Comparison of the toxic effects of two duncecap larkspur (*Delphinium occidentale*) chemotypes in mice and cattle

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**Objective**—To compare the toxic effects of a *Delphinium occidentale* chemotype containing N-(methylsuccinimido) anthranoyllycotoconine (MSAL)-type alkaloids and a *D. occidentale* chemotype lacking MSAL-type alkaloids in mice and cattle.

**Animals**—225 male Swiss Webster mice and 11 Black Angus steers.

**Procedures**—4 collections of larkspur containing MSAL-type alkaloids and 4 collections of larkspur lacking MSAL-type alkaloids were used. From each collection, total alkaloid extracts (0.05 to 0.20 mL) were administered via tail-vein injection in 27 to 29 mice. Dried, finely ground plant material from 1 collection with and 1 collection without MSAL-type alkaloids (doses equivalent to 3.76 mg of total alkaloids/kg) were each administered to 8 cattle via oral gavage in a crossover experiment; 3 cattle received a single dose equivalent to 150.4 mg of total alkaloids/kg (no MSAL-type alkaloids). In mice, clinical effects were monitored; in cattle, heart rate was monitored before (baseline) and 24 hours after treatment. At the 24-hour time point, cattle were exercised as a measure of muscle weakness.

**Results**—In mice, mean LD₅₀ associated with alkaloid extracts prepared from plants that did or did not contain MSAL-type alkaloids was 2.3 and 54.2 mg/kg, respectively. In cattle at 24 hours after treatment, plant material containing MSAL-type alkaloids significantly increased heart rate from baseline and was associated with exercise-induced collapse; plant material lacking MSAL-type alkaloids had no similar effects.

**Conclusions and Clinical Relevance**—Taxonomic classification of *D. occidentale* alone was not a good indicator of the toxic risk to grazing cattle. (Am J Vet Res 2011;72:706–714)

**Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>BW</td>
<td>Body weight</td>
</tr>
<tr>
<td>MDL</td>
<td>7,8-methylenedioxylycoctonine</td>
</tr>
<tr>
<td>MH⁺</td>
<td>Protonated molecular ion</td>
</tr>
<tr>
<td>MLA</td>
<td>Methyllycaconitine</td>
</tr>
<tr>
<td>MSAL</td>
<td>N-(methylsuccinimido) anthranoyllycotoconine</td>
</tr>
<tr>
<td>m/z</td>
<td>Mass-to-charge ratio</td>
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</table>

Larkspurs (*Delphinium* spp) are poisonous plants that grow on rangelands in the western United States and Canada. They are responsible for major losses to the cattle industry and are the subject of extensive research.¹⁻⁴ Total cost to the livestock industry from cattle deaths attributed to larkspur poisoning is estimated to be millions of dollars annually.² Larkspurs have been categorized into 3 groups principally on the basis of their height: low larkspurs, plains larkspurs, and tall larkspurs. The tall larkspurs are responsible for a greater number of cattle losses than either the plains or low larkspurs.

Larkspur-induced poisoning in cattle has been attributed to the diterpenoid alkaloids that can compose up to 3% of the plant dry weight. There are 2 main structural groups of norditerpene alkaloids: the MSAL-type and the MDL-type norditerpenoid alkaloids.⁶ On the basis of the LD₅₀ of the individual compounds in mice, the MSAL-type alkaloids are approximately 20 times as toxic as the MDL-type alkaloids.⁷⁻⁸ Acute larkspur poisoning has been attributed to the MSAL-type alkaloids,¹³ and plants that have a high content of the MSAL-type alkaloids are thought to be the most toxic to cattle. The concentrations of these alkaloids have been used as a predictor of plant toxicity.¹¹ The most abundant constituent of the MSAL-type alkaloids in the tall larkspurs is MLA.¹²

An interesting observation has been the identification of 2 alkaloid profiles in *Delphinium occidentale*.¹²¹³ One alkaloid profile lacked or was associated with very small amounts of the MSAL-type alkaloids, whereas the other alkaloid profile was associated with large amounts of the MSAL-type alkaloids. More recently, it was shown that *D. occidentale* has 2 definable chemo-
types that should differ in potential toxicity and that the 2 chemotypes have distinct geographic boundaries.13 The purpose of the study reported here was to compare the toxic effects of a *D. occidentale* chemotype containing MSAL-type alkaloids with those of a *D. occidentale* chemotype lacking MSAL-type alkaloids in mice and in cattle.

**Materials and Methods**

**Plant material—** *Delphinium occidentale* representing the chemotype containing the MSAL-type alkaloids was collected in the following stage during July 2008 at 2 locations near Victor, Idaho (latitude, 41° 40.929’ N; longitude, 112° 00.224’ N) and near Fairview, Utah (latitude, 39° 49.929’ N; longitude, 111° 21.143’ W) and in the flowering stage during July 2008 near Baggs, Wyo (latitude, 43° 04.567’ N; longitude, 107° 15.997’ W) and near Afton, Wyo (latitude, 42° 58.931’ N; longitude, 110° 58.445’ W). Plant materials collected at the 2 locations near Elko, Nev, were combined and processed as 1 collection, as were the materials collected at the 2 locations near Victor, Idaho. Large collections (>50 kg of dry weight) were harvested near Logan, Utah, and Victor, Idaho; smaller collections (approx 0.5 kg of dry weight) were harvested at the other 6 locations.

Plant materials in the collections harvested near Logan, Utah, and Victor, Idaho, were each air-dried and ground by use of a grinder-mixer until sufficiently fine to pass through a 2.4-mm mesh. After processing, the plant material from each collection was stored in plastic bags away from direct light at ambient temperature (ranging from ~18°C in the winter to 38°C in the summer) in an enclosed shed until used. Plant materials from these 2 collections were used in the mice and cattle experiments. Plant materials from the other 6 locations were air-dried and ground by use of a grinding mill until sufficiently fine to pass through a 2-mm screen. Plant materials from these 6 collections were used in the mice experiments.

**Plant alkaloid analyses—** The larkspur alkaloids used in the study were extracted as previously described.14–16 Samples were analyzed for total alkaloid content and MSAL-type alkaloid content via a previously described method of Fourier transform infrared spectroscopy.17 Samples were also analyzed by use of a mass spectrometry method18 to obtain a mass spectrum of the alkaloid extract from each of the various plant collections for comparison.

**Isolation of alkaloid mixture from *D. occidentale*—** Individual portions (50 g) of the dried and ground plant material were defatted by extraction with hexane (1 L) for 24 hours. Each extract was filtered and the plant material was dried in a fume hood. The plant material was then extracted with methanol (1 L) for 24 hours and filtered. The methanol extract was then evaporated under reduced pressure (rotovaporation). The gum residue was washed successively with 1% sulfuric acid and chloroform (total volume of 75 mL each) and added to a separatory funnel. After mixing, the chloroform layer was drained and the aqueous acid solution was washed an additional 2 times with 100 mL of chloroform. The aqueous solution was cooled in an ice bath, and the pH was adjusted to 9 by the addition of concentrated ammonium hydroxide solution. The aqueous solutions were then extracted 3 times with chloroform (volumes of 100, 80, and 80 mL in succession) in a separatory funnel. The combined extracts were dried over anhydrous sodium sulfate and filtered, and then the solvent was evaporated under reduced pressure. The crude alkaloid was rinsed with a small amount of chloroform, and the solvent was again evaporated under reduced pressure in a 100-mL round-bottomed flask; the crude alkaloid mixture formed an amorphous solid material that was removed by scraping from the flask.

**Mice experiments—** Mice experiments were performed under veterinary supervision with the approval and supervision of the Utah State University Institutional Animal Care and Use Committee. Total alkaloid extracts were suspended in physiologic PBS solution, and the pH was lowered with hydrochloric acid to achieve solubility. Subsequently, ammonium hydroxide was added to the solutions to increase the pH to as close to physiologic pH (5.5 to 7.0) as possible while still retaining solubility. The solutions were stored in sterile injection vials at 4°C until use. The LD<sub>50</sub> of each solution was determined in male Swiss Webster mice (mean ± SD weight, 23 ± 2 g). A volume of 0.05 to 0.2 mL of the solution of each alkaloid extract from each of the 8 plant collections was injected IV into the coccygeal vein of 27 to 29 mice. The doses administered were based on the MSAL-type alkaloid content of the plant collections containing the MSAL-type alkaloids or the total alkaloid content of the plant collections lacking the MSAL-type alkaloids. The mice were observed for up to 10 minutes for clinical effects (eg, labored breathing and muscle tremors) and death.

**Cattle experiments—** Cattle experiments were performed under veterinary supervision with the approval and supervision of the Utah State University Institutional Animal Care and Use Committee. Eleven 2-year-old Black Angus steers (mean ± SD weight, 492 ± 28 kg) were used in the study. Eight cattle were treated with plant material from the collections harvested near Victor, Idaho (plants containing MSAL-type alkaloids [treatment A]), and Logan, Utah (plants lacking MSAL-type alkaloids [treatment B]), in a crossover experiment. For 4 animals, the sequence of treatments was BAB; for the other 4 animals, the sequence of treatments was BABA. Each treatment A (plant material collected near Victor, Idaho) involved administration via oral gavage of a dose of 8.8 mg of MSAL-type alkaloids/kg (ie, 37.6 mg of total alkaloids/kg); each treatment B (plant material collected near Logan, Utah) involved
administration via oral gavage of a dose of 37.6 mg of total alkaloids/kg of BW (no MSAL-type alkaloids).

The remaining 3 cattle were administered 1 dose of plant material from the collection harvested near Logan, Utah (plants with no MSAL-type alkaloids). The dose administered via oral gavage was 150.4 mg of total alkaloids/kg of BW (ie, 4x treatment of the B dose).

Prior to treatment, the cattle were maintained on alfalfa-grass hay with a standard mineral supplement. Food was withheld from the cattle overnight (approx 16 to 18 hours) prior to each treatment day. On each treatment day, each animal was weighed and then restrained in a squeeze chute; the cattle had been habituated to this procedure previously to minimize stress. Baseline heart rate of the animal was recorded immediately prior to the administration of the single larkspur dose (0 hours); the dried, finely ground larkspur was administered in approximately 8 L of tap water via oral gavage. After treatment, each bovid was visually observed for the development of clinical signs (eg, muscular weakness and lateral recumbency) every 2 hours for the first 10 hours. Twenty-four hours after administration of the larkspur dose, each bovid was again restrained in a squeeze chute and heart rate was assessed. After the 24-hour heart rate measurement was obtained, the bovid was exercised as a measure of muscle weakness, a common clinical sign in larkspur-poisoned animals. A 3-week interval was allowed to elapse after each dose within a treatment sequence before the next dose was administered in the crossover experiment. This 3-week

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**Figure 1**—Map of the geographic distribution of the 2 *Delphinium occidentale* chemotypes (ie, plants that contain MSAL-type alkaloids or that lack or contain very small amounts of MSAL-type alkaloids) within the known growing areas of this species (Oregon, Idaho, Montana, Wyoming, Nevada, Utah, and Colorado). Locations of the plant collections used in a study to compare the toxic effects of the 2 *D. occidentale* chemotypes in mice and cattle are marked on the map.
washout period allowed > 99% of the deltaline and MLA to be eliminated (on the basis of clearance times).

Heart rate monitoring—Heart rate was monitored as outlined previously. Briefly, data were recorded and signals were amplified with a biological signals amplifier. Heart rate was monitored by use of repositional monitoring electrodes that were secured in place with a gel-based formulation of cyanoacrylate adhesive. The leads were placed as described by Chen et al, with the positive electrode placed on the region of the right scapula and the negative electrode on the sternum near the heart; a ground electrode was attached to the perineum. The heart rate signal was amplified with a 0.1-Hz high-pass filter, and digital band-pass filter with a high cutoff frequency of 45 Hz and a low cutoff frequency of 0.1 Hz. The cyclic measurements’ feature of a software package was used to calculate heart rate (in beats/min). The heart rate in each bovid was allowed to stabilize (typically for a period of 5 minutes) before analysis. After stabilization, a 5-minute period of heart rate was analyzed. Five-minute periods of heart rate measurement were analyzed prior to and 24 hours after administration of each larkspur dose to each animal.

Exercise test—The study cattle were tractable, halter broken, and trained to be led by use of a tractor. Groups of 4 haltered cattle were placed 0.8 m apart behind a tractor with a specially devised 2.5-m tow bar and 0.6-m lead rope. The cattle were attached to the lead rope with a quick-release safety snap to allow for rapid release from the tow bar. Cattle were exercised behind a tractor at 4.8 to 6.4 km/h in a large, open area until they collapsed as a result of larkspur-induced muscle weakness (a nontreated control bovid would not collapse from muscle weakness under these conditions) or for a maximum time of 35 minutes. If a bovid collapsed, the tractor was stopped, the time to collapse was recorded, and the animal was quickly unhooked from the lead rope (elapsed time, ≤ 30 seconds); after the collapsed animal was released from the tractor, the exercise period was continued for the remaining animals. This procedure was continued until all cattle collapsed or they exercised for a maximum of 35 minutes. The collapsed bovids were temporarily recumbent; during the period of recumbency, they were attended by other personnel until they were ambulatory and could be led back to their pen, typically within 20 to 30 minutes.

Data analysis—All data are expressed as mean ± SD. The LD₅₀ of each total alkaloid extract was determined by use of a modified up-and-down method. This method is preferred because the number of animals required is minimized. The LD₅₀ values were calculated by use of a statistical analysis package with logistic regression analysis. Heart rate was analyzed by use of a mixed linear model of the difference between baseline and the posttreatment heart rate. The model included animal, sequence, period, and treatment by animal within sequence as a random factor. Least squares means were calculated, and differences in the least squares means after a significant (P < 0.05) F test result were obtained used to compare treatments. A χ² analysis was used on the binomial response variable (ie, collapse or no collapse during the exercise tests). For all analyses, a value of P < 0.05 was considered significant.

Results

Plant chemical analysis—Electrospray mass spectra of alkaloid extracts from samples of the 8 D. occidentale collections used in the study were obtained (Figure 2). Deltaline (MH⁺ m/z = 508) was the major MDL-type alkaloid in the 8 D. occidentale extracts. In the 4 extracts that represented collections of D. occidentale containing the MSAL-type alkaloids, MLA (MH⁺ m/z = 683) was the major MSAL-type alkaloid. No MSAL-type alkaloids were detected in the other 4 D. occidentale extracts that represented collections of D. occidentale lacking or containing very little MSAL-type alkaloids. Fourier transform infrared spectroscopy revealed that total alkaloid content ranged from 8 to 21 mg/g of dried plant material, whereas the MSAL-type alkaloid content ranged from 0 to 6.3 mg/g of dried plant material (Table 1).

Mice experiments—To assess the acute toxic effects of each of the 8 D. occidentale collections, a total alkaloid extract of each collection was prepared. The alkaloid profile of the total alkaloid extract of each collection (data not shown) was similar to the corresponding electrospray mass spectrum of each plant collection (Figure 2). The LD₅₀ of extracts from each plant collection was determined as a mean value for groups of 27 to 29 mice by use of a modified up-and-down method. For the 4 alkaloid extracts from plants containing MSAL-type alkaloids, LD₅₀ values in mice ranged from 6.2 to 9.8 mg of total alkaloids/kg (Table 2). The LD₅₀ values of the 4 alkaloid extracts from plants lacking MSAL-type alkaloids ranged from 42.7 to 60.8 mg of total alkaloids/kg. On the basis of the LD₅₀ from each plant collection and the alkaloid content of the plant material, the amount of plant material required to achieve the LD₅₀ was calculated. For plants containing the MSAL-type alkaloids, the amount of plant material required to achieve the LD₅₀ was 0.4 to 0.9 g; this amount was significantly less than the required amount of plant material from the collections lacking MSAL-type alkaloids (2.7 to 4.1 g). Among the plant collections with MSAL-type alkaloids, the amount of plant material required to achieve the LD₅₀ in mice decreased as plant concentrations of the MSAL-type alkaloids increased. For example, the D. occidentale collection from near Elko, Nev, contained 6.3 mg of MSAL-type alkaloid/g of dried plant material, and 0.4 g of that plant material was required to achieve the LD₅₀ whereas the D. occidentale collection from near Wilsal, Mont, contained 2.7 mg of MSAL-type alkaloid/g of dried plant material, and 0.9 g of that plant material was required to achieve the LD₅₀. Similarly, among the plant collections that lacked the MSAL-type alkaloids, the amount of plant material required to achieve the LD₅₀ in mice decreased as plant concentrations of total alkaloids increased.

Cattle experiments—For purposes of the study, we considered an effective dose of plant material as the dose that would significantly increase the heart rate and
Figure 2—Electrospray mass spectra obtained from 8 collections of *D. occidentale*; plant material was collected near Wilsal, Mont (A); Twin Falls, Idaho (B); Victor, Idaho (plants from 2 locations combined; C); Elko, Nev (plants from 2 locations combined; D); Baggs, Wyo (E); Fairview, Utah (F); Logan, Utah (G); and Afton, Wyo (H). The MH+ m/z of deltaline (the major MDL-type alkaloid), MLA (the major MSAL-type alkaloid), and reserpine (an internal standard) are labeled (apply to all spectra). Notice that spectra A through D were derived from collections of *D. occidentale* plants that contain MSAL-type alkaloids and spectra E through H were derived from collections of *D. occidentale* plants that lack MSAL-type alkaloids.
elicit clinical signs of larkspur poisoning. On the basis of previous studies performed in our laboratory, the effective dose in Angus cattle is approximately 8 mg of MSAL-type alkaloids/kg; at this dose, administration of larkspur material causes an increase in heart rate and induces muscle weakness (generally not to the extent that the animal becomes recumbent).

Two groups of 4 cattle were used in the crossover experiment. Each bovid received 3 treatments of plant material at 3-week intervals. Each treatment was a single dose of plant material from the collections harvested near Victor, Idaho (chemotype containing MSAL-type alkaloids [treatment A]), or Logan, Utah (chemotype lacking MSAL-type alkaloids [treatment B]), and the sequence of treatment administration was ABA for 4 animals and BAB for the other 4 animals. Each treatment A (8.8 mg of MSAL-type alkaloids/kg [ie, 37.6 mg of total alkaloids/kg]) and each treatment B (37.6 mg of total alkaloids/kg of BW [no MSAL-type alkaloids]) were administered via oral gavage. At completion of the experiment A and the chemotype that lacks MSAL-type alkaloids (treatment B) in a crossover experiment.

Table 1—The MSAL-type alkaloid, MDL-type alkaloid, and total alkaloid contents* of 8 collections of Delphinium occidentale used in a study to compare the toxic effects of 2 D occidentale chemotypes in mice and cattle.

<table>
<thead>
<tr>
<th>Location at which D occidentale plants were collected</th>
<th>MSAL-type alkaloids (mg/g)</th>
<th>MDL-type alkaloids (mg/g)</th>
<th>Total alkaloids (mg/g)</th>
<th>MDL:MSAL concentration ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wilsal, Mont</td>
<td>2.7</td>
<td>8.0</td>
<td>10.7</td>
<td>3.0</td>
</tr>
<tr>
<td>Twin Falls, Idaho</td>
<td>2.9</td>
<td>5.3</td>
<td>8.2</td>
<td>1.8</td>
</tr>
<tr>
<td>Victor, Idaho</td>
<td>4.5</td>
<td>13.8</td>
<td>18.3</td>
<td>3.1</td>
</tr>
<tr>
<td>Elko, Nev</td>
<td>6.3</td>
<td>11.1</td>
<td>17.4</td>
<td>1.8</td>
</tr>
<tr>
<td>Baggs, Wyo</td>
<td>0</td>
<td>14.7</td>
<td>14.7</td>
<td>NA</td>
</tr>
<tr>
<td>Fairview, Utah</td>
<td>0</td>
<td>21.3</td>
<td>21.3</td>
<td>NA</td>
</tr>
<tr>
<td>Logan, Utah</td>
<td>0</td>
<td>20.2</td>
<td>20.2</td>
<td>NA</td>
</tr>
<tr>
<td>Afton, Wyo</td>
<td>0</td>
<td>15.1</td>
<td>15.1</td>
<td>NA</td>
</tr>
</tbody>
</table>

*Plant material samples were analyzed for alkaloid contents by use of a Fourier transform infrared spectroscopy method. The values represent the milligrams of alkaloid per gram of dried plant material. NA = Not applicable.

Table 2—The LD50 values* for total alkaloid and MSAL-type extracts of plant materials and calculated amount of plant material required to achieve the LD50 for each of 8 collections of D occidentale following injection of a volume† of 0.05 to 0.2 mL into the coccygeal vein of mice.

<table>
<thead>
<tr>
<th>Location at which D occidentale plants were collected</th>
<th>Total alkaloids LD50 (mg of total alkaloids/kg)</th>
<th>MSAL-type alkaloids LD50 (mg of MSAL-type alkaloids/kg)</th>
<th>Amount of plant required to achieve the LD50 (g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wilsal, Mont</td>
<td>9.6 ± 0.8†</td>
<td>2.4 ± 0.2†</td>
<td>0.9</td>
</tr>
<tr>
<td>Twin Falls, Idaho</td>
<td>6.2 ± 0.6†</td>
<td>2.2 ± 0.2†</td>
<td>0.8</td>
</tr>
<tr>
<td>Victor, Idaho</td>
<td>9.8 ± 0.4†</td>
<td>2.4 ± 0.1†</td>
<td>0.5</td>
</tr>
<tr>
<td>Elko, Nev</td>
<td>6.2 ± 1.4†</td>
<td>2.2 ± 0.5†</td>
<td>0.4</td>
</tr>
<tr>
<td>Baggs, Wyo</td>
<td>60.8 ± 2.8†</td>
<td>NA</td>
<td>4.1</td>
</tr>
<tr>
<td>Fairview, Utah</td>
<td>58.1 ± 2.4†</td>
<td>NA</td>
<td>2.7</td>
</tr>
<tr>
<td>Logan, Utah</td>
<td>55.3 ± 7.1†</td>
<td>NA</td>
<td>2.7</td>
</tr>
<tr>
<td>Afton, Wyo</td>
<td>42.7 ± 6.0†</td>
<td>NA</td>
<td>2.8</td>
</tr>
</tbody>
</table>

*The LD50 value of each total alkaloid extract was determined by use of a modified up-and-down method.† The doses of the plant materials administered were based on the MSAL-type alkaloid content of the plant collections containing the MSAL-type alkaloids or the total alkaloid content of the plant collections lacking the MSAL-type alkaloids. †Dose the basis of the LD50 for each plant collection and the alkaloid content of the plant material, the amount of plant material required to achieve the LD50 was calculated. **Within a column, values with different superscript letters differ significantly (P < 0.05).

Table 3—Mean ± SD heart rate and time to exercise-induced collapse in 8 cattle following administration (via oral gavage [0 hours]) of plant materials from each of 2 collections of D occidentale (representative of the chemotype that contains MSAL-type alkaloids [treatment A] and the chemotype that lacks MSAL-type alkaloids [treatment B]) in a crossover experiment.

<table>
<thead>
<tr>
<th>Location at which D occidentale plants were collected</th>
<th>Dose (mg/kg)</th>
<th>Heart rate* (beats/min)</th>
<th>Exercise to collapse†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Treatment</td>
<td>Total alkaloids</td>
<td>MSAL-type alkaloids</td>
</tr>
<tr>
<td>Victor, Idaho</td>
<td>B</td>
<td>37.6</td>
<td>8.8</td>
</tr>
<tr>
<td>Logan, Utah</td>
<td>A</td>
<td>37.6</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Cattle received a single dose of one of the D occidentale chemotypes on 3 occasions; the sequence of treatments for 4 animals was ABA, whereas the sequence of treatments was BAB for the other 4 animals (doses administered at 3-week intervals [a total of 12 doses of each treatment were administered to the group]). *Heart rate was monitored before (baseline) and 24 hours after administration of each dose; data represent the mean ± SD value for 4 to 8 animals. †At 24 hours after each dose, cattle were exercised until collapse or for a maximum of 35 minutes; the time from start of exercise to collapse was recorded. Values in parentheses indicate the number of doses after which treated cattle collapsed. **Value is significantly (P < 0.05) different from the baseline value. NA = Not applicable.
Results of the crossover experiment indicated no sequence ($P > 0.36$) or period effects ($P > 0.37$) on heart rate; however, there was a treatment effect ($P > 0.001$) on heart rate. Treatment of the 8 cattle with the chemotype containing MSAL-type alkaloids increased mean ± SD heart rate from 74.5 ± 7.7 beats/min at baseline to 99.8 ± 13.5 beats/min at 24 hours after dose administration (Table 3). Treatment of the 8 steers with the chemotype lacking MSAL-type alkaloids did not increase heart rate 24 hours after dosing; the mean baseline value was 77.4 ± 11.2 beats/min, compared with 84.2 ± 8.7 beats/min at 24 hours after dose administration.

In addition to changes in heart rate, cattle were monitored for clinical signs of larkspur poisoning including muscle weakness, trembling, and a staggering gait; cattle underwent an exercise test at 24 hours after dose administration as a measure of muscle weakness. All cattle administered plant material containing the MSAL-type alkaloids had developed muscular weakness, trembling, and a staggering gait at the 24-hour time point. None of the cattle administered plant material lacking the MSAL-type alkaloids developed any clinical signs after any dose. All cattle administered plant material containing the MSAL-type alkaloids collapsed within 35 minutes of starting the exercise test (Table 3), whereas none of the cattle administered plant material lacking MSAL-type alkaloids collapsed or developed any clinical signs of poisoning during the exercise test ($\chi^2$ analysis; $P < 0.001$). In cattle treated with the chemotype containing MSAL-type alkaloids, mean time to collapse from start of the exercise test was 17 minutes (range, 1 to 34 minutes).

Because administration of the chemotype lacking MSAL-type alkaloids (treatment B) did not result in an increase (from baseline) in heart rate or any clinical signs of poisoning after each dose in the 8 cattle, the dose was increased by a factor of 4 and administered in a single-dose experiment to 3 other cattle to further evaluate its relative toxicity. This high dose (130.4 mg of total alkaloids/kg) was chosen because it represented the maximum amount of plant material and water that can be safely administered to the animals in a single bolus dose. Treatment of the 3 cattle with this high dose of the chemotype lacking MSAL-type alkaloids did not result in an increase (from baseline) in heart rate at 24 hours after dose administration; mean baseline heart rate was 70.5 ± 10 beats/min, compared with a value of 79.9 ± 10.9 beats/min at the 24-hour time point. In addition, no clinical signs of poisoning were observed during the 24-hour posttreatment period in these animals, in contrast to findings (ie, muscular weakness, trembling, and a staggering gait) from the cattle that received a dose of 8.8 mg of MSAL-type alkaloids/kg.

**Discussion**

Results of previous studies7–9 in mice have indicated that the MSAL-type alkaloids are much more toxic than the MDL-type alkaloids. The MSAL-alkaloids are primarily responsible for acute larkspur poisoning,10 and plants that have a high content of MSAL-type alkaloids are thought to be the most toxic to cattle. Current management recommendations for grazing cattle on larkspur-containing ranges are based primarily on the concentration of the MSAL-type alkaloids in larkspur.411 Recent studies in cattle12 and mice13 have revealed that the MDL-type alkaloids can potentiate the toxicity of the MSAL-type alkaloids. *Delphinium occidentale*, a tall larkspur, has 2 unique chemotypes that grow within distinct geographic boundaries; one chemotype contains MSAL-type alkaloids and the other lacks MSAL-type alkaloids, thereby potentially differing in toxicity.13 Consequently, the objective of the present study was to assess and compare the toxic effects of the 2 chemotypes of *D. occidentale* in mice and cattle.

In the present study, the *D. occidentale* collections were representative of the geographic distribution of each chemotype; 4 collections were composed of plants that contained MSAL-type alkaloids, and 4 collections were composed of plants that did not contain MSAL-type alkaloids. The major distinguishing feature among the 8 collections was the presence or absence of MLA. Deltaline was the major MDL-type alkaloid in all 8 populations; however, minor differences were observed in the presence (and abundance) or absence of other MDL-type alkaloids.

The total alkaloid extracts elicited different responses in mice depending on the concentrations of MSAL-type and total alkaloids. As expected, plants with the MSAL-type alkaloids were more toxic to mice than were plants lacking the MSAL-type alkaloids. Furthermore, these results were consistent with the hypothesis that less plant material is required for a lethal dose when plants contain more MSAL-type alkaloids. In addition, no differences were observed in the LD$_{50}$ of the total alkaloid extracts from plant material containing the MSAL-type alkaloids (2.2 to 2.4 mg of MSAL-type alkaloids/kg). These findings were similar to the LD$_{50}$ of a total alkaloid extract of *Delphinium barbeyi* collected near Manti, Utah (2.0 mg of MSAL-type alkaloids/kg).14 This was not unexpected because the MDL- and MSAL-type alkaloid contents and ratios in *D. barbeyi* collected near Manti, Utah, are similar to the contents and ratios in the 4 collections of plants containing MSAL-type alkaloids in the present study.

The LD$_{50}$ of the total alkaloid extract from Afton, Wyo (42.7 mg of total alkaloids/kg), was lower than the LD$_{50}$ values of the other 3 extracts from plants that lack the MSAL-type alkaloids, which were all similar (53.3 to 60.8 mg of total alkaloids/kg). The LD$_{50}$ values of all 4 of these extracts were lower than what one may have expected because the LD$_{50}$ of individual MDL-type alkaloids range from 70 to 120 mg/kg.29 This discrepancy may be attributable to the presence of other MDL-type alkaloids that are yet to be purified (and for which LD$_{50}$ values have not been determined) and that may have a lower LD$_{50}$ than the MDL-type alkaloids evaluated to date. Alternatively, mixtures of MDL-type alkaloids may act synergistically to decrease the LD$_{50}$ values of these extracts, as suggested by the report of Welch et al.9 Another possibility is the presence of trace amounts...
of other compounds in the total alkaloid extract, which may potentiate toxicity.

In the present study, cattle were given doses of 1 collection of each Delphinium occidentale chemotype to determine whether there were differences in toxic effects of the chemotypes. Cattle responses to doses of the plant materials that did or did not contain MSAL-type alkaloids were markedly different; the plant material lacking MSAL-type alkaloids elicited no clinical response, whereas both heart rate and a measure of muscle function (ie, time to collapse when exercised) were greatly affected by the plant material containing MSAL-type alkaloids. The results from our study corroborate data from previous studies,\(^2\),\(^3\) which indicated that an increase in heart rate is a good indicator of toxicosis. Furthermore, the present study revealed that exercise-induced muscle fatigue (as measured by the time from start of exercise to collapse) was a reliable quantitative measure of larkspur toxicity. There were 3 observations worthy of particular mention from the cattle experiments. The first observation was the time and order of collapse of the 4 animals that received 2 separate doses of treatment A (the plant material containing the MSAL-type alkaloids). After the second dose, all 4 animals collapsed in the same order as they did following the first dose and 3 of the 4 animals collapsed within 3 minutes of the time at which they collapsed following the first dose. This indicates that exercise-induced muscle fatigue (as measured by the time from start of exercise to collapse) is a sensitive measure of larkspur toxicosis and is reproducible among animals. The second observation was the fact that no clinical signs of poisoning were observed in cattle after administration of a dose of treatment B (plant material that did not contain MSAL-type alkaloids), even when a high dose (150.4 mg of total alkaloids/kg) was administered. The third observation was the fact that the effective dose (8.8 mg of MSAL-type alkaloids/kg) of the \(D\) occidentale collection from near Victor, Idaho, required to elicit clinical signs was similar to the effective dose (8.0 mg of MSAL-type alkaloids/kg) of the \(D\) barbeyi collection from Manti, Utah.\(^2\),\(^3\) Both collections have similar ratios of MDL- to MSAL-type alkaloid concentrations, suggesting that the MDL-type alkaloids potentiate the toxic effects of \(D\) occidentale as they do in \(D\) barbeyi.\(^2\),\(^3\) In addition, it suggests that the norditerpenes MSAL-type and not other undefined secondary compounds are responsible for the observed toxic effects.

The results of the present study indicated that the relative toxic effects of the 2 \(D\) occidentale chemotypes (determined by the presence or absence of the MSAL-type alkaloids) are significantly different in cattle. It should be mentioned, however, that these data were derived after cattle were given a single bolus dose of ground plant material. A single bolus dose of ground plant material does not adequately represent the conditions under which animals graze, ingest larkspur, and are subsequently poisoned. There are 3 distinct thresholds involved in tall larkspur toxicosis.\(^2\),\(^3\) The first is a subclinical toxicosis that results in reduced tall larkspur consumption for 1 to 3 days, but with no overt clinical signs or decreased consumption of other forage. The second is a short-term toxicosis that results in overt clinical signs including decreased forage consumption for several days; however, no long-term effects are observed. The third is a potentially fatal toxicosis, in which severe clinical signs develop that may result in death. Furthermore, grazing cattle typically consume larkspur in a cyclic fashion over several days, with 1 or 2 days of high consumption followed by several days of reduced consumption during a period of detoxification.\(^2\),\(^3\) Thus, future studies are needed to investigate various dosing regimens and forms of plant material that may mimic actual grazing situations before definitive livestock management recommendations are made. In addition, further studies should include plant collections representing both chemotypes from other geographic locations to determine whether the relative responses to administration in cattle and mice are similar to those detected in the present study. Plants of each chemotype from different locations may have slight differences in their alkaloid profiles, which may potentially influence toxicity.

The results of the present study confirmed that the toxic effects caused by MSAL-type alkaloids such as MLA are greater than those caused by MDL-type alkaloids; thus, MSAL-type alkaloids are the primary factors responsible for the toxicity of larkspur. Consequently, ingestion of \(D\) occidentale that contains MSAL-type alkaloids is a distinct risk to the well-being of cattle. Alternatively, \(D\) occidentale that contains only the MDL-type alkaloids is much less toxic, and the corresponding risk to grazing cattle appears to be much lower. Further research in cattle will be necessary to refine grazing management recommendations when either of the \(D\) occidentale chemotypes is present on rangelands. It is important to mention that taxonomic classification of larkspur plants alone is not a good indicator of the toxic risk to grazing cattle.

\begin{itemize}
  \item a. Gehl Mix-Allow model 55, Gehl Co, West Bend, Wis.
  \item b. Thomas Scientific, Wiley Mill, Swedesboro, NJ.
  \item c. Simonsen Laboratories Inc, Gilroy, Calif.
  \item d. Octal Bioamp amplifier, AD Instruments Inc, Colorado Springs, Colo.
  \item e. Red Dot model 2670 repositionable monitoring electrodes, 3M Corp, Saint Paul, Minn.
  \item f. Henkel Consumer Adhesive Inc, Avon, Ohio.
  \item g. ADI Chart Software Package, AD Instruments Inc, Colorado Springs, Colo.
  \item h. Proc Probit SAS, version 9, SAS Institute Inc, Cary, NC.
  \item i. SAS, version 9.2, SAS Institute Inc, Cary, NC.
\end{itemize}

\section*{References}

6. Olsen JD, Manners GD, Pelletier SW. Poisonous proper-
ties of larkspur (Delphinium spp.). Collect Bot (Barcelona) 1990;19:141–151.