The effect of administering multiple doses of tall larkspur (Delphinium barbeyi) to cattle

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ABSTRACT: Larkspurs (Delphinium spp.) are one of the most serious toxic plant problems on foothill and mountain rangelands in the western United States. A considerable amount of research has been conducted over the years in both field and pen settings. The results of these research efforts have significantly increased our understanding of the poisoning of cattle by larkspurs. However, most of the pen studies conducted thus far have used a dosing regimen of a single bolus dose, which does not accurately mimic the manner by which cattle are poisoned by larkspur while grazing. Consequently, the objective of this study was to evaluate the acute toxicity of tall larkspur (Delphinium barbeyi collected near Manti, UT) when administered in multiple doses, with the intent to identify a no observable adverse effect level (NOAEL). The adverse effect selected for this study was muscle weakness to the point the cattle could no longer remain ambulatory as would be required in a grazing environment, thus becoming sternally recumbent when exercised. Hereford steers were administered various doses of tall larkspur at 12-h intervals for 4 d or until they showed marked signs of muscle weakness. The results suggest that a dose of 2 mg kg\(^{-1}\)d\(^{-1}\) N-(methylsuccinimido) anthranoyllycocotonine (MSAL)-type alkaloids is the NOAEL for a tall larkspur population with a norditerpenoid alkaloid profile containing 4 mg MSAL-type alkaloids/g plant material and 12 mg non-MSAL-type alkaloids/g plant material. Additionally, a computer model was generated to simulate multiple-dosing regimens at the various doses as well as different dosing regimens. The results from this study suggest that a 500-kg steer can consume a daily dose of 1.25 kg of fresh tall larkspur (with a similar alkaloid profile) without becoming severely poisoned (suffering from muscle weakness to the point of recumbency). Additionally, these results indicate that a serum concentration of approximately 355 ng methyllycaconitine/mL may represent a toxic threshold.

Key words: cattle, computer modeling, Delphinium, diterpenoid alkaloids, larkspur, methyllycaconitine

INTRODUCTION

Larkspurs (Delphinium spp.) are one of the most serious toxic plant problems on foothill and mountain rangelands in the western United States (Nielsen and Ralphs, 1988; Pfister et al., 1999). A number of valuable management recommendations have been made based on years of research into larkspur toxicity in livestock (Pfister et al., 1999, 2002). However, in many of these studies, cattle were treated with a single bolus dose of finely ground plant material. A single bolus dose of finely ground plant material does not accurately represent the conditions under which animals are poisoned on the range. Research has demonstrated that there are 3 distinct thresholds involved in tall larkspur toxicosis (Pfister et al., 1997). First, a subclinical toxicosis occurs that results in reduced tall larkspur consumption for 1 to 3 d but no overt signs nor overall reductions in consumption of other forage. Second, a short-acting toxicosis occurs with overt clinical signs, such as muscle weakness, which results in reduced food intake for several days but no long-term effects. Third, a potentially fatal toxicosis occurs with severe clinical signs that may result in death. It has been proposed that cyclic consumption enables cattle to generally regulate larkspur consumption below the second threshold in a typical

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range setting, which allows most cattle the opportunity to use an otherwise nutritious plant (Pfister et al., 1997). Consequently, studies need to be conducted using dosing regimens that more realistically mimic the multiple-dose exposures that cattle experience in grazing situations. Therefore, in this study, we performed a dose–response study wherein multiple doses of tall larkspur were administered to steers at 12-h intervals, with the objective to identify how much tall larkspur cattle can consume daily without serious adverse effects. Additionally, computer modeling was performed to simulate various multiple-dosing regimens.

MATERIALS AND METHODS

All animal work was done under veterinary supervision with the approval and supervision of the Utah State University Institutional Animal Care and Use Committee.

Plant Material

*Delphinium barbeyi* was collected in the early flowering stage during July 2003 near Manti, UT (39°03.154’ N, 111°30.752’ W) at an elevation of approximately 3,000 m; USDA-ARS Poisonous Plant Research Laboratory [Logan, UT] collection 03-12. The plant material was air dried and ground to pass through a 2.4-mm mesh using a Gehl Mix-All model 55 (Gehl Company, West Bend, WI). After processing, the ground plant material was stored in plastic bags away from direct light at ambient temperature in an enclosed shed until use. The norditerpenoid alkaloids are stable under these conditions.

Plant Alkaloid Analyses

The toxicity of larkspur plants is due to norditerpenoid alkaloids, which occur as 2 chemical structural types: the \(N\)-(methylsuccinimido) anthranoyllycoctonine (MSAL)–type, such as methyllycaconitine (MLA), and the non-MSAL-type alkaloids, including deltaline (Pfister et al., 1999; Panter et al., 2002). The plant was analyzed for total norditerpenoid alkaloid content and MSAL-type alkaloid content immediately before dosing using a Fourier-transform infrared spectroscopy (FTIR) method previously described (Gardner et al., 1997). This method measures the concentration of MSAL-type alkaloids and the concentration of total norditerpenoid alkaloids. Consequently, the concentrations of the non-MSAL-type alkaloids were calculated by subtracting the concentration of MSAL-type alkaloids from the concentration of total norditerpenoid alkaloids. The plant was also analyzed to obtain a mass spectrum of the alkaloid content, using a mass spectrometry method previously described (Gardner et al., 1999).

Positive electrospray ionization mass spectra of alkaloid extracts indicated that deltaline (\(m/z = 508\)) was the major non-MSAL alkaloid with MLA (\(m/z = 683\)) as the major MSAL alkaloid (Fig. 1). The FTIR analysis determined that this collection contained 16 mg/g of total alkaloids with 4 mg/g of MSAL-type alkaloids. Thus, the plant had a 3:1 ratio of non-MSAL- to MSAL-type alkaloids. The concentration of the MSAL-type alkaloids was used as the basis for calculating the doses that were administered to the animals.

Animal Experiments

Twelve yearling Hereford steers were used in this study. The steers weighed 330 ± 19 kg at the beginning of the study and 376 ± 20 kg by the end of the study. The animals were tractable and halter broken before the study began. The steers were maintained on alfalfa/grass hay with a salt–mineral supplement. The steers were fasted overnight before each morning dose. The steers were

![Figure 1. Representative electrospray mass spectrum of the norditerpenoid alkaloids in the tall larkspur (*Delphinium barbeyi*) population used in this study. The major ions detected include deltaline (MH\(^+\) \(m/z = 508\)) and methyllycaconitine (MLA; MH\(^+\) \(m/z = 683\)). MSAL = \(N\)-(methylsuccinimido) anthranoyllycoctonine.](image)
weighed and then restrained in a squeeze chute; steers were habituated to this procedure to minimize stress. The dried finely ground larkspur was administered via oral gavage in approximately 8 L of tap water.

The animals were monitored for the development of clinical signs for 5 d. Twenty-four hours after the first dose, the animals were exercised to measure muscle weakness to the point of becoming nonambulatory, a common clinical sign in larkspur-poisoned animals (Cook et al., 2011). Groups of 4 haltered steers (trained to this procedure before the beginning of the experiment) were placed 0.6 m apart behind a tractor with a specially devised 2.5-m tow bar and 0.6-m lead rope. The steers were attached to the lead rope with a quick release safety snap to allow for rapid release from the tow bar. The steers were led behind a tractor at 5 to 6 km/h in a large open area for 20 min or until they became nonambulatory as a result of larkspur-induced muscle weakness (nontreated control steers remain ambulatory under these conditions). If an animal showed muscular weakness, the tractor was stopped and the animal was quickly unhooked from the lead rope (elapsed time ≤ 30 s). After the affected animal was released from the tractor, the exercise period was continued for the remaining animals. During the period of recumbency, the affected steers were attended by other personnel until they were ambulatory and could be led back to their pen, typically within 20 to 30 min. This exercise procedure was repeated every 24 h, immediately after the morning dosing, with any individuals that had not previously demonstrated overt muscle weakness.

All 12 steers were dosed 1 time with tall larkspur at 8 mg MSAL/kg BW. This allowed for the verification that all 12 of the steers used in this study were susceptible to larkspur poisoning. The steers were given at least a 3-mo washout period afterward to ensure that no residual effects would remain from the first dosing. A 3-mo washout period allowed for complete elimination of the larkspur alkaloids, based on published elimination rates (Green et al., 2009; 2011), before using the steer again.

After the washout period, the steers were randomly divided into 3 groups of 4 steers each. Each group of steers were administered multiple doses of tall larkspur at 4, 2, and 1 mg MSAL kg⁻¹-dose⁻¹ at 12-h intervals, which corresponded to daily doses of 8, 4, and 2 mg kg⁻¹-d⁻¹, respectively. Each animal continued receiving the next dose until it had either shown overt muscle weakness or until it had received 8 doses, which corresponded to 4 d of exposure. Previous research has demonstrated that if cattle are not severely poisoned by tall larkspur, they will self-regulate their intake after 3 to 4 d such that they will not become poisoned (Pfister et al., 1997). This dose–response experiment was conducted to determine the no observable adverse effect level (NOAEL; the dose where none of the steers would demonstrate signs of severe muscle weakness).

**Serum Methyllycaconitine Analyses**

Blood was collected using jugular venipuncture from each steer every 12 h, immediately before the time of dosing. Serum was separated from red blood cells and stored frozen at −20°C. Matrix-matched standards were prepared for MLA as follows: a stock solution of MLA was prepared at 1 mg/mL in ethanol and then 0.020 mL was diluted with 0.980 mL ethanol to provide a 20 µg/mL solution. A 0.050-mL aliquot was added to 1.950 mL of sera from a nontreated steer and serially diluted with steer sera to give matrix standards at 500, 250, 125, 62, 31, 16, and 8 ng/mL MLA. Serum samples were thawed, vortexed, and then centrifuged at 3,000 × g for 5 min at room temperature. For the sera samples and the matrix standards, a 0.500-mL aliquot was taken and placed in a 1.5-mL Eppendorf tube. An equal volume of acetonitrile (0.500 mL) containing 250 ng/mL reserpine, an internal standard, was added to each sample. Samples were vortexed for 10 to 15 sec and then centrifuged at 16,000 × g for 10 min at room temperature. A 0.75-mL aliquot was then removed to a 1.5-mL autosample vial for analysis.

For the analysis of MLA, an LCQ Advantage Max (ThermoFinnigan, San Jose, CA) mass spectrometer coupled with a Surveyor Autosampler Plus and MS Pump Plus (Thermo Scientific) was used in line with a Betasil C18 column (100 by 2.1 mm; 5-µm particle size; Keystone Scientific, Bellefonte, PA) with a guard column (10 by 2.1 mm) of equivalent phase. The column was eluted with a binary solvent gradient using 0.5% acetic acid/0.05% trifluoroacetic acid (solvent A) and acetonitrile (solvent B) at a flow rate of 0.400 mL/min and the following gradient mixture with time: 5 to 15% solvent B (0–1 min), 15 to 75% solvent B (1–8 min), 75% solvent B (8–10 min), 75 to 5% solvent B (10–11 min), and 5% solvent B (11–16 min). The flow from the column was connected to a positive electrospray ion source. The mass spectrometer was set to scan selected tandem mass spectrometry experiments during the following time segments: for 4.5 to 5.5 min, MLA parent ion m/z = 683.3 with collision-induced dissociation (CID) fragmentation power of 35%, and from 5.5 to 10 min, reserpine parent ion m/z = 609.2 with CID fragmentation power of 33%. Reconstructed ion chromatograms used the following selected ions: for MLA, m/z = 619.3, m/z = 651.3, and m/z = 665.3, and for reserpine, m/z = 397.1 and m/z = 448.2. Reserpine was used as an internal injection standard and calibration and quantitation was completed using peak areas from reconstructed ion chromatograms for MLA versus reserpine.
1,000 simulations of 10 doses with an 8- or 12-h dosing interval. The input values were absorption rate ($k_a = 0.260/h$), elimination rate ($k_d = 0.044/h$), and volume of distribution ($V_d = 4,015,105$ mL) with error factors of 0.69, 0.51, and 0.48, respectively.

RESULTS AND DISCUSSION

Tall larkspurs with an MSAL-type alkaloid content of 3 mg/g or higher are considered to be acutely toxic to cattle (Pfister et al., 2002). However, based on current research, it is believed that, for the most part, under normal conditions, cattle can self-regulate their intake such that they do not become lethally poisoned (Pfister et al., 1997). Rather, there appears to be some unknown factors that lead to cattle consuming too much too fast that results in death of the animal. Consequently, even though the numerous research studies that have been performed in the past with a dosing regimen of a single bolus dose of finely ground plant material have been extremely informative, they lacked the multiple exposure scenario that likely occurs with cattle in a grazing environment. Therefore, the objective of this study was to evaluate the acute toxicity of tall larkspur (D. barbeyi collected near Manti, UT) when it is administered in multiple doses, with the intent to identify a NOAEL. The adverse effect selected for this study was muscle weakness to the point the cattle could no longer walk as would be required in a grazing environment, thus becoming sternal recumbent on exercise.

All 12 steers used in this study were first dosed 1 time with 8 mg MSAL-type alkaloids/kg BW (Table 1). Dosing steers with this collection of D. barbeyi (containing 16 mg/g of total alkaloids with 4 mg/g of MSAL-type alkaloids; Fig. 1) resulted in significant marked muscle weakness in all 12 of the steers, to the point that none of the steers could remain ambulatory for 20 min 24 h after dosing (Table 2). Five of the steers were not able to walk at all, and the remaining 7 steers walked for an average of 5.2 min before they could no longer walk due to muscle weakness.

All 4 steers in the 8 mg kg$^{-1}$·d$^{-1}$ (4 mg kg$^{-1}$·dose$^{-1}$) group received only 2 doses, as they all showed marked signs of muscle weakness by the 24-h time point (Table 2). Conversely, none of the 4 steers in the 2 mg kg$^{-1}$·d$^{-1}$ (1 mg kg$^{-1}$·dose$^{-1}$) group developed marked muscle weakness, even after 8 doses (4 d), as they were able to successfully walk for 20 min each day (Table 2). There was more variation in the response of the steers dosed with 2 mg kg$^{-1}$·dose$^{-1}$. One steer developed significant muscle weakness after 3 doses whereas it took 5 doses in 1 steer, with the other 2 steers developing muscle weakness after 4 doses. These results suggest that a dose of 2 mg kg$^{-1}$·d$^{-1}$ is the NOAEL for a tall larkspur population with a similar norditerpenoid alkaloid profile (Fig. 1).

Computer modeling of multiple oral doses of tall larkspur was performed using the package PKfit developed for the software program R. The input values used for the model were obtained from previously published data (Green et al., 2011); the values included the $k_a$ of 0.260/h, the $k_d$ of 0.044/h, and the $V_d$ of 4,015,105 mL. In general, the simulated serum MLA concentrations model the actual data well (Fig. 2 and 3). The model fit the actual data quite well through the 12-h time point (Fig. 2). It is not known how well the model would fit additional doses, as these experiments cannot be performed because multiple doses of 8 mg/kg would likely be lethal to cattle. Similarly, the model for the 8 mg kg$^{-1}$·d$^{-1}$ (4 mg kg$^{-1}$·dose$^{-1}$) fit the actual data very well at the 12-h time point but could not be further validated as only 2 doses were administered (Fig. 3). For the

Table 1. Tall larkspur dosing regimen, including number of doses, and steer BW as well as alkaloid and plant doses

<table>
<thead>
<tr>
<th>Group</th>
<th>No.doses</th>
<th>Steer weight, kg</th>
<th>Total MSAL, mg/kg</th>
<th>Plant, g/dose</th>
<th>Total plant, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 mg kg$^{-1}$·dose$^{-1}$</td>
<td>1</td>
<td>330 ± 19</td>
<td>8</td>
<td>659 ± 38</td>
<td>659 ± 38</td>
</tr>
<tr>
<td>4 mg kg$^{-1}$·dose$^{-1}$ (8 mg kg$^{-1}$·d$^{-1}$)</td>
<td>2</td>
<td>374 ± 22</td>
<td>8</td>
<td>374 ± 22</td>
<td>748 ± 44</td>
</tr>
<tr>
<td>2 mg kg$^{-1}$·dose$^{-1}$ (4 mg kg$^{-1}$·d$^{-1}$)</td>
<td>3 to 5</td>
<td>368 ± 17</td>
<td>6 to 10</td>
<td>184 ± 8</td>
<td>738 ± 164</td>
</tr>
<tr>
<td>1 mg kg$^{-1}$·dose$^{-1}$ (2 mg kg$^{-1}$·d$^{-1}$)</td>
<td>8</td>
<td>387 ± 22</td>
<td>8</td>
<td>97 ± 6</td>
<td>774 ± 45</td>
</tr>
</tbody>
</table>

1 Total N-(methylsuccinimido) anthranoyllycoctonine (MSAL) represents the total amount of MSAL alkaloids/kilogram administered to each group. Similarly, total plant represents the total amount of plant material administered to each group. The 8 mg kg$^{-1}$·dose$^{-1}$ group received only 1 dose ($n = 12$). Doses for all multiple dose groups were administered at 12-h intervals ($n = 4$ per group). Data represent means ± SD.

Computer Modeling

Computer modeling of serum MLA after multiple doses of tall larkspur was performed using the R (version 3.1.2; http://www.r-project.org/about.html) package PKfit (version 1.2.4; http://cran.r-project.org/web/packages/PKfit/index.html). PKfit is a nonlinear regression program that is designed to perform model/curve fitting and model simulations for pharmacokinetics (Lee et al., 2005). The kinetic data used for the model input was obtained from data previously published (Green et al., 2011). The simulations were generated using a model that was 1 compartment, extravascular, multiple doses, first-order absorption and elimination rates, and no lag time. The error type for the Monte Carlo simulations was uniform error X true value. The model consisted of 1,000 simulations of 10 doses with an 8- or 12-h dosing interval. The input values were absorption rate ($k_a = 0.260/h$), elimination rate ($k_d = 0.044/h$), and volume of distribution ($V_d = 4,015,105$ mL) with error factors of 0.69, 0.51, and 0.48, respectively.
The data obtained in this study indicate that a daily 72 h, whereas the actual serum MLA values began to were similar. Finally, the model for the 2 mg kg \(-1\) dose \((8 \text{ mg kg}^{-1} \text{d}^{-1})\) simulated the actual data very well for 36 h (Fig. 3). However, after 36 h, the model output continued to increase, as it should for a first-order kinetic model until a steady state is reached at approximately 72 h, whereas the actual serum MLA values began to plateau and even decrease slightly. This suggests that after 36 h, some physiological change had occurred in the steers such that either the amount of MLA absorbed or eliminated had changed and that a steady state was reached sooner than what the model predicted.

The results presented in this study demonstrate a clear dose–response effect with multiple doses of tall larkspur in cattle. More doses of plant material were required to induce muscle weakness in the steers as the dose of MSAL-type alkaloids decreased (Table 2). The data obtained in this study indicate that a daily dose of less than 2 mg/kg MSAL-type alkaloids will not cause severe muscle weakness to the point that the animals become recumbent. Based on both the computer simulations and the actual data, it appears that a serum MLA concentration of approximately 355 ng/mL may represent a toxic threshold, under the conditions used herein (Fig. 4). In all of our studies using a similar dosing protocol, a poisoned animal (muscle weakness to the point of recumbency) had a serum MLA concentration greater than 355 ng/mL (Fig. 4). In this regard, the steer from the 4 mg kg \(-1\) \(\text{d}^{-1}\) (2 mg kg \(-1\) \(\text{d}^{-1}\)) group that received only 3 doses had a serum MLA concentration of 385 ng/mL at the 36-h time point, the time at which signs of muscle weakness and recumbency were noticed. However, it is possible that the threshold for serum MLA in a poisoned animal is lower than 355 ng/mL as there is a lack of data for animals with serum MLA concentrations between approximately 250 and 350 ng/mL.

We used a 12-h dosing interval for this study because cattle grazing in large pastures typically have major periods of grazing near sunrise and sunset (Ruckebusch and Bueno, 1978; Gregorini et al., 2006). However, cattle will intermittently graze during other times during the day (Ruckebusch and Bueno, 1978). Cattle consume larkspur at several time points around dawn, midday, and dusk depending on weather (Pfister et al., 1988a,b). Therefore, in addition to performing computer simulations of serum MLA concentrations of 12-h intervals (representing morning and evening consumption of larkspur), we also ran simulations using 8-h intervals, which would represent morning, afternoon, and evening consumption (Fig. 5). For these simulations, we modeled 2 different scenarios. In 1 scenario, cattle consumed 1 mg kg \(-1\) \(\text{d}^{-1}\), which would bring their daily total dose to 3 mg/kg. This resulted in

<table>
<thead>
<tr>
<th>Group</th>
<th>Time point</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
<th>96 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 mg kg (-1) dose (\text{d}^{-1}) (\times 2)</td>
<td>0/12</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>4 mg kg (-1) dose (\text{d}^{-1}) ((8 \text{ mg kg}^{-1} \text{d}^{-1}))</td>
<td>0/4</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>2 mg kg (-1) dose (\text{d}^{-1}) ((4 \text{ mg kg}^{-1} \text{d}^{-1}))</td>
<td>4/4</td>
<td>0/4</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>1 mg kg (-1) dose (\text{d}^{-1}) ((2 \text{ mg kg}^{-1} \text{d}^{-1}))</td>
<td>4/4</td>
<td>4/4</td>
<td>4/4</td>
<td>4/4</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\)The time points indicate the time after the first dose. The data represents the number of animals at each time point that were able to walk for 20 min while being towed at 6 km/h behind a tractor.

\(^2\)The 8 mg kg \(-1\) dose \(\text{d}^{-1}\) group received only 1 dose.
a serum MLA concentration of approximately 500 ng/mL, well above the possible toxic threshold. Conversely, according to the model, if the cattle consumed 0.67 mg kg⁻¹·dose⁻¹, or 2 mg kg⁻¹·d⁻¹, they would maintain a serum MLA concentration below the toxic threshold. This data demonstrates that the overall daily intake is critical and should be maintained under 2 mg/kg for animals to avoid intoxication. Based on the alkaloid profile of this population of tall larkspur (4 mg MSAL-type alkaloids/g plant material), a dose of 2 mg kg⁻¹·d⁻¹ corresponds to a daily intake of 250 g of tall larkspur on a dry weight basis for a 500-kg animal, which, assuming that freshly harvested tall larkspur is approximately 80% water, would correspond to 1.25 kg of fresh plant material. Therefore, based on the results of this study, we propose that a 500-kg steer could consume up to 1.25 kg of fresh tall larkspur with this concentration of alkaloids without becoming severely poisoned.

It is interesting to note that serum MLA concentrations reached a plateau and even began to decrease after 36 h (Fig. 3), or even earlier in poisoned animals, when the alkaloid concentrations should have increased for another 36 h until a steady state should have occurred. A steady state of a toxin is reached in the serum when the amount of the toxin being absorbed is equal to the amount of the toxin be cleared (Shargel and Yu, 1993). The time it takes to reach a steady state is controlled by the elimination half-life, with 93.8% of steady state reached in 4 half-lives (Shargel and Yu, 1993; Shen, 2008). Accordingly, if the k_el for MLA is 18 h (Green et al., 2011), then a steady state would be reached at approximately 72 h.

Oftentimes when administering multiple doses, the assumption is made that previous doses will not have any effect on the kinetics of future doses, which is known as the principle of superposition. The principle of superposition assumes that early doses of a toxin do not affect the toxicokinetics of subsequent doses (Shargel and Yu, 1993; Wang and Ouyang, 1998). However, there are a number of reasons why early doses can change the toxicokinetics of subsequent doses, including any physiological or biochemical changes that would alter the absorption, metabolism, elimination, or toxicodynamics of the toxin.

The norditerpenoid alkaloids found in larkspurs are antagonists of nicotinic acetylcholine receptors (nAChR; Dobelis et al., 1999; Welch et al., 2013). Nicotinic acetylcholine receptors play an important role in gastrointestinal (GI) tract motility highlighted by the fact that nAChR antagonists can cause constipation (Mandl and Kiss, 2007). In this regard, one of the common clinical signs noted in cattle poisoned by larkspur is a decreased motility of the GI tract, including constipation. Consequently, in the case of the norditerpenoid alkaloids found in tall larkspur, one can postulate that early exposure to these alkaloids may decrease GI tract motility, thus reducing subsequent absorption of the alkaloids. Further work is required to determine why MLA does not appear to follow a traditional first-order, 1-compartment kinetic profile in a multiple-dosing regimen (Green et al., 2009; 2011).

We recognize that a shortcoming of this study is the small sample size of each group, and as such, the data should be interpreted with caution. With that said, the steers we selected for this study have been shown to be susceptible to larkspur poisoning in general (Green et al., 2014), and these specific 12 steers were all found to be very susceptible, as none of them could walk for more than 12 min after a typical larkspur challenge. Consequently, the data from this study should represent a conservative estimate of a toxic threshold and NOAEL.

Another caveat of this study was the fact that we used dried finely ground plant material. We believe that this form of plant material is likely absorbed much quicker in comparison with plant material consumed via normal grazing. However, it has been our experience that cattle will not readily consume fresh plant material in a pen setting. We have placed fresh tall larkspur in the manger of cattle that had been fasted for 18 h and they would not eat it over a 6-h period (K.D. Welch, unpublished data). Additionally, a controlled dose–response study such as this cannot be conducted in a field setting because it would be impossible to know the exact amount of tall larkspur the cattle would consume. Bite counts give a good understanding of relative dietary preferences over time, but counting bites will not provide sufficient information to determine the quantity of plant ingested as required for a dose–response study. Consequently, at this time, the only feasible way to control the exact dose is to administer dried finely ground plant material via oral gavage.
In conclusion, the results from this study suggest that cattle can ingest 2 mg of MSAL-type alkaloids kg\(^{-1}\) \(\text{d}^{-1}\) of tall larkspur (containing 16 mg/g of total alkaloids with 4 mg/g of MSAL-type alkaloids without becoming poisoned to the point of muscle weakness leading to recumbency. The results and inferences presented in this study are likely valid only for tall larkspur populations that have an alkaloid profile similar to the population used for this study (Fig. 1). Previous research has shown that the ratio of non-MSAL- to MSAL-type alkaloids has an important impact on the toxic potential of tall larkspur populations (Welch et al., 2010, 2012). Consequently, the total concentration of norditerpenoid alkaloids and specifically the concentration of non-MSAL-type and MSAL-type alkaloids needs to be known to better understand the toxic potential of a given larkspur population. Even so, the modeling of the toxicokinetic aspects of larkspur alkaloids can provide important information that elucidates previously unknown relationships between grazing cattle and consumption of various larkspur species (Pfister et al., 1997) and provides hypotheses for testing in future work.

**LITERATURE CITED**


