

Fetotoxicity of *Astragalus lentiginosus* (Locoweed) in Spanish Goats

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Introduction

Locoweed species—*Astragalus* and *Oxytropis* spp. that contain the indolizidine alkaloid swainsonine—are widely distributed on rangelands in North and South America, Mexico, China, and other rangelands throughout the world. Other range plant species including *Sida* spp. (Seitz et al. 2005), *Ipomoea* spp. (Damir et al. 1987), and *Swainsona* spp. (Laws and Anson 1968) from Brazil, Africa, and Australia, respectively, also contain swainsonine and other metabolic toxins that poison livestock and have been problematic for ranchers. Swainsonine poisons livestock by inhibiting the metabolic enzymes lysosomal α -mannosidase and mannosidase II (Dorling et al. 1980). Inhibition of these enzymes results in abnormal oligosaccharide accumulation in cellular lysosomes with accompanying characteristic neurovisceral vacuolation in multiple organ systems (Stegelmeier et al. 1995).

Clinically, locoweeds cause intention tremors, generalized depression, nervousness, proprioceptive deficits, aberrant behavior, reproductive dysfunction, emaciation, and death (James et al. 1970). Poisonings with similar etiologies have been reported in Brazil and Mozambique from *Ipomoea carnea* (Damir et al. 1987, De Balogh et al. 1999) and in Australia from Darling Pea (*Swainsona galegifolia*) (Hartley 1978). Swainsonine is found in these plant species and is believed to be partially responsible for the reported toxicoses. The manifestation of clinical effects varies somewhat, depending on the animal species involved. Additionally, locoweeds cause embryo and fetal death, abortions, generalized reproductive dysfunction, and occasional birth defects. There has been a substantial amount of research in sheep fed

locoweeds at various stages of gestation (James 1971, Panter et al. 1999); however, there has been relatively no research done on goats, particularly pregnant Spanish goats. Thus, the purpose of this study was to describe the clinical effects of locoweed ingestion on pregnant goats, focusing with ultrasound on the embryo/fetotoxic effects during the late first trimester and early-second trimester of pregnancy using Spanish goats.

Materials and Methods

Nine female Spanish goats were hand-mated to like bucks and divided into two groups. Five pregnant does were dosed twice daily with finely ground *Astragalus lentiginosus* via oral gavage beginning on day 30 of gestation. The plant dosage was calculated to deliver 8 mg swainsonine/kg body weight (BW)/day (140-230 g dry ground *Astragalus lentiginosus*). The *Astragalus lentiginosus* (USDA-ARS Poisonous Plant Research Lab accession #1998-02) was collected just south of St. Johns, AZ, and contained 0.17% swainsonine, dry weight. Four similarly bred goats were dosed with equivalent amounts of ground alfalfa hay as negative controls and treated identical to the locoweed-treated goats. Gestation day 30 was considered day 0 of treatment. Blood samples were collected from each goat in a vacutainer vial via the jugular vein on days 1-7, and every 5 days thereafter or until fetal death was confirmed. Blood samples were maintained at room temperature for 30-45 minutes, after which serum was separated by centrifugation at 2,300 rpm. Serum was collected and frozen at -20°C and later analyzed for swainsonine and progesterone. Serum

swainsonine is an indicator of locoweed exposure and serum progesterone is an indicator of fetal viability.

Fetal movement was observed by ultrasound every 5 days beginning on day 30 of gestation, and fetal activity was assessed using visual evaluation. An Aloka Model 900 ultrasound with a 5 mhz abdominal transducer was used for ultrasonography observations. Briefly, an echogenic gel was applied to the abdomen of each goat on either side of the udder and 2-5 cm anterior. Once the fetus was observed, a 5- min scan was performed and recorded to videotape for future analysis. Later, fetal activity was evaluated from the video tapes and each voluntary movement was counted and recorded. The fetal heartbeat was noted to confirm fetal viability, but no attempt was made to determine the fetal heart rate.

Swainsonine Analysis

Serum swainsonine concentrations were determined using a method previously published with some modification (Stegelmeier et al. 1995). Briefly, 0.6 mL of serum was combined with 0.3 mL sodium acetate (0.25 M, pH 4.0), vortexed, and boiled for 10 min. The supernatant was removed and 75 μ L was put into a multiwell plate in duplicate with 15 μ L of 0.0008 U/well Jack Bean α -mannosidase (Sigma Chemical Co., St. Louis, MO) and 10 μ L of 10 mM p-nitrophenyl- α -D-mannosidase (Sigma Chemical Co., St. Louis, MO). The mixture was incubated for 30 min at 37°C, after which 100 μ L glycine (2.5 M, pH 10.3) was added to stop the reaction. The amount of swainsonine (α -mannosidase inhibition) in the test samples was then determined photometrically at 405 nm using a microplate reader and microplate manager software (Bio-Rad 3550 UV, Bio-Rad laboratories, Melville, NY). An analysis of swainsonine standards with concentrations ranging from 1,000 to 1.2 ng/mL was performed in a similar manner using blood serum from normal, unexposed animals spiked with known amounts of swainsonine. The control swainsonine concentrations were verified by capillary gas chromatography using previously described methods (Molyneux et al. 1989). Samples higher than the linear range of the standard curve (>800 ng/mL) were diluted with sodium acetate (0.25 M, pH 4.0). The standards for these assays were similarly modified by diluting the normal serum. Serum α -mannosidase activity is inversely related to swainsonine concentration as shown in figure 1.

Progesterone Analysis

Serum progesterone levels were determined by radioimmunoassay using a non-extraction, solid phase I-125 radioimmunoassay kit (Diagnostic Products Corporation, Los Angeles, CA). The Coat-A-Count progesterone kit is highly specific with low cross-reactivity to other steroids. Specific cross-reactivity is listed in the procedure manual (Diagnostic Products Corporation). Inter- and intra-assay coefficients of variation were 6.15% and 4.25%, respectively. Progesterone kits were validated at 0.5, 2.5, 5, 10, 20, and 40 ng/mL.

Results

Locoweed-fed goats developed mild proprioceptive deficits, as manifested by difficulty stepping over a small wooden barrier, within 9 to 10 days of treatment. These clinical signs became more severe over time, and treated goats became lethargic, were hesitant to move, exhibited pronounced intention tremors, and displayed severe proprioceptive deficits. Several animals developed severe rear limb weakness and partial paresis. These animals were euthanized and necropsied.

Serum swainsonine was rapidly elevated in treated goats, reaching concentrations of > 400 ng/mL after the first day of treatment and peaking above 600 ng/mL (figure 1). Serum α -mannosidase levels were not measured in this study but have been shown to be inversely related to serum swainsonine levels. No swainsonine was detected in sera from control goats.

Serum progesterone significantly ($P<0.05$) declined in locoweed-fed goats after 5 days on locoweed, beginning on gestation day 35 and continuing throughout the treatment period (figure 2). This was indicative of the negative effect of locoweed on the fetus, placenta, ovary, and other structures important in the maintenance of pregnancy in goats.

All pregnant goats, including treatment and controls, were evaluated with ultrasound and had viable embryos (embryonic vesicles appeared normal) on day 30 when the treatments began. Embryonic/fetal movement was detected in most animals between days 36 and 38, which is typical when movement first begins in sheep and goats (Panter et al. 1990). Fetal movement was similar between control and locoweed-fed goats until after day 40 of gestation ($P>0.05$; figure 3).

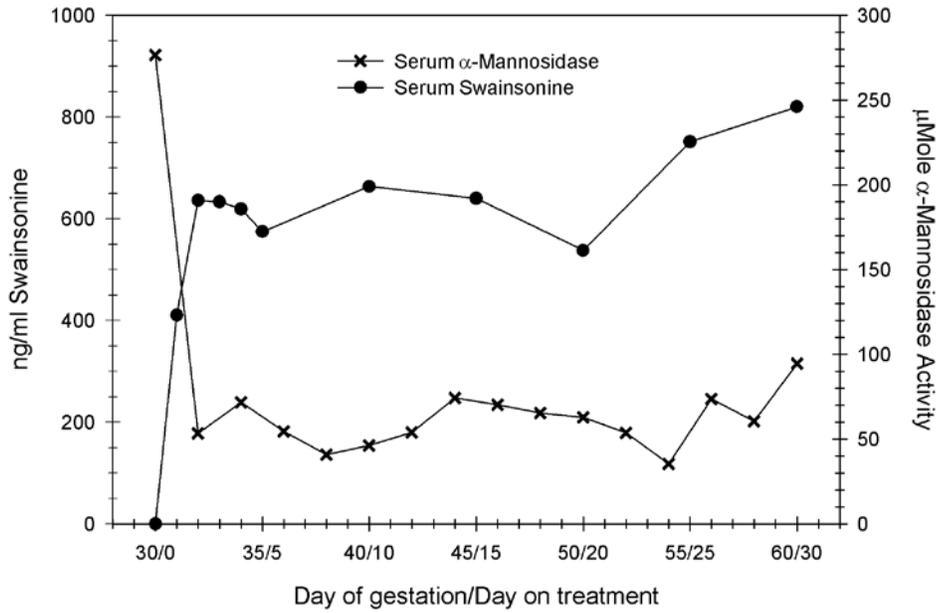


Figure 1. Serum swainsonine concentrations over time in goats dosed with 8 mg swainsonine/kg BW/day as ground *Astragalus lentiginosus*. Swainsonine was determined using a competitive binding assay against Jack Bean α -mannosidase (Stegelmeier et al. 1995). Locoweed-induced decline in α -mannosidase is adapted from Panter et al. (1999).

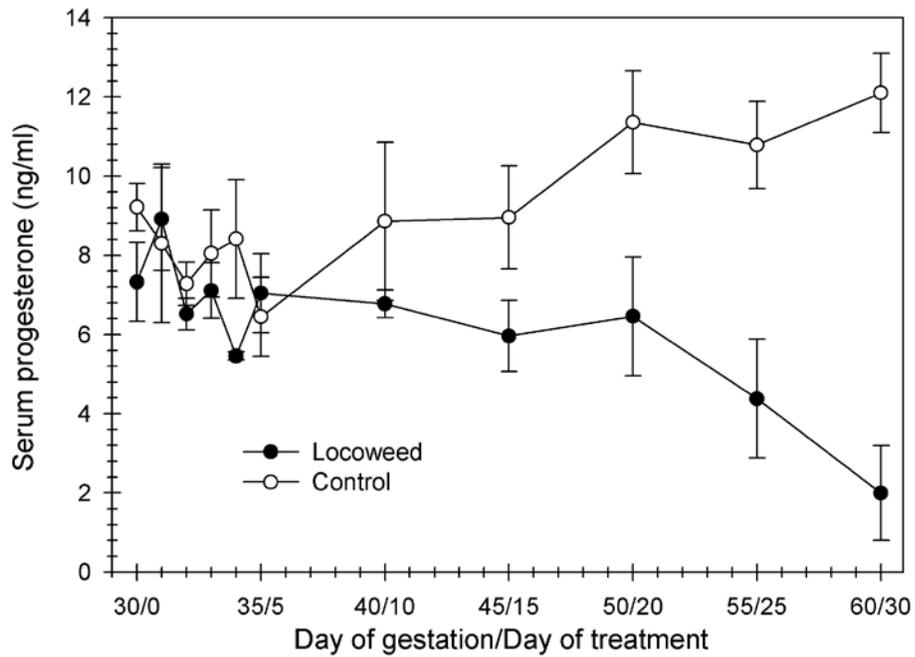


Figure 2. Serum progesterone concentrations (ng/ml) in goats treated with locoweed compared with controls. Declining serum progesterone levels are consistent with the fetal loss shown in figure 3.

Fetal death was observed by ultrasound in one goat on day 40 of gestation (10 days after treatment began). Fetal movement in the surviving fetuses was reduced ($P < 0.05$) compared with controls, and fetal death in 2 more locoweed-treated goats was observed on gestation day 45 and the fourth on gestation day 55 (figure 3). Fetal movement remained normal in control goats throughout pregnancy, and goat kids were within normal parameters at birth.

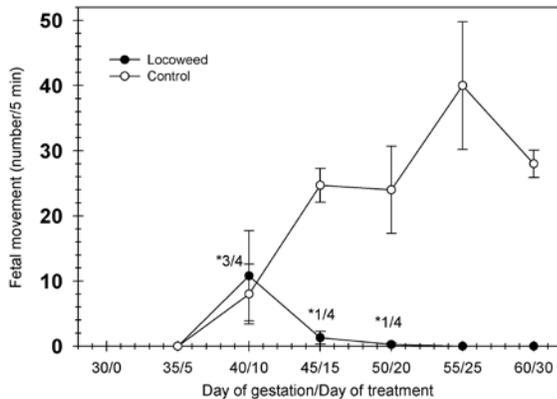


Figure 3. Comparison of fetal activity between locoweed-treated and control goats. Fetal viability and number of movements in real time were visually determined from video-recorded ultrasound images. Fetal movement first begins between 35 and 38 days of gestation in goats. The values shown in the figure represent the number of viable fetuses/total fetuses detected at a given ultrasound time

All fetuses from goats fed locoweed were negatively impacted, and all fetuses died between 10 and 25 days after the beginning of locoweed treatment (figure 3). As early as treatment day 10 in one goat (day 40 of gestation), no fetal heart beat was evident by ultrasound and the fetus was dead. Fetal death was observed in the other 4 goats on treatment days 15 (2 animals) and 20. Ultrasound evaluation revealed dead fetuses in various stages of resorption, and follow-up necropsy confirmed that fetuses were in advanced stages of autolysis. Histologically, all of the treated does had neurovisceral vacuolation in tissues characteristic of locoweed poisoning. The fetuses did not show the typical neurovisceral vacuolation; however, their tissues were in advanced stages of decomposition and were of little histological value.

Discussion

In this study, Spanish goats and their fetuses were determined to be very sensitive to locoweed

poisoning at the doses fed during the first trimester of pregnancy. This is important as many animals, including sheep, cattle, and goats, are exposed to locoweed during early gestation, and field reports often indicate that many females recycle during the breeding season or are not pregnant at the end of the breeding season. Pregnant Spanish goats fed locoweed exhibited clinical signs similar to those described in horses, i.e., ataxia, severe proprioceptive deficits, excitement, muscular tremors, lumbar paresis, and aberrant behavior. These clinical signs had a relatively rapid onset, indicating the severity of the intoxication, and the rear limb paralysis was typically seen when animals were excited or stressed. Rear limb paralysis has not been a commonly reported clinical effect from intoxication by swainsonine-containing locoweeds but has been reported for nitro-bearing species (Mathews 1940, James et al. 1980). Detailed histopathology from this study will be reported elsewhere.

Locoweed exposure in utero also had severe effects on fetal goats. Fetal movement was depressed, indicative of fetotoxicity. Fetal death occurred as early as 10 days after treatment began and all fetuses were dead by 25 days. The fetuses were autolytic and the ultrasound observations superimposed on the progesterone levels suggested that fetal death may be multifaceted, i.e. directly affecting the fetus (fetal death) or affecting the placenta. Locoweed given to pregnant sheep and goats has been shown to affect fetal and placental development (Van Kampen and James 1971, Panter et al. 1987, Hafez et al. 2007). Gross histological findings in sheep include fluid accumulation in the placenta (hydrops allantois, hydrops amnii), altered cotyledonary development, with fetal death followed by abortions. Panter et al. (1987) also showed that fetal heart rate was reduced and fetal heart contractions were irregular and weak. Fetal cardiac insufficiency and right heart failure may contribute to the fluid accumulation in the fetus and placenta (Panter et al. 1999) and cause fetal death and abortion (Panter et al. 1987). James (1971, 1976) fed ewes locoweed during specific gestational time periods and studied lamb fetal development. Sheep fetuses exposed to locoweed in utero before 100 days of gestation did not have any lesions associated with locoweed intoxication (James 1971, 1976). Cytoplasmic vacuolization occurred in lambs whose dams were fed locoweed from 100 to 120 days of gestation (James 1971).

Conclusions

This research suggests that Spanish goats are very sensitive to the negative effects of locoweed on reproduction. The early reduction in fetal movement and fetal death is indicative of this sensitivity. Also, the clinical signs of poisoning were pronounced relatively early but slightly different from what has been reported, i.e. the rear-end paresis and severe proprioceptive deficits. Additional studies of the hormonal, placental, and early fetal lesions in poisoning are needed to better understand the mechanism of locoweed-induced fetal death. This work demonstrates that dosage of swainsonine ingested is an important aspect in predicting risk of poisoning. We know that low doses of swainsonine will also induce reproductive dysfunction; however, ingestion must occur over a longer period of time as expected. This threshold dosage is understood in many species but has not been fully described in goats and will require further research.

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