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Comparison of Sheep and Goats to the Acute Toxic Effects of Foothill Death Camas

Abstract

Death camas (*Zigadenus* spp) is a perennial forb found throughout the western United States, which is known to kill both sheep and cattle. In a previous study, goats appeared to be somewhat resistant to the adverse effects of death camas. Therefore, the objective of this study was to directly compare the susceptibility of goats and sheep to the acute toxic effects of death camas. Sheep and goats were dosed at 0.5, 1.0, 2.0, 4.0, and 6.0 g death camas per kg BW. The data presented in this manuscript suggest that goats are more susceptible to death camas than sheep. There were no differences in the serum concentrations of zygadenine in sheep versus goats. There was a difference between goats and sheep in the severity of observed clinical signs of poisoning. This is highlighted by the fact that five goats from the two highest doses died, whereas none of the sheep died. Consequently, when grazing goats in death camas infested pastures as much caution, if not more, should be taken than one would with sheep. Additionally, the data presented in the study suggests that goats can be used as a small ruminant model to study the toxic effects of death camas.

Keywords

Zigadenus, death camas, zygacine, zygadenine, sheep, goats

Cover Page Footnote

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INTRODUCTION

Death camas (*Zigadenus* spp) is a native, cool-season, bulbous perennial forb found throughout the western United States (Burrows and Tyrl, 2013). Death camas starts growing early in the spring and is toxic to cattle and sheep (Kingsbury, 1964). The toxic alkaloids in death camas are steroidal alkaloids (Knight and Walter, 2001; Burrows and Tyrl, 2013). Zygacine is often the most abundant alkaloid in foothill death camas and is believed to be the primary toxic component (Majak *et al.*, 1992; Welch *et al.*, 2011). Recent research has demonstrated the detection of four steroidal alkaloids, zygacine, zygadenine, 3-angeloylzygadenine, and 3-veratroylzygadenine in foothill death camas (*Z. paniculatus*), with zygacine representing greater than 50% of the total steroidal alkaloid content in plants at different phenological stages over the growing season (Stonecipher *et al.*, 2020). However, a recent study demonstrated no difference in the toxicity of two chemotypes of foothill death camas that contained different amounts of zygacine when dosed orally to sheep (Stonecipher *et al.*, 2022). Investigation into the metabolism of zygacine in the rumen, liver, and blood of sheep and cattle using *in-vitro* and *in-vivo* systems demonstrated that zygacine is rapidly metabolized to zygadenine (Lee *et al.*, 2024). Consequently, zygadenine is likely the alkaloid that reaches general circulation to elicit its toxic effects in ruminants.

Livestock losses to death camas have been reported in numerous species including cattle (Smith and Lewis, 1991; Collet *et al.*, 1996) and sheep (Panter *et al.*, 1987), with the largest losses generally occurring in sheep (Kingsbury, 1964). Sheep are primarily affected because of their tendency to select forbs, particularly in the early spring when there is little other plant growth (Panter *et al.*, 1987). Clinical signs of poisoning are similar for all animal species studied. The clinical signs observed in animals poisoned by death camas include ataxia, muscular weakness, trembling, incoordination, discharge of frothy saliva from the mouth and nose, vomiting, dyspnea, collapse, and death. Pathological lesion include gross lesions of severe pulmonary congestion and subcutaneous hemorrhage in the thoracic region with microscopic lesions of pulmonary congestion and edema (Panter *et al.*, 1987).

In a recent study to develop methods to aide in the diagnosis of animals poisoned by death camas, goats were dosed with death camas as a small ruminant model for method development purposes (Lee *et al.*, 2020). In that experiment, the goats demonstrated less clinical signs of poisoning than what had been noted in sheep given a similar dose in a previous experiment (Welch *et al.*, 2013). Thus, we hypothesized that goats may be more resistant to the toxic effects of death camas than sheep. The objective of this study was to compare the susceptibility of goats and sheep to the acute toxic effects of death camas.

MATERIALS AND METHODS

Death camas was collected in the late vegetative stage in May 2020, near Logan, UT (41.568°N, 111.900°W) at an elevation of approximately 1,650 m. A voucher specimen was deposited at the Poisonous Plant Research Laboratory Herbarium (#4799). Only the above ground parts of the plants were collected. 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 dried ground plant material was stored in plastic bags away from direct sunlight at ambient temperature in an enclosed shed until use, with plant alkaloid analyses performed immediately prior to use.

The procedure for the analysis of zygacine in dry ground plant material by high performance liquid chromatography – high resolution mass spectrometry (HPLC-HRMS) was reported previously (Lee et al, 2024). Dry ground death camas plant material (50 mg) was weighed into 2.0 ml screw cap microcentrifuge tubes (Fisher Scientific, Waltham, MA). Methanol (1.0 ml) was added to the microcentrifuge tubes and the sample extracted by mechanical rotation (16 h) using the Rugged Rotator (Glas Col, LLC). The sample was centrifuged (20 min., 16,000 x *g*). The sample was diluted by the addition of an aliquot (5 µL) of the methanol supernatant in into a 2 mL autosampler vial containing 995 µL water: methanol (95:5; v:v).

Cross-bred western white-faced sheep and Spanish goats were housed in outdoor pens and maintained on alfalfa. All experimental procedures involving livestock were conducted under veterinary supervision with the approval of the Utah State University Institutional Animal Care and Use Committee (USU IACUC protocols #12558 and #12559).

Weanling goats and sheep weighing 30 ± 3 kg and 45 ± 5 kg, respectively, were maintained on alfalfa hay in their normal outdoor paddocks. None of the animals used in this study had prior exposure to death camas. Dried, finely ground death camas was administered via oral gavage in approximately 2-3 liters of tap water at 0.5, 1.0, 2.0, 4.0 and 6.0 g plant material per kg BW with 4 animals per species per dose. Two additional groups of 4 goats and 4 sheep each were dosed at 1.0 and 2.0 g/kg followed by an exercise protocol to exacerbate stress on the animals. Fatigue and weakness were assessed by exercising the animals for 10 min every h for 8 h after dosing. The animals were exercised in groups of four by walking them back and forth in a 40 m alley at a rate of approximately 3 km/h for 10 min. If an animal became fatigued to the point that it could not maintain a 3 km/h pace, it was allowed to fall behind the others. The number of animals that could not walk for 10 min for each group was noted.

Blood was collected into red top vacutainer tubes via jugular venipuncture at 0, 4, 8, and 24 h after dosing. Blood was allowed to clot at room temperature. Serum was separated from red blood cells by centrifugation at 1500 x g for 20 min and stored frozen at -20ºC. The serum was analyzed for zygadenine as described previously (Lee *et al.*, 2024) with slight modifications. Briefly, sera samples (0.5 mL) were transferred into microcentrifuge tubes containing 0.5 mL acetonitrile. This solution was vortexed (20 s) and centrifuged (16,000 x *g*, 30 min). An aliquot of the supernatant (100 µL) was removed and diluted into a 2 mL HPLC autosampler vial containing 900 µL of a 0.05 µg/mL riddelliine in 2% ethanol in distilled water internal standard solution.

Samples were injected (5 µL) onto a Betasil C18 (100 x 2.1 mm i.d.) reversed phase HPLC column (ThermoFisher Scientific Co., Waltham MA, USA) protected by a guard column of the same phase. The alkaloids were eluted from the column with a gradient flow consisting of 5 mM ammonium formate, 0.1% formic acid (A) and acetonitrile (B) at a flow rate of 0.500 mL/min. The mobile phase program was 5% B for 0.5 min followed by a linear gradient to a composition of 40 % B at 3.0 min. The mobile phase was delivered and samples injected using an Ultimate 3000 HPLC (Thermo Scientific, San Jose, CA, USA) and the column eluent was connected to the heated electrospray source of an Q-Exactive Orbitrap high resolution mass spectrometer (Thermo Scientific, San Jose, CA, USA) calibrated per the manufacturer's instructions and with a *m/z* scan range 100 – 800, resolution 70000, microscans 1, sheath gas flow 35, auxiliary gas flow 10, spray voltage 4 kV, capillary temperature 320 °C, S lens RF field 55, and auxiliary gas temperature 300 °C. Chromatographic peaks were identified by generating reconstructed HPLC-HRMS chromatograms with the calculated MH^+ molecular weight of alkaloids to 5 decimals places and with a mass tolerance of 10 ppm. Under these conditions riddelliine and zygadenine, eluted at 2.4 and 2.5 min, respectively. The concentrations of zygadenine in sheep and goat sera were quantitated against six-point zygadenine standard curves over the range of 0.078 – 2.0 µg/mL in sheep and goat sera, respectively.

Statistical comparisons of serum alkaloid profiles were performed by three-way ANOVA using SigmaPlot for Windows (version 14.0, SPSS Inc., Richmond, CA). The alkaloid concentrations were plotted using SigmaPlot for Windows (version 14.0, SPSS Inc., Richmond, CA).

RESULTS AND DISCUSSION

For this study, foothill death camas (*Zigadenus paniculatus*) was collected near Logan, Utah during the late vegetative phenological stage. Sheep losses to death camas typically occur in the spring when the death camas plants are in the mid to

late vegetative phenological stage of growth (Panter *et al.*, 1987). This time also coincides with the time that the plant material is most toxic (Stonecipher *et al.*, 2020). The predominant alkaloids in the death camas plant material used for this study were zygacine and zygadenine and suspected isomers of zygacine and zygadenine (Figure 1A). The concentration of zygacine in the mixed dried ground plant material was 7.9 mg zygacine per gram of plant material (on a dry weight basis).

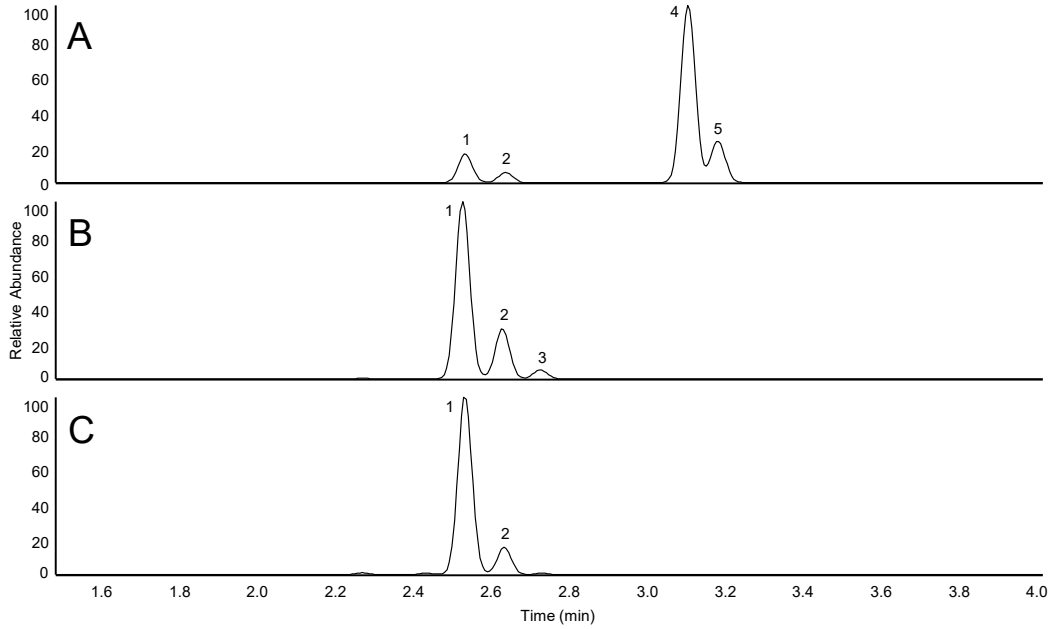

Figure 1. Extracted HPLC-HRMS ion chromatograms (*m/z* = 494.31123, 536.32179) from: (A) methanol extract of death camas (*Zigadenus paniculatus*) plant material; (B) Serum from a sheep dosed at 2.0 g/kg at 8 h post dosing; (C) Serum from a goat dosed at 2.0 g/kg at 8 h post dosing. Peak *m/z* identifications: 1 (MH^+ = 494.31123, zygadenine); 2 (MH^+ = 494.31123), suspected zygadenine isomer; 3 (MH^+ = 494.31123), suspected zygadenine isomer; 4 (MH^+ = 536.32179, zygacine); 5 (MH^+ = 536.32179) suspected zygacine isomer.

To assess the acute toxicity of death camas to sheep and goats, a dose response study was conducted with both sheep and goats dosed with death camas at 0.5, 1.0, 2.0, 4.0 and 6.0 g plant / kg BW. Both sheep and goats were administered the same dose of death camas on the same day so as to directly compare the two species. The most common clinical signs of poisoning observed in both the goats and sheep were frothing, vomiting, lethargy, weakness, dyspnea, difficulty moving, and death. There was a clear dose dependent increase in severity of the signs observed in both sheep and goats (Table 1). The results from this experiment demonstrated that both

goats and sheep tolerate doses of 0.5 and 1.0 g/kg with only minor frothing and vomiting occurring with some lethargy (Table 1). However, as the dose of death camas increased above 1 g/kg, more severe clinical signs of poisoning were observed. Both sheep and goats responded similarly up to 2 g/kg. However, at 4 g/kg ($P=0.02$) and 6 g/kg ($P<0.001$) the goats developed more severe clinical signs of poisoning than did the sheep. This is highlighted by the fact that one goat died at the 4 g/kg dose and all four goats died at the 6 g/kg dose, whereas none of the sheep died.

Table 1. Comparison of clinical signs observed* in sheep and goats after dosing with death camas.

Dose (g/kg)	Goat				Sheep			
	2 h	4 h	8 h	24 h	2 h	4 h	8 h	24 h
0.5	0.50	0.25	0.00	0.00	0.25	0.00	0.00	0.00
1.0	0.50	0.00	0.00	0.00	0.00	0.75	0.00	0.00
2.0	1.25	1.50	1.25	0.50	0.75	1.25	1.50	0.25
4.0	0.75	2.00	2.25	3.00	1.00	1.75	1.75	1.75
6.0	2.00	2.50	3.75	4.00	1.25	2.50	3.00	2.75

* Each animal was evaluated for clinical signs of poisoning using the following scale. The data represent the average score for all four animals in each group at each time point.
0 = no clinical signs of poisoning observed
1 = minor frothing, vomiting and lethargy
2 = moderate frothing, vomiting and weakness
3 = severe frothing, vomiting, and unwilling to stand
4 = death

A dose of 1.0 g/kg corresponds to approximately 30 and 45 g of total plant material (dry plant) or 150 and 225 g (fresh plant) for animals the size of the goats and sheep used in this study. A dose of 4.0 g/kg corresponds to approximately 120 g of total plant material (dry plant) or 600 g (fresh plant) for goats the size used in this study. An average mature death camas plant in the vegetative phenological stage, weighs about 10 g (wet weight of the above ground parts). Consequently, this data suggests that a 30 kg goat can eat approximately 15 death camas plants without much risk for poisoning, whereas if they eat 60 or more plants in a short time they may die.
The purported mechanism of action for the death camas alkaloids is the inhibition of a sodium channel that is found primarily in the cardiac nerves (Knight and Walter, 2001; Burrows and Tyrl, 2013). Consequently, the clinical signs of poisoning are related to deficiencies in the cardiovascular system of the poisoned

animal. Many times, sheep are poisoned by death camas in foothill, or mountain, rangelands. Consequently, often times at the same time the sheep are consuming a toxic dose of death camas they are being trailed up steep mountain slopes, which would increase the burden of the cardiovascular system. Therefore, in an attempt to exacerbate the toxic effects of death camas we subjected two groups of four sheep and goats to an exercise protocol. One group received death camas at 1 g/kg while the other was dosed at 2 g/kg. Every hour for 8 h after dosing, starting at 30 min post dosing, the animals were exercised by walking them back and forth along a 40 m alley for 10 min. The increased stress of the exercise protocol did appear to slightly exacerbate the severity of the clinical signs observed in both sheep and goats (Table 2). However, at these doses, even with the increased stress, most of the animals had completely recovered by 8 h with only one sheep still showing minor signs of poisoning at 24 h.

Table 2. Comparison of clinical signs observed* in sheep and goats that were exercised after dosing with death camas.

Dose (g/kg)	Goat				Sheep			
	2 h	4 h	8 h	24 h	2 h	4 h	8 h	24 h
1.0	1.25	0.50	0.00	0.00	1.25	1.25	0.00	0.00
2.0	1.25	1.75	0.50	0.00	0.75	1.25	1.00	0.75

* Each animal was evaluated for clinical signs of poisoning using the following scale. The data represent the average score for all four animals in each group at each time point.
0 = no clinical signs of poisoning observed
1 = minor frothing, vomiting and lethargy
2 = moderate frothing, vomiting and weakness
3 = severe frothing, vomiting, and unwilling to stand
4 = death

For the most part, at these doses, the exposure to death camas did not affect the ability of the sheep and goats to walk normally (Table 3). Only two goats were affected at the 1 g/kg dose 1.5 h after dosing. Both of those animals quickly recovered and were able to successfully walk for 10 min for the remainder of the test. Only one goat had difficulty walking at the 2 g/kg dose beginning 3.5 h after dosing. However, this goat was more severely affected as it was not able to walk for 10 min for another 5 h. The sheep responded similarly to the exercise stress as the goats, in that only one sheep was unable to walk for 10 min at each time at the1 g/kg and again one sheep at the 2 g/kg dose. Also similar, the sheep dosed at 1 g/kg was only weak for 2 h, while the affected sheep at the 2 g/kg dose was unable to

walk for 10 min from 3.5-8.5 h post dosing. Blood was collected from all the animals immediately prior to dosing and at 4, 8, and 24 h post dosing. Previous research has shown that in sheep dosed with ground plant material very little zygacine is detected in the blood, as it is quickly metabolized to zygadenine (Lee *et al.*, 2024). Pannels B and C of Figure 1 represent the HPLC-HRMS chromatogram of the alkaloids detected in serum of a goat and sheep 8 h after being dosed with 2 g death camas / kg BW. Note, zygacine, the predominant alkaloid in the plant material (Figure 1A), is not the predominant alkaloid in the serum of the goats or sheep in this study. Consistent with previous published data (Lee *et al.*, 2024) zygadenine was the predominant alkaloid detected in the sera of both the sheep and goats in this study (Figure 1B and 1C). Therefore, the sera were analyzed for zygadenine content (Figure 2). In all animals, there was an increase in the serum zygadenine content up to the 8 h time point, with little change, or a slight decrease by 24 h. Overall, there was not a species x dose x time effect (P = 0.981). Additionally, overall, there was not a difference in the serum zygadenine concentration between sheep and goats (P = 1.0). The concentration of zygadenine in the serum of the sheep dosed at 0.5 g/kg in this study is consistent with that reported previously (Lee *et al.*, 2024).

Table 3. Comparison of the effect of death camas treatment on exercise-induced muscle weakness in goats and sheep.

Time (h)	Goat		Sheep	
	1.0 (g/kg)	2.0 (g/kg)	1.0 (g/kg)	2.0 (g/kg)
0.5	0	0	0	0
1.5	2	0	0	0
2.5	0	0	0	0
3.5	0	1	0	1
4.5	0	1	0	1
5.5	0	1	1	1
6.5	0	0	1	1
7.5	0	1	0	1
8.5	0	0	0	1

The data represent the number of animals at each time point that were not able to walk at 3 km/h for 10 min. Each group consisted of four animals. Animals were walked in a 40 m alley every h for 8 h post dosing, beginning at 30 min after dosing.

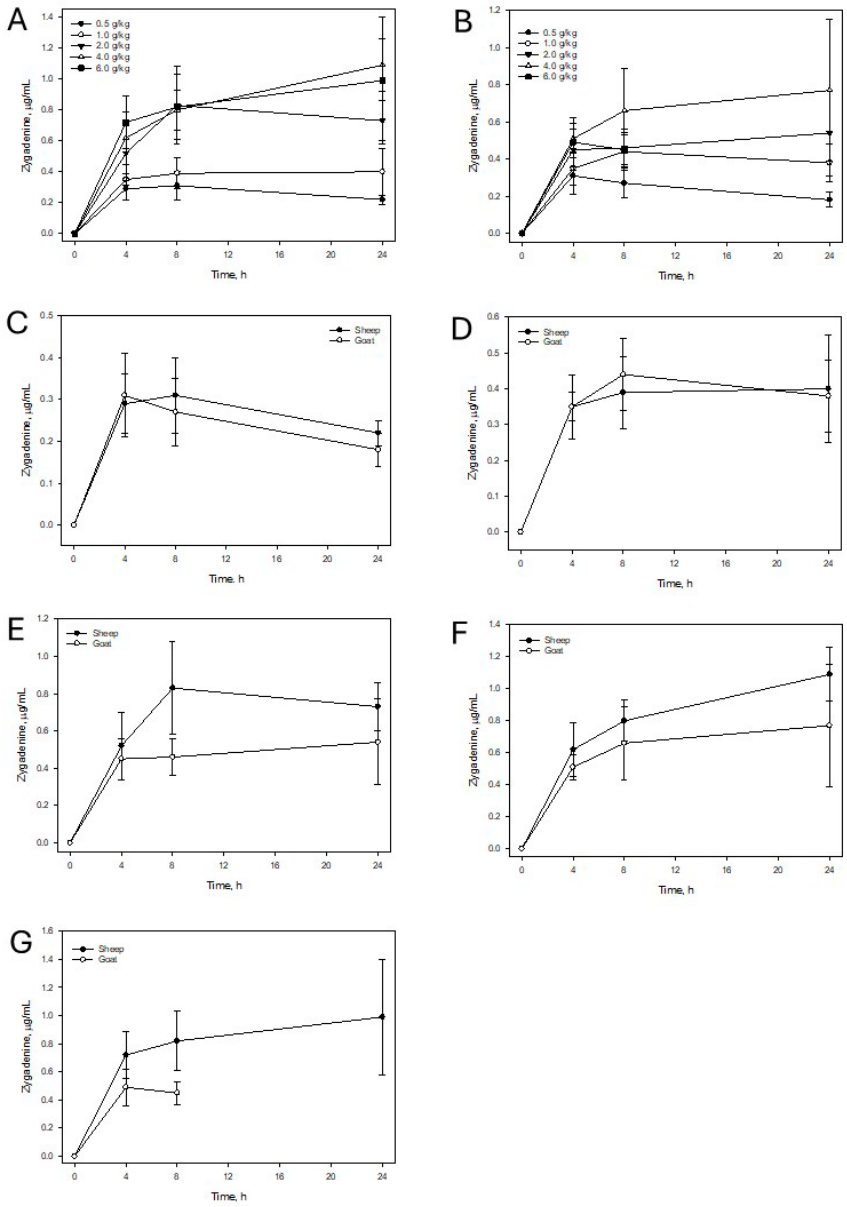

Figure 2. Comparison of the serum concentrations of zygadenine in sheep and goats dosed with death camas at 0.5, 1.0, 2.0, 4.0, and 6.0 g plant per kg BW. Panel A) sheep all doses, B) goats all doses, C) sheep and goats at 0.5 g/kg, D) sheep and goats at 1.0 g/kg, E) sheep and goats at 2.0 g/kg, F) sheep and goats at 4.0 g/kg, and G) sheep and goats at 6.0 g/kg. Note in panel G that no samples were collected from goats at 24 h as all of the goats in this group were dead by 24 h.

In conclusion, the data presented in this manuscript demonstrate that goats are not more resistant to death camas than sheep. In fact, they appear to be more susceptible under the conditions of this study, and the doses administered, highlighted by the fact that only goats died, whereas none of the sheep died. Consequently, any rancher that may consider grazing goats in death camas infested pastures should use as much caution, if not more, than they would with sheep. Additionally, the data presented in the study, suggests that goats can be used as a small ruminant model to study the toxic effects of death camas.

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