

Research Note

Consumption of Low Larkspur (*Delphinium nuttallianum*) by Grazing Sheep

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Abstract

Low larkspur (*Delphinium nuttallianum* Pritz.) poisoning causes serious economic loss to livestock producers that graze cattle on foothill and mountain ranges in western North America. In general, all *Delphinium* spp. are five times less toxic to sheep than to cattle. Because low larkspurs are less toxic to sheep than cattle, grazing sheep before cattle on rangelands with dense populations of low larkspur can reduce larkspur density and risk of poisoning to grazing cattle. All previous published work on sheep and larkspur interactions has involved tall larkspurs. This series of studies was conducted to determine if sheep would consume sufficient low larkspur to reduce subsequent risk to cattle. Four summer trials were conducted in Collbran, Colorado, and Soda Springs, Idaho on pastures with dense (> 9 plants \cdot m⁻²) low larkspur populations. In all trials, sheep ate very little low larkspur ($< 0.5\%$ of bites). During one final trial using high sheep density (two sheep \cdot 0.015 ha⁻¹ for 9 d), sheep consumed little low larkspur, but animals appeared to trample much of the low larkspur. Toxic alkaloid concentrations in low larkspur ranged from 1.1 mg \cdot g⁻¹ to 1.6 mg \cdot g⁻¹ in all trials. The use of sheep to graze low larkspurs to reduce subsequent consumption by grazing cattle does not appear to be a viable option.

Resumen

La toxicosis ocasionada por el *Delphinium* de porte bajo (*Delphinium nuttallianum* Pritz.) causa serias pérdidas económicas a productores ganaderos cuyos rodeos pastorean en pastizales de piedemonte y montaña del oeste de los Estados Unidos. En general, las especies de *Delphinium* son cinco veces menos tóxicas para ovinos que para bovinos. Dado que *Delphinium nuttallianum* es menos toxica para ovinos que para bovinos, el pastoreo con ovinos previo al pastoreo de bovinos en pastizales con poblaciones densas de esta especie podría reducir el riesgo de toxicosis en bovinos en pastoreo. Todos los trabajos previamente publicados sobre las interacciones entre ovinos y *Delphinium* han involucrado a las especies de porte alto de este género. Esta serie de estudios se condujo para determinar si los ovinos podrían consumir suficiente cantidad de *Delphinium nuttallianum* como para reducir el riesgo de toxicosis subsecuente en bovinos. Se realizaron cuatro ensayos durante el verano en Collbran, Colorado, y Soda Springs, Idaho en potreros con poblaciones densas (> 9 plantas \cdot m⁻²) de *Delphinium nuttallianum*. En todos los ensayos los ovinos consumieron cantidades muy pequeñas de *Delphinium nuttallianum* ($< 0.5\%$ de bocados). Durante un ensayo final, altas densidades de ovinos (dos ovejas \cdot 0.015 ha⁻¹ por 9 días), consumieron poco *Delphinium nuttallianum*, pero los animales aparentemente pisotearon la mayor parte de las plantas de esta especie. La concentración de alcaloides tóxicos en *Delphinium nuttallianum* osciló entre 1.1 mg \cdot g⁻¹ y 1.6 mg \cdot g⁻¹ en todos los ensayos. El uso de ovinos para consumir *Delphinium nuttallianum* y reducir el riesgo de toxicosis subsecuente en bovinos no aparenta ser una alternativa viable.

Key Words: alkaloids, diet selection, larkspur, poisonous plants, sheep

INTRODUCTION

Low larkspur (*Delphinium nuttallianum* Pritz.) poisoning causes serious economic loss to livestock producers grazing cattle in the western United States and Canada (Pfister et al. 1999; Pfister et al. 2003). Cattle death losses to larkspur are estimated to be 3% to 15% annually in areas where larkspurs are abundant (Nielsen et al. 1994). Low larkspurs contain a number of toxic diterpenoid alkaloids, including the primary toxic alkaloid, methyllycaconitine (MLA). The risk of poisoning is related to the concentration of toxic alkaloids (Gardner and Pfister 2007), and the amount and rate of consumption by cattle (Pfister and Gardner 1999).

The primary result of larkspur intoxication is neuromuscular paralysis. Larkspur alkaloids reversibly inhibit action potential generation though the blockade of postsynaptic nicotinic acetylcholine receptors (nAChRs) in skeletal muscle (Dobelis et al. 1999). Larkspurs are five times less toxic in sheep compared to cattle (Olsen 1978). The i.v. dose of purified MLA that provokes clinical signs is 2 mg \cdot kg⁻¹ body weight in calves vs. 10 mg \cdot kg⁻¹ in sheep given a single injection (K. E. Panter, unpublished data, 1998). Sheep might be less susceptible to larkspur because the binding affinity of alkaloids to nAChRs is lower in sheep than in cattle (Stegelmeier et al. 1998).

Because low larkspurs are less toxic to sheep than cattle, grazing sheep before cattle on rangelands with dense populations of low larkspur can reduce larkspur density and risk of poisoning to grazing cattle. Marsh et al. (1934) remarked that sheep sometimes eat low larkspur (*Delphinium nelsonii* Greene, now *D. nuttallianum*) but no experiments were

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conducted. Results from grazing sheep ahead of cattle to reduce larkspur losses have been highly variable, and all experiments have been done with tall larkspurs (*Delphinium barbeyi* [Ralphs et al. 1991], *Delphinium occidentale* [Ralphs et al. 1991; Ralphs and Olsen 1992], *Delphinium glaucescens* [Ralphs et al. 1991]). In some studies sheep have grazed tall larkspur and reduced risk to cattle (Aldous 1917; Ralphs and Olsen 1992), whereas in other trials sheep have not eaten tall larkspur (Aldous 1917; Ralphs et al. 1991). Cattle primarily eat low larkspur after flowering (Pfister and Gardner 1999). Thus, the objective of this study was to determine if sheep would eat low larkspur (*D. nuttallianum*), particularly flowers, in sufficient quantities to reduce subsequent risk of grazing by cattle.

MATERIALS AND METHODS

Animals and Management

The sheep used in these studies were one-year-old crossbred white-faced ewes raised at the US Sheep Experiment Station near Dubois, Idaho. These animals likely encountered low larkspur (*D. nuttallianum*) as lambs grazing on mountain rangelands near Dubois, Idaho. In all studies, sheep were introduced into experimental pastures when low larkspur was in full flower. Bite counts (% of bites) were used in all studies to document diet selection. Beginning at 0700 every day, individual animals were focally sampled (Altmann 1974) in a predetermined random order. Each animal was observed in turn for 5 min. Bites were categorized as grasses, other forbs, shrubs, and low larkspur. In Trials 2 to 4, bites of lupine (*Lupinus* spp.) also were recorded. After observing all animals, the process was repeated during all active grazing periods until about 1900 (dusk), when sheep were placed in a corral for the night. Typically each animal was observed for 30–40 min each day. Water and trace mineral salt were freely available. All procedures were approved by the Institutional Animal Care and Use Committee at Utah State University, Logan.

Trial 1: Collbran, Colorado (2001)

The objective of this trial was to measure larkspur intake by sheep in pastures with high and low densities of low larkspur. The study was located at lat 39°14.355'N, long 107°37.077'W at 2548-m elevation near Collbran, Colorado. This location was a montane meadow dominated by white sagebrush (*Artemisia ludoviciana* Nutt. var. *ludoviciana*); other major species included mountain brome (*Bromus marginatus* Nees ex Steud.), orchard grass (*Dactylis glomerata* L.), sticky geranium (*Geranium viscosissimum* Fisch. & Mey), and Hood's sedge (*Carex hoodii* Boott). Eight sheep were randomly assigned in pairs to four 450-m² paddocks. Low larkspur density was determined by counting plants in 300 0.5-m² plot frames placed every other meter along three to six transects across the study pastures. Two paddocks had a high density of low larkspur plants (10.1 plants · m⁻²) and two pastures had a low density of low larkspur plants (0.8 plants · m⁻²). Paddocks were contiguous, and specifically chosen and delineated with electric fence based on larkspur density. Vegetation composition except for low larkspur was similar in all paddocks.

Sheep grazed in the paddocks for 9 d (30 May 2001 to 7 June 2001).

Trials 2 and 3: Soda Springs, Idaho (2004 and 2005)

The objective of these two trials was to determine the influence of low larkspur phenology on consumption by sheep. The study site (lat 42°51'10.2"N, long 111°19'2.1"W; elevation 1962 m) was a mix of wet and dry meadow, and the dominant vegetation was low larkspur, Wyoming sagebrush (*Artemisia tridentata* Nutt. var. *wyomingensis* [Beetle & Young] Welsh), silvery lupine (*Lupinus argenteus* Pursh), dandelion (*Taraxacum officinale* Weber ex Wiggers), sedge (*Carex scopulorum* T. Holm), Kentucky bluegrass (*Poa pratensis* L.), bulbous bluegrass (*Poa bulbosa* L.), camas (*Camassia quamash* [Pursh] Greene), and sheep fescue (*Festuca ovina* L.).

In 2004, 14 sheep grazed on a 2-ha pasture with a high density (9.0 plants · m⁻²) of low larkspur for 21 d from 3–9 June (vegetative stage) and 10–23 June (flower stage). In 2005, 12 sheep grazed on an adjacent 2-ha pasture for 21 d (17–28 June, flower stage; 29 June–7 July, pod stage); larkspur density was 9.3 plants · m⁻².

Trial 4: Soda Springs, Idaho (2006)

The objective of this trial was to determine if animal density influenced sheep consumption of low larkspur. This site was adjacent to the location of the previous two trials. Six contiguous paddocks with abundant low larkspur (average density 9.7 plants · m⁻²) were delineated using electric fence. Three of six paddocks were 150 m² and the other three paddocks were 600 m². All paddocks were grazed by two sheep from 6–14 June.

Alkaloid Concentration of Larkspur

Ten randomly selected low larkspur plants were collected at the beginning of each trial in each paddock to determine toxic alkaloid concentrations. Plants were dried for 24 h at 40°C in a forced-air oven, and ground through a 1-mm screen in a Wiley mill. A single composite sample representing the alkaloid concentration at the beginning of each trial was made using the ground plant material. Larkspur samples were prepared for analysis by extracting 100 mg with 4 mL methanol for 18 h. A known amount of calibration standard (reserpine, 100 µg) was added and the samples mixed for 5 min followed by rotation in a centrifuge. An aliquot (50 µL) was then added to 1 mL of methanol · 20 mM⁻¹ ammonium acetate (50:50) in an auto-sampler vial. Toxic alkaloids were quantified using flow injection electrospray ionization mass spectroscopy (Gardner et al. 1999).

Forage Quantity

Forage availability was measured during each study by clipping to ground level all nonwoody plant material in 30 0.5-m² plots randomly placed throughout each pasture; this material was separated into grasses, other forbs, and low larkspur, dried at 40°C for 48 h, and weighed. Lupine was not present in Colorado, but was a major species in Idaho, and thus was clipped separately because of its large biomass, and also because it is potentially toxic to sheep.

Table 1. Forage availability ($\text{kg} \cdot \text{ha}^{-1} \pm \text{SE}$) during low larkspur grazing studies during summers, 2001, 2004, 2005, and 2006.

Year/Date	Grass	Other forbs ¹	Lupine	Low larkspur
2001				
30 May	321 ± 50	511 ± 24	—	45 ± 5
7 June	315 ± 35	485 ± 45	—	32 ± 7
2004				
2 June	169 ± 60	313 ± 19	449 ± 81	33 ± 6
22 June	164 ± 26	356 ± 52	564 ± 216	4 ± 0
2005				
17 June	349 ± 42	487 ± 50	149 ± 38	32 ± 7
23 June	219 ± 20	581 ± 42	348 ± 72	15 ± 3
30 June	240 ± 21	401 ± 40	238 ± 42	0 ± 0
2006				
Large (low animal density) pastures ²				
6 June	338 ± 63	737 ± 86	48 ± 36	19 ± 7
10 June	561 ± 82	779 ± 118	115 ± 60	18 ± 5
14 June	720 ± 77	914 ± 73	146 ± 85	13 ± 2
Small (high animal density) pastures ²				
6 June	329 ± 71	540 ± 126	32 ± 19	27 ± 13
10 June	115 ± 15	197 ± 43	48 ± 12	10 ± 3
14 June	21 ± 5	32 ± 5	38 ± 10	2 ± 1

¹All other forbs except for low larkspur and lupine species. Shrubs were not included in forage availability measurements.

²Means for three "large" pastures of 600 m² each, and means for three "small" pastures of 150 m² each; all pastures held two sheep and were grazed for 9 d. The decline in low larkspur biomass was due to trampling and senescence, not consumption.

Statistical Analysis

The primary dependent variable in all trials was percent of total daily bites composed of low larkspur. The repeated measures model for Trial 1 included larkspur density (high vs. low), pasture (i.e., replication), day, and interactions. Trials 2 and 3 were combined and analyzed using a model that included phenology as the treatment effect and years as replications. The repeated measures model for Trial 4 included animal density (high vs. low), pasture (i.e., replication), day, and interactions. In all cases, animals were a random factor in the mixed linear model analysis using the procedures of SAS (SAS Institute Inc., Cary, NC; Version 9.1 for Windows).

RESULTS

Trial 1: Collbran, Colorado (2001)

Sheep ate very small amounts of low larkspur (0.4% of bites as low larkspur); other forbs and grasses comprised 64% and 35% of sheep bites, respectively. There was no effect ($P > 0.8$) of low larkspur density on sheep bites of low larkspur (data not shown). The toxic alkaloid concentration in low larkspur was $1.5 \text{ mg} \cdot \text{g}^{-1}$ (dry weight). Forage availability for all trials and locations is given in Table 1.

Trials 2 and 3: Soda Springs, Idaho (2004 and 2005)

There was no effect ($P > 0.6$) of plant phenology on low larkspur consumption by sheep (data not shown), because sheep ate almost no larkspur during either year. During summer 2004, sheep ate primarily grass (58% of total bites), other forbs (42% of bites), and a very small amount of low larkspur (0.1% of total bites). During 2005, sheep primarily ate other forbs (83% of bites), grass (17%), and only a few bites of low larkspur. Toxic alkaloid concentration in low larkspur was $1.6 \text{ mg} \cdot \text{g}^{-1}$ and $1.1 \text{ mg} \cdot \text{g}^{-1}$ in 2004 and 2005, respectively.

Trial 4: Soda Springs (2006)

There was no effect ($P > 0.8$) of animal density on low larkspur consumption, because sheep ate essentially no larkspur in either treatment. Sheep diets were primarily grasses (80% of bites) and other forbs (20%). Toxic alkaloid concentration in low larkspur was $1.4 \text{ mg} \cdot \text{g}^{-1}$.

DISCUSSION

There are no documented losses of sheep to tall or low larkspur; thus there has been continual interest for many years by livestock producers with persistent cattle losses in using sheep to reduce risk for subsequent cattle grazing in larkspur-dominated areas. Marsh et al. (1934) reported anecdotally that sheep eat low larkspur in quantities sufficient to reduce risk to cattle, but did no research on the subject. Ralphs et al. (1991) found that sheep consumption of three different tall larkspur species (*D. barbeyi*, *D. occidentale*, and *D. glaucescens*) was highly variable; further, the most reliable use of sheep was the manipulation of herds to trample dense tall larkspur patches. Ralphs and Olsen (1992) reported variable results with sheep eating substantial tall larkspur during one year, but little the next year. Ralphs (2005) found that sheep could be conditioned to graze tall larkspur, but the amount consumed and the timing of consumption was not sufficient to prevent potential cattle poisoning.

In these trials, sheep ate little or no low larkspur, indicating that sheep are not a viable management tool to reduce subsequent risk to cattle grazing on rangelands with dense populations of low larkspur. Our interest centered primarily on documenting sheep consumption of low larkspur during the flower stage of growth for two related reasons. First, cattle primarily eat low larkspur during or after flowering (Pfister and Gardner 1999), and consumption of flowering plants, or even flowers alone, by sheep would reduce toxic biomass available to cattle. Second, defoliation of flowers by grazing wounds the low larkspur plants, and wounded plants probably have an increase in ethylene production, which is reported to accelerate senescence (Kuroda et al. 2003). Alkaloid concentrations in tall larkspurs decline with senescence (Ralphs et al. 2000). Therefore, removal of low larkspur flowers by sheep might accelerate senescence, and possibly reduce risk for subsequent cattle grazing. Because of the lack of consumption by sheep we did not conduct manual defoliation studies to test this hypothesis. When cattle consume low larkspur at any phenological stage, they typically consume each individual plant in a single bite, often breaking off the plant at ground level.

The alkaloid concentration of larkspur greatly influences acceptability to sheep. We found previously that sheep

consumption of tall larkspur was negatively influenced by higher total and toxic alkaloid concentrations (Pfister et al. 1996). During these low larkspur grazing trials, alkaloid concentrations in low larkspur were similar over time and across sites, ranging from $1.1 \text{ mg} \cdot \text{g}^{-1}$ to $1.6 \text{ mg} \cdot \text{g}^{-1}$. Because these alkaloid concentrations are relatively low, these studies provide no evidence that low larkspur alkaloids negatively influence consumption by sheep. At times, however, low larkspurs might have very high alkaloid concentrations (e.g., $> 8 \text{ mg} \cdot \text{g}^{-1}$, Pfister et al. 2003). In Canada, Majak (1993) reported high concentrations of MLA (up to $8.7 \text{ mg} \cdot \text{g}^{-1}$) in vegetative low larkspur (*D. nuttallianum*), declining to $2 \text{ mg} \cdot \text{g}^{-1}$ in flowering plants. We speculate that high alkaloid concentrations would largely deter sheep consumption of low larkspur (Pfister et al. 1996).

Sheep might be deterred from eating some range plants because of conditioned aversions (Burritt and Provenza 2000). However, it is unlikely that any type of conditioned aversion from previous exposure to larkspur as lambs influenced the results of this study. Sheep are unaffected by larkspur when fed or dosed with larkspur plant material, even at high doses ($> 50 \text{ g} \cdot \text{kg}^{-1}$ body weight per day for 7 consecutive days; Marsh et al. 1916). Sheep show typical symptoms of toxicity from i.v. injections of purified larkspur alkaloids, but only at high doses (≥ 5 times) relative to cattle (K. E. Panter, unpublished data, 1998).

Low larkspur phytomass declined in each pasture, not from consumption by sheep, but from senescence with maturity, and from sheep trampling on the dense plants. Low larkspurs are small, hollow-stemmed plants often growing in dense patches, and our observations indicate that injury from trampling leads to rapid desiccation.

Not only did sheep eat minute quantities of low larkspur in the flower or pod stages of growth, but greatly increasing animal density did not influence consumption of low larkspur by sheep. The primary result of increased sheep density appeared to be trampling of low larkspur on the small pastures in comparison to the larger pastures. Based on this visual observation, it might be possible to reduce cattle risk by grazing sheep ahead of cattle and concentrating bands of sheep on dense patches of low larkspur, forcing sheep to trample the plant, similar to recommendations made for tall larkspur management (Ralphs et al. 1991).

IMPLICATIONS

Under free-grazing conditions, sheep ate very little low larkspur during four grazing trials. The use of sheep to graze low larkspurs to reduce subsequent consumption by grazing cattle does not appear to be a viable option. Our observations suggest that sheep can be used to trample dense low larkspur populations.

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LITERATURE CITED

- ALDOUS, A. E. 1917. Eradicating tall larkspur on cattle ranges in the national forests. USDA Farmers Bulletin 826. 23 p.
- ALTMANN, J. 1974. Observational study of behavior: sampling methods. *Behaviour* 49:227–267.
- BURRITT, E. A., AND F. D. PROVENZA. 2000. Role of toxins in intake of varied diets by sheep. *Journal of Chemical Ecology* 26:1991–2005.
- DOBELIS, P., J. E. MADL, J. A. PFISTER, G. D. MANNERS, AND J. P. WALROND. 1999. Effects of *Delphinium* alkaloids on neuromuscular transmission. *Pharmacology and Experimental Therapeutics* 291:538–546.
- GARDNER, D. R., K. E. PANTER, J. A. PFISTER, AND A. P. KNIGHT. 1999. Analysis of toxic norditerpenoid alkaloids in *Delphinium* species by electrospray, atmospheric pressure chemical ionization, and sequential tandem mass spectrometry. *Journal of Agriculture and Food Chemistry* 47:5049–5058.
- GARDNER, D. R., AND J. A. PFISTER. 2007. Toxic alkaloid concentrations in low and plains larkspurs (*Delphinium* spp.). *Rangeland Ecology and Management* 60:441–446.
- KURODA, S., M. HAKATA, Y. HIROSE, M. SHIRAIISHI, AND S. ABE. 2003. Ethylene production and enhanced transcription of an ethylene receptor gene, ERS1, in *Delphinium* during abscission of florets. *Plant Physiology and Biochemistry* 41:812–820.
- MAJAK, W. 1993. Alkaloid levels in a species of low larkspur and their stability in rumen fluid. *Journal of Range Management* 46:100–103.
- MARSH, C. D., A. B. CLAWSON, AND H. MARSH. 1916. Larkspur poisoning of livestock. USDA Bureau of Plant Industry Bulletin 365. 91 p.
- MARSH, C. D., A. B. CLAWSON, AND H. MARSH. 1934. Larkspur or poison weed. USDA Farmers Bulletin 988. 12 p.
- NIELSEN, D. B., M. H. RALPHS, J. O. EVANS, AND C. A. CALL. 1994. Economic feasibility of controlling tall larkspur on rangelands. *Journal of Range Management* 47:369–372.
- OLSEN, J. D. 1978. Tall larkspur poisoning in cattle and sheep. *Journal of the American Veterinary Medical Association* 173:762.
- PFISTER, J. A., AND D. R. GARDNER. 1999. Consumption of low larkspur (*Delphinium nuttallianum*) by cattle. *Journal of Range Management* 52:378–383.
- PFISTER, J. A., D. R. GARDNER, K. E. PANTER, G. D. MANNERS, M. H. RALPHS, B. L. STEGELMEIER, AND T. K. SCHOCH. 1999. Larkspur (*Delphinium* spp.) poisoning in livestock. *Journal of Natural Toxins* 8:81–94.
- PFISTER, J. A., D. R. GARDNER, B. L. STEGELMEIER, K. HACKETT, AND G. SECRIST. 2003. Catastrophic cattle loss to low larkspur (*Delphinium nuttallianum*) in Idaho. *Veterinary and Human Toxicology* 45:137–139.
- PFISTER, J. A., G. D. MANNERS, D. R. GARDNER, K. W. PRICE, AND M. H. RALPHS. 1996. Influence of alkaloid concentration on acceptability of tall larkspur (*Delphinium* spp.) to cattle and sheep. *Journal of Chemical Ecology* 22:1147–1168.
- RALPHS, M. H. 2005. Conditioning sheep to graze duncceap larkspur (*Delphinium occidentale*). *Rangeland Ecology and Management* 58:628–631.
- RALPHS, M. H., J. E. BOWNS, AND G. D. MANNERS. 1991. Utilization of larkspur by sheep. *Journal of Range Management* 44:619–622.
- RALPHS, M. H., D. R. GARDNER, AND J. A. PFISTER. 2000. Functional explanation for patterns of norditerpenoid alkaloid levels in tall larkspur (*Delphinium barbeyi*). *Journal of Chemical Ecology* 26:1595–1606.
- RALPHS, M. H., AND J. D. OLSEN. 1992. Prior grazing by sheep reduces waxy larkspur consumption by cattle: an observation. *Journal of Range Management* 45:136–139.
- STEGELMEIER, B. L., K. E. PANTER, J. A. PFISTER, L. F. JAMES, G. D. MANNERS, D. R. GARDNER, AND M. H. RALPHS. 1998. Experimental modification of larkspur (*Delphinium* spp.) toxicity. In: T. Garland and A. C. Barr [eds.]. Toxic plants and other natural toxicants. Wallingford, Oxon, United Kingdom: CABI Publishing. p. 205–210.