The Texas Journal of Agriculture and Natural Resources 30:1-15 (2017)

Bitterweed Ingestion May Impact Reproductive Potential in Rambouillet Lambs and Ewes

Derrick K. Fuchs[1]
Dan C. Dusek[1]
Cody B. Scott[*1]
Micheal W. Salisbury[1]
Corey J. Owens[1]
Richard Brantley[2]

[1]*Department of Agriculture, Angelo State University, San Angelo, TX*
[2]*Director of Surface Interests, University Lands, Midland, TX*

ABSTRACT

Bitterweed (*Hymenoxys odorata*) is a cool season annual forb that causes toxicosis in sheep. However, limited knowledge is available on bitterweed's effect on reproduction in sheep. Trial 1 assessed the effect of bitterweed on fetal development. Eight mature Rambouillet ewes were dosed with ground bitterweed and 8 did not receive bitterweed. Fetal survival and birth weights were recorded. For Trial 2, 14 Rambouillet ewe lambs were divided into two treatments with half dosed with bitterweed with hormonal changes recorded to determine the impact on reproductive development. Trial 3 determined if bitterweed affected sperm production in Rambouillet ram lambs. Twenty-five rams were allocated into either the bitterweed treatment or the control. For all trials, treatments were dosed with ground bitterweed (0.2% BW) mixed in distilled water. Intake of a novel food was paired with bitterweed dosing to assess toxicosis. Serum metabolite concentrations were monitored to assess soft tissue damage from toxicosis. Bitterweed toxicosis reduced intake and elevated serum metabolite concentrations in all three trials. Bitterweed ingestion did not affect fetal survival, vigor or birth weights, but did impact ewe lamb reproductive development as evident from the lower ($P < 0.05$) luteinizing hormone levels. In Trial 3, bitterweed ingestion did not impact sperm production.

Key Words: *Hymenoxys*, aversive, LH, intake, serum

INTRODUCTION

Bitterweed contains hymenoxon, a sesquiterpene lactone, which can cause chronic, acute, or subacute toxicosis (Hill et al. 1979; Terry et al. 1983; Kim et al. 1987). Symptoms range from stiffness to soft tissue damage in the lungs, kidneys, and liver and even into the central nervous system depression and eventual death if intake exceeds estimated lethal levels (LD_{50} = 0.264% BW) (Witzel et al. 1974; Hill et al. 1979; Calhoun et al. 1981).

[*] Corresponding author: Cody.Scott@angelo.edu.

Several methods have been used to help contain the spread of the plant and prevent bitterweed toxicity. Spraying stands of bitterweed with herbicides, such as 2, 4-D, are effective in controlling the plant when infestations are heavy, but spraying is often economically unfeasible (Bunting and Wright 1974; Ueckert and Calhoun 1988). Feed additives have also been used to help reduce the likelihood of bitterweed toxicity. Activated charcoal, Santaquin, and L-cysteine all reduce the likelihood of bitterweed toxicosis (Poage et al. 2000). Activated charcoal is difficult to mix because of its fine particle size and volatility (Dollahite et al. 1973). Both Santaquin and L-cysteine reduce the palatability of the feed to the point where sheep will not consume sufficient levels to circumvent toxicosis (Calhoun et al. 1989; Kim et al. 1981, 1982).

Rotational grazing and moderate stocking rates reduce the likelihood of bitterweed toxicosis (Ueckert and Calhoun 1988). Even when grazing management strategies are implemented to minimize the impact of grazing, sheep may be unable to meet their nutritional requirements during winter when bitterweed may be the only readily digestible forage available (Whisenant and Ueckert 1982; Pfeiffer and Calhoun 1988). Sheep typically avoid bitterweed because ingestion results in aversive postingestive feedback and the formation of a conditioned food aversion (Calhoun et al. 1981; Poage et al. 2000). Because of the lack of alternative forage, sheep are often forced to consume bitterweed, resulting in toxicosis and death thereafter.

Unfortunately, little is known of bitterweed's effects on reproductive functions and development of livestock when sub-lethal levels are consumed. Given that bitterweed toxicosis causes central nervous system depression and soft tissue damage throughout the body, it seems likely that bitterweed could affect reproductive performance/development in sheep as well. Central nervous system (CNS) depression and soft tissue damage from bitterweed ingestion could potentially interfere with hormone levels in both rams and ewes and cause reproductive problems. Accordingly, the objective of this study was to determine if bitterweed reduces reproductive performance of both ewes and rams.

MATERIALS AND METHODS

All trials were conducted at the Angelo State University (ASU) Management Instruction and Research (MIR) Center, San Angelo, TX (Lat: 31.38, Lon: 100.5). For Trial 1, 16 mature (mixed-aged), bred Rambouillet ewes were used. Fourteen yearling Rambouillet ewes were used in Trial 2. Twenty-five Rambouillet ram lambs (six months old) were utilized in Trial 3. All sheep used in the experiments were placed in individual pens (1 m X 1.5 m) during testing. Water and a calcium/phosphorous mineral with trace elements were provided *ad libitum*. Alfalfa pellets (2.5% BW) were fed daily to meet maintenance requirements in Trials 1 and 2 (NRC 2007). For Trial 3, ram lambs received a grain-based ration (RAM 20) used at the MIR Center (Table 1). RAM 20 (2.5% BW) was utilized instead of alfalfa pellets because rams were scheduled to be used for breeding purposes after the trial. Before and after testing, sheep were housed on wheat pasture with fresh water and calcium/phosphorous mineral with trace elements was provided *ad libitum*. All research protocols used in these studies were approved by the Angelo State University Institutional Animal Care and Use Committee.

Bitterweed was collected from December (germination) through March (flowering). Samples were air-dried, mixed, and ground to ensure a uniform distribution of hymenoxon in bitterweed fed to the sheep. Hymenoxon levels vary depending on phenological stage and growing conditions (Pfeiffer and Calhoun 1988). Air-dried

bitterweed was ground to pass through a 1 mm screen, then added to 500 mL of distilled water immediately before dosing and administered via gastric tube daily for three to four days, depending on the trial. Intake of barley and the basal diet (alfalfa or RAM 20) were measured daily to assess the presence of toxicosis. The level used in this study (0.2% BW) was chosen to induce toxicosis while avoiding potential death (Calhoun et al. 1981). Hymenoxon levels vary throughout the growing season, but remain potentially lethal through flowering if consumed as sufficient levels (Ueckert and Calhoun 1988). Similarly, we stopped dosing with bitterweed after three to four days to reduce the likelihood of causing death. Given that sheep were dosed with bitterweed, we had to feed a novel food source (rolled barley) so that the aversive feedback experienced in the gastrointestinal tract from bitterweed could be associated with the flavor of a food (Poage et al. 2000). A novel food was used because aversions form faster to novel foods (Provenza 1995).

Table 1. Ingredient and nutrient content of the ration (RAM 20) used to meet maintenance requirements of rams in Trial 3. Data reported herein was on an as-fed basis.

Ingredient	Percent (%) in the Feed
Sorghum grain	45.0
Cottonseed meal	10.0
Soybean hulls	22.5
Alfalfa pellets (dehy)	17.0
Cane molasses	3.5
Premix[1]	2.0
Nutrient Content	
Crude protein	14.8
Digestible protein	10.0
Digestible energy (mcal/kg)	2.8
Crude fiber	14.1
TDN	63.0

[1] Premix includes: Lasalocid, calcium, salt, manganese, zinc, selenium, copper, Vitamins A, D, and E.

Trial 1. The objective of Trial 1 was to determine if bitterweed ingestion affects fetal development. Ewes were examined using radio ultrasound on Days 100 to 110 of gestation to determine pregnancy status and number of offspring carried. Ewes with twins were equally distributed across treatments. Pregnant ewes were then exposed to a seven-day adjustment period in which all ewes were placed in individual pens and fed alfalfa pellets at 2.5% BW. Alfalfa pellets were also fed throughout bitterweed dosing. Following the adjustment period, ewes were fed 200 g of a novel grain source (rolled barley) for 15 min a day for two days. Prior to dosing with bitterweed, we had to make sure that ewes were consuming barley so that the taste of barley could be paired with any postingestive feedback experienced by the ewes.

Beginning on Day 3 and continuing for four days thereafter, half of the ewes were dosed via oral gavage with bitterweed daily immediately after feeding barley. Control ewes were not dosed. Gastrointestinal malaise is only associated with taste (Provenza 1995). When lambs were dosed with distilled water while others were dosed with Lithium Chloride, which causes internal malaise, dosing with distilled water had no impact on intake (Provenza et al. 1994). Prior to dosing, ground bitterweed was mixed with 500 mL of distilled water to facilitate dosing.

Intake of rolled barley was measured throughout dosing with bitterweed to assess toxicosis. Dosing with bitterweed resulted in the formation of a conditioned food aversion to a novel food source within a couple of days in a previous study (Poage et al. 2000). Severe bitterweed toxicity also causes a decrease in overall intake (Ueckert and Calhoun 1988). Alfalfa intake was also recorded daily to assess toxicity.

As a secondary measure of toxicosis, blood samples were taken via jugular veni-puncture to assess changes in serum levels of several key metabolites. Blood samples were collected using jugular veni-puncture on the day before dosing and on 24, 48, and 60 h following dosing. Thirty minutes after collection, samples were placed in a centrifuge (3,000 rpm for 20 min) to separate the serum and then stored in a freezer at -80 °C. Following completion of the study, the samples were sent to the Texas Veterinary Medical Diagnostic Laboratory in College Station, TX and analyzed for blood urea nitrogen (BUN), creatinine, serum aspartate transaminase (AST), and gamma glutamytransferase (GGT). Changes in these serum metabolite levels were indicative of bitterweed toxicity in other studies (Calhoun et al. 1981; Poage et al. 2000; Frost et al. 2003).

Following bitterweed dosing, all ewes were placed on wheat pasture through lambing. Ewes were checked daily. Once lambs were born, ewes and lambs were penned and the number of lambs born and birth weights were recorded. A vigor score (1 through 5; 1 = weak, 5 = vigorous) was also assigned by two observers for each lamb born. The number of lambs born/ewe, birth weights, and body weights taken 28 d later were used to measure offspring health and vigor.

Trial 2. The objective of Trial 2 was to determine if bitterweed ingestion interferes with reproductive activity or development of yearling ewe lambs. Fourteen Rambouillet ewe lambs were exposed to the same experimental protocol used in Trial 1 with seven dosed with bitterweed. Fourteen days following bitterweed dosing, ewes' estrous cycles were synchronized with two injections of prostaglandin F2 alpha (15 mg) (UpJohn Pharmaceuticals, Kalamazoo, MI) with the second injection administered nine days after the first injection. Seven days following the second injection, ewe lambs underwent a gonadotropin releasing hormone (GnRH) challenge. Blood samples were taken 15 min before and at the time of GnRH injection to establish a baseline serum level for luteinizing hormone (LH) concentrations. Immediately thereafter, lambs were injected intravenously with 12.5 mg of GnRH (Cystorelin, Rhode Meriux, Inc., Athens, GA). Blood samples were then collected every 15 min for 2 h then at 30 min intervals for an additional 2 h. All blood samples were centrifuged at 3,000 rpm for 20 min at 4 ºC. Serum was then harvested and stored in vials at -80 ºC until analyzed by radioimmunoassay described by Schneider and Hallford (1996). Samples were analyzed at the New Mexico State University Endocrinology Lab, Las Cruces, NM. Serum was also analyzed for BUN, creatinine, AST, and GGT as a secondary measure of toxicosis. Serum samples were sent to the Texas Veterinary Medical Diagnostic Lab, College Station, TX.

Trial 3. The objective of Trial 3 was to determine the effect of bitterweed ingestion on reproductive performance of Rambouillet ram lambs. Twenty-five recently weaned Rambouillet rams were randomly allocated to two treatments. Rams in Treatment 1 were dosed with bitterweed at 0.2% BW using oral gavage while rams in the control treatment were not dosed with bitterweed. Prior to dosing, rams were placed in individual pens and allowed a 14-day adjustment period.

The same feeding protocol (pairing intake of rolled barley with bitterweed dosing) was used in Trial 3. In addition, intake of the basal diet of RAM 20 was monitored prior to and throughout dosing with bitterweed.

Semen quality (concentration, motility, color) was compared among treatments by artificially ejaculating rams throughout the study. Thereafter, semen samples were collected every 14 d over a 56-day period after the cessation of dosing with bitterweed. For each semen quality characteristic, scores were assigned from 1 through 7, with 1 being the lowest score and 7 being the highest score using the procedures described in Yates et al. (2010). Serum samples were again analyzed for AST, GGT, BUN, and Creatinine by the Texas Veterinary Medical Diagnostic Lab, College Station, TX.

Statistical Analysis. For Trial 1, intake of rolled barley, alfalfa intake, and serum metabolite levels were analyzed using repeated measures analysis of variance with day as the repeated measure and ewes (replication) nested within treatments (dosing with or without bitterweed). Differences among means were assessed by least significant differences when $P < 0.05$. Covariance structures were compared to determine the appropriate structure for each model. Autoregressive order-1 was used for all analyses. Data were analyzed with the statistical package JMP (SAS Institute 2007).

In Trials 2 and 3, intake and serum data were analyzed in the same manner as in Trial 1. Weight change and sperm data were also compared between treatments in Trial 3 using the same statistical model. For LH concentrations in Trial 2, a ewe lamb was considered one experimental unit. Serum LH was analyzed using a split plot analysis of variance. Treatment differences were tested with animals within treatment as the error term.

Ewes vary in the rate at which LH is released. Some release large amounts in short pulses while others release small amounts over extended periods of time. The total amount of LH may not vary, but because of the variations in the rate of release of LH among individuals, treatment means are often skewed (Salisbury et al. 2000). Thus, area under the response curve is commonly accepted as a more accurate assessment of LH response to GnRH challenges. Accordingly, we also assessed LH release was as the area under the response curve (0 min to 240 min) by trapezoidal summations and the resulting area under the curve units were analyzed using the GLM procedure of SAS.

RESULTS

Trial 1. Ewes dosed with bitterweed in Trial 1 experienced toxicosis as evident from the significant treatment x day ($P < 0.05$) interaction for both barley and alfalfa intake (Figs. 1a and 1b). Rolled barley intake increased daily until dosing began on Day 4 of the study.

Thereafter, intake of rolled barley decreased ($P < 0.05$) daily for those ewes dosed with bitterweed (Fig. 1a). On the final day that barley intake was recorded, ewes dosed with bitterweed refused to eat barley. Alfalfa intake of ewes dosed with bitterweed decreased ($P < 0.05$) following the first dosing and continued to decrease throughout the trial (Fig. 1b). Ewes in the control group initially were reluctant to consume barley. However, after the third day of feeding, ewes readily accepted barley and continued to consume barley throughout the study. Alfalfa intake of ewes in the control group remained constant throughout the trial.

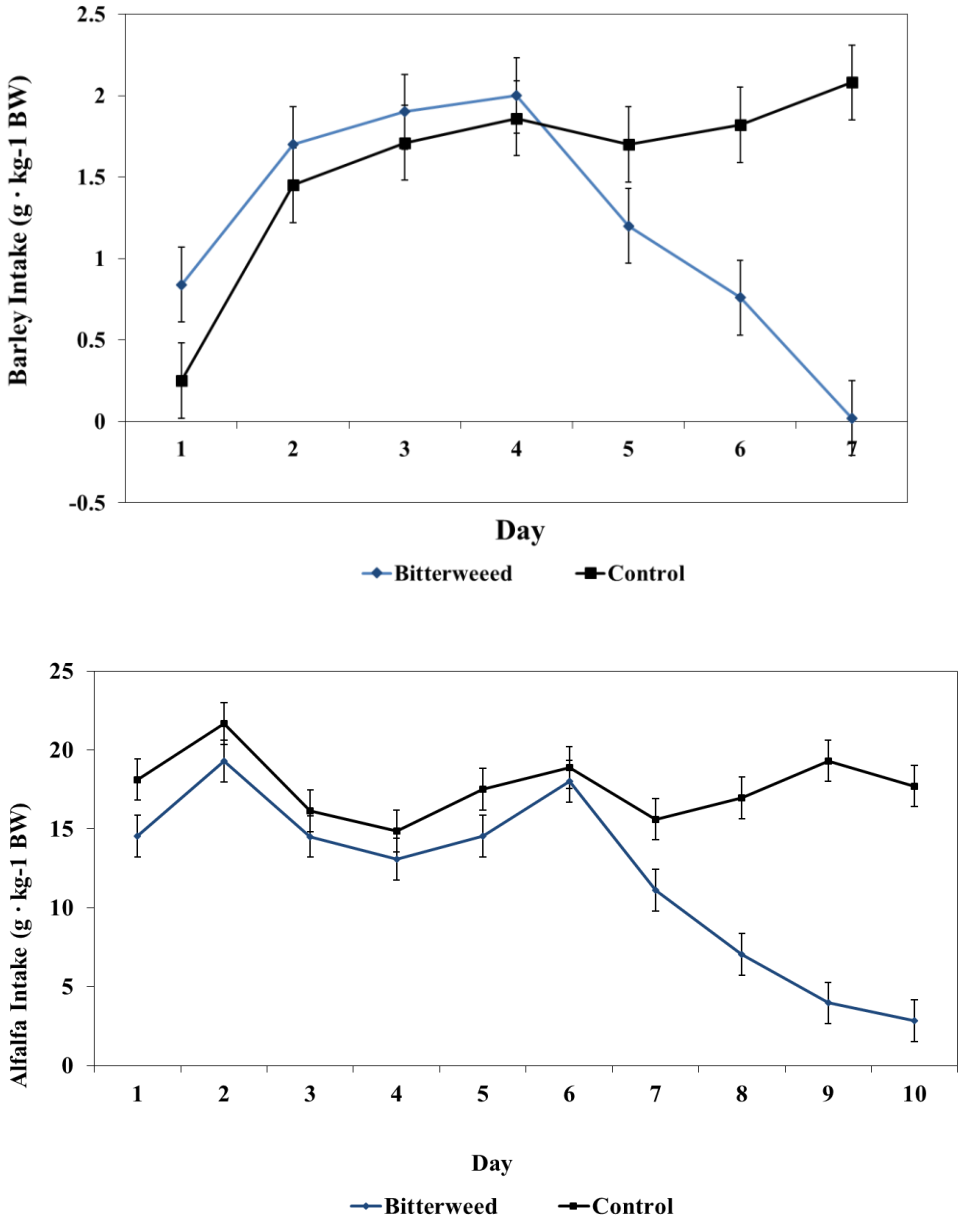

Figure 1. Intake (g · kg^{-1} BW) of rolled barley (a) and alfalfa pellets (b) when ewes were offered barley following dosing with bitterweed in Trial 1. Dosing began on Day 4.

Serum metabolite samples collected during this experiment were also indicative of toxicosis. Serum concentrations of creatinine, serum aspartate transaminase (AST), gamma glutamytransferase (GGT), all increased ($P < 0.05$) above normal ranges by the end of the study (Table 2). Blood urea nitrogen differed across days of dosing but remained within normal range for healthy animals.

Dosing with bitterweed did not affect fetal development in this study. Number of births, birth weight, and 28 d weight were similar between ewes dosed with bitterweed and ewes not dosed. Eight lambs were born to ewes in each treatment. Mean birth weights for the treatment and the control groups were 3.5 kg and 4.0 kg, respectively. Mean 28 d weights for the treatment and the control groups were 14.9 kg and 16.4 kg, respectively. Overall vigor at birth of the offspring from the two groups was also not affected by the treatment. Two lambs from the treatment group died post-partum and one ewe from the control group aborted her offspring. Death of the ewe and lambs appeared unrelated to the study.

Table 2. Changes in serum metabolite levels during Trial 1. All ewes were sampled before (Day 1), during (Days 2 and 3), and after (Day 4) dosing with bitterweed.

Serum metabolite	Day				Normal Range[1]
	1	2	3	4	
Treatment					
Serum Aspartate Transaminase (u/l)	135.7^{d}	170.3^{c}	324.5^{b}	499.5^{a}	51-130
Gamma Glutamytransferase (u/l)	52.7^{c}	51.9^{c}	95.7^{b}	234.4^{a}	34-82
Creatinine (mg/dl)	1.2	1.1	1.4	1.6	0.3-1.3
Blood Urea Nitrogen (mg/dl)	20.6^{c}	17.7^{d}	31.4^{a}	28.6^{b}	12-32
Control					
Serum Aspartate Transaminase (u/l)	81.0^{e}	88.1^{e}	82.8^{e}	92.6^{e}	51-130
Gamma Glutamytransferase (u/l)	56.0^{c}	54.4^{c}	55.0^{c}	56.3^{c}	34-82
Creatinine (mg/dl)	1.1	1.1	1.1	1.1	0.3-1.3
Blood Urea Nitrogen (mg/dl)	19.8^{c}	16.8^{d}	18.1^{cd}	17.4^{d}	12-32

$^{a-d}$ Means for each serum metabolite with different superscripts differed ($P > 0.05$).
[1]Normal range for healthy sheep as reported by the Texas Veterinary Medical Diagnostic Lab, College Station, Texas.

Trial 2. Ewe lambs dosed with bitterweed in Trial 2 also experienced toxicosis. The treatment x day interaction was significant for both barley and alfalfa intake (Figs. 2a and 2b). Intake of rolled barley increased after initial exposure, but decreased ($P < 0.05$) once ewe lambs in the treatment group were dosed with bitterweed (Fig. 2a). Alfalfa intake of ewes in the treatment group also decreased ($P < 0.05$) following bitterweed dosing (Fig. 2b). As in Trial 1, barley intake increased initially and remained high for ewe lambs in the control group. Alfalfa intake of ewes in the control group was constant throughout the trial.

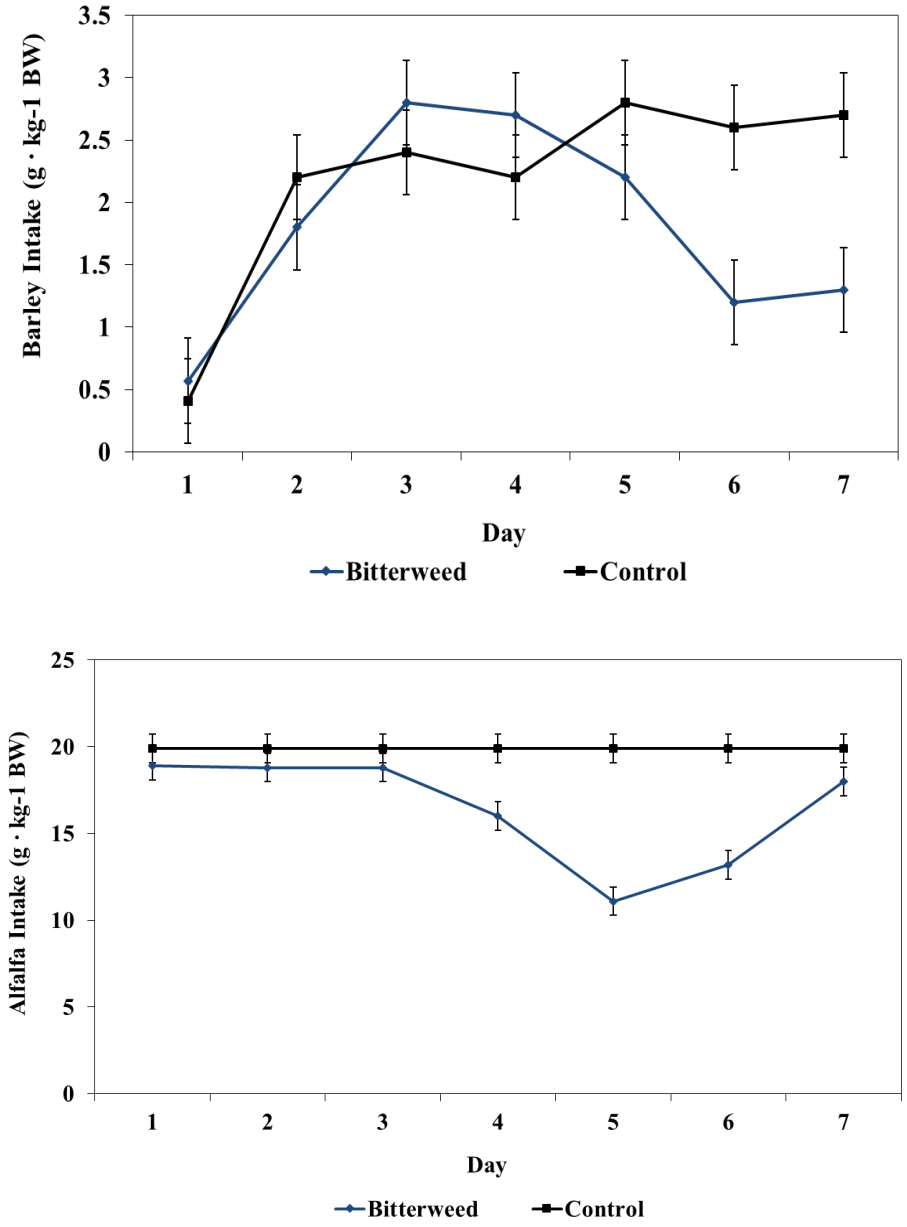

Figure 2. Intake (g · kg^{-1} BW) of rolled barley (a) and alfalfa pellets (b) when ewe lambs were offered barley following dosing with bitterweed in Trial 2. Dosing began on Day 4.

Serum metabolite samples collected during Trial 2 were also indicative of toxicosis. However, in Trial 2 only BUN and creatinine changed ($P < 0.05$) across days of dosing (Table 3). Results of the GnRH challenge indicated that ewe lambs dosed with

bitterweed had lower ($P < 0.05$) LH concentrations and the area under the response curve was lower (Table 4).

Table 3. Changes in serum metabolite levels during Trial 2. All ewe lambs were sampled before (Day 1), during (Days 2 and 3), and after (Day 4) dosing with bitterweed.

Serum metabolite	Day				Normal Range[1]
	1	2	3	4	
Treatment					
Serum Aspartate Transaminase (u/l)	91.9	88.1	82.9	105.0	51-130
Gamma Glutamytransferase (u/l)	61.7	60.0	59.4	60.3	34-82
Creatinine (mg/dl)	0.8[b]	1.0[a]	0.9[ab]	0.8[b]	0.3-1.3
Blood Urea Nitrogen (mg/dl)	20.4[d]	25.0[a]	25.6[a]	24.6[ab]	12-32
Control					
Serum Aspartate Transaminase (u/l)	76.3	78.7	75.6	73.7	51-130
Gamma Glutamytransferase (u/l)	59.0	61.4	57.1	57.8	34-82
Creatinine (mg/dl)	0.8[b]	0.9[ab]	0.8[b]	0.9[ab]	0.3-1.3
Blood Urea Nitrogen (mg/dl)	20.4[d]	21.6[c]	21.6[c]	22.3[c]	12-32

[a-c]Means for each serum metabolite with different superscripts differed ($P > 0.05$).
[1]Normal range for healthy sheep as reported by the Texas Veterinary Medical Diagnostic Lab, College Station, Texas.

Table 4. Mean serum LH concentrations and area under the response curve of Rambouillet ewe lambs following a GnRH challenge.

	Treatment		SEM
	Dosed with Bitterweed	Control	
LH (ng/ml)	9.9[b]	17.7[a]	3.7
AUC[1]	2,383.7[b]	4,254.4[a]	799.3

[1]Area under the curve (AUC) was calculated using the trapezoidal summation method and the resulting areas were analyzed as a one-way analysis of variance. Area was calculated from 0 min to 240 min post GnRH injection.
[a-b] Means within rows with different superscripts differ ($P < 0.05$).

Trial 3. Dosing with bitterweed reduced intake of rolled barley and the basal diet in Trial 3 (treatment X day interaction differed, $P < 0.05$) (Figs. 3a and 3b). Initially, rams were reluctant to consume rolled barley. However, by Day 9, both treatments were consuming all of the barley offered. To facilitate acceptance of rolled barley, the amount of the basal ration was reduced from 2.5% BW to 2.0% BW on Day 7. Thereafter, rams consumed all of the rolled barley offered in both treatments. Dosing began on Day 10. Rams dosed with bitterweed consumed less barley than rams in the control treatment. By Day 14 of the study (three days of dosing with bitterweed), rams refused to eat barley. RAM 20 intake for rams dosed with bitterweed declined on Days 13 and 14. Dosing with bitterweed stopped after three days of dosing to avoid death of rams from bitterweed toxicosis. One ram died on Day 15 of the study apparently from bitterweed toxicosis.

All rams dosed with bitterweed lost weight (initial 54.8 kg, final 52.2 kg; SEM = 2.5), but weight change was similar ($P > 0.05$) between treatments and when initial to final

weights were compared, initial and final weights were similar for rams in the control group (initial 55.8 kg, final 58.4 kg; SEM = 2.4). Serum metabolites differed between treatments and across days of feeding (treatment X day interactions were significant, $P < 0.05$). Concentrations of each increased for rams dosed with bitterweed especially when comparing beginning and ending values (Table 5). By the end of the study, serum metabolite concentrations differed ($P < 0.05$) and were above normal range for healthy animals.

Semen concentration, color and motility were similar ($P > 0.05$) among treatments, but differed across days of collection. Semen color score was 2.4 for both treatments. Motility scores were 2.4 and 2.3 for rams dosed with bitterweed and the control rams, respectively. Concentration was 98.2% for rams dosed with bitterweed and 84.3% for rams not dosed. Concentration, color, and motility improved across days of collection for both treatments as rams sexually matured. The treatment × day interactions for concentration, color, and motility did not differ ($P > 0.05$).

Table 5. Average serum metabolite levels for rams dosed with bitterweed or not dosed (control) in Trial 3. Samples were collected before dosing (initial), 24 hrs after dosing began (middle), and at the end of the study.

Treatment/Serum Metabolite	Serum Collection			
	Initial	Middle	End	Normal Range[1]
Dosed with bitterweed				
Serum Aspartate Transaminase(U/l)	105.6[b]	236.1[b]	435.4[a]	51-130
Gamma Glutamytransferase (U/l)	72.9[b]	136.8[b]	241.2[a]	34-82
Creatinine (mg/dl)	0.6[c]	1.2[b]	1.6[a]	0.3-1.3
Blood Urea Nitrogen (mg/dl)	17.2[b]	33.1[a]	37.1[a]	12-32
Control				
Serum Aspartate Transaminase (U/l)	104.8	80.2	82.6	51-130
Gamma Glutamytransferase (U/l)	69.7	72.4	74.5	34-82
Creatinine (mg/dl)	0.6	0.7	0.8	0.3-1.3
Blood Urea Nitrogen (mg/dl)	19.9	17.2	16.7	12-32

[a-b] Means within rows with different superscripts differed ($P < 0.05$).

[1]Normal range for healthy sheep as reported by the Texas Veterinary Medical Diagnostic Lab, College Station, Texas.

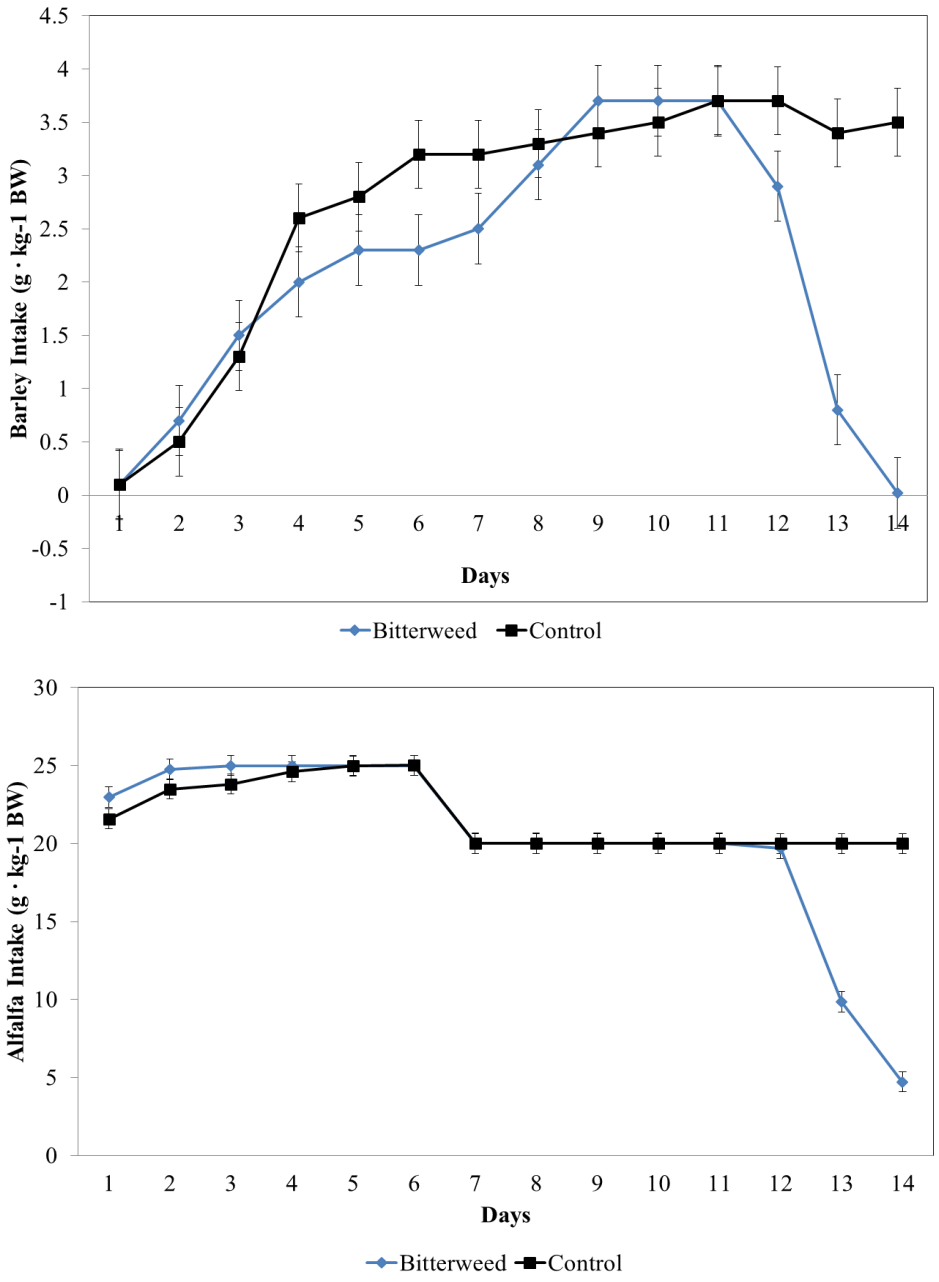

Figure 3. Intake (g · kg^{-1} BW) of rolled barley (a) and RAM 20 (b) for rams dosed with bitterweed or not dosed (control) in Trial 3. Dosing began on Day 11.

CONCLUSION AND DISCUSSION

Sheep in all three trials experienced bitterweed toxicosis. Sheep reduced intake of the novel food (barley) that was paired with bitterweed dosing and reduced intake of the basal ration (alfalfa or RAM 20) once dosing began. Once bitterweed toxicosis is initiated, sheep will reduce total intake, apparently because of gastrointestinal distress (Calhoun et al. 1981). Other observed signs of toxicosis include central nervous system depression and mucoid nasal discharge. Mild to moderate renal and hepatic damage is also a common finding in sheep sub-acutely poisoned by bitterweed ingestion (Witzel et al. 1977).

The level of toxicosis experienced by sheep in this study was apparently sufficient to cause some soft tissue damage (probably renal and hepatic injury) as evident of changes in serum metabolite concentrations (Calhoun et al. 1981; Frost et al. 2003). When soft tissue damage occurs as a result of plant-induced toxicosis, levels of AST, GGT, BUN, and creatinine are typically altered (Cornelius 1989). Concentrations of AST, GGT, and creatinine were elevated above the range for healthy animals in this study as well. When Calhoun et al. (1981) dosed sheep with increasing levels of bitterweed, serum total protein and albumin decreased while blood urea nitrogen, creatinine, and bilirubin increased. In addition, AST concentrations increased at the highest levels of bitterweed dosing. Results of this study agree with those of Calhoun et al. (1981) that intake and serum metabolite concentrations are altered because of bitterweed ingestion and subsequent toxicosis.

Bitterweed toxicity did not affect fetal development in Trial 1. There were no differences in the number of births, birth weight, vigor score, or 28-day weight of lambs from treatment or control ewes. However, bitterweed altered the LH and hormone profiles which may have a deleterious effect on reproduction in sheep consuming bitterweed. Lower LH concentrations may be in response to some damage to the hypothalamus and/or the anterior pituitary; these two locations are responsible for the release of reproductive hormones.

It appears that consuming bitterweed does not interfere with sperm production in Rambouillet yearling rams in this study. All rams were apparently suffering from soft tissue damage apparently because of bitterweed-induced toxicosis. One ram died during the study. The ram was dosed with bitterweed and died before the end of the study. The post-mortem examination indicated bitterweed toxicosis as a possible cause of death. Susceptibility to bitterweed toxicosis varies among individual sheep even within the same flock (Ueckert and Calhoun 1988). For instance, dosing with 0.264% BW of bitterweed resulted in the death of four out of 10 sheep while others showed no signs of toxicity even as dosing level increased (Calhoun et al. 1981).

Bitterweed ingestion typically occurs when sheep are unable to meet nutrient requirements on pasture (Ueckert and Calhoun 1988). Most landowners report higher incidences of bitterweed toxicosis in yearling ewe and ram lambs that have higher nutrient requirements because of demand for growth. If male or female yearling animals are unable to meet their nutritional requirements, reproductive performance could be compromised regardless of the direct impact of toxin ingestion. At the very least, libido should decline as males experience toxicosis or nutrient stress. In addition, if nutrient intake is compromised, reproductive efficiency will probably suffer in females as well.

Most current management approaches to avoid bitterweed toxicosis typically rely on grazing management decisions to reduce the likelihood of nutrient stress and bitterweed ingestion. When stocking rates are excessive, range condition typically declines. The production and diversity of desirable plants is suppressed, while toxic plants become more

numerous. As a result, the probability that livestock will consume toxic plants increases. Merrill and Schuster (1978) reported that sheep death losses from bitterweed occurred during 12 of 20 years at heavy stocking rates of sheep alone. Conversely, in a moderately stocked, four-pasture deferred-rotation system grazed with cattle, sheep, and goats there were no death losses due to bitterweed poisoning over a 20-year period. The combination of cattle, sheep, and goats reduces the intraspecific competition for forage, thus decreasing the likelihood that sheep would consume sufficient amounts of bitterweed to experience toxicosis. In addition, maintaining moderate stocking rates promotes increases in species composition and forage production (Taylor et al. 1993a, 1993b).

Proper supplementation strategies, especially during winter when bitterweed toxicosis typically occurs, should reduce the likelihood of toxicosis. Protein sources that provide a source of sulfur-containing amino acids (cysteine, methionine) improve rumen degradation of hymenoxon. Once hymenoxon is absorbed into the blood stream, it travels to the liver where it can be partially oxidized and conjugated with gluconurides for excretion (Terry et al. 1983). Protein sources that consist of amino acids that escape rumen degradation and are absorbed in the small intestine may provide substrates of conjugation in the liver (Bundick 2008). Irrespective of the type of protein fed, providing additional nutrients to meet animal requirements will reduce the likelihood of consuming bitterweed.

Bitterweed toxicity does not appear to reduce the number of lambs born, lamb birth weights, or vigor. Conversely, bitterweed ingestion by yearling ewes may reduce their reproductive activity. Thus, yearling ewes should be maintained on bitterweed-free rangelands when possible. Producers should attempt to minimize bitterweed toxicity in mature and yearling rams as well. Although the study did not show any effect on reproduction in males, bitterweed ingestion could cause production and growth problems as well as livestock death when it is consumed at a toxic level. The key to avoiding bitterweed toxicosis is providing sufficient forage for livestock. Producers should maintain proper stocking rates and avoid stands of bitterweed especially with replacement ewes or rams that have higher nutritional requirements because of additional nutrients required for growth. Proper supplementation during the dormant season should further reduce the likelihood of bitterweed toxicosis.

ACKNOWLEDGEMENTS

Research reported herein was supported by University Lands and the Angelo State University Management, Instruction, and Research Center.

REFERENCES

Bundick CJ. 2008. Supplementation with onions to increase intake by sheep. M.S. Thesis, Angelo State University, San Angelo, Texas. 32 p.

Bunting SC, Wright HA. 1974. Controlling bitterweed with fall and winter applications of 2,4-D amine. *Journal of Range Management* 27:381-382.

Calhoun MC, Ueckert DN, Livingston CW, Baldwin Jr. BC. 1981. Effects of bitterweed (*Hymenoxys odorata*) on voluntary feed intake and serum constituents of sheep. *American Journal of Veterinary Research* 42:1713-1717.

Calhoun MC, Baldwin Jr. BC, Kuhlmann SW, Kim HL. 1989. Experimental prevention of bitterweed (*Hymenoxys odorata*) poisoning of sheep. *American Journal of Veterinary Research* 50:1642-1646.

Cornelius CE. 1989. Liver Function. *In*: J.J. Kaneko (Ed.) Clinical biochemistry of domestic animals. Academic Press, San Diego, CA. p 364-397.

Dollahite JW, Rowe LD, Kim HL, Camp BJ. 1973. Control of bitterweed (*Hymenoxys odorata*) poisoning in sheep. Texas Agricultural Experiment Station Progress Report PR-3149.

Frost RA, Scott CB, Walker JW, Hartmann FS. 2003. Effects of origin, experiences early in life, and genetics on bitterweed consumption in sheep. *Applied Animal Behaviour Science* 84:251-264.

Hill DW, Kim HL, Camp BJ. 1979. Qualitative analysis of hymenoxon in plant tissue. *Journal of Agricultural and Food Chemistry* 27:885-886.

Kim HL, Anderson AC, Terry MK, Bailey Jr. EM. 1981. Protective effect of butlylated hydroxyanisole on acute hymenoxon and bitterweed poisoning. *Research Communications in Chemical Pathology and Pharmacology* 33:365-368.

Kim HL, Anderson AC, Herrig BW, Jones LP, Calhoun MC. 1982. Protective effects of antioxidants on bitterweed (*Hymenoxys odorata* DC) toxicity in sheep. *American Jounal of Veterinary Research* 43:1945-1950.

Kim HL, Safe SH, Calhoun MC. 1987. Quantitation of hymenoxon and related sesquiterpene lactones. *Journal of Agricultural and Food Chemistry* 35:891-895.

Merrill LB, Schuster JL. 1978. Grazing management practices affect livestock losses from poisonous plants. *Journal of Range Management* 31-351-354.

National Research Council [NRC]. 2007. Nutrient Requirements of Small Ruminants: Angora, Dairy, and Meat Goats. The National Academy Press, Washington, D.C. 362 p.

Pfeiffer FA, Calhoun MC. 1988. Effects of environmental, site and phenological factors on hymenoxon content of bitterweed (*Hymenoxys odorata*). *Journal of Animal Science* 65:1553-1562.

Poage III GW, Scott CB, Bisson MG, Hartmann FS. 2000. Activated charcoal attenuates bitterweed toxicosis in sheep. *Journal of Range Management* 53:73-78.

Provenza FD. 1995. Postingestive feedback as an elementary determinant of food preference and intake in ruminants. *Journal of Range Management* 48:2-17.

Provenza FD, Ortega-Reyes L, Scott CB, Lynch JJ, Burritt EA. 1994. Antiemetic drugs attenuate food aversions in sheep. *Journal of Animal Science* 72:1989-1994.

Salisbury MW, Ross TT, Krehbiel CR, Melton LL, Schultz CL, Hallford DM. 2000. Development and reproductive performance in Suffolk and whitefaced lambs consuming medium-quality forage and supplemented with two levels of undegradable intake protein. *Sheep and Goat Research Journal* 16:37-45.

SAS Institute. 2007. JMP User's Guide, Version 7.0 SAS Institute Inc., North Carolina.1035 p.

Schneider FA, Hallford DM. 1996. Use of rapid progesterone radioimmunoassay to predict pregnancy and fetal numbers in ewes. *Sheep and Goat Research Journal* 12:33-38.

Taylor Jr. CA, Garza Jr. NE, Brooks TD. 1993a. Grazing systems on the Edwards Plateau of Texas: are they worth the trouble? I. Soil and vegetation response. *Rangelands* 15:53-57.

Taylor Jr. CA, Garza Jr. NE, Brooks TD. 1993b. Grazing systems on the Edwards Plateau of Texas: are they worth the trouble? II. Livestock Response. *Rangelands* 15:57-60.

Terry MK, Williams HG, Kim HL, Post LO, Bailey Jr. EM. 1983. Ovine urinary metabolites of hymenoxon, a toxic sesquiterpene lactone isolated from *Hymenoxys odorata* DC. *Journal of Agricultural and Food Chemistry* 31:1208-1210.

Ueckert DN, Calhoun MC. 1988. Ecology and toxicology of bitterweed (*Hymenoxys odorata*). *In:* James LF, Ralphs MH, Nielson DB, editors. The Ecology and Impact of Poisonous Plants on Livestock Production. Westview Press, Colorado. Pages 131-143.

Whisenant SG, Ueckert DN. 1982. Factors influencing bitterweed seed germination. *Journal of Range Management* 35:243-245.

Witzel DA, Rowe LD, Clark DE. 1974. Physiopathologic studies on acute *Hymenoxys odorata* (Bitterweed) poisoning in sheep. *American Journal of Veterinary Research* 35:931-934.

Witzel DA, Jones LP, Ivie GW. 1977. Pathology of subacute bitterweed (*Hymenoxys odorata*) poisoning in sheep. *Veterinary Pathology* 14:73-78.

Yates DT, Salisbury MW, Ross TT, Anderson H. 2010. Effects of Tasco-14 supplementation on growth and fertility traits in young male Boer goats experiencing heat stress. *Texas Journal of Agriculture and Natural Resources* 23:12-18.