Development of a PCR-Based Detection for Delphinium Species in Poisoned Cattle

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**Introduction**
- Delphinium spp. (i.e., larkspur) poisoning are a significant cause to livestock losses worldwide (Nielsen et al. 1994).
- Larkspur contains norditerpene alkaloids such as methyllycaconitine (MLA) that cause neuromuscular paralysis by blocking nicotinic acetylcholine receptors (Green et al. 2011).
- Clinical signs of larkspur poisoning include: labored breathing, increased heart rate, muscle tremors, fatigue, and death (Pfister et al. 1999).
- Determining the cause of death is essential in preventing future losses.
- Alkaloids in rumen content can be detected using Mass Spectrometry, but MS is not available in all veterinary labs.
- Primer Chain Reaction (PCR) is an easily accessible tool that may allow detection of genetic material in rumen.
- Studies have shown that plant DNA is degraded through the digestive tract, but fragmented DNA was detectable (Chowdhury et al. 2004).
- The objective of this study was to evaluate the use of PCR as an alternative diagnostic tool to determine if larkspur could be detected within rumen contents.

**Methods**
- **Primer Design**
  - Primers designed by aligning ITS region using Sequencer from multiple Delphinium species and Aconitum species.

**Methods (continued)**
- **In Vivo Digestion**
  - *D. occidentale* (5 g) in nylon digestion bags (Ankom tech) with weight placed in rumen of cannulated cows.
  - Time frame 4-48 hours.
- **In Vitro Digestion**
  - Rumen fluid extracted from cannulated cow.
  - Plant samples in digestion bags placed in Daisy II anaerobic digestion vessel.
  - Time frame 8-46 hours.
- **In Vivo/In Vitro Digestion**
  - In vivo digestion for 8 hours followed in vitro digestion up to 84 hours in Daisy II incubator.
  - Time frame 8-72 hours.
- **Dilutions**
  - Different ratios of Larkspur (g): Alfalfa (g) were prepared: 5:0, 1:2, 1:3, 1:5, 3:1, 11:5, 1:24, 1:49.
  - Samples placed in cannulated cow rumen for 8 hours.
- **DNA Extraction and PCR**
  - DNA was extracted from 150 mg of samples using 2R plant/sedimentary mini prep kit (Zymo Research Corp).
  - PCR was performed using Bio-Rad Dyad PCR Thermal Cycler (Bio-Rad Laboratories Inc.).
  - PCR conditions were as follows: a) an initial denaturation step for 3 min at 94°C, b) followed by 10 cycles of 30 s at 94°C, 45 s at 60°C, and 30 s at 72°C, c) followed by 25 cycles of 30 s at 94°C, 45 s at 54°C, and 30 s at 72°C, and d) a final extension of 5 min at 72°C.
  - Samples were analyzed using 1% agarose gel and visualized using Kodak Image Station 2000RT Imager (Eastman Kodak, Rochester NY).
  - All experiments were conducted at the USDA-Poisonous Plant Research Laboratory (Logan, UT) under veterinary supervision.

**Results**
- Figure 3. Agarose gel electrophoresis showing specificity of DNA primers. 10000 bp marker (LD) shown as a reference. Lanes: 1, *D. occidentale* (Logan, UT); 2, *D. nuttallianum*; 3, D. andersonii; 4, D. glaucescens; 5, D. scoparium; 6, D. camusum; 7, D. barbeyi; 8, D. occidentale; 9, A. columbianum; and 10, M. sativa.
- Figure 4. Agarose gel electrophoresis showing amplicons from the in vivo and in vitro coupled rumen digestion. 10000 bp marker (LD) shown as a reference. Triplicates of undigested positive control (+ctrl) and digested (8-72h) *D. occidentale*. Negative control (-ctrl) representing A. columbianum and M. sativa.
- Figure 5. Agarose gel electrophoresis showing amplicons from undigested positive control (+ctrl) and the in vivo rumen digestion from different ratios of larkspur to alfalfa. 10000 bp marker (LD) shown as a reference. Ratios represent grams of larkspur : grams of alfalfa. Negative control (-ctrl) representing A. columbianum and M. sativa.

**Conclusion**
- Larkspur specific primers were developed.
- Larkspur can be detected 48-72 hours after ingestion and death of an animal.
- This experiment exposed larkspur to extreme conditions compared to normal grazing by submersing them in the rumen fluid.
- Normal larkspur would have been protected from degradation by the rumen mat above the ruminal dorsal sac.
- PCR method can detect larkspur in 2% composition of larkspur compared to a normal 5-10% composition in typical larkspur poisoning.
- Results suggest that PCR based method has potential as a diagnostic tool.
- Studies should be conducted using qPCR to quantify the amount of larkspur in the rumen content in order to infer a stronger association to cause of death with larkspur poisoning.
- Other PCR based detection methods for more toxic plants such as water hemlock, or oleander could be developed.

**References**

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