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Benedict T. Green

USDA-ARS, ben.green@usda.gov


Jim Pfister

Dale Gardner

Kevin Welch

Daniel Cook

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Cover Page Footnote

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Dynamics of larkspur (*Delphinium barbeyi*) pellet consumption and tolerance of the inhibitory effects of larkspur alkaloids on muscle function in cattle.

Benedict T. Green, James A. Pfister, Dale R. Gardner, Kevin D. Welch, and Daniel Cook

USDA-ARS Poisonous Plant Research Laboratory, Logan UT 84341
Email: Ben.Green@usda.gov

Abstract: Larkspurs (*Delphinium* spp.) are toxic native plants on foothill and mountain rangelands in western North America, which poison cattle grazing on those rangelands. The purpose of this study was to examine in a laboratory setting, the subclinical effects of larkspur intake and toxicosis, by allowing larkspur susceptible and resistant cattle to self-select the amount of larkspur consumed in pellet form. We hypothesized that there would be differences in short term (9 – 11 day) pellet consumption between susceptible and resistant animals. Two trials were completed, each with larkspur resistant and susceptible Angus steers, and larkspur-alfalfa pellets at a 12% and 6% larkspur for trial 1 and trial 2, respectively. There were no differences in pellet consumption between the two groups in either trial. The cattle were averted to the 12% larkspur-containing pellet in trial 1. During trial 2, the susceptible and resistant steers differed in serum concentrations of methyllycaconitine (MLA) on days 9 to 12 but did not have significantly different exercise times (38.0 ± 3 min and 27.2 ± 6.5 min for resistant and susceptible steers, respectively). Larkspur alkaloids were potent aversive agents in cattle when fed as a 12% larkspur-containing pellet. The lack of differences in responses between susceptible and resistant cattle were attributed to the development of pharmacodynamic tolerance to larkspur alkaloids by the actions of larkspur alkaloids at nicotinic acetylcholine receptors.

Key Words: Angus, *Delphinium*, methyllycaconitine, diterpenoid alkaloid, larkspur, poisoning/toxicity

Introduction: Larkspurs (*Delphinium* spp.) are native plants on foothill and mountain rangelands in western North America. On these rangelands, larkspurs are grazed by cattle during the spring and summer (Pfister et al., 1997). The clinical outcome of larkspur poisoning is dependent on the genetic background of the cattle, the amount of plant consumed, the rate of consumption, and the concentrations of toxic norditerpene alkaloids in the plants (Pfister et al., 1999, 2002; Green et al., 2019). Identifying cattle which are resistant to larkspur intoxication might improve grazing animal welfare on these rangelands and reduce economic losses to poisonous plants.

Research suggests that there are significant differences between cattle breed responses to larkspur (Green et al., 2014). When four breeds of cattle representing *Bos taurus* and one breed representing *Bos indicus* were orally dosed with dried ground larkspur and exercised by walking 24 hours after dosing, *Bos taurus* dairy breeds were much more resistant to poisoning than *Bos taurus* beef breeds or a *Bos indicus* breed. In that study, Angus cattle were unique because they had a large distribution of individual walk times with some of the Angus unaffected/resistant to the larkspur dose. Other Angus cattle had exercise intolerance and were susceptible to the larkspur. As a result of this work, larkspur-resistant and susceptible Angus cattle were selected for use in this study.

Larkspur poisoning in cattle is the result of norditerpene alkaloid blockade of nicotinic acetylcholine receptors (nAChR) at post-synaptic neuromuscular junctions (Aiyar et al., 1979; Benn and Jacyno, 1983; Green et al., 2013b; Welch et al., 2013). This blockade of nAChR leads to the clinical signs of larkspur poisoning; they include: muscle weakness, trembling and lack of coordination, rapid heart rate, sternal recumbency (i.e., lying on brisket and unable to stand) followed by lateral recumbency (i.e., unable to maintain an upright posture even when lying down), bloating, and death.

There are two principal types of norditerpene alkaloids produced by the plant, the *N*- (methylsuccinimido) anthranoyllycoctonine type (MSAL), such as methyllycaconitine (MLA), and the non-MSAL-type such as deltaline (Green et al., 2019). Both types of alkaloids are found in most larkspur species which poison cattle, but MSAL-type alkaloids are much more toxic than non-MSAL-type alkaloids and are thought to be the driving force behind plant toxicity (Gardner et al., 1999; Pfister and Gardner, 1999; Pfister et al., 2002). Recent research has also shown non-MSAL-type alkaloids exacerbate the toxicity of MSAL-type alkaloids in mice and cattle (Cook et al., 2015; Welch et al., 2008, 2010, 2012), and that there is substantial animal-to-animal variation in breeds of cattle for resistance and susceptibility to larkspur (Green et al., 2014, 2019). The clinical signs of poisoning and serum alkaloid toxicokinetic parameters from these experiments have been consistent whether they are based upon a single dose or a series of four oral doses given 12 hours apart (Green et al., 2011, 2012, 2013a, 2019; Welch et al., 2015a,b).

However, there is no information available in the literature about short term larkspur exposure in an experimental setting nor is there information about the responses of short term-exposed larkspur resistant or susceptible cattle.

Researchers at the Poisonous Plant Research Laboratory have performed field studies with larkspur susceptible and resistant cattle (Pfister et al., 2018). Results from our field work suggest that there are differences in diet selection between larkspur susceptible and resistant cattle with larkspur resistant cattle consuming more larkspur. Herbivores are known to self-regulate their intake of plant secondary metabolites to keep the physiological responses to plant alkaloids within a tolerable limit (Foley and Moore 2005; Iason 2005). This self-regulation presents itself as temporal cyclicity in that the animals follow periods of ingestion of toxin-containing food followed by periods of low intake that permits recovery from the effects of the plant secondary metabolites (Pfister et al., 1999, 2002, 2011; Stapley et al., 2000). For example, Pfister et al., (2018) reported cattle grazing on larkspur containing rangelands with serum MLA concentrations from 613 to 1162 ng/ml displayed signs of poisoning. It has been hypothesized that cyclic consumption of larkspur enables cattle to regulate body alkaloid concentrations below a toxic threshold (Pfister et al., 1997). Further research is needed to understand the effects of sub-acute larkspur intake in cattle. Thus, the purpose of this study was to examine, in a laboratory setting, the subclinical effects of larkspur intake and toxicosis rather than acute effects, by allowing larkspur susceptible and resistant cattle to self-select the amount of larkspur consumed. We hypothesized that there would be differences in short term consumption patterns between susceptible and resistant animals.

Materials and Methods:

Animals

All procedures for these trials were conducted under veterinary supervision and were given prior approval by the Institutional Animal Care and Use committee (IACUC) at Utah State University.

Trial 1 (12% larkspur pellet)

Twelve, 1 yr. old Angus steers were purchased from a commercial herd in western Montana. At 10 months of age, steers were screened for susceptibility to larkspur poisoning using an oral dosing and exercise protocol (Cook et al., 2011, Green et al., 2014). Resistant animals were categorized as those with an exercise time of ≥ 20 minutes; susceptible animals were those with an exercise time of < 10 minutes. Mean (\pm SEM) exercise time for steers in Trial 1 was 33.5 ± 2.3 minutes and 7.3 ± 2.2 minutes for resistant and susceptible steers, respectively. When this trial began steers ($n=6$ /group) weighed 506 ± 33 kg and 507 ± 35 kg for resistant and susceptible steers, respectively.

Larkspur consumption by grazing cattle often ranges from 6 to 12% during summer (Pfister et al., 1988a,b, 1991). A 12% larkspur pellet was made by a commercial feed mill with *D. barbeyi* harvested from mountain rangelands in central Utah (N lat 39° 03' W long 111° 30'), air-dried, ground to pass a 2 mm screen, and stored at ambient temperature in plastic bags until used. The ground larkspur (12%) was mixed with ground alfalfa hay (88%) to provide the 12% pellet. Subsamples of the larkspur-containing pellets were taken during each day of the trial, composited, and used for alkaloid and nutritional analysis. The pellets had an average alkaloid concentration of 0.69 mg/g MSAL-type alkaloids, and 2.2 mg/g total alkaloids.

Steers were placed into 3 x 6 m indoor pens for a 9-day adaptation period and given ad libitum access to commercial alfalfa pellets during the autumn season. Alfalfa pellet intake was measured over a 6-day period before the larkspur-containing pellets were provided to the animals, after which each day the steers were provided ad libitum larkspur-containing pellets; pellets were weighed into feeding troughs at 0715, replenished as needed during the day, and refusals weighed out at 0700 the following morning.

Trial 2 (6% larkspur pellet)

This trial used different steers than Trial 1; these steers were 1 yr. old Angus steers purchased from commercial herds in western Montana. The steers weighed 416 ± 16 kg and 445 ± 18 kg for resistant and susceptible steers, respectively (n=5/group). The steers had been gentled, trained to walk on a lead rope, and tested to determine their response to larkspur. The mean times to become exercise intolerant were 31.5 ± 2.7 min and 0.4 ± 0.4 min, respectively for resistant and susceptible steers.

Larkspur-containing pellets (6%) were commercially made and modified in that 5% molasses was added to the pellets in addition to 6% ground larkspur (*D. barbeyi*) from the same location as in Trial 1. Steers were fed commercial alfalfa pellets for a 9-day baseline period in order to compare alfalfa pellet intake with intake when offered larkspur-containing pellets during the autumn season. In addition, because the larkspur-containing pellets had 5% molasses, before offering the steers the larkspur-containing pellets, they were given a 6-day adaptation period with commercial alfalfa pellets with 5% molasses added. After this adaptation period, the larkspur-containing pellets were offered ad libitum to the steers for 11 days. Pellets were always offered in excess of what steers consumed the previous day, and refusals weighed the following day. During the period when larkspur-containing pellets were offered, blood samples were taken each morning at 0700 via jugular venipuncture. Blood was also collected if animals showed any clinical signs during the trial. Further, on day 9 of the larkspur-containing pellet period, steers were exercised at 0800 to 0900 for 30 min by walking them at 4-6 kph to

assess their state of intoxication; blood samples were taken from steers showing clinical signs during the exercise period.

Nutritional analysis of alfalfa and larkspur pellets

Subsamples of pellets were taken on a daily basis throughout the trials, composited, ground to pass a 1-mm screen in a Wiley mill (Thomas Scientific, Swedesboro, NJ), and analyzed for dry matter (DM), crude protein (CP; Nitrogen x 6.25; LECO FP-528 Nitrogen Analyzer, LECO Corp., St. Joseph, MI), neutral detergent fiber (NDF; ANKOM Fiber Analyzer system, ANKOM Technology, Macedon, NY), and in vitro true digestibility (IVTD; ANKOM Daisy II system, ANKOM Technology, Macedon, NY). The NDF procedure was modified by addition of heat-stable amylase (Sigma Chemical, St. Louis, MO). All analyses are reported on a DM basis.

Alkaloid analysis of pellets and sera

Larkspur pellets were analyzed for norditerpene alkaloids, specifically the more toxic MSAL-type and then total combined alkaloids. Ground plant material (100 mg) was extracted with 5 mL of 80% ethanol for 16 hrs. A 0.100 mL aliquot of the extract was added to 0.900 mL of 50% methanol in a 1.5 mL autosample vial. Calibration standards were prepared from stock solutions (1 mg/mL) of MLA and deltaline that were diluted to 50 ppm in ethanol. An aliquot (0.200 mL) of each standard was added to 1.60 mL of 50% methanol to give 5 ppm standards that was serially diluted to give standards at 5, 2.5, 1.25, 0.625, 0.312, 0.156, 0.078 and 0.039 $\mu\text{g/mL}$. Samples were then analyzed by reverse-phase liquid chromatography/mass spectrometry as previously described (Gardner and Pfister 2009). Concentration of MSAL alkaloids were measured from combined peak areas of MLA and related MSAL alkaloids from selected extracted ion chromatograms (m/z 669 and 683) versus the MLA standard curve. Total alkaloids included the MSAL plus the concentration of deltaline and related non-MSAL alkaloids measured from selected ion chromatograms (m/z 494, 508, 536 and 578) using the deltaline standard curve.

Serum samples were analyzed for the major toxic alkaloid, MLA. 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 diluted with 0.980 mL ethanol to provide a 20 ppm solution. A 0.050 mL aliquot was added to 1.950 mL of blank cow sera and serially diluted with cow 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 for 5 min. 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 (internal standard) was

added to each sample. Samples were vortexed for 10-15 sec and then centrifuged at 16000 x G for 20 min. A 0.75 mL aliquot was then removed to a 1.5 mL autosample vial for analysis.

To quantify MLA in sera, a Velos Pro (Thermo Scientific) mass spectrometer coupled with an Agilent 1260 autosampler and MS pump plus (Agilent Technologies) was used in-line with a Hypersil Gold C18 column (100 x 2.1 mm) with a guard column of equivalent phase. The column was eluted with a binary solvent gradient using 0.1% formic acid (solvent A) and acetonitrile (solvent B), at a flow rate of 0.400 mL/min and the following gradient mixture with time: 15% B (0-1 min); 15-75% B (1-8 min); 75% B (8-10 min); 75-15% B (10-11 min); 15% B (11-16 min). The flow from the column was connected to a heated electrospray ion source. The mass spectrometer was set to scan selected MS/MS experiments during the following time segments: (0 - 5.5) MLA parent ion m/z 683.3 with CID fragmentation power of 28; and (5.5 – 10 min) reserpine parent ion m/z 609.2 with CID fragmentation power of 28. Reconstructed ion chromatograms used the following selected ions for MLA (619.3, 651.3 and 665.3) and for reserpine (397.1 and 448.2). Quantitation was completed using peak areas from reconstructed ion chromatograms for MLA versus reserpine.

Statistical analysis

Intake of larkspur-containing pellets was analyzed using a linear mixed model (PROC MIXED) in SAS (v.9.4, SAS Institute Inc., Cary, NC). The model included treatment (resistant vs. susceptible animals), day, and the treatment x date interaction. Animals and day were random factors in the model. A similar model that included hours post-exposure (trial 1) or trial day (trial 2) was used for serum MLA concentrations. Compound symmetry or autoregressive were the best fitting covariance structures. Least square means were used for all comparisons, and the PDIF procedure in SAS 9.4 was used with preplanned comparisons to evaluate the treatment x day interaction. The limit for statistical significance was set at $P < 0.05$.

Results:

Larkspur and Alfalfa pellets

The chemical composition and digestibility of the pellets offered in the experiment is given in Table 1. Larkspur and alfalfa hay are similar in nutritional value (Pfister et al., 1988a, 1989), and thus the larkspur-containing pellets used in this study were nutritious.

Table 1. Chemical composition and digestibility (%)¹ of the pellets used in the larkspur (*Delphinium barbeyi*) trials.

Item	CP	ND	
		F	IVD
Trial 1			
Larkspur pellets ²	20.1	41.3	76.4
Alfalfa pellets	22.7	44.4	74.1
Trial 2			
Larkspur pellets	22.0	40.1	75.4

¹ Dry matter basis; CP = crude protein, NDF = neutral detergent fiber, IVD = in vitro digestibility

² Pellets in Trial 1 were 12% larkspur and 88% alfalfa hay; pellets for Trial 2 were pellets were 6% larkspur, 89% alfalfa hay, and 5% molasses (by weight; as fed basis).

Trial 1

Steers classified as resistant consumed 2.14 g/kg BW compared to 2.16 g/kg BW for steers categorized as susceptible ($P > 0.95$) on the first day of exposure to the larkspur-containing pellets. All the animals ate the larkspur-containing pellets within the first 12 hours after the initial exposure. On the 2nd day of Trial 1, consumption of larkspur-containing pellets dropped to essentially zero (Figure 1) for both treatment groups and continued near zero for 3 days (days 2 to 4). At that point, the steers were again offered ad libitum access to alfalfa pellets during a 3-day recovery period (days 5 to 7), and consumption recovered to normal levels (Figure 1). When larkspur-containing pellets were again reintroduced to the steers (days 8 and 9), consumption again decreased to near zero (days 8 to 9; Figure 1). Only 1 steer in the susceptible group ate a measurable quantity of larkspur-containing pellets when offered on day 8.

Fifty percent (3/6) of susceptible steers and 16% (1/6) of resistant animals displayed overt signs of larkspur intoxication on the morning of day 2. Those clinical signs, such as tremors and periodic collapse into sternal recumbency, persisted for all 3 of the susceptible animals during the entire day until early evening on day 2. Post-ingestive concentrations in sera of MLA (Figure 2) did not differ over 72 hours between susceptible and resistant animals (treatment effect, $P \geq 0.12$), nor was there a treatment x time interaction ($P \geq 0.5$) for either alkaloid in sera. MLA concentrations peaked at 24 h post-consumption, and measurable amounts of MLA were in sera at 72 h post-consumption. The serum MLA concentration at 12 h post-exposure for the single steer that consumed the pellets on day 9 (Figure 2) peaked at 362 ng/mL.

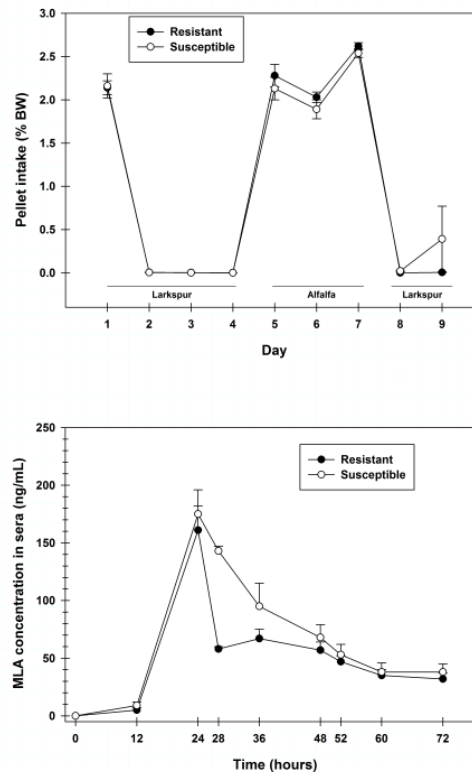


Figure 1. Trial 1 A: feed intake for 8 days, and B: MLA concentration in sera for 72 h for 6 larkspur susceptible and 6 larkspur resistant Angus steers fed a 12% larkspur alfalfa pellet.

Trial 2

There was a trend ($P = 0.07$) for resistant steers to consume more 6% larkspur-containing pellets compared to susceptible steers (Fig. 2a), but there was no day \times treatment interaction ($P = 0.57$). By comparison, there was a day \times treatment interaction for MLA concentrations in sera ($P = 0.003$; Figure 2b). Susceptible and resistant steers differed in concentrations of MLA in sera on days 9 to 12 ($P < 0.01$). There was also a trend towards a treatment difference on day 8 ($P = 0.09$). One susceptible steer became sternally recumbent in the pen on day 8.

On day 9 all steers were evaluated for their ability to walk and to determine if they would show clinical signs during exercise, using the standard exercise protocol as previously outlined (Green et al., 2019).

On day 9 the exercise times were 38.0 ± 3 min and 27.2 ± 6.5 min for resistant and susceptible steers, respectively (t-test, $P = 0.11$). Only one of the resistant steers showed clinical signs after 30 min of walking; the serum MLA

concentration for this steer at this time point was 167 ng/mL. Three susceptible steers showed clinical signs at 25, 11, and 5 min. Their mean concentration of MLA in sera was 841 ± 112 ng/mL.

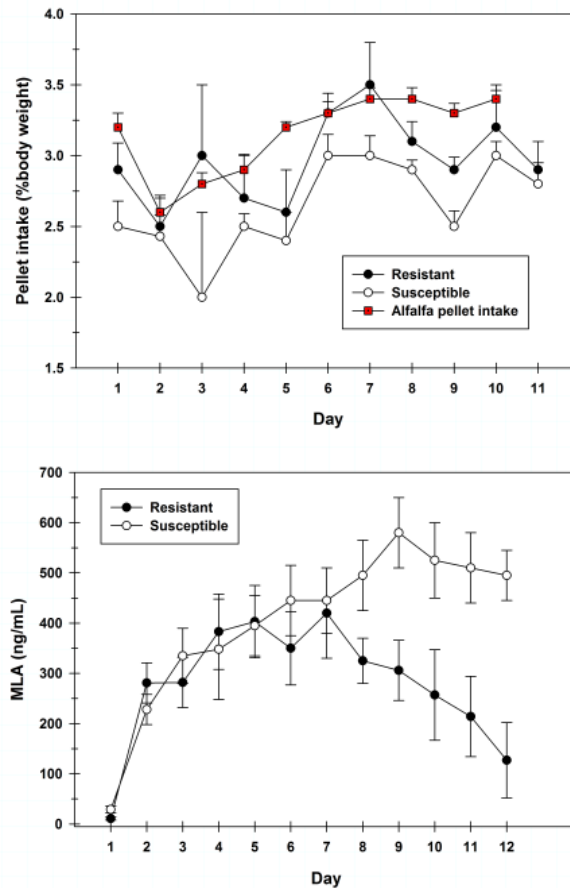


Figure 2. Trial 2, A: larkspur-containing pellet intake for 11 days (circles), alfalfa pellet intake for 10 days (red square), and B: MLA in sera for 6 larkspur susceptible and 6 larkspur resistant Angus steers fed a 6% larkspur 5% molasses alfalfa pellet.

Discussion: The purpose of this study was to examine, in a controlled setting, the subclinical effects of larkspur intake by allowing larkspur susceptible and resistant cattle to self-select the amount of larkspur consumed in pellet form. We tested the hypothesis that there would be differences in short term pellet consumption patterns between susceptible and resistant animals due to post-ingestive consequences of larkspur alkaloids in the pellets. We did not see any significant differences in pellet consumption between the two groups in either trial.

In the first trial, the steers consumed a large quantity of larkspur-containing pellets, and quickly developed a conditioned taste aversion to 12% larkspur-containing pellets (Ralphs and Olsen 1992, Pfister et al., 1997). After a single exposure on day 1 neither the resistant or susceptible steers would accept the 12% pellets even when hungry. When switched to alfalfa pellets after 3 days of low or no consumption of the 12% larkspur-containing pellets, steers ate normal amounts of feed. However, when switched back again to larkspur-containing pellets on day 8, steers refused the larkspur-containing pellets, with 1 exception. There were no differences in pellet intake between resistant and susceptible steers. Field studies with free-grazing cattle have concluded that animals at times develop strong taste aversions to fresh larkspur (Ralphs et al., 2001). We concluded from this initial experiment with a 12% larkspur-containing pellet that the conditioned taste aversion to the larkspur content was very strong, and essentially overwhelmed any differences between the susceptible and resistant animals.

To correct for the taste aversion to 12% larkspur-containing pellets, we decreased the larkspur content of the pellets by half to 6% and included 5% molasses for trial 2 (Figure 2). Flavors like molasses are used by the dairy industry to stimulate feed intake (Harper et al., 2016). For that reason, we chose to add 5% molasses to the 6% larkspur-containing pellet. Feeding alfalfa-molasses pellets without larkspur for 10 days indicated that steers had some initial variability in pellet consumption, but after the first 2 days, intakes gradually increased, and unlike larkspur-containing pellet intake, there was no indication of cyclic consumption (Pfister et al., 1997, 1999, 2011) with alfalfa pellets. In Trial 2, steers from both treatment groups showed cyclic consumption during this 11-day trial, and there was a tendency for resistant steers to consume more larkspur-containing pellets compared to the susceptible steers, but it was not significantly different ($P = 0.07$) (Figure 2a).

In trial 2, blood samples were taken daily from each steer. MLA concentrations in the sera of resistant steers were different from the susceptible steers from day 9 to day 12. Interestingly, MLA concentrations in sera did not reflect trends in pellet intake with the cycling of pellet consumption continuing as serum MLA concentrations continued to change in each group of steers (Figure 2). The serum MLA concentrations in resistant steers gradually decreased for the last 5 days of the study while pellet intake remained above 2.75% of body weight. Conversely, the susceptible steers ate somewhat lesser amounts of larkspur-containing pellets and had greater serum MLA concentrations. This result suggests toxicokinetic differences between the two groups of cattle with the resistant steers more effectively clearing the larkspur toxins than susceptible animals. However, these toxicokinetic differences did not result in differences in exercise times between the two groups of cattle on day 9 of the experiment. The exercise times were not significantly different (38.0 ± 3 min and 27.2 ± 6.5 min for resistant and

susceptible animals, respectively). This was unexpected because the steers were selected for the study based upon a single alkaloid dose resulting in mean times to exercise intolerance of 31.5 ± 2.7 min and 0.4 ± 0.4 min, respectively for resistant and susceptible animals. Field studies examining larkspur consumption patterns and serum alkaloid concentrations of grazing resistant and susceptible cattle have also noted a disconnect between susceptibility and serum MLA concentrations (Pfister et al., 2018). Further, Welch et al., (2015b), using a multiple dosing schedule, suggested a toxic threshold for serum MLA of 355 ng/mL in intoxicated cattle. However, in trial 2 the susceptible steers had serum MLA concentrations nearly 2x this level, with few overt clinical signs of intoxication. Taken together, these observations suggest that an alternative mechanism besides a change in larkspur alkaloid toxicokinetics must be occurring.

One possible explanation for lack of differences in exercise times between the two groups was the development of pharmacodynamic tolerance to larkspur alkaloids by the steers. Dumas and Pollack (2008) define pharmacodynamic tolerance as when increasing amounts of drug must be dosed to maintain a physiological effect. We speculate that pharmacodynamic tolerance to MLA occurs through the upregulation of cellular responses and an increase in receptor numbers due to a prolonged blockade of nAChR function. An example of this type of tolerance is the H₂ receptor antagonist famotidine which causes the upregulation of histamine H₂ receptors, and tolerance to its effects on stomach acid secretion (Taekeuchi et al., 1999; McRorie et al., 2014). Previous research at this laboratory supports this speculation. Experiments by Welch et al (2009) with nine different inbred strains of mice documented that differences in the expression of nAChR was associated with differences in susceptibility or resistance to the effects of MLA. Welch et al., (2016) have also speculated that alkaloid/nAChR toxicodynamic differences between cattle, sheep, and goats was responsible for differences in susceptibility to larkspur toxicity between the three species of livestock.

The pharmacology of MLA supports the speculation that the tolerance to larkspur was due to pharmacological actions by the alkaloids at nAChR. MLA is a blocker of acetylcholine binding at muscle-type nAChR (Dobelis et al., 1999), and it competes for binding sites with α -bungarotoxin, a potent neurotoxin (Kukel and Jennings, 1994). MLA causes muscle weakness and neuromuscular paralysis from the blockade of nAChR post-synaptic neuromuscular junctions (Aiyar et al., 1979; Benn and Jacyno, 1983; Alkondon et al., 1992). Drugs that increase the persistence of acetylcholine at neuromuscular junctions such as neostigmine reverse the clinical signs of larkspur poisoning in cattle (Green et al., 2009).

In conclusion, larkspur alkaloids were again demonstrated to be potent aversive agents in cattle when fed as a 12% larkspur-containing pellet. In these trials susceptible and resistant steers did not differ in larkspur-containing pellet consumption. However, in the second trial the susceptible steers showed higher

concentrations of serum MLA than did resistant animals even though exercise times were not different between the two groups of steers. This and other studies of larkspur intoxication in cattle suggests that differences in responses of susceptible and resistant cattle may be attributed to the development of pharmacodynamic tolerance to larkspur alkaloids by the actions of larkspur alkaloids at nAChR. Further work will be required to determine whether there are differences between susceptible and resistant animals in toxicokinetic responses such as serum absorption and elimination.

Conflict of Interest:

The authors declare that there are no conflicts of interest.

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