EFFECTS OF DIGESTED AND UNDIGESTED SNAKEWEED INGESTION ON BLOOD COMPONENTS OF FEMALE SPRAGUE-DAWLEY RATS

R. A. Halalsheh¹, L. J. Yates¹, D. T. Yates², A. F. Montoya¹, and T. T. Ross¹
¹ New Mexico State University, Las Cruces, NM, USA¹, ²The University of Arizona, Tucson, AZ, USA

Abstract: Snakeweed (Gutierizia sarothrea) is a noxious plant infesting rangelands in the western U.S., northern Mexico, and southern Canada. An experiment was conducted and replicated over time (Exp. 1 and 2) to examine effects of snakeweed (SW) ingestion on serum components in female Sprague-Dawley rats. In both experiments, 36 rats were offered either In Vitro ruminally digested (DSW) or undigested (USW) snakeweed. Treatments were 15% digested SW (15% DSW; n = 6), 15% undigested SW (15% USW; n = 6), or 20% digested SW (20% DSW; n = 6) of 5001 Rat Chow® diet. Additionally, each of these rats was assigned a pair-fed, non-snakeweed control 5001 Rat Chow® diet (control; n=6/treatment). Pair-fed control rats were fed based on the intake of their treated pair to eliminate any nutritional variation due to feed intake. The pair-fed control diet was adjusted using corn meal and the 5001 Rat Chow® diet. Additionally, each of these rats was assigned a pair-fed control 5001 Rat Chow® diet (control; n=6/treatment). Pair-fed control rats were fed based on the intake of their treated pair to eliminate any nutritional variation due to feed intake. The pair-fed control diet was adjusted using corn meal and the 5001 Rat Chow® diet to make the diet iso-caloric and iso-nitrogenic. Data were analyzed as a completely randomized design and feed intake as a repeated measures. Rats were fed for 10 d, after which blood samples were collected via heart venipuncture. In Exp. 1, daily feed intake and BW were similar (P > 0.05) among treatments compared to controls. In Exp. 2, rats consumed 15% DSW and 15% USW had greater (P < 0.05) feed intake than rats consumed 20% DSW. Additionally, rats consumed 15% DSW had greater (P < 0.05) feed intake compared to rats fed 15% USW on d 3, 5, 6 and 9. In Exp. 2, BW decreased (P < 0.05) in all treatments compared to controls. In Exp 1 and 2, serum constituents were similar (P > 0.05) among treatments except for triglycerides which decreased (P < 0.05) in Exp. 2 in rats consuming SW treatments compared to pair-fed controls. This may be an effect of reduced BW in treated rats compared to pair-fed controls, as body fat is most likely being mobilized. These results indicate that SW ingestion causes mild toxic effects in rats.

KEYWORDS: Snakeweed, Sprague-Dawley rat, Enzymes

Introduction
Rangelands in the Western United States contain a wide range of plant species, some of them are of great benefit to animals while others are very toxic. Toxic plant species can cause large economical losses in livestock industry, both directly and indirectly (James et al., 1992). Direct losses include reduce growth rates, low birth rates, weakened newborns, abortion, and increased death loss. Indirect losses include preventative measures, such as feeding supplements and managing livestock to avoid poisoning, as well as decreased forage availability and lower stocking rates.

Snakeweed (Gutierizia sarothrea) is a noxious plant the western United States, northern Mexico and southern Canada (Florez-Rodríguez et al., 1989; Smith et al., 1991). Approximately 60% of New Mexico and 22% of Western Texas have been invaded by snakeweed (Torell et al., 1988). Many methods have been developed to control snakeweed. The most common is herbicidal treatment, however, burning, mowing and biological control are also used (McDaniel, 1991).

Snakeweed poisoning occurs during periods in which forage is scarce. Snakeweed is unpalatable to animals, but under severe conditions like drought when more palatable forage is in short supply, animals are more likely to graze this toxic plant (Gardner et al., 1999). Clinical signs of snakeweed poisoning vary according to the degree of poisoning, but may include loss of appetite and weight loss, reduction in growth rate, low birth weight, weakened newborns, abortion, and increased death loss (James et al., 1980; Dollahite et al., 1957; James et al., 1992; Smith et al., 1994).

Poisoning effect of snakeweed is the result of the presence of compounds such as saponins, alkaloids, terpenes, flavanols and other resinous substances (Smith et al., 1991). A diet containing 20% or more snakeweed will usually lead to poisoning (Smith et al., 1994). Many researchers have found that saponins are the primary toxicant in snakeweed (Dollahite et al., 1962; Kinsbury, 1965; Smith et al., 1994), and multiple studies have shown that rats are sensitive to SW toxicity (Florez-Rodríguez et al., 1989, 1990; Edrington et al., 1990, 1993a, 1993b). Using rats as a model in ruminants may not be appropriate because of the potential changes snakeweed may undergo in the rumen so, SW has been digested before offering it to rats. Rumen and abomasum compartments of ruminant animals have potential to alter compounds found in various feedstuffs due to the presence of microflora in the rumen and low pH in the abomasum (McDonald et al., 2002). This possibility leads to a potential for toxic compounds in SW to be altered in a manner that could render them more or less toxic to the animal. The objective of this study was to examine effects of snakeweed ingestion on certain blood
serum components of female rats fed either ruminally digested or undigested snakeweed.

**Materials and Methods**
All procedures and protocols described below were approved by the New Mexico State University Institutional Animal Care and Use Committee (IACUC). All snakeweed samples were harvested from the Chihuahuan Desert Range Research Center (CDRRC), located 37 km north of Las Cruces, New Mexico. Snakeweed was harvested during the pre-bloom stage via hand clipping. Approximately 5 to 10 cm of the distal portion of the plants were harvested. Snakeweed samples were stored frozen at -20°C and were ground to pass through a 2 mm screen at the time of use.

**In vitro ruminal digestion:** Two salt solutions were used in the *in vitro* digestion. Salt solution A consisted of 7.3 g K2HPO4*3H2O/L de-ionized (DI) water. Salt solution B consisted of 6.9 g KH2PO4, 12.0 g (NH4)2SO4, 12.0 g NaCl, 2.5 g MgSO4*7H2O, and 25 g CaCl2*2H2O. Forty mL of solution A and 40 mL of solution B were mixed together, along with 0.6 g of cestein hydrochloride, 1 mL resazorine (1.0%), and 875 mL DI water to create the complete buffer. Buffer was then adjusted to a pH of 7.0, autoclaved and cooled. Finally, 8% Na2CO3 was added, and the buffer was bubbled with CO2 for 3 min (Russel and Martin, 1984). Rumen fluid was obtained from a canulated Angus cow fed *ad libitum* sorghum hay. The buffer and rumen fluid were mixed at a ratio of 50:50. Ground snakeweed was added at 0.5g per 50 mL of the mixed buffer and rumen fluid. After incubation in a water bath at 39ºC for 24h, the content of each flask was filtered through a 2 mm screen at the time of use.

**Table 1. Nutrient analysis (% dry matter) for digested and undigested snakeweed.**

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment 1</th>
<th>Treatment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry Matter, %</td>
<td>23.9</td>
<td>61.2</td>
</tr>
<tr>
<td>Crude protein, %</td>
<td>10.7</td>
<td>11.3</td>
</tr>
<tr>
<td>Acid detergent fiber, %</td>
<td>37.5</td>
<td>26.5</td>
</tr>
<tr>
<td>Ether extract, %</td>
<td>11.3</td>
<td>11.7</td>
</tr>
</tbody>
</table>

1Ruminally digested snakeweed  
2Undigested snakeweed

Rats were housed at the Small Animal Care Facility at New Mexico State University. Mature female rats were weighed and placed individually in plastic box-type cages. Rats were randomly assigned to 1 of 3 treatments: 15% digested snakeweed (15% DSW), 20% digested snakeweed (20% DSW), and 15% undigested snakeweed (15% USW) with 5001 Rat Chow™ as the remainder of the diet. Each treatment contained 6 rats each treated rat was assigned a pair-fed control. Pair-fed rats received rat chow mixed with corn to make it isenergetic and isonitrogenic. The experiment was replicated over time (36 rats per replicate: 6 per treatment, 18 controls). Treatment diets were offered for 10 d. Initial weight, final weight, and daily feed intake were recorded. After d 10, a blood sample was collected via heart venipuncture for blood serum component analysis and rats were euthanized by decapitation.

**Statistical Analysis**
Data were analyzed using the mixed procedure of SAS (SAS Inst., Inc., Cary, NC). Model effects were treatment, time, and treatment x time, and the animal was considered the experimental unit. Intake was analyzed as a split plot using the mixed procedure of SAS with the repeated measures function. Treatment was in the main plot and day and the interaction were in the sub-plot. For each experiment, intake was compared between snakeweed conditions for each of the 10 treatment days. Where F values were significant (P < 0.05), means were separated by contrast statements.

**Results and Discussion**
In Exp 1, BW was similar (P > 0.05) among all groups over the 10-d treatment period. However, in Exp 2, rats fed 15% DSW and 20% DSW lost more weight (P < 0.05) compared to controls and USW (Table 2). Feed intake was similar (P > 0.05) among all treatments in Exp 1 (Figure 1). However, in Exp 2, 20% DSW reduced (P < 0.05) feed intake compared to other diets except on d 3, 5, 6, and 9, where rats fed 15% USW consumed the least (P < 0.05; Figure 2). Loss in BW associated with similar or reduced feed intake among treatments indicates adverse effects of snakeweed on nutrient metabolism and absorption, as saponins in snakeweed have been reported to decrease growth rate and absorption of nutrients (Cheeke, 1971). Decreased BW and feed intake could also be due to the fact that snakeweed is unpalatable.

In Exp 2, blood serum triglyceride concentrations decreased (P < 0.05) in all treatments compared to pair-fed controls (15% DSW: 78.7 ± 9.9 vs. 163.0 ± 9.9; 15% USW: 90.0 ± 16.7 vs. 158.3 ± 16.7; and 20% DSW: 79.3 ± 21.2 vs. 200.3 ± 21.2, respectively). Reduced triglycerides may be an effect of reduced BW, as body fat metabolism occurs when glycogen is depleted after fasting or losing BW. Rates of body fat synthesis and mobilization are closely related to energy intake (Parmley and McNamara, 1996). Florez-Rodriguez et al. (1989), Edrington et al. (1991), and Edrington (1993) reported similar effects of snakeweed on glucose and triglycerides related to decrease BW after snakeweed consumption. Decreased triglycerides in Exp. 2 may be related to under-nutrition and reduced BW in rats consuming snakeweed.
Implications

Findings from this study indicate that snakeweed ingestion reduces feed intake and BW and causes mild toxicity as indicated by blood serum components. Digested snakeweed elicited the most dramatic changes, and thus further testing of additional serum components is needed to determine if snakeweed causes changes in metabolism and absorption of nutrients.

Literature Cited


Figure 1. Daily feed intake of rats (n = 6) offered ruminally digested snakeweed (DSW) or undigested snakeweed (USW) over a 10-d period in Exp 1. A treatment x day interaction was observed (P < 0.05).

Figure 2. Daily feed intake of rats (n = 6) offered ruminally digested snakeweed (DSW) or undigested snakeweed (USW) over a 10-d period in Exp 2. A treatment x day interaction was observed (P < 0.05). Differing superscripts indicate differing means (P < 0.05).

Table 2. Body Weight (g) of rats fed ruminally digested snakeweed (DSW) and undigested snakeweed (USW) over a 10-d feeding period.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>15%DSW</th>
<th>Control</th>
<th>15%USW</th>
<th>Control</th>
<th>20%DSW</th>
<th>SE^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BW change</td>
<td>-45.3</td>
<td>-34.8</td>
<td>13.0</td>
<td>-8.0</td>
<td>-9.8</td>
<td>-18.9</td>
<td>18.5</td>
</tr>
<tr>
<td>Exp 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BW change</td>
<td>-2.6^a</td>
<td>-25.6^b</td>
<td>27.6^a</td>
<td>-9.7^b</td>
<td>13.9^a</td>
<td>-20.4^b</td>
<td>8.3</td>
</tr>
</tbody>
</table>

^a,bMeans with different superscripts differ (P < 0.05) from controls.
1Pair-fed rats.
2Standard error (n