Crude Protein Supplementation To Reduce Lupine Consumption by Pregnant Cattle in the Scablands of Eastern Washington

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Abstract

Lupinus leucophyllus (velvet lupine) is prevalent in eastern Washington State, and when consumed by pregnant cows, it can cause "crooked calf syndrome." Rangelands in this region are dominated by poor-quality annual grasses. The objective of this study was to determine whether feeding supplemental crude protein (CP) would reduce cattle consumption of velvet lupine during midsummer. Twelve pregnant cows were divided into 2 treatments: 6 controls received no supplement, and 6 cows received a supplement of 44% soybean meal at 4 g/kg body weight per day for 19 days in July 2010. Lupine density was >10 plants/m^2. There was no supplement effect on lupine consumption (P = 0.68), nor was there a day x supplement interaction (P = 0.88). Supplemented cattle took 6.9 ± 0.7% of daily bites as lupine compared to 5.0 ± 0.6% of bites for controls. Dry grass and dry forbs were the major diet components selected by cattle. Cattle ate mostly dry grass during the first 8 days of the trial, but then their consumption of dry forbs increased substantially. Cattle switched from eating green forbs to lupine after about 1 week; this change coincided with a decrease in green forb biomass. Five calves from both treatments were born with mild to moderate birth defects. We conclude that CP supplementation had no effect on cattle consumption of lupine under these supplement and abundant-forage conditions. The combined effect of supplementation and drought (i.e., forage scarcity) on lupine consumption by cattle has not been determined.

Keywords: cattle, lupine, protein supplementation

Introduction

Lupines (*Lupinus* spp.) are prevalent in the rugged Scabland region of eastern Washington State. Rangelands in this region are dominated by annual grasses, including *Bromus tectorum* (cheatgrass) and *Taeniatherum caput-medusae* (medusahead), and a number of weedy forbs, such as *Chondrilla juncea* (skeletonweed) and *Amsinckia lycopsoides* (fiddleneck). Lupines are deep-rooted legumes that are grazed in mid- to late summer, when annual grasses are dormant and other forbs have been depleted by grazing. One dominant lupine species is *Lupinus leucophyllus* (velvet lupine), which contains the teratogenic alkaloid anagyrine. When pregnant cows graze velvet lupine between days 40 and 100 of gestation, they are at risk of having deformed offspring, so-called "crooked calves." The incidence of crooked calf syndrome on ranches where velvet lupine is prevalent is estimated to be about 5% per year. Some years the incidence is much higher, typically when lupine populations are very dense from increased winter and spring precipitation (Panter et al. 2009).

Our studies on this type of rangeland indicate that grazing cattle prefer graminoids, particularly green grass when available (Lopez-Ortiz et al. 2007, Pfister et al. 2008). However, at times cattle will consume relatively large amounts of forbs (>60% of the diet) depending on the forage milieu (Lopez-Ortiz et al. 2007, Pfister et al. 2008). Some studies have suggested that cattle may include large amounts of forbs in their diets because of forbs' high protein content (Pieper and Beck 1980, Holechek 1984).

Altering the nutritional status of cattle may influence their forage preference (Heady 1964). Protein supplementation can influence an animal's nutritional status (Kartchner 1980). However, protein supplementation has had limited success in influencing botanical diet selection by grazing cattle on grass-forb rangelands (Judkins et al. 1985). In contrast, Odadi et al. (2013) reported that protein supplementation reduced forb grazing by cattle in African savanna during the dry season. For ruminants grazing on shrub-dominated rangelands (e.g., sagebrush), providing supplemental protein and/or energy to detoxify terpenes may greatly enhance shrub use by ruminants (Villalba et al. 2006, Dziba et al. 2007).

If protein supplementation could reduce selection of plants such as toxic lupine by grazing livestock, then it could be a useful tool to reduce the risk of crooked calf syndrome on Scabland rangelands. During a typical summer in the Scablands, all grasses and many forbs become dry and senescent, with resultant decreases in concentrations of crude protein, compared to forages that remain green later into the summer. Grazing cattle typically increase their consumption of lupine in midsummer (Ralphs et al. 2006). Pregnant heifers require approximately 9% to 10% crude protein (CP) in their diet to gain 0.75 kg/day (NRC 2000). Our hypothesis was that cattle would reduce consumption of still-green lupine in midsummer after the grasses had senesced if the cattle were supplemented with high-quality protein. Thus, the purpose of this trial was to determine whether providing a CP supplement to pregnant cattle would alter their selection of velvet lupine during midsummer grazing.

Materials and Methods

The grazing study was conducted from July 8 to July 26, 2010, near Washtucna, WA (46° 48' 12.8" N 118° 17' 08.5" W; 529 m elevation). Vegetation on the study site was dominated by cheatgrass and medusahead; other annual species included fiddleneck, tumblemustard *(Sisymbrium altissimum)*, tansy mustard *(Descurainia pinnata)*, prickly lettuce *(Lactuca serriola)*, and black mustard *(Brassica nigra)*. Velvet lupine and vetch *(Vicia cracca)* were abundant in all pastures.

All procedures were approved by the Utah State University Institutional Animal Care and Use Committee and were conducted under veterinary supervision. Twelve 2-year-old Hereford x Angus (479 kg ± 13 kg body weight) cows were time bred so that they were between 40 and 60 days pregnant at the start of this study. They were verified pregnant by rectal palpation before the study began and palpated upon return to Logan, UT, shortly after the end of the study. The 12 cows were divided into 2 treatments: 6 controls receiving no supplemental feed and 6 cows receiving a supplement of 44% soybean meal at 4 g/kg body weight per day. Supplemental feeding began 10 days before the grazing trial was started.

A lupine-infested pasture was subdivided into 6 smaller pastures such that lupine was abundant in all pastures, and 2 cows from the same treatment were placed into each of the 3 replicate pastures. Cattle were placed into a corral at night at 1900 h each day, with water and salt provided ad libitum. Supplement was fed to each animal individually in the corral during the evening. Lupine consumption was determined using bite counts. Each morning at 0530 h, cattle were released in pairs into their respective pastures to graze, and diets were determined using bite counts. Bite counts were taken on individual animals during active grazing periods; categories for bite counts were lupine (subcategories: whole plant, leaves, flowers, and pods), dry grass, green grass, dry forbs (other than lupine), and green forbs. Each animal was observed for a 5-min period, then the observer moved to the next animal. In this manner, the observer rotated through the entire group of animals several times each day. Animals were accustomed to the procedure, and the observer could remain within 1 to 2 m of the animals without disturbing their grazing. Typically, about 30 min of daily observation time was recorded for each animal.

Forage availability and quality were evaluated by weekly clipping of plants to ground level in 10 plots (0.25 m^2) within each of the 6 pastures. Clipped material was sorted into dry grass, green grass, lupine, other green forbs, and other dry forbs. The clipped material was dried at 40 °C for 48 hours and weighed to determine available forage (kilograms per hectare). The same plant material was also retained for nutritional analysis. Lupine density was determined at the beginning of the study by placing 150 quadrats, 0.5 m^2, along 3 pace transects that trisected all of the pastures, and counting all lupine plants within each plot.

Five individual clipped samples of each forage category for each time point were ground to pass a 1-mm screen in a Wiley mill and analyzed for dry matter, CP (N x 6.25; LECO FP-528 Nitrogen

Analyzer, LECO Corp., St. Joseph, MI), neutral detergent fiber (NDF) (ANKOM Fiber Analyzer system), and in vitro true digestibility (ANKOM Daisy II system). The NDF procedure was modified by addition of heat-stable amylase (Sigma Chemical, St. Louis, MO).

The alkaloid composition of lupine (Lee et al. 2007) was determined on 10 randomly selected whole plants collected weekly. Extraction of *L. leucophyllus* was done by weighing 100 mg of ground plant material into a 10-mL screw-top test tube equipped with Teflon-lined caps. Five milliliters of 1*N* hydrochloric acid (HCl) and 4 mL chloroform ($CHCl_3$) were added to the test tubes, which were placed in a mechanical shaker for 15 min, then centrifuged to separate the aqueous and organic phases. The aqueous fraction was transferred to a clean test tube and basified to a pH of between 9 and 9.5 by drop-wise addition of concentrated ammonium hydroxide (NH_4OH). The basified solution was extracted twice with $CHCl_3$ (4 mL, then 2 mL). The combined $CHCl_3$ fractions were filtered through anhydrous sodium sulfate (Na_2SO_4) and then dried under a stream of nitrogen (N_2) at 50 °C to yield the crude alkaloid fraction.

The alkaloid fraction extracted from *L. leucophyllus* was reconstituted in 4 mL of methanol containing 1.3 µg/mL caffeine (internal standard). A portion (~1 mL) was transferred to 1.5-mL gas chromatograph (GC) autosample vials, and 2 µL were injected into an HP 5890 GC (HP 5890, Agilent, Palo Alto, CA) equipped with a split/splitless injector, FID detector, and a J&W DB-5 (30 m x 0.33 mm i.d.) capillary column. Injector temperature was 250 °C and operated in the splitless mode. Split vent flow rate was 60 mL/min and purged after 1 min. Oven temperature was programmed: 100°C for 1 min; 100 to 200°C at 50°C/min; and 200 to 320°C at 5°C/min. Caffeine was used as an internal standard, and plant alkaloid peaks were quantified against a 6-point anagyrine standard curve over the range of 25 to 800 µg/mL anagyrine in methanol prepared by serial dilution.

Climatic data before and during the study were taken from a National Climate Data Center weather station in Endicott, WA, and a local meteorological station in Washtucna, WA. Analysis of all data was performed using SAS (SAS Institute Inc., Cary, NC). The bite-count data were checked for normality and transformed using an arcsine square root transformation typically used for proportions ranging from 0 to 100%. All means shown here are nontransformed means. The bite-count data were analyzed using a mixed linear model (Proc Mixed in SAS), with pasture as the experimental unit; animals as a random factor nested within pasture and treatment; treatment; days; and interactions. The probability-of-difference option was used for preplanned comparisons.

Results

The mean maximum, minimum, and average temperatures during the study period were 33, 18, and 25 °C, respectively. There was no precipitation during the study. Normal January-to-June precipitation in Washtucna is 14.5 cm (NOAA 2002); during 2010, precipitation during this period was 14% above normal.

Lupine was abundant and uniformly distributed across the pastures. Mean (± SE) lupine density for plants of all phenological growth stages and across all 6 small pastures was 10.8 ± 1.0 plants/m^2. There was ample dry grass during the study, but virtually no green grass in the pastures (table 1). Conversely, there was an abundance of green forbs. Much of the green forb component was *V. cracca*, which eventually changed to the dry-forb category, with a few other weedy plants such as rush skeletonweed also present.

The anagyrine concentration in the lupine population was near or above 0.2% during the study

Table 1. Forage availability (kg/ha ± SEM) of green and dry grasses and forbs, and lupine *(Lupinus leucophyllus)* in eastern Washington during July 2010.

Date	Dry Grass	Green Grass	Dry Forbs	Green Forbs	Lupine
July 8	701 ± 102	18 ± 9	29 ± 15	524 ± 63	766 ± 126
July 15	497 ± 39	0	85 ± 14	206 ± 37	372 ± 68
July 22	210 ± 33	0	156 ± 27	185 ± 22	281 ± 53

Table 2. Alkaloid concentration (%) of lupine *(Lupinus leucophyllus)* in eastern Washington during July 2010.

Date	Anagyrine	Lupanine	Total alkaloids
July 8	0.21	0.09	1.06
July 15	0.24	0.03	0.85
July 22	0.19	0.03	0.81

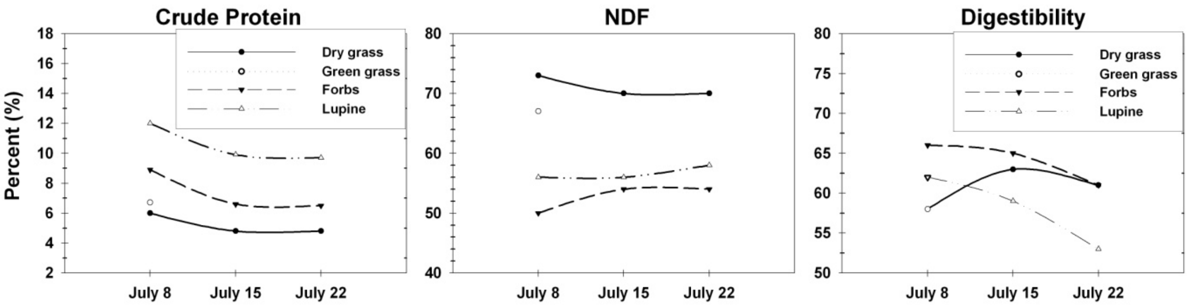

Figure 1. Nutrient content (% of dry matter) in dry grass, green grass, other forbs, and lupine *(Lupinus leucophyllus)* in eastern Washington during July 2010. Crude protein is N x 6.25; NDF = neutral detergent fiber; digestibility = in vitro true digestibility.

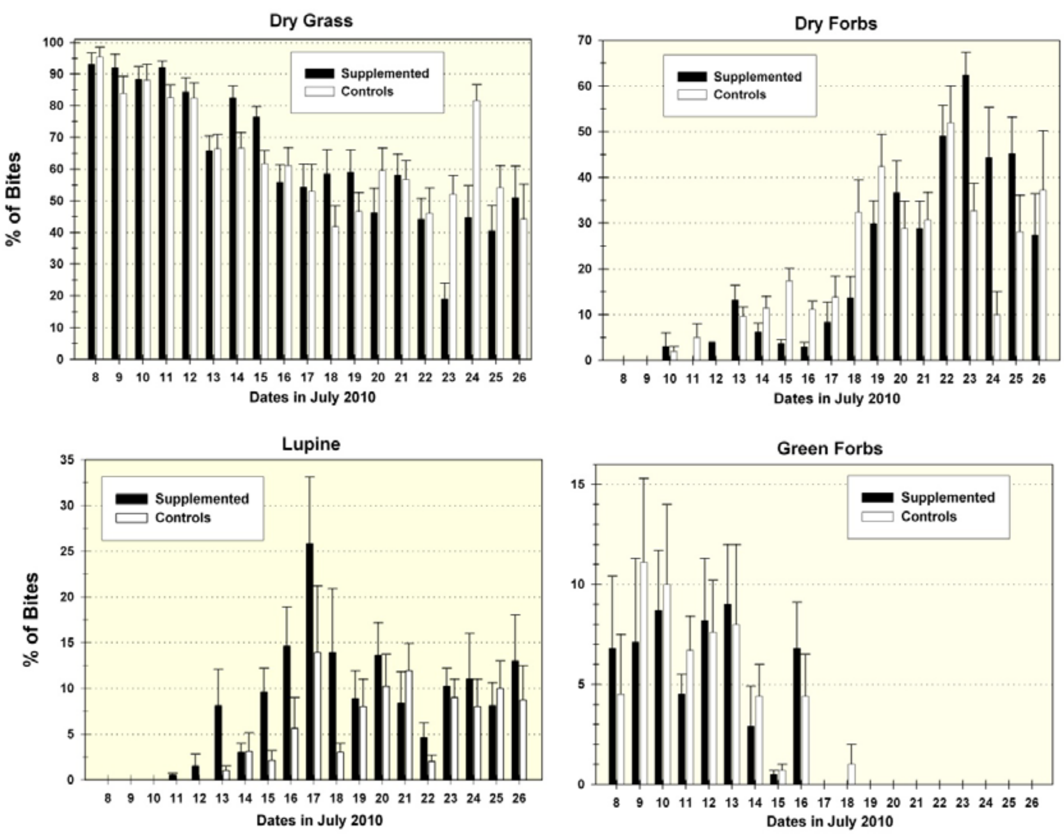

Figure 2. Diet selection (% of bites ± SE) by cattle during July 2010 when receiving either a protein supplement (soybean meal) or no supplement (controls) while grazing a lupine-infested rangeland in eastern Washington.

period (table 2). Total alkaloid concentrations exceeded 0.85% on the three sampling dates.

Over the study period, the average CP content was 5.3% for dry grass, 6.7% for dry forbs, and 7.7% for green forbs (including vetch). These values are in contrast to the average CP content of vetch alone (8.4%) and lupine (10.5%). Dry grass contained the highest concentrations of NDF (>70%), whereas lupine and green forbs contained less NDF (50–60%). As the lupine matured during the study, digestibility decreased from >60% to slightly above 50%; Digestibility of green forbs and dry grass was >60% at the start of the study and decreased only slightly during the study period (figure 1).

Dry grass and dry forbs were the major diet components during much of the study (figure 2). Cattle ate mostly dry grass during the first 8 days of the trial, then dry grass consumption became more variable (figure 2). There was a day x supplement interaction ($P = 0.04$), with control animals eating more dry grass on days 23 and 24. The consumption of forbs such as *V. cracca* increased substantially in the latter portion of the trial, but there was no treatment effect or treatment x day interaction (P = 0.08). There was no treatment (i.e., supplement) effect on lupine consumption (P = 0.68), nor was there a day x supplement interaction (P = 0.88). Numerically, supplemented animals ate substantially more lupine on several days during the trial (figure 2), but animal-to-animal variability was high, and no statistical differences were found in lupine consumption between supplemented and control animals. Overall, supplemented cattle took 6.9 ± 0.7% of daily bites as lupine compared to 5.0 ± 0.6% of bites for control cattle. Interestingly, cattle began to switch from green forbs to lupine in their diets after about 1 week on the study pastures, and this switch coincided with a decrease in available green forb biomass about midway through the trial.

One pregnant cow resorbed her fetus during the grazing study. Five other calves were born with mild to moderate arthrogryposis, scoliosis, and/or rotational defects (i.e., impaired leg movement) in the front legs (table 3). No cleft palates were noted. There was no apparent relationship between supplementation and the occurrence of birth defects.

Table 3. Deformities in calves born to pregnant cows which grazed on lupine-infested pasture from July 8 to 26, 2010, within the gestational day 40 to 70 window for birth defects. + signifies slight defects, and ++ indicates moderate defects. S= Supplement; C=Controls. No cleft palates were noted.

		Deformity		
Cow #	Treat-ment	Arthro-gryposis	Scoliosis	Leg movement
62	S	+		+
59	S			+
61	S			+
36	C		++	
57	C	++		+

Discussion

There was ample plant biomass for cattle diet selection throughout the entire study period; near the end of the study, there were declining but still-sufficient amounts of green and dry forbs, dry grass, and lupine across the pastures. It was apparent that the vegetation, including lupine and other forbs, responded favorably in terms of biomass to the wetter-than-normal winter and spring conditions. This site was selected specifically because of the abundant lupine population during some years. Because of the intentional timing of the study, the grasses were nearly all senesced when the study began. By the last week of the study, virtually all lupine plants on the study site were at least partially dry from the seasonally hot temperatures (>38 °C) and desiccating wind. Under these forage and climatic conditions, there was no effect of CP supplementation on lupine consumption by pregnant cattle.

As noted in other studies (Ralphs et al. 2006, Pfister et al. 2008), lupine is not a preferred forage in these annual rangelands, but cattle eat lupine after the grasses are senesced. Further, in this study, cattle increased lupine consumption and greatly diminished consumption of green forbs about midway through the study period. This dietary switch coincided with increased consumption of dry forbs, particularly vetch, the majority of which senesced during this period on the study site. Lopez-Ortiz et al. (2007) and Ralphs et al. (2011) also found that cattle selected lupine only after availability of annual grasses and other forbs declined. Our observations indicate that cattle typically began to graze lupine plants near the top, eating floral parts, pods, leaves, and stem, then often regrazed the same plants several times, eventually eating the plants down to short, bare stalks.

One can speculate that the cattle may have responded differently to the supplement if lower precipitation had resulted in reduced biomass for other forbs, particularly the relatively palatable vetch. The abundant vetch was consumed when green, and cattle continued to consume the vetch after it senesced. However, our previous experience in this region suggests that lupine populations are also depressed during drought (Pfister et al. 2008). Lupine density at this site was >10 plants/m^2, which contrasts greatly with lupine density measured at another nearby site during a moderate drought (1-2 plants/m^2; Pfister et al. 2008). The effectiveness of any supplementation regime to reduce lupine consumption by cattle during drought in the Scablands region is not known. However, recurring drought conditions in the Scablands region since 1997 have reduced lupine populations and diminished the incidence of crooked calf syndrome (Panter et al. 2009). Gay et al. (2007) also noted an association between lupine density in pastures and the reported incidence of “crooked calves.”

It is interesting to note that some “weedy” forbs such as rush skeletonweed are relatively palatable for cattle on these degraded annual rangelands; the stemmy skeletonweed, for example, is listed as a noxious weed in eastern Washington and elsewhere. However, at times cattle consumed relatively large quantities of this weed. On these annual rangelands, cattle also consumed large, but declining, quantities of cheatgrass and also some of the very coarse medusahead. We did not distinguish between consumption of the various annual grasses in our bite counts, but in the absence of other, more palatable green grasses, cattle readily accepted the dry annual grasses, primarily cheatgrass, as forage.

The CP content of lupine and vetch was numerically higher than other available forages such as dry grass or dry forbs. However, it is important to note that these CP concentrations are taken from clipped plant material, and numerous studies have shown that cattle can select a diet substantially higher in nutritional composition than is shown in available plant material by clipping (Weir and Torell 1959, Galt et al. 1969, Coleman and Barth 1973). That was undoubtedly true for cattle in this study as well. Thus, we speculate that even unsupplemented cattle in this study were able to meet their minimal nutritional needs for CP (about 9% CP in diet) without grazing toxic lupine on this annual grassland site during midsummer.Soybean meal is a rich source of protein, with both rumen degradable protein (RDP, 65% of CP) and rumen undegradable protein (RUP, 35% of CP). Many forage grasses contain mostly RDP (Buckner et al. 2013), at levels similar to those in soybean meal. There is no information available on the relative RDP and RUP concentrations of Scabland forbs or lupine. It is unclear whether using a protein supplement that would provide more RUP (e.g., blood meal and feather meal) would have altered the results of this study.

The anagyrine concentrations in lupine of about 0.20% found in this study are either similar or slightly higher than those noted in other studies in this area for whole plant material (Ralphs et al. 2006, Pfister et al. 2008). Floral parts and pods are typically highest in anagyrine (Ralphs et al. 2006), but these were not analyzed separately in this study. At the start of this study, pods were present but were rapidly drying and shattering. Even though cattle ate considerable lupine over a 14-day period, the birth defects we noted in calves the following spring were mild to moderate, suggesting that the lupine exposure was perhaps too brief in this study to cause severe birth defects. Another consideration is that the cattle were penned overnight during the study in order for us to use the bite-count method, and it may be that such interruptions in lupine grazing allowed circulating anagyrine concentrations to decline sufficiently to reduce the severity of defects. There is currently little information available about the anagyrine dose and duration necessary to cause defects.

We conclude that supplemental protein had no effect on lupine consumption by pregnant cattle. This study was conducted under conditions of abundant forage, including lupine and other forbs, and these conditions undoubtedly had a major impact on the findings. With normal precipitation patterns in conjunction with overgrazing, it is possible that the depletion of forbs by grazing may not go hand in hand with lupine depletion, and under this scenario the likelihood of a positive effect from protein supplementation may increase. It is unknown whether cattle grazing on lupine-infested rangelands during drought would alter their lupine consumption if given a CP supplement, because lupine populations also decline during drought along with other forage classes.

Acknowledgments

We thank Kermit Price for assistance with all aspects of the study from laboratory to the field, Chuck Hailes for anagyrine analysis, and Rex Probst for animal care and transportation during the study.

References

Buckner CD, Klopfenstein TJ, Rolfe KM, et al. 2013. Ruminally undegradable protein content and digestibility for forages using the mobile bag in situ technique. Journal of Animal Science 91:2812-2822.

Coleman SW, Barth KM. 1973. Quality of diets selected by grazing animals and its relation to quality of available forage and species composition of pastures. Journal of Animal Science 36:754-761.

Dziba LE, Provenza FD, Villalba JJ, Atwood SB. 2007. Supplemental energy and protein increase use of sagebrush by sheep. Small Ruminant Research 69:203-207.

Galt HD, Theurer B, Ehrenreich JH, et al. 1969. Botanical composition of diet of steers grazing a desert grassland range. Journal of Range Management 22:14-19.

Gay CC, Panter KE, Motteram ES, et al. 2007. Risk factors for lupine-induced crooked calf disorder in east-central Washington state. *In* KE Panter, TL Wierenga, JA Pfister, eds., Poisonous Plants: Global Research and Solutions, pp. 156–164, CABI, Wallingford, UK.

Heady HF. 1964. Palatability of herbage and animal preference. Journal of Range Management 17:76-82.

Holechek JL. 1984. Comparative contribution of grasses, forbs, and shrubs to the nutrition of range ungulates. Rangelands 6:261-263.

Judkins MB, Krysl LJ, Wallace JD, et al. 1985. Intake and diet selection by protein supplemented grazing steers. Journal of Range Management 38:210-214.

Kartchner RJ. 1980. Effects of protein and energy supplementation of cows grazing native winter range forage on intake and digestibility. Journal of Animal Science 51:432-438.

Lee ST, Cook D, Panter KE, et al. 2007. Lupine induced “Crooked Calf Disease” in Washington and Oregon: Identification of the alkaloid profile in *Lupinus sulfureus, Lupinus leucophyllus,* and *Lupinus sericeus.* Journal of Agriculture and Food Chemistry 55:10649-10655.

Lopez-Ortiz S, Pfister JA, Launchbaugh K, Gay CC. 2007. Effects of forage availability and body condition on intake of lupine *(Lupinus leucophyllus)* by grazing cattle. Professional Animal Scientist 23:459-466.

[NOAA] National Oceanographic and Atmospheric Administration. 2002. Climatography of the United States No. 81, 1971-2000: Monthly normals. National Climatic Data Center, Asheville, NC.

[NRC] National Research Council. 2000. Nutrient Requirements of Beef Cattle, 7th rev. ed. The National Academies Press, Washington, DC.

Odadi WO, Karachi MK, Abdulrazak SA, Young TP. 2013. Protein supplementation reduces non-grass foraging by a primary grazer. Ecological Applications 23:455-463.

Panter KE, Motteram ES, Cook D, et al. 2009. Crooked calf syndrome: Managing lupines on rangelands of the Channel Scablands of east-central Washington state. Rangelands 31:10-15.

Pfister JA, Lee ST, Panter KE, et al. 2008. Effects of experience and lactation on lupine consumption by cattle. Rangeland Ecology and Management 61:240-244.

Pieper RD, Beck RF. 1980. Importance of forbs on southwestern ranges. Rangelands 2:35-36.

Ralphs MH, Panter KE, Gay CC, et al. 2006. Cattle consumption of velvet lupine *(Lupinus leucophyllus)* in the Channel Scablands of eastern Washington. Rangeland Ecology and Management 59:204-207.

Ralphs MH, Pfister JA, Panter KE, et al. 2011. Influence of grazing pressure on cattle consumption of the teratogenic plant velvet lupine. Professional Animal Scientist 27:101-108.

Villalba JJ, Provenza FD, Olson KC. 2006. Terpenes and carbohydrate source influence rumen fermentation, digestibility, intake, and preference in sheep. Journal of Animal Science 84:2463-2473.

Weir WC, Torell DT. 1959. Selective grazing by sheep as shown by a comparison of the chemical composition of range and pasture forage obtained by hand clipping and that collected by esophageal fistulated sheep. Journal of Animal Science 18:641-649.

Submitted: 12/15/2013
Revised: 1/9/2014
Accepted: 1/13/2014