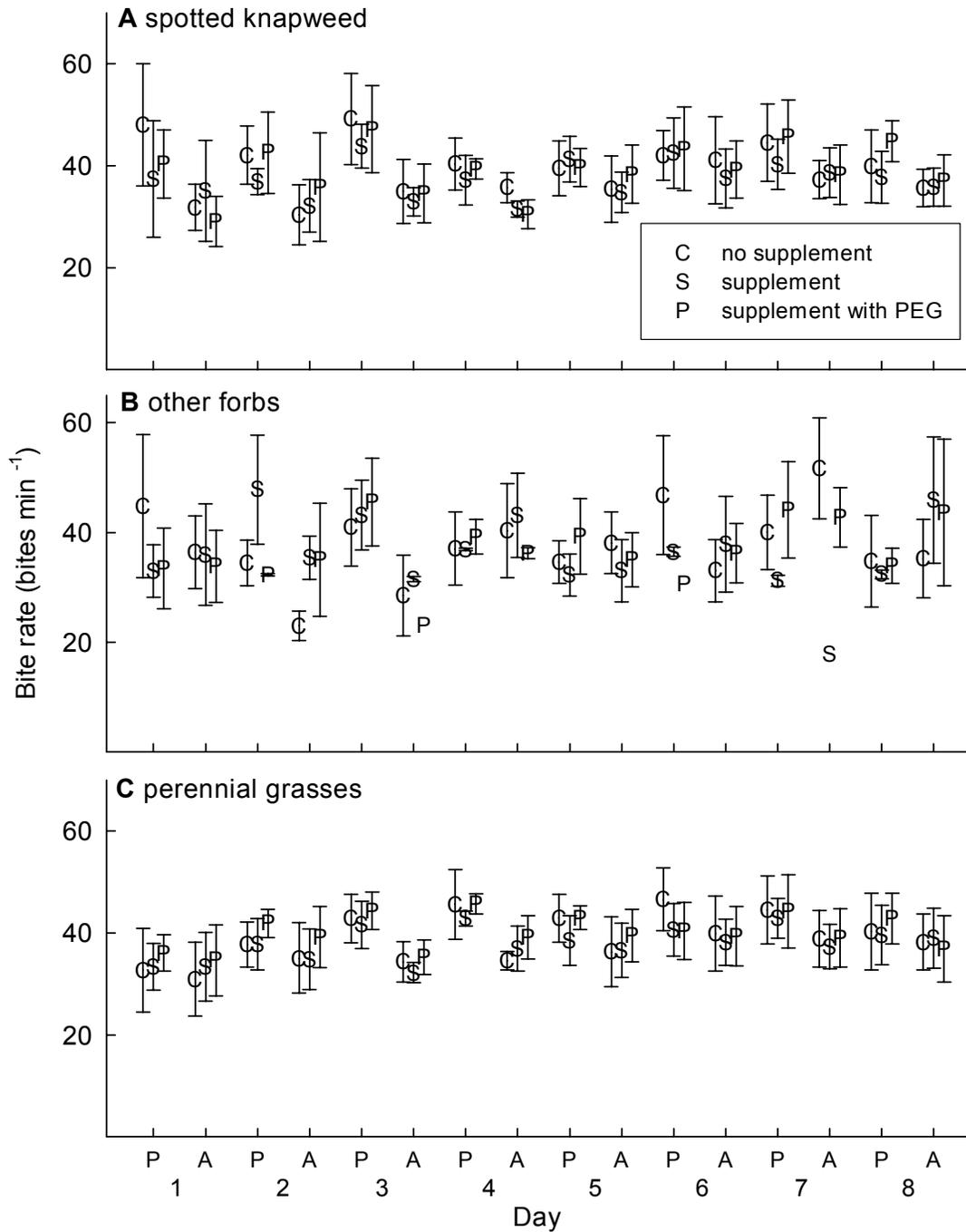


FIG. 5. In the field, bite rates (bites min⁻¹) of spotted knapweed (A), other forbs (B), or perennial grasses (C) for yearling ewes provided with no supplement (C), a nutrient/energy supplement (S), or a nutrient/energy supplement with PEG (P) observed during evening (P) and morning (A) grazing periods for 8 days beginning July 7, 2005. Means \pm 1 S.E.

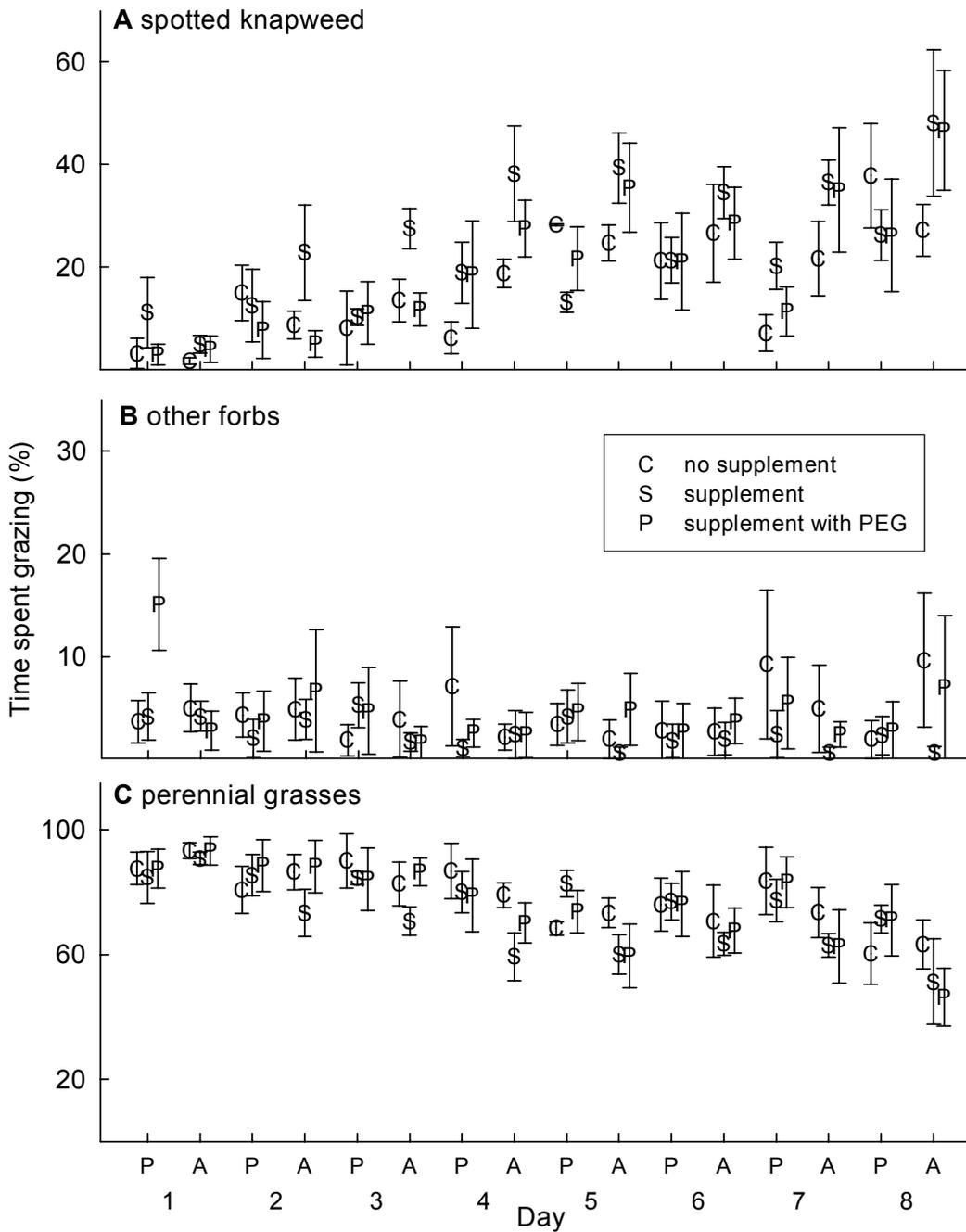


FIG. 6. In the field, time spent grazing (% relative to total grazing time) spotted knapweed (A), other forbs (B), or perennial grasses (C) for yearling ewes provided with no supplement (C), a nutrient/energy supplement (S), or a nutrient/energy supplement with PEG (P) observed during evening (P) and morning (A) grazing periods for 8 days beginning July 7, 2005. Means \pm 1 S.E. Note y-axis scales differ.

Supplement intake did not vary between S and P treatments or among blocks. On average, each ewe consumed $0.34 \text{ kg} \pm 0.07 \text{ S.E.}$ of supplement daily during the 7 d trial. Ewe weight changes were similar for all treatments. On average, ewes did not gain or lose any weight during this mid-July trial. Forage use was similar among treatments in this trial. Sheep used $71\% \pm 1.3 \text{ S.E.}$ of the grasses and $51\% \pm 2.5 \text{ S.E.}$ of the spotted knapweed.

Field Trial 2 (August 16-22)

Bite rates for spotted knapweed and grass varied during the 6-d trial (day, $P = 0.0004$, day, $P = 0.0002$, respectively; Fig. 7). Percent time grazing spotted knapweed and grass varied by day (day, $P < 0.0001$, $P < 0.0002$, respectively; Fig. 8) but not in a consistent manner. Bite rate and percent time grazing of other forbs were not estimated for every grazing period because sheep grazed few other forbs during this trial.

Daily supplement intake did not differ between treatments but differed among blocks ($P = 0.05$). On average, per head per day, ewes from Block 1 consumed the least supplement ($0.01 \text{ kg} \pm 0.01 \text{ S.E.}$), ewes from Block 2 were intermediate ($0.08 \text{ kg} \pm 0.01 \text{ S.E.}$), and those from Block 3 consumed the most supplement ($0.13 \text{ kg} \pm 0.03 \text{ S.E.}$) during the 6 d trial. Ewe weight changes varied by treatment (treatment, $P = 0.13$). C and S ewes (1.2 kg, 0.9 kg, respectively) gained weight, whereas P ewes lost 0.8 kg on average. Forage use was similar among treatments in this trial. Sheep used $36\% \pm 1.3 \text{ S.E.}$ of the grass and $57\% \pm 2.3 \text{ S.E.}$ of the spotted knapweed.

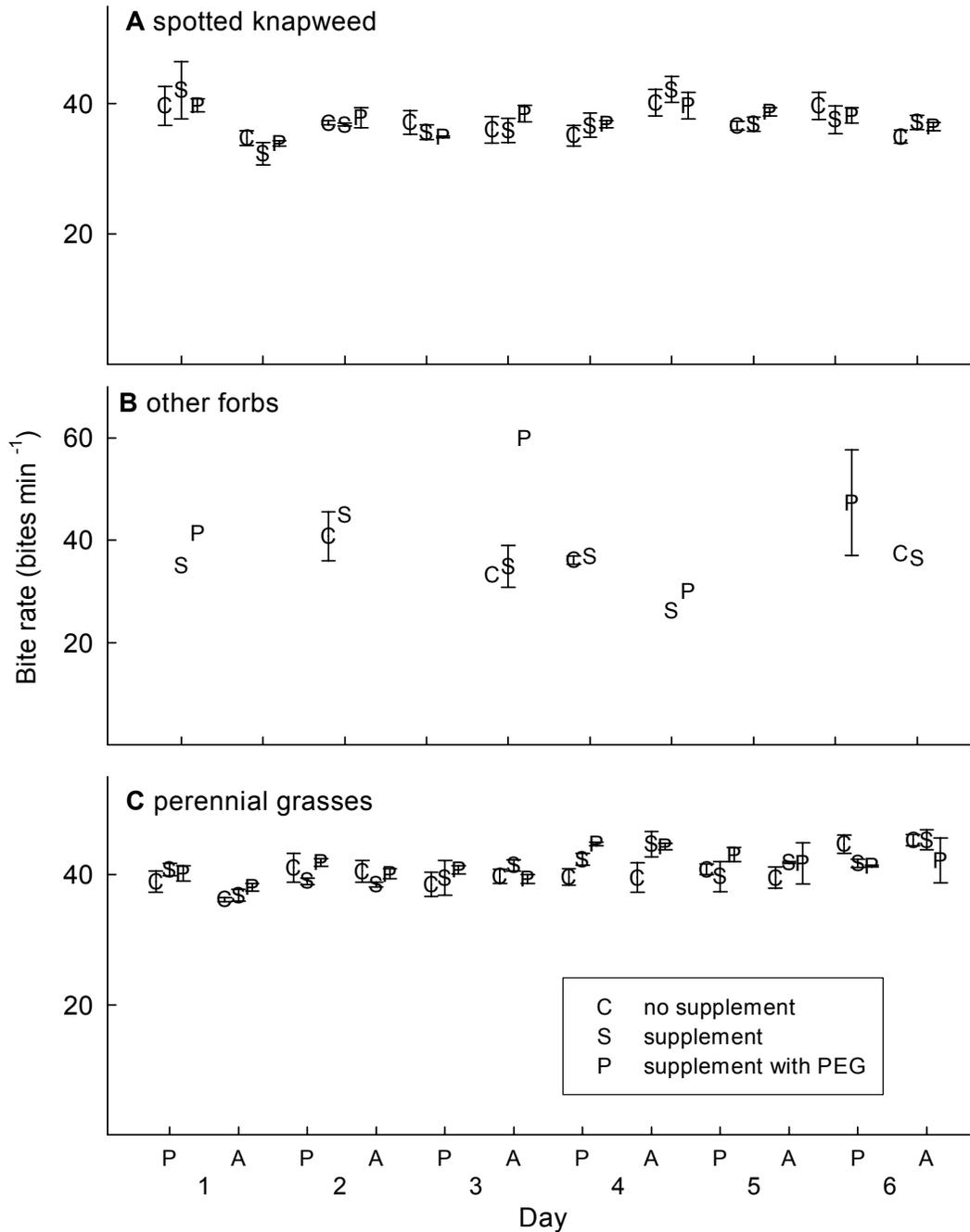


FIG. 7. In the field, bite rates (bites min⁻¹) of spotted knapweed (A), other forbs (B), or perennial grasses (C) for yearling ewes provided with no supplement (C), a nutrient/energy supplement (S), or a nutrient/energy supplement with PEG (P) observed during evening (P) and morning (A) grazing periods for 6 days beginning August 16, 2005. Means \pm 1 S.E. Note y-axis scales of A and C differ from B.

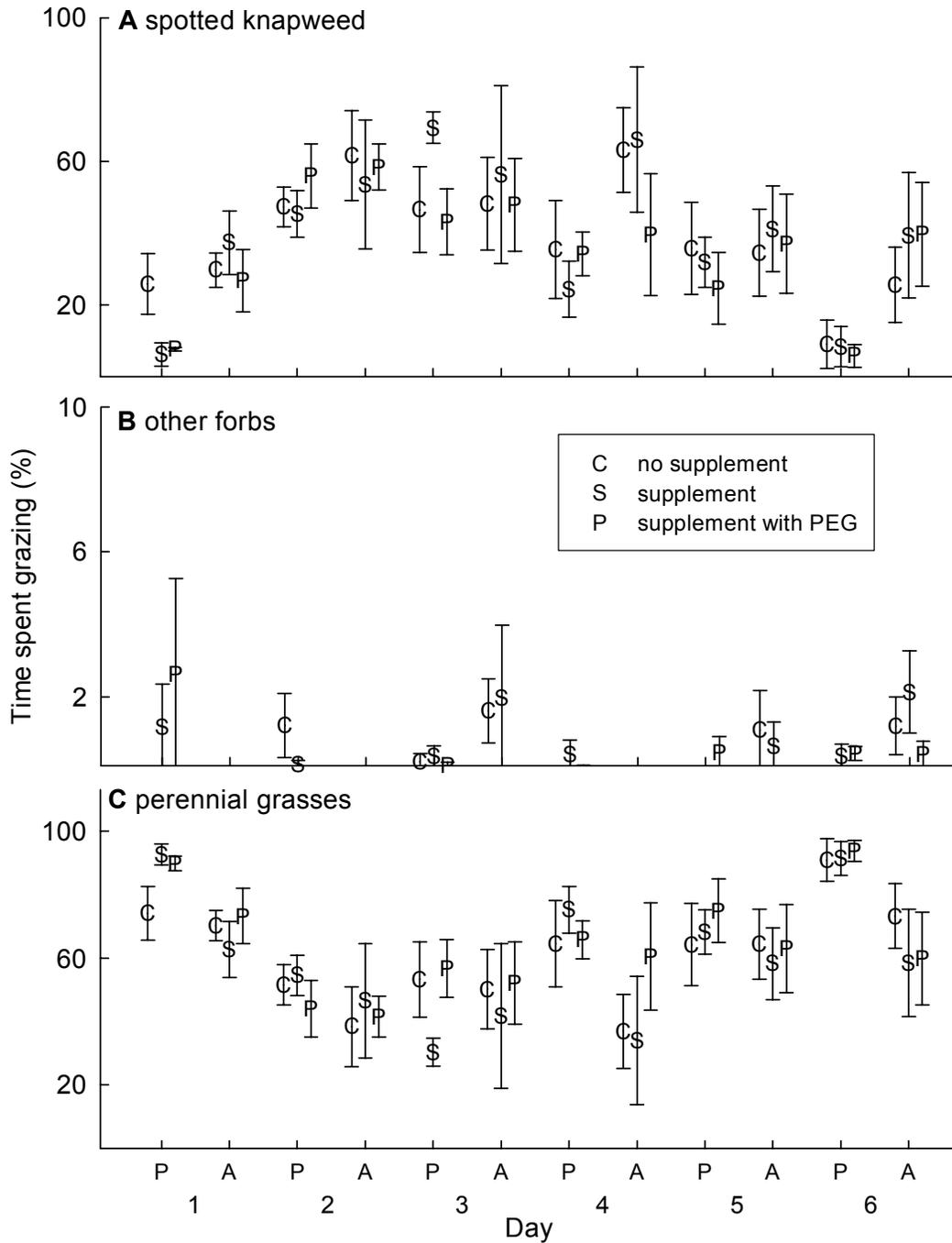


FIG. 8. In the field, time spent grazing (% relative to total grazing time) spotted knapweed (A), other forbs (B), or perennial grasses (C) for yearling ewes provided with no supplement (C), a nutrient/energy supplement (S), or a nutrient/energy supplement with PEG (P) observed during evening (P) and morning (A) grazing periods for 8 days beginning August 16, 2005. Means \pm 1 S.E. Note y-axis scales differ.

Discussion

Drylot Trial (June 10-18)

Time spent consuming spotted knapweed and fresh-cut grass varied little by day. On day 2 of the trial, heavy rain limited intake and time spent consuming spotted knapweed and fresh-cut grass (M. Cheeseman, personal observation). However, in general, time spent consuming spotted knapweed was low (< 30%), and time consuming fresh-cut grass was high (> 70%). This indicates that ewes preferred fresh-cut grass over spotted knapweed. Based on forage analysis, spotted knapweed had similar IVDMD, but had lower NDF and ADF compared with the alternative fresh-cut grasses; however, this forage was not preferred. Spotted knapweed may have been less preferred than the alternative fresh-cut grass for the following reasons. First, ewes may have limited their time consuming spotted knapweed because it was a novel forage. Ruminants are less likely to consume novel forages; however, ruminants will sample small amounts of novel forages and regulate their intake based on post-ingestive feedback (Thorhallsdottir et al, 1987; Burritt and Provenza, 1989). Negative PIF may cause a ruminant to reduce their intake or avoid a plant species (Provenza et al., 1990). Second, if the secondary compound cnicin caused negative post-ingestive consequences in the rumen, spotted knapweed intake may also have been low. However, spotted knapweed does not negatively affect sheep rumen microbes *in vitro* when it constitutes less than 30% of the diet (Olson and Kelsey, 1997). Rumen microbes and ewes were probably not negatively affected by the amount of spotted knapweed consumed in the drylot.

Unexpectedly, intake of spotted knapweed was lower when ewes were provided with the nutrient/energy supplement, with and without PEG, in the drylot. Ewes were probably not substituting supplement for part of their basal diet. Substitution occurs when herbivores replace forage intake with supplement intake (Caton and Dhuyvetter, 1997). Supplement was offered for only 6 hours each day to limit substitution (Titgemeyer et al., 2004) and ewes did not consume large enough quantities of the supplement for substitution to occur. The reason for lower intake of spotted knapweed by the supplemented treatments is not known. However, the ewes provided supplement with PEG had much lower intake than ewes provided supplement without PEG. The low intake of spotted knapweed by the ewes provided supplement with PEG probably caused the low average of the supplemented treatments.

In previous research, herbivore response to PEG has varied. Polyethylene glycol can increase intake of forages containing 3 - 9% tannins (Ben Salem et al., 2000, 2002; Bhatta et al., 2004). In some cases, providing PEG does not increase intake but increases crude protein and fiber digestibility of plants containing secondary compounds (Ben Salem et al., 1999; Moujahed et al., 2005). However, when the delivery or dose of PEG is not appropriate to bind with the secondary compounds in the target plant (Ben Salem et al., 2003), or if other nutritious alternatives are available (Titus et al., 2000), intake of the target plant may not be affected.

The effects of supplements containing PEG have not been tested with forages containing cnicin. In my trial, ewes provided supplement with PEG consumed less spotted knapweed than ewes provided supplement without PEG and non-supplemented

ewes. Polyethylene glycol is a non-digestible polymer (Bauman et al., 1971); however, it may have had unknown dietary effects. Supplement intake did not differ between the supplemented groups, so substitution of supplement for forage should not have affected intake by ewes provided supplement with PEG. Polyethylene glycol may not bind to cnicin as expected at the dose provided in the supplement block, which may explain why it did not enhance spotted knapweed intake in the drylot.

Field Trial 1 (July 7-15)

Ewes spent more time grazing spotted knapweed and less time grazing grass as the 8-d trial progressed. Percent time grazing spotted knapweed was also somewhat cyclic. Negative effects of secondary compounds are greater when compounds accumulate in the rumen or ruminant. Herbivores cycle their intake of forages containing secondary compounds to mitigate the cumulative negative effects of some secondary compounds (Pfister et al., 1997).

Time spent grazing spotted knapweed may have been low at the beginning of the trial because spotted knapweed was a novel forage to yearling ewes in the field. Although ewes were initially exposed to spotted knapweed in the drylot, the form of spotted knapweed (growing rather than chopped and provided in a pile) was novel. Greater time spent consuming spotted knapweed towards the end of the trial may also indicate that rumen microbial populations were adjusting to the plant and the secondary compound cnicin. Ruminants can increase intake of forages containing secondary compounds if rumen microorganisms adjust to digest the compounds in the plant without negative post-ingestive consequences (Allison et al., 1977; Odenyo et al., 1997).

In general, ewes provided supplement without PEG tended to spend more time grazing spotted knapweed than control ewes. Providing supplemental nutrients, energy, and protein can increase intake and digestibility of forages containing secondary compounds (Banner et al., 2000; Villalba et al., 2002a, b). The supplement may have enhanced the digestion of spotted knapweed and increased intake of the plant. However, although time spent grazing spotted knapweed was greater, percent use of spotted knapweed, based on an ocular estimate, was similar among treatments.

Ewes provided supplement with PEG spent less time grazing spotted knapweed and more time grazing grass than ewes provided supplement without PEG. Supplement intake was similar between supplemented treatments; however, ewes consuming the supplement block containing 12.5% PEG consumed less nutrients, energy, and protein than ewes consuming supplement without PEG. Providing 12.5% more nutrients, energy, and protein in the supplement block instead of PEG may benefit ewes grazing spotted knapweed more than the anti-toxicant. The effects of the anti-toxicant may have been reduced if ewes were not consuming large quantities of spotted knapweed and the secondary compound cnicin, or if the dose or delivery of PEG was not appropriate to enhance sheep intake of spotted knapweed in July.

Field Trial 2 (August 16-22)

Time spent grazing spotted knapweed was greater at the start of Field Trial 2 than at the start of Field Trial 1. Ewes may have been less neophobic of spotted knapweed in the field after being exposed to it during Trial 1. The low quality of the other forages available may also have caused ewes to consume more spotted knapweed.

Time spent grazing spotted knapweed was not as cyclic as in Field Trial 1. In Field Trial 2, ewes were selectively consuming flower buds from spotted knapweed plants (M. Cheeseman, pers. observation). Ewes may have been selecting buds because of their intermediate cnicin concentration and high CP content; spotted knapweed buds have less cnicin than leaves, but more cnicin than stems (Olson and Kelsey, 1997). However, the CP of buds is higher than the CP of stems (Olson and Kelsey, 1997; Chapter 2). Ewes were not selectively grazing a particular spotted knapweed plant part during Field Trial 1 (M. Cheeseman, pers. observation) and the change in cnicin intake may have affected how ewes cycled their intake of spotted knapweed in Field Trial 2.

Time spent grazing varied more during this trial than in Field Trial 1. The large variation may have been caused by differences in grazing behavior among blocks, which may reflect differences in supplement intake among blocks. Ewes consuming more supplement may have spent more time grazing spotted knapweed than ewes with low supplement intake. However, time spent grazing also varied more in this trial for the non-supplemented treatment group. Therefore, the large variation may reflect the different sampling procedure used in Field Trial 2. Animals were observed for fewer grazing periods during this trial ($n = 3$ in Field Trial 1, $n = 2$ in Field Trial 2), and the lower sample size may have caused the greater variation.

Unexpectedly, supplement, with or without PEG, did not affect time spent grazing spotted knapweed. I expected ewes provided with supplement to spend more time grazing spotted knapweed than non-supplemented ewes because of the low quality of alternative forages during this trial. Entire spotted knapweed plants were also of low

quality during this trial; however, ewes can consume higher quality diets by grazing selective plant parts (Meyer et al., 1957), such as spotted knapweed flower buds which are of higher quality than entire spotted knapweed plants (Olson and Kelsey, 1997).

Supplement intake was much lower during this trial than in Field Trial 1. The limited amount of supplement consumed may not have positively affected rumen microbes, and thereby the negative effects of cnicin were not reduced when spotted knapweed was consumed. Ruminant intake of self-fed block supplements is quite variable, CV values range from 17 - 249% (Bowman and Sowell, 1997). Some animals over-consume supplement whereas others do not consume any; few animals consume the target amount of supplement each day (Mulholland and Coombe, 1979; Lobato and Pearce, 1980). Providing supplement in another form, such as a pellet, may minimize the variation in intake among ewes and treatments (Bowman and Sowell, 1997; Taylor et al., 2002)

Supplement with PEG was expected to enhance time spent grazing spotted knapweed during this trial. Cnicin was potentially greater in this field trial than in Field Trial 1, based on previous research (Locken and Kelsey, 1987). If PEG bonded with cnicin and reduced its negative effects on rumen microbes, time spent grazing spotted knapweed should have been greater. However, the supplement with PEG did not enhance time spent grazing spotted knapweed. This indicates that the dose and delivery of PEG were not appropriate to increase intake of spotted knapweed in August or that intake of spotted knapweed was too low for the anti-toxicant to benefit ewes and alter grazing behavior.

Based on visual estimates, percent use of spotted knapweed was somewhat higher and percent use of grasses was somewhat lower in this trial than in Field Trial 1. Time spent grazing spotted knapweed was also somewhat higher during this trial; however, time spent grazing does not accurately reflect percent canopy removed or frequency of grazed plants (Olson and Wallander, 2003). In my study, grazing behavior was recorded only during morning and evening grazing periods and did not represent time spent grazing during an entire 24-h period. Ewes may have been consuming spotted knapweed mid-day or late evening when grazing was not observed. I did not estimate percent use for other forbs because forbs were a small part of the plant community and were grazed in limited quantities by sheep during this trial.

In this trial, ewes provided with supplement either gained less weight than control ewes or lost weight. Supplements can be used to reduce the weight loss when animals are consuming low quality forages (Cohen, 1974; Titgemeyer, 2004), and supplements with PEG increase ADG when animals consume tannin-rich forages (Ben Salem et al., 2000, 2002; Bhatta et al., 2004, 2005). However, in my trial supplemented ewes lost weight, possibly because of their limited supplement intake combined with the low quality of the forages available.

Forage Analysis and Vegetation Measurements

During the Drylot Trial, spotted knapweed had much lower fiber, but similar CP to fresh-cut grass. However, IVDMD of spotted knapweed and fresh-cut grass were similar. In general, forages low in fiber are more digestible than forages high in fiber (Van Soest, 1994). Despite its low fiber but similar CP, the low IVDMD of spotted

knapweed relative to fresh-cut grass may indicate the negative effects of cnicin on rumen microbes. Cnicin concentrations of spotted knapweed plants range from 1.2 – 1.9% in June (Kelsey and Mihalovich, 1987; Locken and Kelsey, 1987). Cnicin negatively affects rumen microbial activity and mass and can reduce the efficiency of rumen microbes (Olson and Kelsey, 1997; Chapter 2).

Spotted knapweed had lower NDF and higher CP than grasses during the July trial; however, grass had slightly higher IVDMD than spotted knapweed, again indicating the negative effect of cnicin on rumen microbes. Cnicin concentrations in spotted knapweed plants average 1.3% in July (Locken and Kelsey, 1987).

Spotted knapweed and grass had low nutritive value during the August trial. However, IVDMD of spotted knapweed was much lower than grass. Again, this may indicate greater cnicin concentrations in the plant. Cnicin concentrations of spotted knapweed plants can increase from July to August (Locken and Kelsey, 1987) and rumen microbial function is less when cnicin concentrations in spotted knapweed plant parts are high (Olson and Kelsey, 1997).

For all analyses, entire spotted knapweed plants were harvested and assayed for nutritional content and IVDMD. However, cnicin concentrations and nutritive values differ in spotted knapweed plant parts. Leaves and buds have higher cnicin concentrations (3.86% and 1.95%, respectively) than stems (0.12%) (July sample; Olson and Kelsey, 1997), but leaves and buds also have much higher CP and lower ADF and NDF than stems (Olson and Kelsey, 1997; Chapter 2). Sheep have small, mobile mouthparts and can select individual plant parts (Meyer et al., 1957). Therefore, nutritive

values determined from entire plants may not reflect sheep nutrient intake when consuming spotted knapweed.

Conclusions

Supplemented sheep grazing is probably not an effective method to reduce the spread of spotted knapweed on rangelands. Sheep will consume spotted knapweed, however, providing a nutrient/energy supplement, with or without PEG, does not enhance time spent grazing this plant.

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CHAPTER 4

CATTLE TRIALS

Introduction

In the northwestern United States, invasive plant species displace native vegetation and decrease forage (Watson and Renney, 1974; Tyser and Key, 1988). Spotted knapweed (*Centaurea maculosa* Lam.), an invasive species from Eurasia, infests over 1.7 million ha in Montana (Montana Weed Management Task Force, 2005) and costs millions of dollars in forage losses each year (Bucher, 1984; Hirsch and Leitch, 1996). Many alternatives are being considered to reduce the number and spread of invasive plant species. Chemical herbicides are the most common method of control (DiTomaso, 2000). Livestock grazing may offer a cost-effective method of control for some rangeland weeds (Williams et al., 1996).

Increasing herbivores' intake of an undesirable species to a higher percentage of their diet may help control some species. Sheep grazing can control some undesirable species (Bowes and Thomas, 1978; Sharrow and Mosher, 1982; Ralphs et al., 1991; Bell et al., 1996). Because cattle are larger than sheep and consume more total dry matter each day, they may also have the potential for controlling some undesirable species.

Some wildlife and livestock species graze spotted knapweed (Wright and Kelsey, 1997; Olson and Wallander, 2001), while some livestock, including cattle and horses, avoid spotted knapweed and prefer native plants. For example, cattle will consume forbs, but prefer grasses (Grant et al., 1885; Ruyle and Bowns, 1985) and may consume less

spotted knapweed simply because it is a forb. However, if cattle consumed more spotted knapweed, they may be able to reduce the spread of this invasive species. Because repeated sheep grazing can reduce the number of spotted knapweed plants and recruitment of young plants into the community (Olson et al., 1997), grazing by cattle may also help to manage this invasive species if cattle intake of spotted knapweed can be enhanced.

Based on traditional measures, spotted knapweed is a nutritious forage for ruminants (Kelsey and Mihalovich, 1987; Olson and Kelsey, 1997; Wright and Kelsey, 1997). However, spotted knapweed contains the bitter-tasting (Suchy and Herout, 1962) sesquiterpene lactone, cnicin (Locken and Kelsey, 1987; Olson and Kelsey, 1997; Wright and Kelsey, 1997). Cnicin is present in all aboveground plant parts, although concentrations are higher in leaves and buds than stems (Olson and Kelsey, 1997). Cnicin negatively affects cattle rumen microbial activity, efficiency, and mass (Chapter 2), and thereby may cause negative post-ingestive feedback (PIF) (Freeland and Janzen, 1974). Ruminants avoid or reduce their intake of plant species associated with negative PIF (Provenza et al., 1990).

Introducing supplemental nutrients and energy into the rumen system increases microbial populations and activity (Freeland and Janzen, 1974; Garg and Gupta, 1992; Srinivas and Gupta, 1997; Moujahed et al., 2000), and may reduce the negative effects of some secondary compounds (Hess et al., 2004). Ruminant intake of plants containing secondary compounds is greater when supplemental nutrients, energy, and protein are provided (Banner et al., 2000; Villalba et al., 2002a, b). Supplements can also enhance

weight gain or reduce weight loss when herbivores consume low quality forages (Cohen, 1974; Mulholland and Coombe, 1979; Titgemeyer et al., 2004). Supplement blocks, like Sweet 14% (Ridley Block Operations; Table 12), contain nutrients and energy that may increase rumen microbial populations and activity, and thereby increase digestion and intake of spotted knapweed.

Anti-toxicants can bind with secondary compounds and reduce their negative effects on rumen microbes and ruminants (Smith, 1992). The anti-toxicant polyethylene glycol (PEG) binds to the hydroxyl groups of tannins (Jones and Mangan, 1977) and reduces the negative effects of these secondary compounds. The effects of PEG have not been assessed with other secondary compounds but cnicin has several hydroxyl groups and may also bind with PEG. Ruminants provided with 5 - 300g PEG consume more tannin-rich forage in feeding (Silanikove et al., 1996,1997; Villalba and Provenza, 2001, Villalba et al., 2002a) and grazing trials (Titus et al., 2001; Bhatta et al., 2002; Landau et al., 2002). In some cases, providing PEG does not increase intake of the target plant but increases crude protein and fiber digestibility of plants containing secondary compounds (Ben Salem et al., 1999; Moujahed et al., 2005). Cattle provided with PEG may consume more spotted knapweed if PEG binds to cnicin and reduces the negative effects of this compound on rumen microbes.

Polyethylene glycol is more effective when delivered in a supplement block than when delivered in drinking water, sprayed on forage, or provided in concentrate (Ben Salem et al., 1999, 2002). A self-fed, free-access supplement may provide small doses of nutrients, energy, and PEG if animals consume the supplement several times throughout

the day. Polyethylene glycol is more effective *in vitro* when delivered in several small doses rather than when added all at once (Getachew et al., 2001). Herbivores provided with a supplement containing PEG consumed more tannin-rich forage in feeding (Moujahed et al., 2000; Ben Salem et al., 2000, 2002; Bhatta et al., 2005) and grazing trials (Bhatta et al., 2004). Providing a nutrient/energy supplement with PEG may enhance cattle intake of spotted knapweed if the dose of PEG provided binds with cnicin.

My objectives were to determine if cattle provided with a nutrient/energy supplement, with or without PEG, would consume more spotted knapweed: 1) in a drylot (Drylot Trial), 2) in the field during July (Field Trial 1), and 3) in the field during August (Field Trial 2).

Methods and Materials

Drylot Trial (June 21-25)

On June 20, 2005, 18 beef heifers (mean BW = 461.1 kg \pm 4.5 S.E.) from the Montana State University research herd were moved to the university nutrition center feedlot, near Bozeman, Montana. Heifers were randomly assigned to 3 treatment groups (n = 6 per group): 1) no supplement (C), 2) nutrient/energy supplement (S), and 3) nutrient/energy supplement with PEG (P).

Heifers (n = 2) were assigned to treatments and penned in a feedlot (n = 3 blocks; pen size was 73 m² per treatment in each block). Cattle consumed dry grass hay for 1 d, and had free access to their assigned supplement at this time. For the next 5 d, heifers were provided supplement tubs at 0800 h. At 1200 h each heifer was offered a choice of

2000 g (678 g DM basis) chopped spotted knapweed (*C. maculosa*), and 4000 g (1202 g DM basis) mixed perennial grass, which was primarily smooth brome (*Bromus inermis* L.). Forages were fresh-cut each morning before the trial. Two pairs of piles (spotted knapweed, fresh-cut grass) were placed in the feed bunk in each pen to allow both heifers access to grass or spotted knapweed and avoid agonistic behavior between the heifers.

Observers were randomly assigned to each block (n = 6 heifers). Three observers recorded animal feeding behavior with Polycorder Dataloggers (Omnidata, Logan, Utah) for 30 min. Behaviors were classified as: consuming spotted knapweed or fresh-cut grass, using supplement, and other (traveling, watering, etc.). After 30 min, intake was determined by collecting and weighing orts from each pen. Around 1500 h, supplement tubs were removed and weighed to determine supplement intake. Heifers were then fed a high quality alfalfa grass hay at 2% BW. Animals had access to fresh water and salt at all times.

Field Trial 1 (July 21-27)

Grazing behavior was observed for 7 d in July at a field site near Bozeman, Montana (45°36'N, 111°06'W). The mean annual precipitation is 45.7 – 55.9 cm year⁻¹ and the elevation is about 1622 m. The soil is a fine-loamy over sandy or sandy-skeletal mixed, superactive, frigid Typic Argiustoll with 0 - 4% slopes (USDA, 2002). Heifers (n = 18, mean BW = 450 kg ± 4.9 S.E.) that had previous experience with the supplement and fresh-cut, chopped spotted knapweed in the feedlot at the Nutrition Center (Drylot Trial) were used in this trial. Heifers were randomly assigned to treatments (n = 6 per

treatment; C, S, P). Using portable electric fence, three replicate blocks (3716 m²) were created and sub-divided into three lanes. Treatments were randomly assigned to lanes.

Heifers were transported to the field site, and acclimated to the site and fence conditions for 1 d. For the next 7 d, grazing behavior was observed for approximately 90 min during morning (0530 h) and evening (1800 h) grazing periods using focal animal sampling (Altmann, 1974). Three trained observers were randomly assigned to blocks for the entire trial. Observers recorded each animal's bite rate and feeding behavior for six, 3 min periods using a Polycorder Datalogger (Omnidata, Logan, Utah). Observers rotated through animals within their block so that observation periods were non-consecutive for each heifer. Behaviors were classified as: grazing spotted knapweed, other forbs, or perennial grasses, ruminating, using supplement, and other. Ewes had access to fresh water, salt, and their assigned supplement tub (except controls) at all times. Supplement tubs were weighed daily to determine group intake for heifers assigned supplement. Heifers were weighed after the trial to determine weight change. Percent use of spotted knapweed and perennial grasses were determined by ocular estimates after this trial.

Field Trial 2 (August 9-13)

The grazing trial was repeated in August for 5 d at the Applegate Ranch near Deerlodge, Montana (46°23'N, 112°38'W). The mean annual precipitation is 38.1 – 48.3 cm year⁻¹ and the elevation is about 1533 m. The soil is a Clayey-skeletal, mixed, superactive, frigid Typic Argiustoll with 0 – 4% slopes (USDA, 2003). Yearling cattle (n = 12) that had some experience grazing spotted knapweed but no experience with the

supplement were used in this trial. Cattle were randomly assigned to a treatment group ($n = 4$ per group; C, S, P). Using portable electric fence, 3 replicate blocks (5806 m²) were created and sub-divided into 3 lanes. Treatments were randomly assigned to lanes. Cattle could not be contained in one block because several of the trial cattle did not respect the power fence. Therefore, only 2 blocks were observed in this trial. Methods for observing behavior remained the same, except only 2 observers recorded animal behavior. Observers were randomly assigned to a block for the entire trial. Percent use of spotted knapweed and perennial grasses were determined by ocular estimates after this trial.

Forage Analysis and Vegetation Measurements

Spotted knapweed and grass samples were collected 3 times during the Drylot Trial, whereas only 1 sample of basal diet grass hay was collected. During each field trial, species composition was determined by the Daubenmire canopy cover method (USDA-USDI, 1996) in each lane ($n = 3$ per block) along three, 30 m transects using a 0.1 m² Daubenmire frame at 1 m intervals. To determine biomass, the standing crop of current years growth was clipped in 3 randomly located 0.1 m² plots adjacent to each block, and separated into forage type (spotted knapweed, other forbs, and grasses). All collected samples were oven-dried at 60° C for 48 h, weighed, and ground in a Wiley mill through a 1 mm screen. Forages were analyzed for DM, CP, and ash using standard methods (AOAC, 2002). Acid detergent fiber (ADF) and neutral detergent fiber (NDF) were analyzed using an ANKOM fiber analyzer (Ankom Technology Corp., Fairport,

NY; Van Soest et al., 1991). *In vitro* dry matter disappearance was determined using a Daisy Incubator (Ankom Technology Corp., Fairport, NY; Harris, 1970).

Nutritive value of spotted knapweed and grasses did not vary by day during the Drylot Trial (Table 7). The hay provided as a basal diet was of high quality. Fresh-cut grass had higher CP and IVDMD than spotted knapweed. Spotted knapweed had lower NDF concentrations than fresh-cut grass.

Table 7. Dry matter (DM), neutral detergent fiber (NDF), acid detergent fiber (ADF), crude protein (CP), organic matter (OM), and *in vitro* dry matter disappearance (IVDMD) of grass hay, fresh-cut grass, and spotted knapweed sampled during the cattle Drylot Trial

Forage and date collected	DM (%)	NDF (%)	ADF (%)	CP (%)	OM (%)	IVDMD (%)
Grass Hay	95.27	44.65	32.06	16.26	91.73	65.44
Fresh-cut grass						
6/21/05	96.66	63.98	33.18	15.58	89.61	71.26
6/24/05	96.20	67.40	35.09	15.39	88.96	64.89
6/25/05	96.34	67.69	36.20	14.07	89.44	59.30
Spotted Knapweed						
6/22/05	95.07	45.24	30.98	8.87	92.71	60.67
6/24/05	94.84	49.13	32.02	10.14	92.13	57.79
6/25/05	94.56	48.94	31.70	10.54	91.59	54.16

In July, species composition and biomass were similar among blocks (Table 8). Overall, spotted knapweed averaged 28% species composition and 10% canopy cover. Average biomass was $751.8 \text{ kg ha}^{-1} \pm 6.3 \text{ S.E.}$

Nutritive value of forages did not vary among blocks in July (Table 9). Spotted knapweed had lower NDF and slightly higher OM content than grasses. Other forbs had the greatest CP, but also the greatest ADF during this trial.

Table 8. Canopy cover and species composition (\pm S.E.) of forage types by block during cattle Field Trial 1

Forage	Block	Canopy cover (%)	Species composition (%)
Spotted knapweed	1	10.8 \pm 4.2	32.6 \pm 12.9
	2	10.0 \pm 3.6	27.7 \pm 9.1
	3	10.3 \pm 2.1	26.2 \pm 3.8
Grasses	1	20.1 \pm 4.6	59.3 \pm 12.7
	2	22.7 \pm 2.8	64.0 \pm 6.2
	3	22.7 \pm 0.9	59.5 \pm 4.0
Other forbs	1	2.8 \pm 0.2	8.2 \pm 0.3
	2	3.0 \pm 1.2	8.4 \pm 3.0
	3	5.6 \pm 1.8	14.3 \pm 3.9

Table 9. Neutral detergent fiber (NDF), acid detergent fiber (ADF), crude protein (CP), organic matter (OM), and *in vitro* dry matter disappearance (IVDMD) (\pm S.E.) of spotted knapweed, grasses, and other forbs sampled during cattle Field Trial 1

Forage	NDF (%)	ADF (%)	CP (%)	OM (%)	IVDMD (%)
Spotted knapweed	46.9 \pm 1.6	31.1 \pm 1.1	6.9 \pm 0.5	94.3 \pm 0.6	56.3 \pm 2.6
Grasses	65.1 \pm 0.7	35.0 \pm 0.9	6.3 \pm 0.1	89.8 \pm 0.9	55.5 \pm 3.1
Other forbs	57.3	39.4	8.7	92.43	*

* Not enough sample to analyze IVDMD

In August, species composition and biomass were similar between blocks (Table 10). Overall, spotted knapweed averaged 24% species composition and 6% canopy cover. Average biomass was 473.6 kg ha⁻¹ \pm 3.3 S.E.

Nutritive value of forages did not vary among blocks in August (Table 11). Spotted knapweed had lower NDF and higher CP than grasses. Other forbs were intermediate in NDF, CP, and OM but had the greatest ADF of all forage types.

Table 10. Canopy cover and species composition (\pm S.E.) of forage types by block during cattle Field Trial 2

Forage	Block	Canopy cover (%)	Species composition
			(%)
Spotted	1	7.2 \pm 2.5	27.3 \pm 9.3
knapweed	2	4.3 \pm 2.6	21.8 \pm 1.3
Grasses	1	9.9 \pm 1.6	32.8 \pm 2.4
	2	9.2 \pm 1.4	50.5 \pm 5.5
Other forbs	1	11.5 \pm 3.0	39.9 \pm 9.6
	2	6.2 \pm 2.0	27.8 \pm 4.7

Table 11. Neutral detergent fiber (NDF), acid detergent fiber (ADF), crude protein (CP), organic matter (OM), and *in vitro* dry matter disappearance (IVDMD) (\pm S.E.) of spotted knapweed, grasses, and other forbs sampled during cattle Field Trial 2

Forage	NDF (%)	ADF (%)	CP (%)	OM (%)	IVDMD (%)
Spotted					
knapweed	50.0 \pm 0.1	33.1 \pm 0.2	6.3 \pm 0.3	94.1 \pm 0.1	52.0 \pm 1.4
Grasses	71.1 \pm 1.1	38.2 \pm 1.2	5.4 \pm 0.5	92.6 \pm 1.0	47.2 \pm 1.8
Other forbs	62.1	46.7	5.5	92.7	*

* Not enough sample to analyze IVDMD

Statistical Analysis

For the Drylot Trial, time spent consuming (%) and intake (% consumed) of spotted knapweed and fresh-cut grass were analyzed with repeated measures ANOVA (PROC Mixed, SAS, 2004). For the field trials, percent time grazing and bite rate were also analyzed by repeated measures ANOVA. The model included effects of treatment as the between factor, and day as the within factor, and their interaction. Random errors due to block ($n = 3$) were included in the model. Treatment groups within blocks were the experimental unit.

Covariance structures (autoregressive order-1 and compound symmetry) were compared to determine which was appropriate for each analysis. Planned contrasts were used to compare treatment means: 1) non-supplemented cattle versus supplemented cattle (C vs. S, P), and 2) cattle provided supplement versus cattle provided supplement with PEG (S vs. P).

A t-test was used to compare supplement intake between S and P treatment groups in each trial. A one-way ANOVA was used to compare supplement intake among blocks.

I present P - values less than 0.15 (Gill, 1981). Data were transformed when the assumption of normality was not met. Actual means are presented in the text, tables, and figures.

Results

Drylot Trial (June 21-25)

Percent time consuming spotted knapweed and grass varied among the treatments by day (treatment x day, $P = 0.02$; Fig. 9A). P cattle spent more time consuming spotted knapweed and less time consuming grass than S cattle ($P = 0.007$).

Spotted knapweed and grass intake varied by day (day, $P = 0.07$, day, $P = 0.02$, respectively; Fig. 9B). Spotted knapweed intake varied by treatment (treatment, $P = 0.05$); C and P cattle tended to consume more than S cattle. P cattle consumed more spotted knapweed than S cattle ($P = 0.02$).

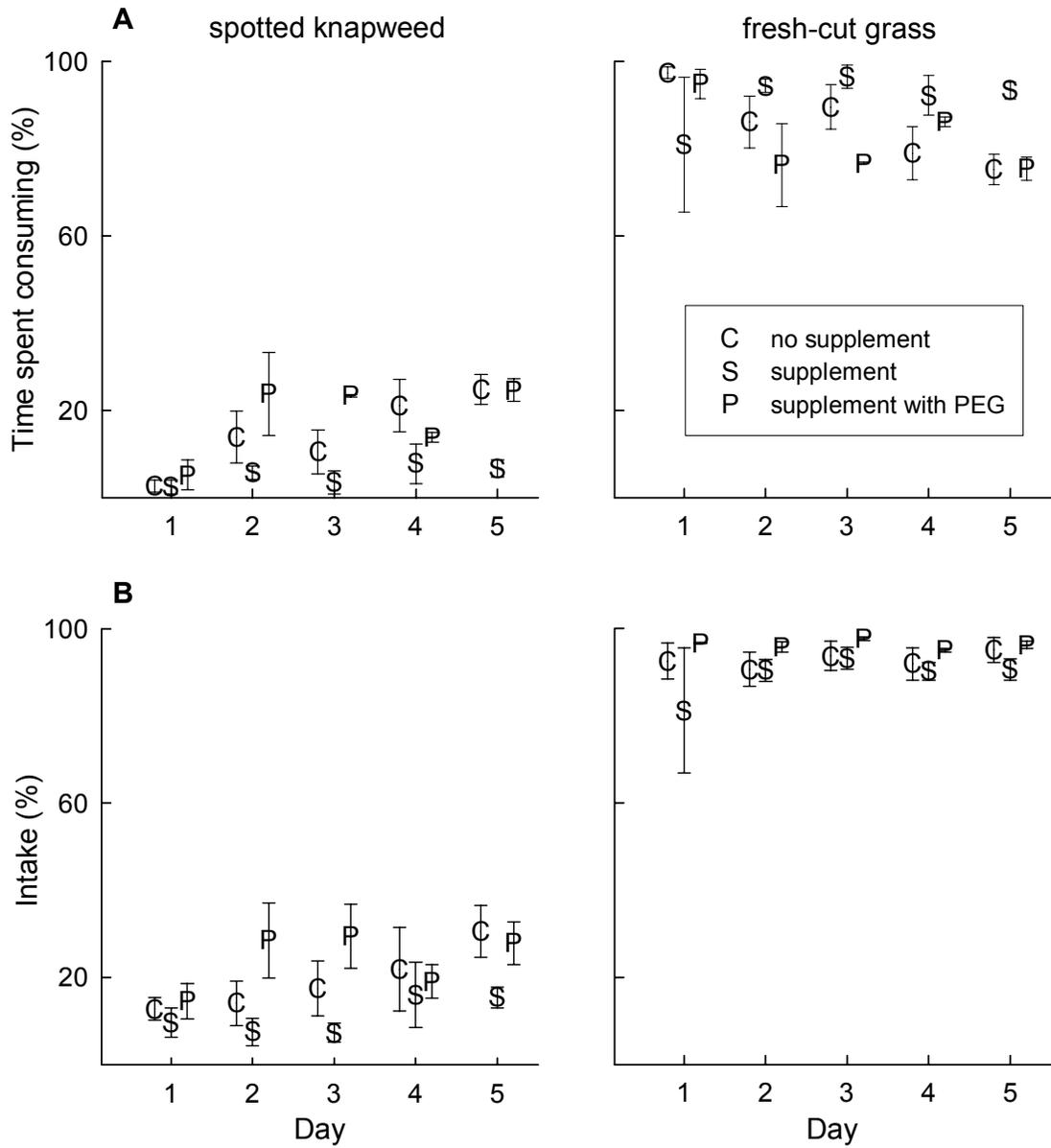


FIG. 9. In the drylot, time spent consuming (A) and intake (B) of fresh-cut spotted knapweed, and fresh-cut grass by heifers provided with no supplement (C), a nutrient/energy supplement (S), or a nutrient/energy supplement with PEG (P) for 5 days beginning June 21, 2005. Means \pm 1 S.E.

Supplement intake differed by treatment ($P = 0.14$). On average, each heifer provided supplement with PEG consumed less supplement per day ($0.2 \text{ kg} \pm 0.1 \text{ S.E.}$) than cattle provided supplement without PEG ($0.4 \text{ kg} \pm 0.1 \text{ S.E.}$) during the 5-d trial.

Field Trial 1 (July 21-27)

Bite rate of cattle consuming grasses varied during the trial (day, $P = 0.0001$; Fig 10). Bite rate of other forbs were not estimated for every grazing period because cattle grazed few other forbs during this trial. Percent time grazing spotted knapweed, grass, and forbs varied during the 6-d trial (day, $P < 0.0001$, $P = 0.0001$, $P = 0.01$, respectively; Fig. 11).

Supplement intake did not differ among blocks or between S and P treatments. On average, each heifer consumed $0.6 \text{ kg} \pm 0.2 \text{ S.E.}$ supplement daily during the 7-d trial. Cattle weight changes did not vary by treatment. Overall, cattle lost $7.3 \text{ kg} \pm 1.6 \text{ S.E.}$ Based on visual estimates, cattle use of spotted knapweed and perennial grasses were similar among treatments in July. On average, cattle used $76.7\% \pm 2.2 \text{ S.E.}$ of the grass and $20.8\% \pm 0.7 \text{ S.E.}$ of the spotted knapweed.

Field Trial 2 (August 9-13)

Bite rate did not vary during the trial (Fig. 12). Bite rate of other forbs were not estimated for every grazing period because cattle grazed few other forbs during this trial. Time grazing spotted knapweed and grass varied by grazing period ($P = 0.0002$, $P = 0.0001$, respectively; Fig 13).

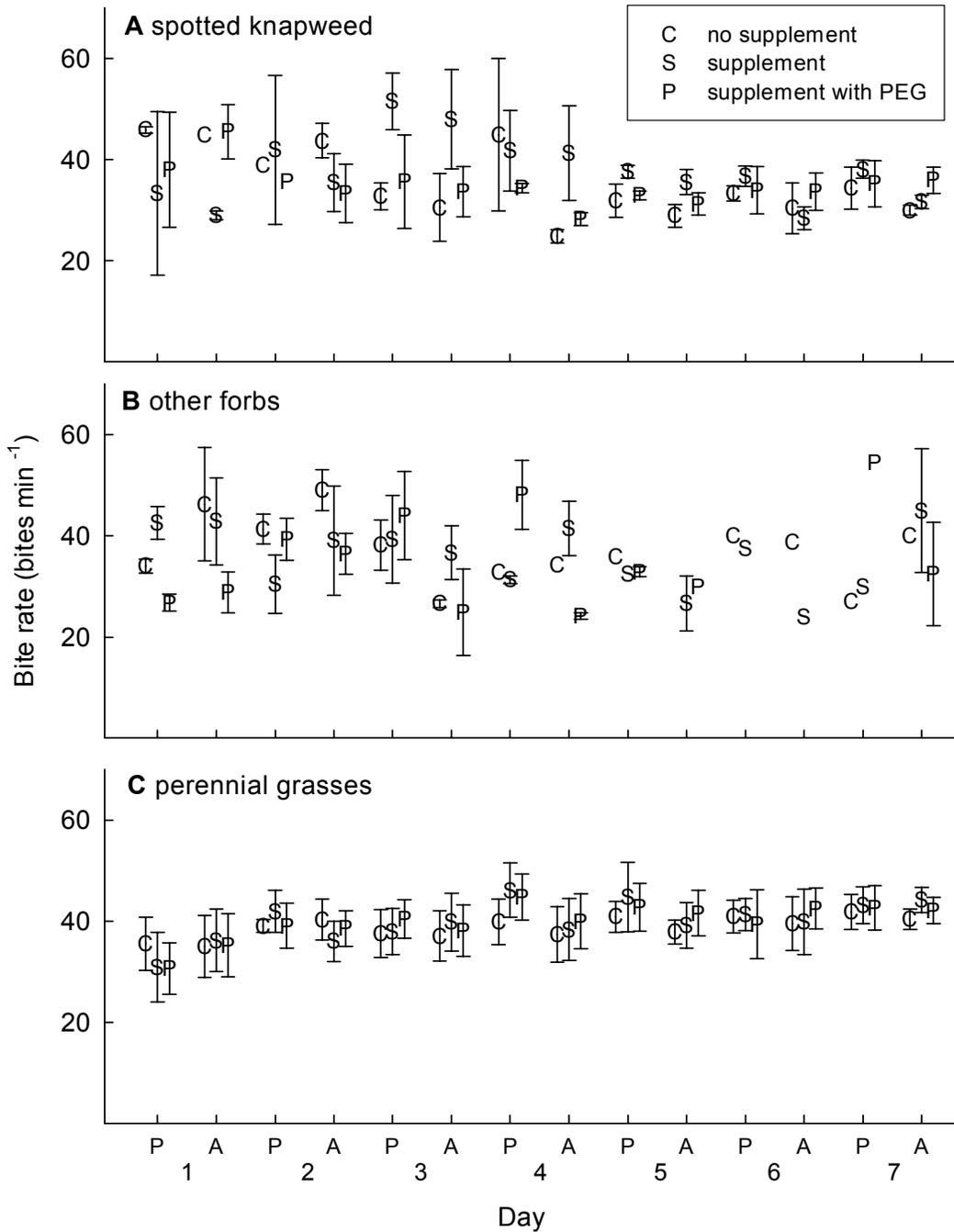


FIG. 10. In the field, bite rates (bites min⁻¹) of spotted knapweed (A), other forbs (B), or perennial grasses (C) for heifers provided with no supplement (C), a nutrient/energy supplement (S), or a nutrient/energy supplement with PEG (P) observed during evening (P) and morning (A) grazing periods for 7 days beginning July 20, 2005. Means \pm 1 S.E.

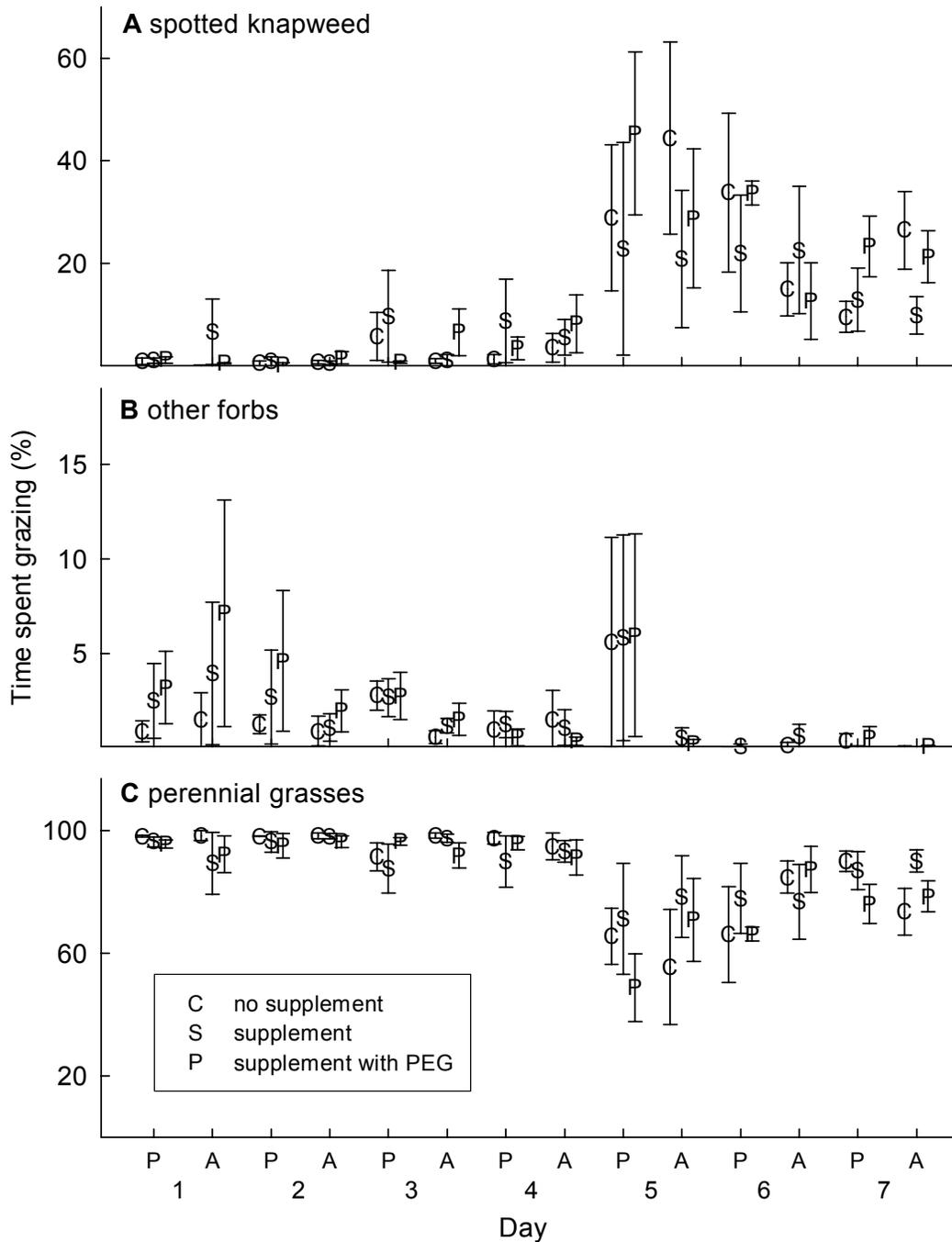


FIG. 11. In the field, time spent grazing (% relative to total grazing time) spotted knapweed (A), other forbs (B), or perennial grasses (C) for heifers provided with no supplement (C), a nutrient/energy supplement (S), or a nutrient/energy supplement with PEG (P) observed during evening (P) and morning (A) grazing periods for 7 days beginning July 20, 2005. Means \pm 1 S.E. Note y-axis scales differ.

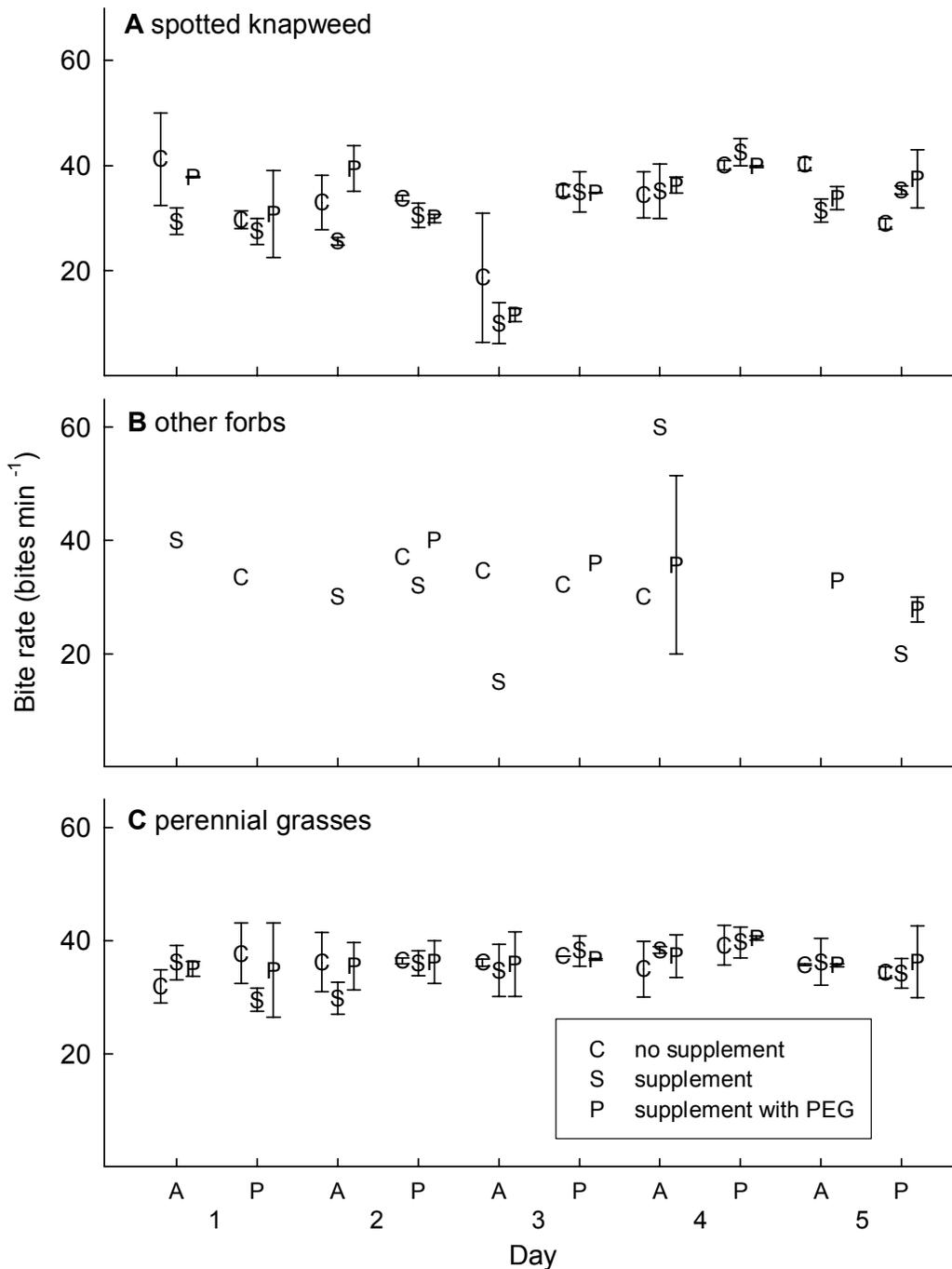


FIG. 12. In the field, bite rates (bites min⁻¹) of spotted knapweed (A), other forbs (B), or perennial grasses (C) for cattle provided with no supplement (C), a nutrient/energy supplement (S), or a nutrient/energy supplement with PEG (P) observed during evening (P) and morning (A) grazing periods for 5 days beginning August 9, 2005. Means \pm 1 S.E.

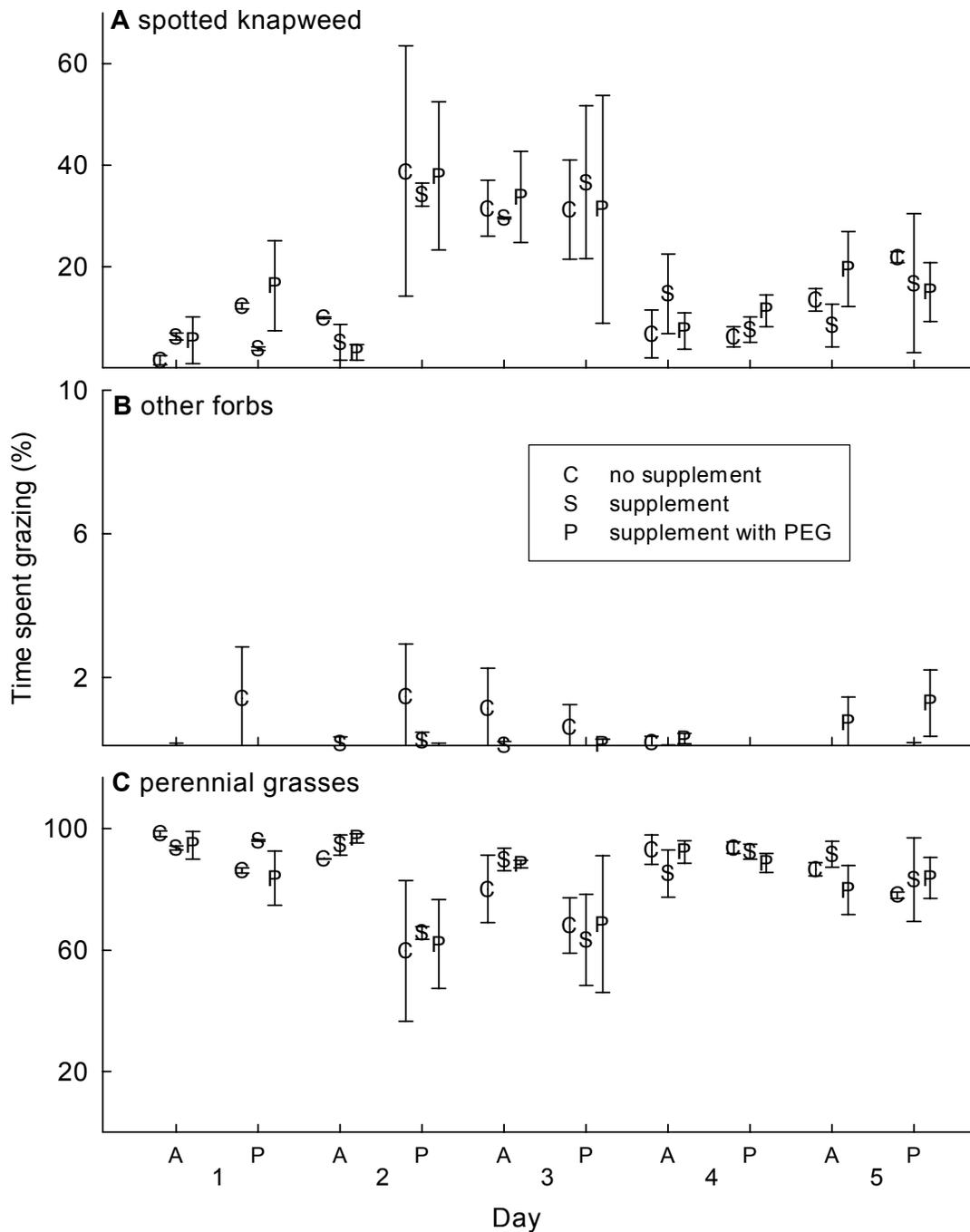


FIG. 13. In the field, time spent grazing (% relative to total grazing time) spotted knapweed (A), other forbs (B), or perennial grasses (C) for cattle provided with no supplement (C), a nutrient/energy supplement (S), or a nutrient/energy supplement with PEG (P) observed during evening (P) and morning (A) grazing periods for 5 days beginning August 9, 2005. Means \pm 1 S.E. Note y-axis scales differ.

Supplement intake did not differ between blocks or S and P treatments. On average, each heifer consumed $0.6 \text{ kg} \pm 0.1 \text{ S.E.}$ supplement daily during the 5-day trial. These cattle, from a local producer, were not weighed before or after the trial. Based on visual estimates, cattle use of forages was similar among treatments in August. Cattle used an average of $33\% \pm 3.3 \text{ S.D}$ of the grass and $25\% \pm 3.1 \text{ S.D}$ of the spotted knapweed.

Discussion

Drylot Trial (June 21-25)

Cattle preferred fresh-cut grass to spotted knapweed in the drylot. Cattle intake and time spent consuming spotted knapweed was always low ($< 40\%$) compared with fresh-cut grass ($> 60\%$). Cattle consumed most or all of the fresh-cut grass before consuming spotted knapweed, and some cattle did not consume any spotted knapweed on some trial days (M. Cheeseman, pers. observation). On d 1, a heifer provided supplement without PEG did not consume any spotted knapweed or fresh-cut grass, causing the large variation in time spent consuming and intake on that day.

Cattle are roughage eaters and prefer grasses to forbs when grazing (Grant et al., 1985; Ruyle and Bowns, 1985). Cattle may have consumed less spotted knapweed than grasses, in part, because it is a forb. Spotted knapweed was also a novel forage to the heifers. Ruminants prefer familiar forages to novel forages and regulate their intake of novel forages based on post-ingestive feedback (Thorhallsdottir, 1897; Burritt and Provenza, 1989). Negative post-ingestive feedback can cause herbivores to avoid plants

(Provenza, 1990). Spotted knapweed can negatively affect cattle rumen microbial activity, efficiency, and mass when presented as 100% of the diet (Chapter 2) and may cause negative PIF. However, the limited amount of spotted knapweed that was consumed probably did not negatively affect rumen microbes or cause negative PIF.

Cnicin, the secondary compound in spotted knapweed, has a bitter flavor (Suchy and Herout, 1962), which may make spotted knapweed less palatable to some herbivores. Some wildlife and livestock consume spotted knapweed despite its bitter flavor (Wright and Kelsey, 1997; Olson and Wallander, 2001); however, cattle may have a stronger aversion to the flavor of spotted knapweed and it may reduce intake of the plant.

Cattle provided supplement without PEG had the lowest intake and spent the least time consuming spotted knapweed during this trial. The supplement was expected to increase digestibility and enhance cattle intake of spotted knapweed. Providing nutrient and energy supplements can increase digestibility and intake of low quality forages (Cohen, 1974; Mulholland and Coombe, 1979; Titgemeyer et al., 2004; Leupp et al., 2005) and forages containing secondary compounds (Banner et al., 2000; Villalba et al., 2002a, b). *In vitro* dry matter disappearance of spotted knapweed plant parts was greater when the nutrient/energy supplement provided in these trials was added to a flask containing cattle rumen microbes (Chapter 2). However, if cattle were consuming limited amounts of spotted knapweed because it was novel or unpalatable, providing the supplement to enhance digestion probably would not enhance intake.

The cattle provided supplement without PEG were probably spending more time consuming supplement and less time consuming forages. Some herbivores spend less

time grazing and more time loafing when provided with a nutrient/energy supplement (Hatfield et al., 1990). Cattle provided supplement without PEG had the spent the least time consuming spotted knapweed and had the lowest total forage intake; however, they also had the greatest supplement intake. This may indicate that they were spending time consuming supplement rather than consuming spotted knapweed, the less preferred forage.

I expected that cattle provided supplement with PEG would have the greatest intake and spend the most time consuming spotted knapweed in the drylot. Providing supplement with PEG in feeding trials increases digestibility and intake of tannin-containing forages (Ben Salem et al., 2000, 2002; Bhatta et al., 2004). However, in this trial, heifers provided supplement with PEG only consumed the most spotted knapweed on d 2 and 3. Unexpectedly, non-supplemented cattle consumed similar amounts of spotted knapweed as cattle provided supplement with PEG during the rest of the trial. The dose and delivery of the anti-toxicant may not have been appropriate to increase intake of spotted knapweed. When the dose of PEG in feed blocks is not appropriate to bind with the secondary compounds in the target plant, intake and digestibility of the plant are not affected (Ben Salem et al., 2003).

Field Trial 1 (July 21-27)

Time spent grazing spotted knapweed was consistently lower than time spent grazing grasses. This was expected because cattle prefer grasses over forbs (Grant et al., 1985; Ruyle and Bowns, 1985). Time spent grazing spotted knapweed was very low during the first 4 d of the trail, but increased considerably towards the end of the trial.

The form of spotted knapweed (growing rather than fresh-chopped in the Drylot Trial) was novel to the heifers and may have limited the time spent grazing spotted knapweed at the beginning of the trial. Animals sample novel forages and adjust their intake of plants based on post-ingestive feedback (Thorhallsdottir, 1897; Burritt and Provenza, 1989). At the beginning of the trial, cattle may have been sampling spotted knapweed. At the end of the trial, cattle may have spent more time consuming spotted knapweed based on positive post-ingestive feedback after sampling.

On d 5 and 6 of the trial, time spent selecting spotted knapweed and grasses varied more than it had on previous days. Heifers may have been cycling their intake of spotted knapweed at different rates. Ruminants cycle their intake of forages containing secondary compounds to mitigate the cumulative negative effects of the compounds (Pfister et al., 1997). Cattle may have begun cycling their intake of spotted knapweed at different times between d 5 and 6, resulting in greater variation of time spent grazing spotted knapweed on those days.

I expected that supplemented cattle would spend more time grazing spotted knapweed than non-supplemented cattle. Although supplemented cattle were consuming supplement throughout the entire trial (M. Cheeseman, pers. observation), time spent grazing spotted knapweed was similar to non-supplemented cattle. The supplement and anti-toxicant may have improved rumen microbial activity and efficiency when spotted knapweed was grazed. However, cattle may not have consumed more spotted knapweed because they prefer grasses to forbs. Nutritious grasses were available as an alternative to spotted knapweed throughout the trial, and this may have limited time spent grazing

spotted knapweed by all treatments. Herbivores provided with an anti-toxicant do not always consume large quantities of plants containing secondary compounds when nutritious alternatives are available (Titus et al., 2000).

All cattle lost weight during this trial. Supplements can reduce weight loss when ruminants consume low quality forages (Cohen, 1974; Mulholland and Coombe, 1979; Titgemeyer et al., 2004). However, previous to this trial, heifers were confined to a drylot and were fed hay ad libitum. The change in diet and activity probably caused cattle to lose weight during this short-term grazing trial.

Field Trial 2 (August 9-13)

Spotted knapweed had greater CP and lower NDF and ADF than grasses; however, cattle spent more time grazing grasses than spotted knapweed during the trial. Again, this may simply reflect that cattle prefer grasses over forbs (Grant et al., 1985; Ruyle and Bowns, 1985).

Cattle used in this trial had some experience grazing spotted knapweed at the trial site (M. Applegate, pers. communication). However, percent time grazing spotted knapweed was still low during the first 3 observation periods, even though spotted knapweed was not novel. During the following 3 observation periods, cattle spent more time grazing spotted knapweed and then it was lower again during the last 4 observation periods. This grazing behavior may, again, indicate cattle cycling their intake of spotted knapweed, and the secondary compound cnicin. Cnicin concentrations of spotted knapweed average 1.8% in August (Locken and Kelsey, 1987).

Unexpectedly, the supplement with or without PEG did not alter cattle foraging behavior. I expected that cattle provided with supplement would spend more time grazing spotted knapweed because of the low nutritive value of alternative forages during this trial. However, supplement use was low at the beginning of the trial (M. Cheeseman, pers. observation) possibly because cattle did not have previous experience with the block supplements (M. Applegate, pers. comm.). Intake of self-fed supplements is quite variable, especially when animals have not been exposed to supplement before (Mulholland and Coombe, 1979; Lobato and Pearce, 1980). Cattle use of the supplement was greater after d 3 of the trial, however, the supplement may not have affected grazing behavior if cattle were cycling their intake of spotted knapweed. The increased intake of supplement may not have affected grazing behavior because of cycling. If the trial had continued, a supplement effect may have become more evident.

I expected that cattle provided with supplement containing PEG would spend the greatest amount of time grazing spotted knapweed. However, low supplement intake at the beginning of the trial may have limited the effects of the anti-toxicant. Also, the PEG dose and delivery may not have been appropriate to increase time spent consuming spotted knapweed in early August.

Forage Analysis and Vegetation Measurements

In the drylot, spotted knapweed had lower CP, IVDMD, and NDF than fresh-cut grass. The lower IVDMD of spotted knapweed relative to grasses may reflect the lower CP of spotted knapweed but could also reflect the cnicin content of spotted knapweed. In June, cnicin concentrations in spotted knapweed range from 1.2 - 1.9% (Kelsey and

Mihalovich, 1987; Locken and Kelsey, 1987). Cnicin negatively affects cattle rumen microbial function (Chapter 2) and may explain spotted knapweeds lower IVDMD relative to fresh-cut grass.

In July, CP, OM, and IVDMD of spotted knapweed and grasses were similar but spotted knapweed had much lower ADF and NDF than grasses. In general, forages low fiber are more digestible than forages high fiber (Van Soest, 1994). Again, the cnicin in spotted knapweed may explain the similar IVDMD between spotted knapweed and grass, despite the lower fiber content of spotted knapweed. Cnicin concentrations in spotted knapweed average 1.3% in July (Locken and Kelsey, 1987). Other forbs had the highest CP during this trial. However, cattle do not prefer forbs (Grant et al., 1985; Ruyle and Bowns, 1985), and consumed them in limited quantities (M. Cheeseman, pers. observation).

In August, spotted knapweed and the perennial grasses had similar CP and IVDMD but spotted knapweed had much lower ADF and NDF than grasses. Again, the low IVDMD of spotted knapweed may indicate the negative effects of cnicin on rumen microbial function (Chapter 2). Cnicin concentrations in spotted knapweed average 1.8% in August (Locken and Kelsey, 1987).

For all analyses, entire plants were harvested and analyzed. However, cnicin concentrations and nutritive values differ among plant parts of spotted knapweed. Leaves and buds have high CP and cnicin concentrations, and low ADF and NDF, whereas stems have low CP and cnicin concentrations, and high ADF and NDF (Olson and Kelsey, 1997). Cattle have large mouths and consume large quantities of forage in each bite

(Hofmann, 1989). During the July and August field trials, cattle did not select individual plant parts of spotted knapweed; however, they also did not consume the entire plant. Cattle would consume the tops of the plants, which included some stem, leaf, and bud material all in one bite (M. Cheeseman, pers. observation). Nutritive values of entire plants probably do not reflect the nutritive value of spotted knapweed consumed by cattle because they were not consuming the entire stem, which is of lower quality than other plant parts (Olson and Kelsey, 1997; Chapter 2).

Conclusions

Supplemented cattle grazing is probably not an effective method to reduce the spread of spotted knapweed on rangelands. Cattle will graze spotted knapweed, however, they prefer to graze perennial grasses, even when provided with a nutrient/energy supplement, with or without PEG.

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CHAPTER 5

SUMMARY

I hypothesized that sheep and cattle rumen microbial activity (gas production), efficiency (IVDMD), and mass (purines) would be: 1) decreased by the secondary compound cnicin, 2) greater at greater rates of the anti-toxicant PEG, and 3) enhanced by a nutrient/energy supplement with or without PEG *in vitro*. My results supported some of these hypotheses (Chapter 2).

The secondary compound cnicin decreased rumen microbial function. Sheep and cattle had lower rumen microbial activity, and efficiency and cattle had lower microbial mass when the secondary compound cnicin was added to hay. The negative effects of cnicin on rumen microbes may cause negative PIF, which may cause herbivores to avoid or reduce their intake of spotted knapweed.

Polyethylene glycol, at the rates provided, had inconsistent effects *in vitro*. Sheep and cattle rumen microbial activity, efficiency, and mass were not affected by different rates of the anti-toxicant. Providing PEG at the rates and delivery method tested in this study would not likely enhance sheep and cattle intake of spotted knapweed.

A nutrient/energy supplement with or without PEG enhanced sheep and cattle rumen microbial response to plant parts of spotted knapweed *in vitro*. Sheep had greater rumen microbial activity when exposed to plant parts of spotted knapweed if the nutrient/energy supplement was added to the flask. Compared with non-supplemented flasks, IVDMD of spotted knapweed and grass hay was greater in supplemented flasks

when cattle rumen fluid was used and IVDMD of spotted knapweed stems and grass hay were greater when sheep rumen fluid was used. Supplement with 12.5% PEG did not further enhance sheep or cattle rumen microbial response to spotted knapweed plant parts. Based on these results, providing grazing sheep and cattle with the nutrient/energy supplement used in this trial may increase microbial function and intake of spotted knapweed.

I hypothesized that sheep provided with a nutrient/energy supplement, with or without PEG, would consume more spotted knapweed: 1) in a drylot, 2) in the field during July, and 3) in the field during August. My results did not support these hypotheses (Chapter 3).

Sheep provided supplement, with or without PEG, in a drylot consumed the least amount of spotted knapweed. Sheep provided supplement with PEG consumed much less spotted knapweed than sheep provided supplement without PEG. Some unknown dietary effects of PEG may have limited intake of spotted knapweed, at the rate of PEG provided in the supplement.

In July, supplement did not increase time spent grazing spotted knapweed. Time spent grazing spotted knapweed was low at the beginning of this trial, probably because spotted knapweed was novel to the ewes in the field although they had been exposed to fresh-cut, chopped spotted knapweed in the drylot. All ewes spent more time consuming the plant at the end of the trial. Sheep provided with supplement without PEG spent more time grazing spotted knapweed than non-supplemented sheep. The nutrients and energy provided in the supplement block may have increased rumen microbial function

and therefore increased time spent grazing spotted knapweed. However, sheep provided with supplement with PEG spent a similar amount of time grazing spotted knapweed as non-supplemented sheep. The dose of the anti-toxicant provided in the supplement may not have been appropriate to bind with cnicin and increase time spent grazing spotted knapweed in the field in July.

In August, time spent grazing spotted knapweed was similar among treatments. The nutrients and energy provided in the supplement block may not have increased rumen microbial function enough to reduce the negative effects of the secondary compound cnicin in spotted knapweed, and thereby increase time spent grazing the plant. However, ewes may not have experienced the positive effects of the supplement on rumen microbial response to spotted knapweed when consuming such limited amounts of the plant. Providing a supplement block with the anti-toxicant PEG also did not increase time spent grazing spotted knapweed. The dose provided in the supplement block may not have been appropriate to bind with the secondary compound cnicin and increase time spent grazing spotted knapweed.

I hypothesized that cattle provided with a nutrient/energy supplement, with or without PEG, would consume more spotted knapweed: 1) in a drylot, 2) in the field during July, and 3) in the field during August. My results did not support these hypotheses (Chapter 4).

In the drylot, spotted knapweed intake was similar among treatments. Heifers provided with supplement without PEG consumed less spotted knapweed and more supplement than other treatments. Heifers provided with supplement with PEG

consumed the most spotted knapweed on d 2 and 3 of the trial, however, this trend was not consistent throughout the trial. Heifers provided with supplement with PEG consumed similar amounts of spotted knapweed as non-supplemented heifers on most days of the trial.

In July, time spent grazing spotted knapweed was similar among treatments. All cattle spent limited time grazing spotted knapweed at the beginning of the trial, probably because spotted knapweed was novel to the heifers in the field. Time spent grazing spotted knapweed increased towards the end of the trial; however, providing supplement did not seem to enhance time spent grazing spotted knapweed. The nutrients and energy in the supplement block may have enhanced rumen microbial function; however, heifers may not have consumed more spotted knapweed, a forb, because of their preference for grasses. The anti-toxicant, provided at 12.5% in the supplement block, also did not enhance time spent grazing spotted knapweed. The dose of PEG in the supplement block may not have been appropriate to increase time spent grazing spotted knapweed by cattle in July.

In August, time spent grazing spotted knapweed was not enhanced by supplement with or without PEG. Although cattle used in this trial had some experience grazing spotted knapweed, the nutrient/energy supplement was novel. Cattle use of the nutrient/energy supplement was low during the first 3 days of the trial, possibly limiting the benefits of the supplement to the cattle. However, even when cattle were using the supplement, it did not enhance time spent grazing spotted knapweed. Again, cattle preference for grasses over forbs may have limited time spent grazing spotted knapweed.

Providing supplement with 12.5% PEG also did not enhance time spent grazing spotted knapweed. The dose of the anti-toxicant may not have been appropriate to increase time spent grazing spotted knapweed in August.

In summary, cnicin negatively affected sheep and cattle rumen microbial function, and may cause negative PIF and possibly could reduce intake of spotted knapweed by grazing ruminants. The nutrient/energy supplement, with or without PEG, benefited sheep and cattle rumen microbes exposed to spotted knapweed plant parts *in vitro* and was expected to increase intake and time spent grazing spotted knapweed. However, the supplement, with or without PEG, did not appreciably increase use of spotted knapweed by sheep or cattle in a drylot, in the field in July, or in the field in August. Sheep and/or cattle intake of spotted knapweed may be enhanced by other methods that reduce the negative effects of the secondary compound cnicin on rumen microbes, and thereby reduce negative PIF associated with the plant.

APPENDIX A

SWEET 14% NUTRIENT ANALYSIS

TABLE 1. SWEET 14% NUTRIENT ANALYSIS*

CP (%)	14
Fat (%)	4
Calcium (Max.%)	1.5
Phosphorus (%)	1
Magnesium (%)	0.3
Potassium (%)	3
Sulfur (%)	0.5
Copper (PPM)	15
Selenium (PPM)	4.4
Zinc (PPM)	25
Vitamin A (IU/LB)	50000
Vitamin D (IU/LB)	5000

* Values provided by Ridley Block Operations

Ingredients: molasses products, plant protein products, hydrolyzed vegetable oil, processed grain by-products, monocalcium phosphate, dicalcium phosphate, calcium carbonate, magnesium oxide, sodium selenite, vitamin A acetate, vitamin D₃ supplement.