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Short communication

Comparison of the serum toxicokinetics of larkspur toxins in cattle, sheep and goats

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ABSTRACT

Larkspurs (*Delphinium* spp.) are a major cause of cattle losses in western North America, whereas sheep are thought to be resistant to larkspur toxicosis. Goats are often used as a small ruminant model to study poisonous plants. In this study, we compared the serum toxicokinetic profile of toxic larkspur alkaloids from *Delphinium barbeyi* in cattle, goats, and sheep. The results from this study indicate that kinetic differences could partially explain species differences in susceptibility to larkspur toxicosis.

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Larkspur plants (*Delphinium* spp.) are abundant in western North America (Burrows and Tyrl, 2013). There are over 60 wild species of larkspur in North America (Kingsbury, 1964; Knight and Walter, 2001). Larkspurs are acutely toxic to cattle, and as such they cause a significant number of cattle death losses every year (Nielsen and Ralphs, 1988), especially on foothill and mountain rangelands (Pfister et al., 2002). There are three types of larkspurs categorized primarily by mature plant height and distribution as tall, low, and plains larkspurs (Pfister et al., 1999). The toxicity of larkspurs is due to norditerpenoid alkaloids including two predominant types, the *N*- (methylsuccinimido) anthranoyllycoctonine (MSAL)–type including methyllycaconitine (MLA) and the non MSAL-type including the 7, 8-methylenedioxylycoctonine (MDL)-type including deltaline (Panter et al., 2002; Pfister et al., 1999).

Larkspurs have been shown to be toxic to horses, although horses will not voluntarily consume sufficient quantities of larkspurs to become poisoned (Marsh and Clawson, 1916). Sheep have been shown to be quite resistant to larkspurs (Fleming et al., 1923; Marsh and Clawson, 1916; Olsen, 1978). Consequently, cattle are the primary livestock species associated with larkspur toxicosis, and thus the effect of larkspurs on cattle has been the focus of the majority of the research efforts regarding larkspur toxicosis. In this study, we compared the serum toxicokinetic profiles of toxic larkspur alkaloids from *Delphinium barbeyi* in cattle, goats, and sheep. Cattle and sheep are the two livestock species that are most often exposed to larkspur-infested rangelands, with cattle being more susceptible and sheep more resistant to larkspur toxicosis. It is possible that differences in the toxicokinetic profile of the toxic larkspur alkaloids could account for some of the differences in susceptibility to larkspur toxicosis between these two livestock species. We also included goats, as goats are often used as a small ruminant model to study poisonous plants (Davis et al., 2015; Panter et al., 1999; Welch et al., 2015b).

Delphinium barbeyi was collected in the early flowering stage during July 2003 near Manti, Utah (N lat 39° 03.154′, W long 111° 30.752′, at an elevation of approximately 3000 m; Poisonous Plant Research Laboratory 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, USA). 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 in the plant material are stable under these conditions. The plant material was analyzed for total norditerpenoid alkaloid content and MSAL-type alkaloid content immediately prior to dosing using a Fourier-transform infrared

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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. This collection of *D. barbeyi* 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 norditerpenoid alkaloids in this collection of *D. barbeyi* were primarily MLA and deltaline (Welch et al., 2015a).

All animal work was done under veterinary supervision with the approval and supervision of the Utah State University Institutional Animal Care and Use Committee. Five Black Angus steers weighing 601 ± 36 kg were maintained on alfalfa hay. The steers were placed in metabolism cages 48 h prior to dosing. A 16 gage indwelling catheter was placed in the jugular vein of each steer as previously described (Green et al., 2009b). A single dose (2 g plant/kg BW) of dried, finely ground larkspur was administered via oral gavage in approximately 10 L of tap water. The catheters were used to sample venous blood at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 18, 24, 36, 48, 60, 72, and 96 h after dosing. Four Western White-faced sheep weighing 42 ± 4 kg and four Spanish goats weighing 23 ± 1 kg were maintained on alfalfa hay in their normal outdoor paddocks. A single dose (2 g plant/kg BW) of dried, finely ground larkspur was administered via oral gavage in approximately 2–3 L of tap water. Blood was collected via jugular venipuncture at 0, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 29, 34, 48, 53, 58, 72, 82, and 96 h after dosing. Serum was separated from red blood cells and stored frozen at −20 °C. The serum was analyzed for MLA and deltaline as described previously (Welch et al., 2015a) with the addition of MS/MS parameters to quantitate deltaline, which include monitoring the MS/MS *m/z* 508 to 476 transition during 4.5–5.5 min in the chromatographic run.

Data are expressed as the mean ± standard deviation. The alkaloid concentrations were plotted using SigmaPlot for Windows (version 12.5, SPSS Inc., Richmond, CA). Statistical analyses were performed using SigmaPlot for Windows (version 12.5, SPSS Inc., Richmond, CA). Statistical comparisons were made using ANOVA with a post-hoc test of significance (Bonferroni) between individual groups. Differences were considered significant when $P < 0.05$. Kinetic profiles were analyzed using standard pharmacokinetic software (PK Solutions 2.0, Summit Research Services, Montrose, CO; 1998). The following parameters were determined: absorption half-life ($A_{1/2}$), elimination half-life ($E_{1/2}$), maximum alkaloid concentration (C_{max}), time to maximum serum concentration (T_{max}), mean residence time (MRT), and area under the curve ($AUC_{0-\infty}$).

All three species of livestock evaluated in this study were dosed with *D. barbeyi* at a dose of 2.0 g plant material/kg BW, which corresponded to 8.0 mg MSAL-type alkaloids/kg BW and 32 mg total norditerpenoid alkaloids/kg BW. This dose has been shown to be well tolerated by cattle, causing significant muscle weakness in some cattle but not mortality (Green et al., 2014). In this study, four of the five cattle showed minimal clinical signs of poisoning as they were restrained in the metabolism crates for the duration of the study. However, one of the five cattle had significant muscle weakness and was given neostigmine 12 h after dosing to prevent possible mortality. Neostigmine can help cattle sustain adequate muscle function after exposure to a toxic dose of larkspur, and help prevent lethal outcomes (Green et al., 2009a). Two of the four goats demonstrated very minimal clinical signs of poisoning, such as minor lethargy 24 h after dosing but appeared completely normal by 28 h after dosing. The other two goats showed no adverse effects. Two of the sheep showed some clinical signs of poisoning, while the other two sheep showed no adverse effects. One of the affected sheep showed minor muscle weakness and lethargy at 34 h post dosing but was normal by 48 h post dosing. The other affected sheep showed more significant muscle weakness from 34 to 58 h post dosing. This was a very surprising finding as sheep are traditionally thought to be resistant to poisoning by larkspur.

A comparison of the serum MLA and deltaline concentrations were made between the three livestock species evaluated in this study (Fig. 1 and Table 1). There was a significant difference in the serum MLA concentration over time between these three animal species ($P < 0.001$). Cattle were found to have a significantly higher concentration of serum MLA than goats ($P < 0.001$) and sheep ($P < 0.001$), while there was no difference in the serum MLA concentrations between sheep and goats ($P = 1.0$) (Fig. 1A). Cattle had a significantly higher C_{max} and AUC for MLA than goats and sheep (Table 1). However, there were no statistically significant differences in the T_{max}, elimination half-life, absorption half-life, or mean residence time for MLA between any of the species (Table 1).

There was little difference in the serum deltaline concentrations between the three species (Fig. 1B). There was a difference between cattle and goats ($P = 0.029$), which is likely explained by the fact that no serum deltaline was observed after 60 h in the goats whereas deltaline was still detected in the serum of the cattle up to 72 h. There were no statistically significant differences observed in any of the deltaline kinetic parameters between any of the three

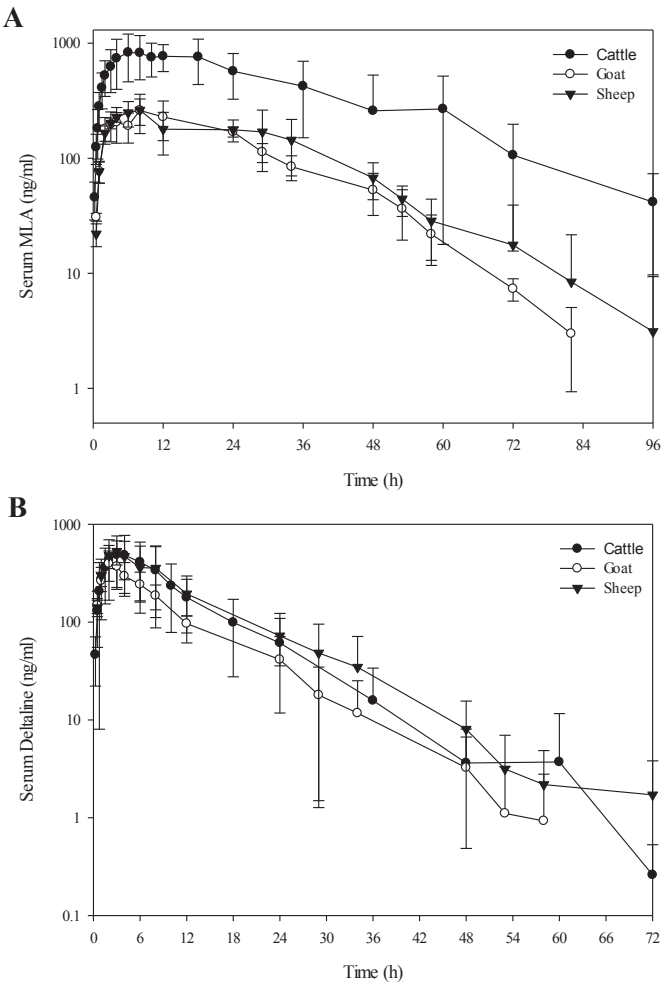

Fig. 1. Comparison of the larkspur serum alkaloid concentrations between cattle, goats, and sheep. Data represent the mean ± SD for (A) methyllycaconitine (MLA) and (B) deltaline. All animals were dosed with *Delphinium barbeyi* at 2.0 g plant material/kg BW. All zero value data points were omitted due to the logarithmic scale.