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An Evaluation of the Susceptibility of Goats to Larkspur Toxicosis

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An Evaluation of the Susceptibility of Goats to Larkspur Toxicosis

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Abstract:

Larkspurs (*Delphinium* spp.) are a major cause of cattle losses in western North America, whereas sheep have been shown to be resistant to larkspur toxicosis. Goats are often used as a small ruminant model to study poisonous plants, even though they can be more resistant to some poisonous plants. It is not known how susceptible goats are to the adverse effects of larkspurs. In this study, we evaluated the susceptibility of goats to larkspur toxicosis by performing a dose-response study. We dosed goats with *D. barbeyi* collected near Cedar City, Utah at 3.3, 4.4, 6.6, 8.8 and 10.0 g plant material per kg body weight. None of the goats, at any of the doses, exhibited clinical signs typical of larkspur poisoning, including no observable muscle weakness. We conclude that goats are resistant to larkspur toxicosis, and thus it is very unlikely that goats would be poisoned by larkspur.

Keywords: Larkspur, Goat, Delphinium, Caprine

Introduction:

Larkspur plants (*Delphinium* spp.) are abundant in western North American mountain rangelands (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; Pfister et al., 2002). The toxicity of larkspurs is due to the more than 18 norditerpenoid alkaloids that are often grouped into two predominant types, the *N*- (methylsuccinimido) anthranoyllycoctonine (MSAL)–type including methyllycaconitine (MLA) and the non MSAL-type including deltaline a 7, 8-methylenedioxylycoctonine (MDL)-alkaloid (Pfister et al., 1999; Panter et al., 2002). The norditerpenoid alkaloids each have varying degrees of affinity for the nicotinic acetylcholine receptors (nAChR)(Macallan et al., 1988; Dobelis et al., 1999). Previous research has demonstrated that the physiological effects of MLA, one of the more abundant toxic alkaloids in many species of larkspur, was attributable to its high affinity to nAChR in muscle and nervous systems (Benn and Jacyno, 1983; Stegelmeier et al., 1998). Methyllycaconitine has been shown to be a potent and selective competitive antagonist with nanomolar affinity at alpha 7 nAChR and micromolar affinity at other nAChR (Ward et al., 1990; Alkondon et al., 1992; Lopez et al., 1998; Daly, 2005).

Larkspurs have been shown to be toxic to horses, although horses are not likely to voluntarily consume sufficient quantities of larkspurs to become poisoned (Marsh and Clawson, 1916). Sheep have been shown to be quite resistant to larkspurs (Marsh and Clawson, 1916; Fleming et al., 1923; 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. Although the mechanism of action for larkspur alkaloids has been described, there is little information on the variation in animal responses to larkspur alkaloids. Anecdotal observations and pilot studies in cattle (Green et al., 2008) indicated that there is variation in response to a debilitating dose of larkspur. Previous research using various strains of mice demonstrated that there is a fairly large animal to animal variability to the toxicity of the larkspur alkaloid MLA, and that this variation found across numerous strains of mice can be attributed to the abundance of nAChR in those strains (Welch et al., 2009). The susceptibility of cattle to larkspur alkaloids acting at nAChR may be due to genetic differences, which cause changes in nAChR number or function (Green et al., 2014). Goats are often used as a small ruminant model to study poisonous plants, even though they can be more resistant to some poisonous plants. It is not known how susceptible goats are to the adverse effects of larkspurs. Thus, the objective of this study was to perform a dose-response study to evaluate the susceptibility of goats to the adverse effects of larkspur.

Materials and Methods:

Delphinium barbeyi was collected in the early flowering stage during July 2007 at an elevation of approximately 3,300 m near Cedar City, Utah, (N lat 37º 40.223′, W long 112º 49.335'; Poisonous Plant Research Laboratory collection No. 07-06). 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 (Cook et al., 2009). The plant material was analyzed for total norditerpenoid alkaloid content and MSAL-type alkaloid content immediately prior to dosing using a flow injection-mass spectrometry method (Gardner et al., 1999). This

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collection of *D. barbeyi* contained 19.3 mg/g of total alkaloids with 13.1 mg/g of MSAL-type alkaloids. The norditerpenoid alkaloids in this collection of *D. barbeyi* were primarily MLA and deltaline (Welch et al., 2010).

All animal work was done under veterinary supervision with the approval and supervision of the Utah State University Institutional Animal Care and Use Committee. One-year old Spanish wether goats weighing 45 ± 10 kg were maintained on their normal basal alfalfa hay diet in their normal outdoor paddocks, with ad libitum access to water and a common mineral salt block. These goats were naïve to larkspur. Dried, finely ground larkspur was administered via oral gavage in approximately 2-3 liters of tap water at 3.3, 4.4, 6.6, 8.8 and 10.0 g plant material per kg BW at 7 am. Three goats were also dosed at 10.0 g/kg twice in the same day, at 7 am and 3:30 pm.

Blood was collected via jugular venipuncture at 0, 8, and 24 h after the 7 am dose. Serum was separated from red blood cells and stored frozen at -20ºC. The serum was analyzed for MLA as described previously (Welch et al., 2015). Additionally, at 8 and 24 h post-dosing the goats were walked back and forth in a 40 m alley for 20 min to facilitate exercise-induced muscle weakness that is associated with larkspur poisoning (Green et al., 2014).

Results and Discussion:

In previous research studies using goats to assess larkspur toxicosis, including the toxicokinetics of the larkspur alkaloids, a collection of *D. barbeyi* from near Manti, UT was primarily used. That collection of larkspur plants contained mostly MLA and deltaline at a concentration of 4 mg MLA and 12 mg of deltaline/g of plant material. In a toxicokinetic study (Welch et al., 2016), goats were dosed with 2.0 g plant/kg BW. Similarly, in another study goats were dosed with 2.2 g plant/kg BW (Lee et al., unpublished observations). These doses of plant material corresponded to a dose of 8 and 8.8 mg of MLA/kg BW respectively. None of the goats in either of those studies exhibited any signs of larkspur toxicosis. Thus, for this study much larger doses of toxic alkaloids were administered. Goats were dosed with a collection of *D. barbeyi* from near Cedar City, UT that contained MLA at a concentration of 13.1 mg/g plant. Thus, the doses of 3.3, 4.4, 6.6, 8.8 and 10.0 g/kg corresponded to a dose of 43, 58, 86, 115, and 131 mg MLA/kg BW. However, even with these very large doses of larkspur plant, and corresponding MLA concentrations, the goats did not exhibit any typical signs of larkspur poisoning, including muscle weakness (Table 1).

Table 1: Dose-response evaluation of larkspur in goats

Plant Dose (g/kg)	**MSAL Dose (mg/kg)**	**Clinical Signs Observed**
3.3	43	None
4.4	58	None
6.6	86	None
8.8	115	None
10	131	None
10 x 2*	262	None

Note: Doses were administered based on the amount of plant material (g/kg).
*This group received one dose of 10 g plant material/kg BW at 7 am and a second dose 3:30 pm.

The goats were exercised by walking them back and forth in a 40 m alley for 20 min to exacerbate muscle fatigue at 8 and 24 h post dosing. All four goats that had been dosed with 10.0 g plant/kg BW were able to easily walk for 20 min at 8 h after dosing. Additionally, blood was collected from these goats at this time. None of the goats exhibited any signs of muscle weakness as they were being handled during the blood collection process. Eight hour post dosing is likely the best time to evaluate goats for adverse effects from larkspur, as previous work (Welch et al.,

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2016) demonstrated that the time to maximum serum alkaloid concentration (T_{max}) in goats is approximately 8 h post dosing.

Due to the fact that no adverse effects were observed from a single dose, 3 goats were dosed again after walking at 8 h with another dose of 10.0 g plant/kg BW. At 24 h after the first dose, or 15.5 h after the second dose, one of the goats was found dead. However, there was no indication that the goat died from larkspur exposure, as there was no indication that the goat had experienced severe muscle fatigue. It is more likely that this goat experienced adverse effects from the large amount of plant material and water that were pumped into its rumen the day before. Although this was not confirmed via necropsy and post-mortem analysis, and is thus speculation on our part. The two remaining goats did not have any observable signs of muscle weakness. They did become very reluctant to walk after about 8 min of walking; however, they did not show any signs of muscle weakness or tremors as is normally observed in cattle that are no longer able to walk after being poisoned by larkspur. Their reluctance to walk may be attributed to them not feeling well from the large volume of material pumped into their rumen. Previous research with cattle, has shown that administering multiple doses of larkspur in a day will exacerbate the adverse effects of the larkspur (Welch et al., 2015). However, even after administering two doses of 10.0 g/kg in one day, there were no obvious signs of muscle weakness in the goats.

Of important note, the total amount of plant material administered to these three goats was 753 ± 80 g of plant. In a previous study, where cattle were dosed with this same collection of *D. barbeyi*, a dose of 665 ± 13 g of plant material was shown to cause adverse signs in cattle including muscle weakness to the point that they could not walk (Welch et al., 2010). This highlights the large difference in the susceptibility of cattle and goats to larkspur toxicosis, as these goats (45 kg

BW) were administered more plant material than what is a toxic dose to a full-grown steer (500 kg BW), without any signs of poisoning.

There are conflicting reports in the literature regarding the susceptibility of sheep to larkspur toxicosis. Some early reports suggested that larkspur could poison sheep in a range setting (Wilcox, 1897; Chesnut and Wilcox, 1901). However, much of the data presented in those studies were circumstantial. In controlled follow up studies, the researchers were unable to poison sheep with various species of larkspur, even when doses 3-5 times greater than doses toxic to cattle were administered (Marsh and Clawson, 1916; Fleming et al., 1923). Later researchers were able to poison sheep with larkspur in a controlled setting, however, at doses ranging from 4-6 times that required to poison/kill cattle (Marsh et al., 1934; Olsen, 1978). Research conducted in our laboratory, has shown that sheep can tolerate doses of the *D. barbeyi* collected near Manti, Utah at 6.4 g/kg BW (unpublished observations), which is 2.3 times greater than a lethal dose reported for cattle (Welch et al., 2012). Taken together, these studies suggest that sheep are much more resistant to larkspur toxicosis than cattle.

One important note to keep in mind is that recent research is bringing to light the tremendous variation in toxicity of different larkspurs, even within a single species at one location versus another (Cook et al., 2009; Cook et al., 2015; Cook et al., 2017). Consequently, it is possible that a specific population of a larkspur species at one location may be toxic, however, that same species, at another location, may be much less toxic (Welch et al., 2010; Cook et al., 2011; Cook et al., 2015). Consequently, the conflicting reports regarding the toxicity of larkspurs in the literature could be in part due to variations in the alkaloid composition of larkspurs from location to location.

Previously, we compared the toxicokinetics of the larkspur alkaloids in cattle, sheep and goats (Welch et al., 2016). The data from that study suggested that one possible explanation for the

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increased resistance of goats and sheep to poisoning by larkspur could be due to differences in the toxicokinetics of the toxic alkaloids. When administered the same dose, cattle had approximately a 3-fold higher maximum serum concentration (C_{max}) and a 4.5-fold higher area under the curve (AUC) than goats and sheep. This indicates that the toxic alkaloids, including MLA, are more bioavailable in cattle. An increased concentration of the alkaloids at the neuromuscular junction would increase the blockade of the nAChR and consequently exacerbate the toxic effect. Also, cattle had a longer elimination half-life (18 vs 11 and 13 h), therefore the toxic alkaloids remained in circulation in cattle longer than in goats and sheep, which would also increase the adverse effects of the toxins.

In addition to differences in toxicokinetics, there are other possible reasons for the differences in susceptibility to larkspur toxicosis between cattle, sheep and goats; including toxicodynamic differences, such as differences in binding efficiency of the larkspur alkaloids at the nAChR in the neuromuscular junction. Species differences in the affinity of MLA for nAChR have been demonstrated (Ward et al., 1990; Stegelmeier et al., 1998). Therefore, it is possible that the larkspur alkaloids may have a lower affinity for goat muscle-type nAChR compared to cattle, and likely similar to sheep. The potential toxicodynamic differences that may also account for species differences in susceptibility to larkspur toxicosis, require further investigation.

In previous reports, cattle experiencing adverse effects of larkspur have been found to have serum MLA concentrations between 800 to 930 ng/mL (Welch et al., 2015; Welch et al., 2016). In this study, the goats had a serum MLA concentration ranging from 608-1342 ng/mL at 8 h post dosing, in a dose-response manner (Figure 1). Consistent with previous reports (Welch et al., 2016), the serum MLA concentration was higher at 8 h compared to 24 h after a single dose. The goats that were administered two doses had an even higher serum MLA concertation at the 24 h time point (Figure 1). This data demonstrates that the goats dosed in this study had similar, or even higher, serum MLA concentrations than poisoned cattle, which further suggests that in addition to toxicokinetic differences in goats, there is likely a toxicodynamic difference in how goats respond to the presence of the toxic larkspur alkaloids, such as MLA.

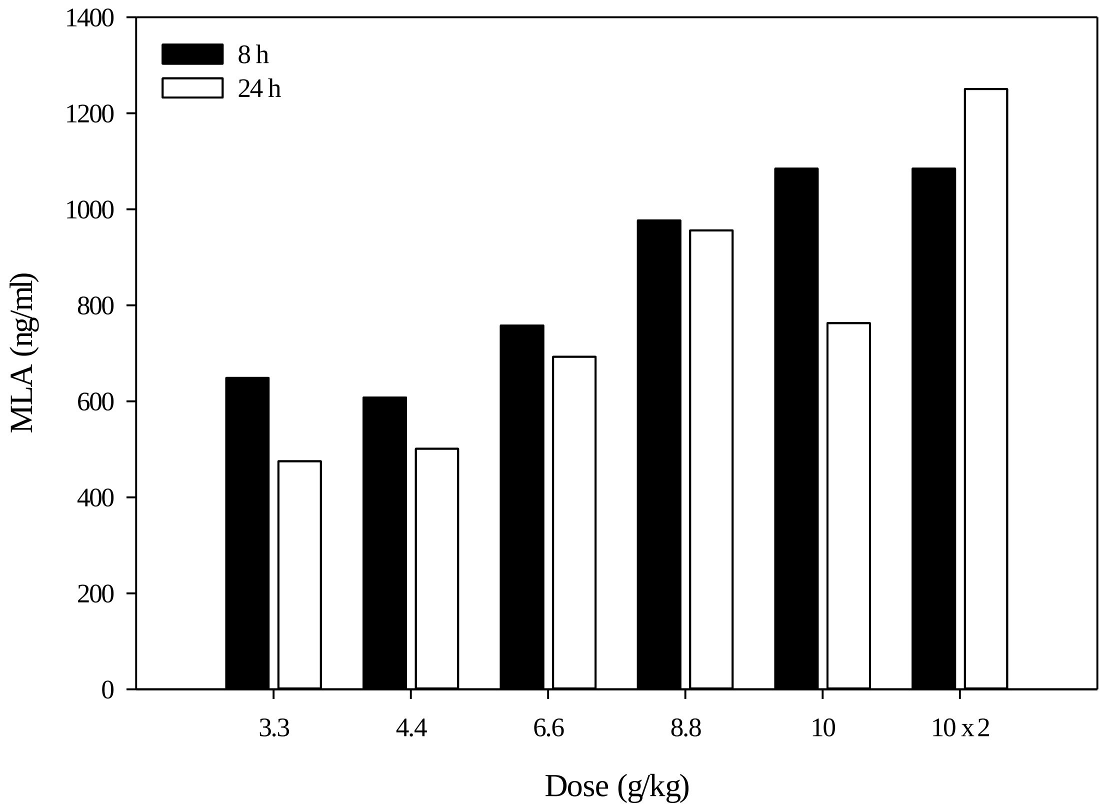

Figure 1. Serum methyllycaconitine (MLA) concentration in goats dosed with larkspur. The time represents the time (h) after dosing.

In summary, the data presented in this study demonstrate that goats are very resistant to the adverse effects of larkspurs. Further work is required to determine what factors, other than differences in the toxicokinetics of the larkspur alkaloids, are responsible for their resistance to larkspur toxicosis.

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Conflicts of Interest:

None.

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