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Toxicology

Lupine-induced crooked calf syndrome: mitigation through intermittent grazing management of cattle

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Abstract

Lupines are responsible for a condition in cattle referred to as "crooked calf syndrome" (CCS) that occurs when pregnant cattle graze teratogenic lupines. A proposed management strategy to limit these types of birth defects includes utilizing an intermittent grazing schedule to allow short durations of grazing lupine-infested areas interrupted by movement to a lupine-free pasture. The objective of this study was to determine if an intermittent schedule of ten continuous days of lupine treatment followed by 5 d off treatment would be sufficient to decrease, or prevent, the incidence of lupine-induced malformations. Continuous dosing of the teratogenic lupine (*Lupinus leucophyllus*) to pregnant cows for 30 d during the most susceptible stage of pregnancy (gestation days 40 to 70) resulted in severe skeletal birth defects in their calves. However, intermittent dosing of the teratogenic lupine demonstrated that interrupted intake of lupine reduced the severity, or eliminated, permanent skeletal malformations in calves born to cows dosed lupine. Toxicokinetic and ultrasound data demonstrated a clear inverse correlation between serum anagyrine (the primary teratogenic alkaloid in some lupines) concentrations in the dam and fetal movement. In the intermittent group, fetal movement quickly returned to normal after lupine feeding stopped and remained normal until lupine treatment resumed. Therefore, interrupting lupine intake for at least 5 d through an intermittent grazing program could reduce the severity of the CCS. Furthermore, this method would allow ranchers to move cattle back into lupine pastures after a brief interruption, which would allow for more efficient utilization of forage resources.

Lay Summary

Lupines are responsible for a condition in cattle referred to as "crooked calf syndrome" (CCS) that occurs when pregnant cattle graze teratogenic lupines. A proposed management strategy to limit these types of birth defects includes utilizing an intermittent grazing schedule to allow short durations of grazing lupine-infested areas interrupted by movement to a lupine-free pasture. The objective of this study was to determine if an intermittent schedule of ten continuous days on lupine treatment followed by 5 d off treatment would be sufficient to decrease, or prevent, the incidence of lupine-induced malformations. The data reported in this study demonstrate that interrupting lupine intake for at least 5 d through an intermittent grazing program could reduce the severity of the CCS. Furthermore, this method would allow ranchers to move cattle back into lupine pastures after a brief interruption, which would allow for more efficient utilization of forage resources.

Key words: anagyrine, cattle, crooked calf syndrome, intermittent grazing, lupine, teratogen

Introduction

Lupines are forbs found in a variety of habitats at all elevations, from lowland deserts to alpine crests (Knight and Walter, 2001). While lupines are responsible for large losses to cattle producers, lupines do have forage value as they are high in protein, and as wild legumes, they are good soil stabilizers and enhance nitrogen availability in the soils for the other grasses and forbs (Burrows and Tyrl, 2001). In some areas, lupines become an important forage source beginning in early to mid-July when the annual grasses and forbs become mature, dry, and are of low nutritional quality (Ralphs et al., 2006). As legumes, lupines are highly nutritious plants with a crude protein content of up to 16% in the vegetative parts of the plant and over 40% in the seeds (Panter et al., 2001). In the channel scablands of central Washington, velvet lupine has been shown to contain 14% crude protein in June, decreasing to 8% to 10% in July, while the other available forages are often less than 6% to 7% in that area during that time of year (Ralphs et al., 2006; Lopez-Ortiz et al., 2007). Thus, lupine in this area is a quality forage for grazing cattle (Ralphs et al., 2006). Consequently, as the availability, or quality, of other forages decreases, grazing livestock will increase their consumption of lupine.

Crooked Calf Syndrome (CCS) is a colloquial term describing a pattern of axial and appendicular skeletal malformations and cleft palate in newborn calves resulting from their mothers grazing certain lupine species during the first trimester of pregnancy (Panter et al., 1999, 2013). Common appendicular skeletal deformities are flexure-type abnormalities characterized by the lateral rotation and bowing of the forelimbs with joint swelling and loss of motion (arthrogryposis), though less severe similar lesions may occasionally involve the hind limbs. Axial skeletal lesions include involvement and spinal column malformations (scoliosis, lordosis, or kyphosis) affecting the cervical, thoracic, lumbar, and sacral vertebrae (Panter et al., 2013). Secondary

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skeletal defects such as rib cage abnormalities, asymmetry of the head, hoof abnormalities, and occasional sacral vertebral defects seen as a kinked tail may also be part of the syndrome (Panter et al., 1999). Cleft palate may occur with or without the accompanying skeletal defects depending on what stage of pregnancy the cows graze lupines (Panter et al., 1990a, 1990b, 1998). These malformations as a whole may also be referred to as multiple congenital contractures.

The skeletal malformations and cleft palate are the result of alkaloid-induced inhibition of fetal movement (Panter et al., 1990a). The incidence and severity of the malformations are dependent on the amount of lupine ingested, which alkaloids are present, and the alkaloid concentration in the plant, how many continuous days lupine is ingested, and the stage of pregnancy when lupine is ingested. The quinolizidine alkaloid anagyrine (Keeler, 1976; Keeler et al., 1976) and some piperidine alkaloids (ammodendrine and N-methyl ammodendrine) cause CCS by reducing fetal movement during this critical period of gestation (Panter et al., 1990a, 1990b).

Field observations, and research results, indicate CCS will not occur with a single exposure or even after a few days of lupine ingestion. Rather a more prolonged continuous exposure is required (Keeler, 1973). Lupine plant material must be ingested daily and for an extended period of time to maintain a relatively constant blood alkaloid concentration before permanent malformations occur (Panter and Keeler, 1992; Panter et al., 1998). Fetal movement has been shown to be inversely related to lupine ingestion and blood alkaloid (anagyrine) concentrations in pregnant cows. Thus, intermittent grazing of lupine may be a management tool to reduce lupine-induced CCS. In this regard, previous research in a goat model demonstrated that intermittent exposure of pregnant goats with teratogenic alkaloids can reduce the malformations observed in the offspring (Welch et al., 2015). Therefore, the objective of this study was to determine if an intermittent schedule of ten continuous days on a lupine treatment, followed by 5 d off treatment, would be sufficient to decrease, or prevent, the incidence of lupine-induced malformations in cattle.

Materials and Methods

All animal procedures were performed under veterinary supervision, and the animal treatment protocol was reviewed and approved by the Utah State University Animal Care and Use Committee under protocol #1481.

Plant

Several collections of *Lupinus leucophyllus* were mixed together to form a composite collection that contained enough plant material for the study. All collections had a similar alkaloid profile, with the profile of the composite collection shown in Figure 1. All collections of plants were collected in the late vegetative phenological stage and allowed to air dry, then ground in a hammer mill to pass through a 2 mm screen, bagged, and stored until use. Almost 60 yr of work with lupine plants at the Poisonous Plant Research Laboratory has shown that the quinolizidine alkaloids in lupines do not degrade in ground plants but remain stable for an extended period of time when stored in cool dry conditions (Cook et al., 2009). Chemical analysis was performed soon after grinding and then again just prior to commencement of the feeding trials to confirm that the alkaloid profiles and concentrations remained the same.

Animals

Fourteen black Angus heifers, 18 to 20 mo old, were naturally mated to a Hereford bull. The last day of standing heat was considered gestation day (GD) 0. The heifers were randomly assigned to one of the three treatment groups as follows; group 1 = continuous lupine ($n = 5$); group 2 = intermittent

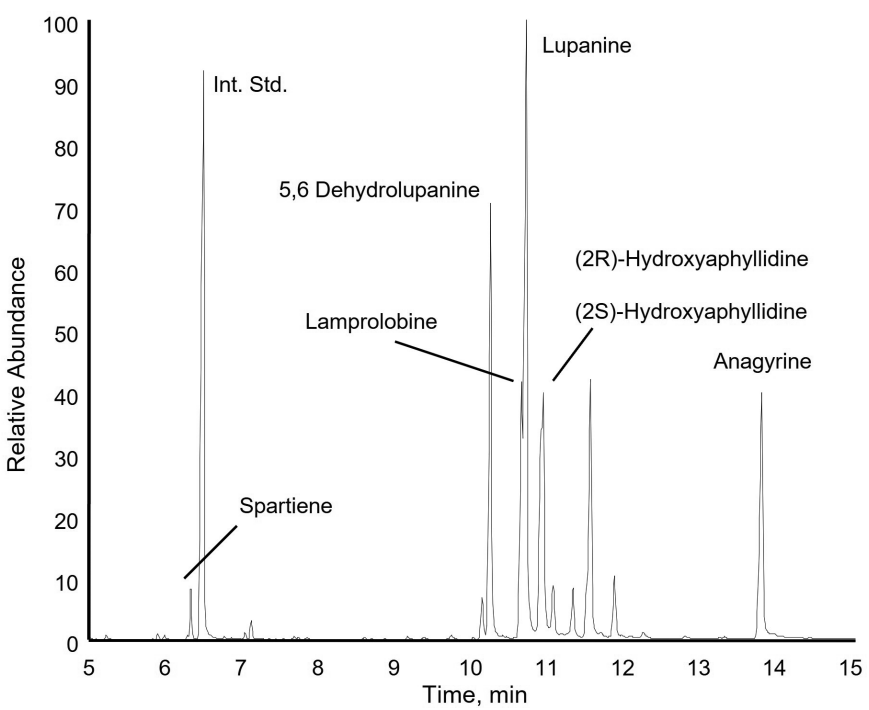

Figure 1. Chromatogram of alkaloids present in the collection of *Lupinus leucophyllus* dosed to cows in this study.

lupine (n = 4) and group 3 = controls (n = 5). All heifers had free access to alfalfa hay, fresh water, and trace mineral salt throughout the treatment period and throughout gestation. The treatment protocols were as follows: group 1, continuous group heifers, were given 1.25 to 1.5 g/kg of ground lupine mixed with approximately 10 liters of tepid tap water to make a slurry. Then using a Frick speculum the slurry was pumped by hand pump directly into the rumen via a plastic stomach tube two times a day at 0700 and 1530 hours for 30 d (GD 40 to 69). Group 2, intermittent group heifers, were similarly treated with 1.25 to 1.5 g/kg ground lupine dosed for 10 days (GD 40 to 49), followed by dosing with 1.25 to 1.5 g/kg ground alfalfa hay for 5 d (GD 50 to 54) followed by ground lupine dosed for 10 d (GD 55 to 64) followed by dosing with ground alfalfa hay for 5 d (GD 65 to 69). Group 3, control group heifers, were dosed with dried ground alfalfa at 1.5 g/kg on the same schedule as the continuous group.

All heifers were observed daily during treatment protocol for any clinical response and for the general health of the animals. At the end of the treatments, all heifers were maintained as a group and had free access to alfalfa hay, fresh water, and trace mineral salt for the remainder of their pregnancies. At the time of parturition, the heifers were observed daily, and when calves were born each calf was weighed and evaluated for overall health as well as any visually observable birth defects.

Blood Sampling Protocol

Blood was collected once a day from all animals via jugular venipuncture to evaluate daily serum alkaloid concentrations from GD 40 to 70. Additionally, blood was collected at 0, 0.25, 0.5, 0.75, 1, 2, 4, and 8 h after the first dose on GD 40 and at 2, 4, 6, 8, 24, 28, 32, 48, 54, and 72 h after the last dose on GD 69 to obtain a kinetic profile of the absorption and elimination of anagyrine, respectively. Two red top 10 mL vacutainer tubes were collected at each time point, allowed to clot for 2 h and centrifuged at 2,300 rpm for 30 min after which serum was collected and stored frozen at –22 °C until analyzed.

Ultrasound Schedule

On GD 40, all 14 heifers were evaluated via ultrasound to confirm pregnancy. Additionally, all heifers were evaluated via ultrasound to monitor fetal movement on GD 50, 60, and 70. The cows in the intermittent group were also evaluated via ultrasound on GD 55. Fetal movement was assessed via transrectal ultrasonography using an Aloka ultrasound with an Aloka 7.5 MHz linear rectal probe (Model SSD 900 V, Aloka Corporation, Wallingford, Connecticut). Visualization and recording of fetal activity were performed each time for a 5-min period. During the evaluation periods, the number of fetal movements per 5 min (movement composed of both flexion and extension of more than 1 appendage or joint) were counted by an experienced observer. Such movements were initiated by the fetus and were easily differentiated from any movements that might be from maternal sources.

Plant Alkaloid Extraction

Plant material was weighed (100 mg) into a 16 mL screw-top glass test tube. Plant material was extracted using a previously reported procedure (Lee et al., 2007b). In brief, the plant material was extracted by mechanical rotation using the Rugged Rotator (Glas Col, LLC) with a mixture of 1 N HCl (4.0 mL) and $CHCl_3$ (4.0 mL) for 15 min. The samples were centrifuged (5 min), and the aqueous layer was removed. An additional 2.0 mL of 1N HCl was added to the test tube containing plant material and $CHCl_3$, extracted again by mechanical rotation (15 min), centrifuged, and the aqueous layer removed. The aqueous portions were combined into a clean 16 mL screw-top glass test tube. The pH of the aqueous layer was adjusted to 9.0 to 9.5 with concentrated NH_4OH. The basic solution was extracted twice with $CHCl_3$, first with 4.0 mL and then with 2.0 mL. The $CHCl_3$ solutions were combined and filtered through anhydrous Na_2SO_4 into a clean 16 mL screw-top glass test tubes and the solvent evaporated under N_2 at 60 °C. The alkaloid fraction extracted 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 GC autosample vials for GC/FID or GC/MS analysis.

GC/FID Analysis

Plant samples were analyzed by GC/flame ionization detector using a Shimadzu GC-2010 gas chromatograph equipped with a Shimadzu AOC-20i autosampler, a J&W DB-5 column (30 m × 0.32 mm, 0.25 µm film thickness) and a flame ionization detector. Samples (1.0 uL) were injected splitless at 250 °C and helium was used as the carrier gas at a constant flow rate of 2.0 mL/min. The column oven was temperature programmed starting at 100 °C for 1 min; increased to 200 °C at 50 °C/min; increased to 260 °C at 5 °C/min; increased to 320 °C at 50 °C/min; and held at 320 °C for 8.8 min for a total run time of 25 min. Anagyrine was quantitated against a five-point standard curve over the range of 25 to 400 µg/mL.

GC/MS Analysis

GC/MS analysis was performed as previously reported (Lee et al., 2007b). In brief, plant samples (2 µL) were analyzed by GC/MS using a Finnigan MAT GCQ equipped with a split/splitless injector and a DB-5MS (30 m × 0.25 mm; J&W Scientific) column. Injection port temperature was 250°C and operated in the splitless mode. Split vent flow rate was 50 mL/min and purged after 0.80 min. Oven temperature was 100 °C for 1 min; 100 to 200 °C at 40 °C/min; 200 to 275 °C at 5 °C/min; and held at 275 °C for 1.5 min. Electron impact ionization (EI) at 70 eV was used with an ion source temperature of 200 °C.

Alkaloid Identification

Sparteine was identified from commercially obtained standards. Anagyrine was identified using an authenticated (MS, NMR) sample from the alkaloid collection of Poisonous Plants Research Laboratory, USDA, ARS, Logan, UT, USA. The remaining alkaloids were determined from correlation of measured retention times to retention indices (RI) calculated by linear extrapolation from RI values generated from known standards and assigned RI numbers from the literature and their CI and EI mass spectra (Wink et al., 1995). Alkaloids were also determined by correlation of measured relative retention times (RR_t) and EI mass spectra to those reported in the literature (Kinghorn and Balandrin, 1984).

Serum Alkaloid Analysis

Sera samples were thawed, thoroughly mixed, and a 50 µL aliquot was transferred to a 1.7 mL polypropylene microcentrifuge tube (ISC Bioexpress, Kaysville, UT USA) containing 450 µL of water. Internal standard solution,

0.5 mL of a 0.01 µg/mL colchicine in acetonitrile, was then added to the microcentrifuge tube, the tube vortexed, and then centrifuged for 10 min. A 250 µL aliquot of the supernatant in the microcentrifuge tube was transferred to an autosample vial containing 750 µL water, the content of the autosample vial was mixed thoroughly prior to HPLC-MS/MS analysis.

Analysis of anagyrine in the samples was accomplished using a Hewlett Packard 1,100 Series HPLC and autosampler system (Palo Alto, CA USA) coupled to a ThermoFinnigan LTQ Velos Pro mass spectrometer (Thermo Finnigan, San Jose, CA USA). Samples (20 µL) were injected with a Hewlett Packard 1,100 Series autosampler onto a Betasil C 8 reversed-phase column (100 × 2.1 mm i.d., 5 µm particle size; Thermo Electron Corporation, Waltham, MA USA) protected by a guard column of the same adsorbent. Anagyrine was eluted from the column with a gradient (0.250 mL/min) consisting of 20 mM ammonium acetate in water (solvent A) and methanol (solvent B). Initial mobile phase conditions were 93:7 (A:B). From 0 to 8 min, the mobile phase composition was changed from 93:7 (A:B) to 75:25 (A:B) using a linear gradient. From 8 to 16 min the mobile phase composition was again changed from 75:25 (A:B) to 0:100 (A:B) using a linear gradient. The mobile phase was then held constant at 0:100 (A:B) for the next 3 min for a total run time of 19 min. The HPLC system was re-equilibrated for 13 min at the initial mobile phase composition of 93:7 (A:B) prior to the next injection. The flow from the HPLC column was connected directly to the heated electrospray ionization (ESI) source of the mass spectrometer with capillary temperature of 275 °C and source temperature of 300 °C. Under these mobile phase conditions, anagyrine was eluted at 15.2 min and colchicine at 17.4 min. Full scan mass data were collected for a mass range of 65 to 300 amu from 1 to 16.5 min and then 110 to 500 amu from 16.5 to 22 min. MS/MS product ion spectra were collected after isolation of a selected precursor ion (±1 amu) and the relative collision energy was manually adjusted to observe significant fragmentation of the selected ion. Anagyrine was used as a standard to quantify anagyrine while colchicine was used as the internal standard.

Anagyrine was prepared in water:internal standard solution (87.5:12.5) to give a five-point standard curve over the range of 7.81 ng/mL—125 ng/mL by serial dilution. Peak areas of anagyrine and colchicine were determined from reconstructed ion chromatograms of the respective fragment ions (anagyrine: m/z = 98, 148; colchicine: 341, 358, and 382).

Statistics Analyses

Statistical comparisons of serum alkaloid profiles were performed using ANOVA with a Bonferroni posthoc test of significance between individual groups using SigmaPlot for Windows (version 14.0, SPSS Inc., Richmond, CA). Correlations were determined with a Pearson Product Moment Correlation analysis using SigmaPlot for Windows (version 14.0, SPSS Inc.). Differences were considered statistically significant at $P < 0.05$.

Results and Discussion

Serum anagyrine concentrations were determined daily during the 30-d dosing regimen. The concentration of anagyrine remained fairly constant throughout the 30 d in the animals in the continuous group, whereas in the animals dosed intermittently the concentration of anagyrine quickly decreased to zero while not being dosed (Figure 2). There was a significant difference between the continuous and intermittent groups ($P < 0.001$) with significant differences on days 51 to 55 and 66 to 69, which corresponded to the days the intermittent group was not dosed. A more extensive kinetic analysis was performed for 8 h after the first dose (uptake) and 72 h after the last dose (elimination; Figure 3). There were no differences in the uptake or elimination of anagyrine between the animals in the continuous and intermittent groups

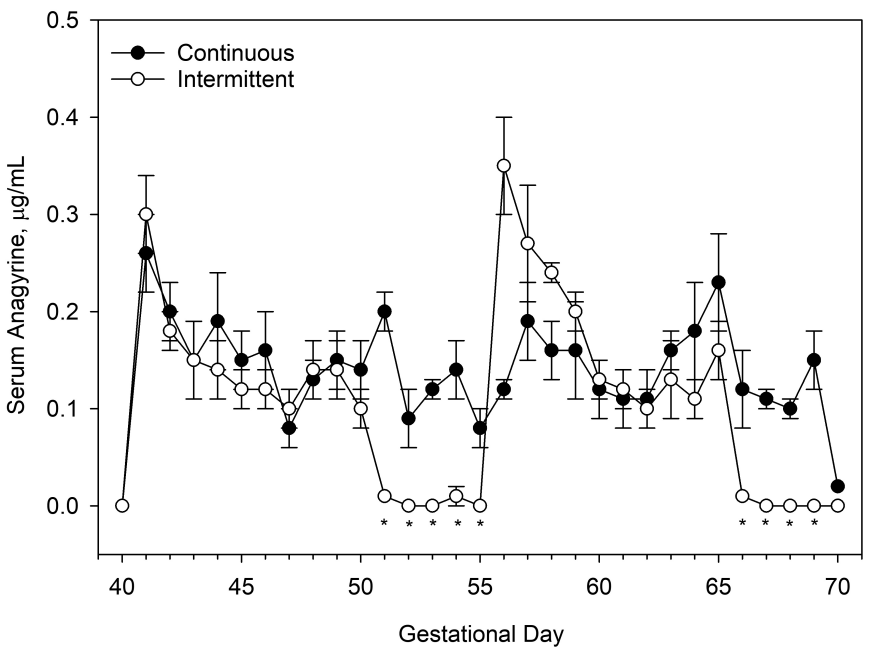

Figure 2. Comparison of the serum anagyrine concentrations during the 30-d dosing regimen between the animals continuously dosed lupine vs. the cows intermittently dosed lupine. * Denotes a significant difference ($P < 0.05$) between the two groups on that day.

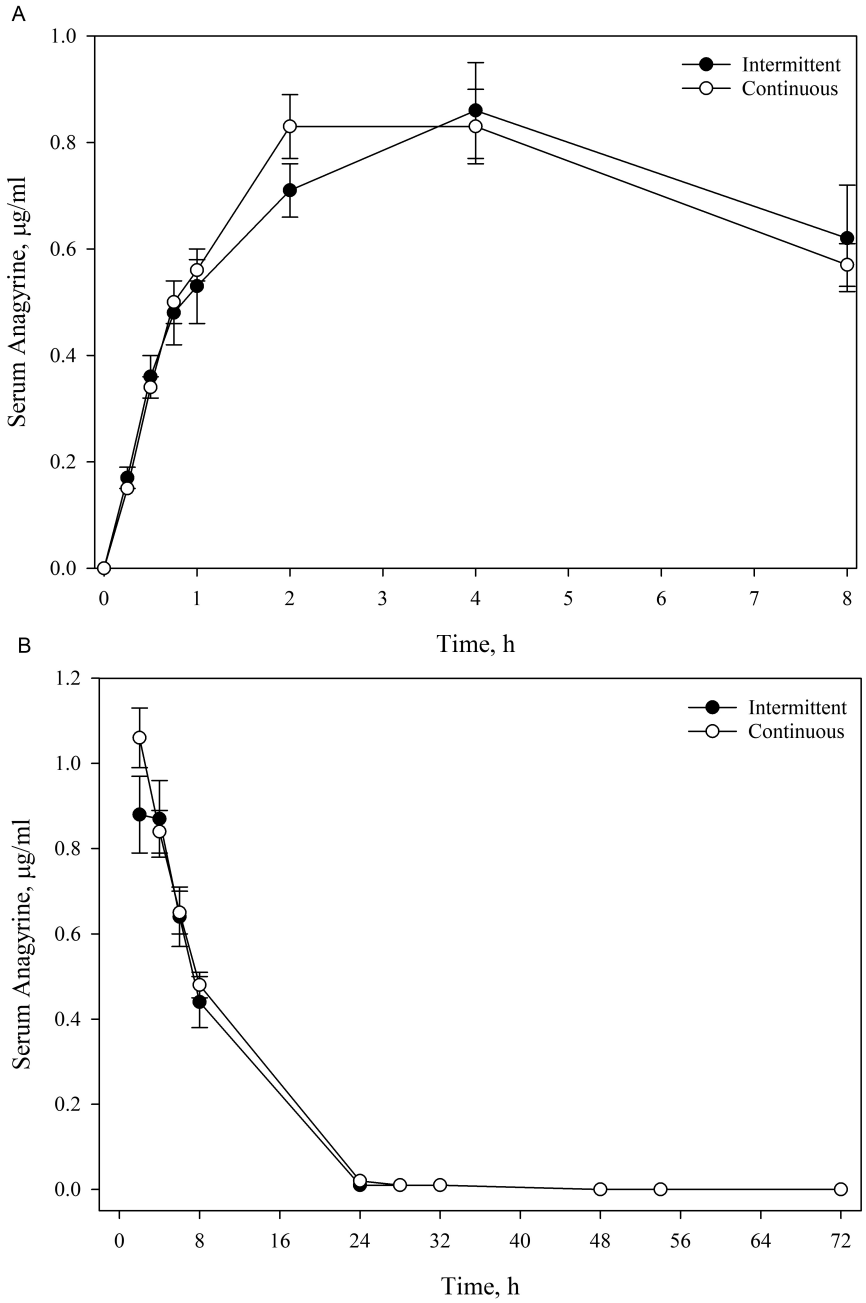

Figure 3. Comparison of the **s**erum anagyrine concentrations over an 8-h period after the first dose of lupine (A) and over a 72 h period after the last dose of lupine (B) of a 30-d dosing regimen between continuously dosed vs. intermittently dosed cows.

($P = 0.907$ first dose and $P = 0.355$ last dose, respectively). These data demonstrate that there was no difference in the concentration of anagyrine in the serum of cattle in the continuous group versus the intermediate group, except when the cattle in the intermittent group were off treatment. The 5 d that the cattle in the intermittent group were off treatment resulted in a rapid decrease in serum anagyrine concentration to baseline concentrations.

The decrease in serum anagyrine concentrations in the intermittent group proved to be important as the concentration of anagyrine in serum has been shown to be inversely correlated with fetal movement (Green et al., 2015a, 2015b; Welch et al., 2015). Fetal movement was observed and quantitated via ultrasound imaging several times throughout the 30-d dosing regimen (Figure 4). On GD 50, after 10 d of lupine exposure for both the continuous and intermittent groups, fetal movement was found to be significantly decreased in both groups ($P < 0.001$). Fetal movement was assessed in the intermittent group on GD 55, after 5 d without lupine exposure. Fetal movement in all of the animals in the intermittent group had returned to normal on GD 55 (Figure 4). Fetal movement was assessed again in all the groups on GD 60, in which, the intermittent group had been back on lupine for 5 d. Fetal movement in the animals in the continuous and intermittent groups were significantly decreased compared to the controls, while the movement in the control group animals was unchanged. An important note was that there was no fetal movement detected in several animals in the continuous group at both

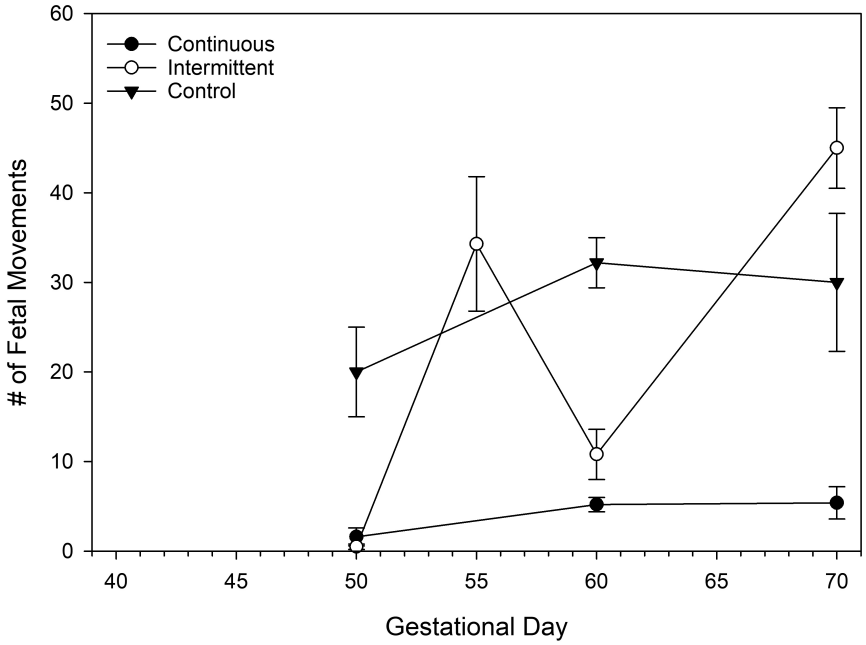

Figure 4. Comparison of the fetal movements in the fetuses from the cows continuously dosed with lupine vs. the cows intermittently dosed with lupine. Animals were treated from gestation days (GD) 40 to 70, with fetal movement data collected on GD 50, 55, 60, and 70.

GD 50 and GD 60. Research has shown that the teratogenic alkaloids that bind to nicotinic acetylcholine receptors can cause embryonic death if the dose is high enough (Welch et al., 2014). However, even though the fetuses in these cows were not showing any movement, they were still alive and were born at full term.

On GD 70 fetal movement was assessed again in all of the groups. This corresponded to the day after the last dose for the continuous group and 5 d after the last dose for the intermittent group. There was still decreased fetal movement in the continuous group, with the intermittent group again back to normal movement. This data suggests that fetal movement in the continuously dosed animals was significantly reduced for over 30 d, while fetal movement in the intermittently dosed animals returned to normal during the periods of non-exposure to lupine, which provided the fetus an opportunity to move normally and for the limbs to develop normally.

All of the calves in the control group were born healthy with no visually observable skeletal defects (Table 1). In contrast, all of the calves in the continuous group were born with visually observable skeletal malformations including severe multiple congenital contractures and severe arthrogryposis. One calf had only moderate limb contractures including contracted knees, which resolved after 4 to 6 wk. This calf was healthy enough to survive, while the other four calves in this group were all euthanized immediately after parturition due to the severity of their malformations (Table 1). One of the calves in the intermittent group was normal and healthy at birth, while the other four calves in this group had minor limb contractures, which all resolved within 2 to 3 wk (Table 1). None of the malformations in the intermittent group were severe enough to compromise the overall health of the calf, thus none were euthanized. Also noteworthy, no difference in number of days to parturition was observed between the groups ($P = 0.112$) nor was there a difference in the body weight of the calves at birth ($P = 0.831$). This data suggests that apart from the skeletal deformities, these calves developed normally.

The most severely deformed calves were all found in the continuous group. A gross evaluation of the most severely deformed calf was as follows. The calf appeared to be fully developed. The hair was clean and with no residual placental material. The cervical vertebrae were deviated to the left with some left axial rotation. The left axial rotation of the spinal column was maintained throughout the thoracic vertebrae. This resulted in rotation and marked deformation of the ribs. The left ribs had a prominent bend about 44° about 3 cm from their vertebral end. The right ribs were straight with less natural curvature. The sternum was prominent and displaced to the left. The lumbar vertebrae had axial rotation to the right bringing the spinal column back past normal with a slight right rotation of the pelvis. All four legs were contracted and would not flex easily due to tendon and muscle tension. The left front leg had a valgus rotation of about 85°. The result of these axial and appendicular skeleton rotations and contractions resulted in the calf maintaining compact position that was difficult to change. The rib malformation conformed to this position. This suggests that there was very little fetal movement for an extended period of time. These results suggest that early malformations may result in continued and sustained alterations in musculoskeletal development. It is likely that the early lack of fetal movement resulted in severe deviations that caused the fetus to return to a set position more frequently even after lupine exposure ended and fetal movement returned.

The data presented in this study demonstrate that there is a strong inverse correlation between serum anagyrine concentration in the animals and the amount of movement in their fetus (Correlation Coefficient = –0.644; Figure 5). While a correlation has been demonstrated, it is not known if there is a threshold for the concentration of anagyrine in the serum of the cow that would result in the loss of fetal movement. Due to the variation in responses of individual animals, and

Table 1. Evaluation of calves at birth for observable skeletal malformations

#	Group	# Days to parturition	Weight, kg	Malformations [*]	Euthanized [†]
1	Control	291	30	Normal	No
2	Control	292	32	Normal	No
3	Control	290	27	Normal	No
4	Control	277	31	Normal	No
5	Control	290	30	Normal	No
6	Continuous	280	26	Severe MCC [‡]	Yes
7	Continuous	280	28	Severe contractures [‖]	Yes
8	Continuous	285	32	Moderate contractures [§]	No
9	Continuous	283	34	Severe MCC [‡]	Yes
10	Continuous	275	30	Severe MCC [‡]	Yes
11	Intermittent	280	33	Minor contractures [¶]	No
12	Intermittent	290	37	Minor contractures [¶]	No
13	Intermittent	284	30	Minor contractures [¶]	No
14	Intermittent	279	25	Normal	No

[*]Calves were visually evaluated for skeletal contracture malformations immediately after birth.
[†]Malformations severe enough to require the calf to be euthanized.
[‡]Severe appendicular and axial contractures and rotations. MCC, multiple congenital contractures.
[‖]Severe appendicular contractures and rotations.
[§]Moderate appendicular contractures and rotations, which resolved after 4 to 6 wk.
[¶]Minor contractions of the limbs and knee joints, which resolved after 2 to 3 wk.

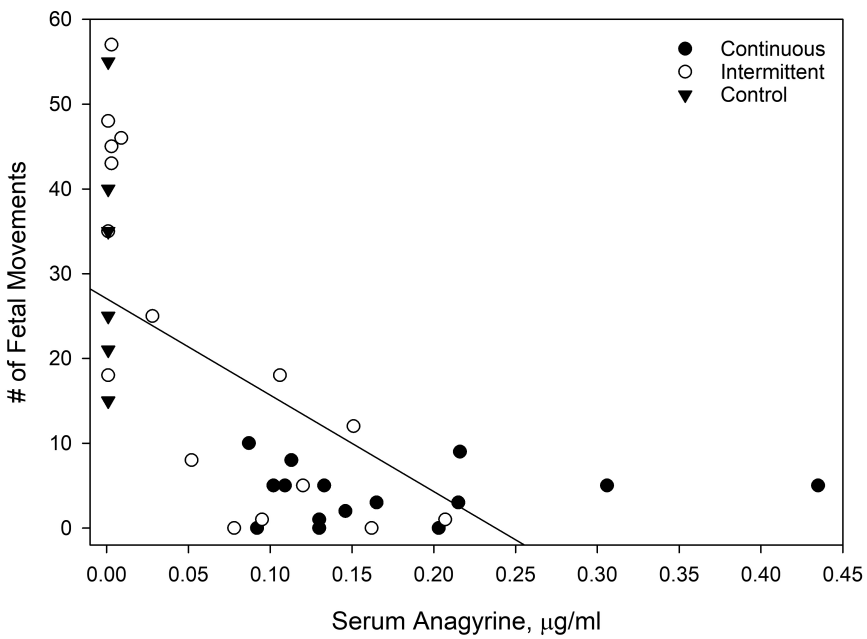

Figure 5. Correlation of serum anagyrine concentration and fetal movement. Serum anagyrine and fetal movement data were collected on gestation days (GD) 50, 55, 60, and 70. Correlation was calculated by Pearson Product Moment Correlation analysis ($P < 0.001$, Correlation Coefficient = −0.644).

between different breeds of cattle, it would likely be very difficult to identify a threshold (Green et al., 2014, 2015a, 2015b, 2024). The data presented in this study supports the hypothesis that anagyrine is a teratogenic alkaloid in some lupines and that anagyrine inhibits fetal movement. This data also supports the theory that extended exposure to lupine results in a protracted loss of movement in the fetus, which results in skeletal malformations. Additionally, this study demonstrates that if the concentration of anagyrine can be reduced, for even a short period of time, to allow normal fetal movement to return, the calf will be born with no, to minor, limb defects. Therefore, the results from this study suggest that an intermittent grazing regimen may be a management tool to help minimize the deleterious results of grazing pregnant cattle in lupine-infested pastures/rangelands.

There are many factors that impact the overall incidence of lupine-induced CCS including periodic large population outbreaks. On many ranches in the northwest part of the United States, a 1% to 5% incidence of CCS is accepted as the cost of "doing business." However, even this level of CCS is economically important and affects the economic sustainability of many producers. Frequently (every 5 to 10 yr), the

incidence exceeds this level and can be catastrophic to many ranches (Lee et al., 2007a). Lupine population cycles and availability of other quality forages are important factors in CCS incidence (Ralphs et al., 2006, 2011). Improving rangeland conditions through restoration efforts using improved grass species or introducing forb species may provide long-term solutions (Stonecipher et al., 2017, 2019, 2021; Spackman et al., 2021). For example, preliminary studies in annual grassland-infested rangelands have demonstrated that improved wheatgrass cultivars and forage Kochia (*Bassia prostrata*) may compete with annual grasses and provide alternative high-quality forages later in the grazing season. Lupine seeds remain viable in the soil for years, or decades, and when optimum weather conditions exist, populations will increase accompanied by an increase in CCS. Weather patterns impact lupine populations and significant emergence of seedlings following a wet winter and spring can be a prelude to a major outbreak of CCS the following season. The nutritional status of cows is also a significant consideration. Cows inadequate to good body condition are less likely to graze lupines and other poisonous plants (Lopez-Ortiz et al., 2004; Lee et al., 2008). While there are other factors involved in CCS, the management suggestions in this paper can be tailored to each region, or individual ranch, and used to reduce the impact of lupine-induced CCS.

Management Guidelines

Reducing losses from lupine-induced CCS requires multiple approaches to be successful. Intermittent grazing is only one method to reduce risk, yet also allows utilization of pastures. A simple rule may be applied: avoid grazing toxic lupines by pregnant cows during the susceptible stages of gestation (GD 40 to 100). Having said this, these rangelands can be better utilized by following a few management guidelines:

(1) Identify potential poisonous plants (in this case, lupines) and determine which specific lupines are present.
(2) Determine the chemical profile of the lupine species and obtain a risk evaluation based on chemical profiles. Measuring alkaloid concentrations early in the growing season (May) will allow for an early prediction of higher incidence of CCS and enable ranchers to strategize alternates to lupine grazing during the summer months.
(3) Evaluate the historical incidence of CCS on the ranch, lupine population cycles, range conditions, and forage quality and availability. Avoid overstocking as this may force cows to start grazing lupines earlier than expected.
(4) Use intermittent grazing, moving cows to lupine-free pastures when lupine grazing is observed, or pull "lupine eaters" as they start to graze lupine plants. This requires intense management and may not be practical for some ranchers. Once seed pods have shattered, cattle can be returned to lupine pastures with a decreased risk of adverse effects occurring.
(5) Adjust breeding schedules to avoid exposing pregnant cows to lupines when they are at highest risk (GD 40 to 100). In areas having a high incidence of CCS over multiple years, moving to fall calving schedules has virtually eliminated CCS.
(6) Restrict access to lupine in late summer when cattle normally begin eating lupine and when anagyrine concentrations are elevated in seed pods.
(7) Graze high-risk lupine pastures with stockers, open heifers or cows, or other livestock species to optimize pasture utilization. Avoid putting pregnant cows in these pastures if possible but, if necessary, rotate to lupine-free pastures frequently. Lupine is nutritious forage, particularly on dormant rangelands dominated by annual grasses. Some ranchers have implemented this management strategy and successfully utilized their lupine-infested range and avoided CCS.
(8) Control lupine with herbicides. While generally not economical on large pastures, smaller ranches with few options may consider controlling lupines with herbicides. Lupine seeds remain viable in the soil for decades; thus, seed reserves are high in soil profiles and will germinate when conditions are right, therefore repeat treatment may be required every few years. Herbicides may also be used to clean up a pasture for use in the intermittent grazing approach for managing lupine.

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Conflict of interest statement

There is no conflict of interest for this work.

Author contributions

KW: Formal analysis, project administration, validation, visualization, writing—original draft, writing—review, and editing. SL: Formal analysis, investigation, methodology, validation, writing—review, and editing. KP: Conceptualization, investigation, methodology, project administration, writing—review and editing. BS: Formal analysis, investigation, validation, writing—review, and editing. CS: Formal analysis, methodology, validation, writing—review, and editing. DC: project administration, resources, supervision, validation, writing—review, and editing.

Ethical statement

The authors certify that all ethical considerations were made for the study and preparation of the manuscript, such that there are no ethical issues with the manuscript.

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