



## Effects of Breed of Sheep and Dietary Onions on Bitterweed (*Hymenoxys odorata* DC) Toxicity

E. S. Campbell<sup>1,3</sup>, T. R. Whitney<sup>2</sup>, C. A. Taylor, Jr.<sup>1</sup>, N. E. Garza<sup>1</sup>

<sup>1</sup> Texas A&M AgriLife Research Center, Sonora, TX 76950

<sup>2</sup> Texas A&M AgriLife Research and Extension Center, San Angelo, TX 76901

<sup>3</sup> Corresponding author: erika@suseahorse.com

### ACKNOWLEDGMENT

Partial support for the research was provided by the Texas Food and Fiber Commission/ Texas Department of Agriculture, Austin, TX.

### Summary

Bitterweed (*Hymenoxys odorata* DC) toxicity is a major cause of death losses in Rambouillet sheep. This study compared the susceptibility of two breeds [Rambouillet and Dorper × Barbados Blackbelly (DBB)], of wool and hair sheep lambs to bitterweed toxicosis; and the potential for cull onions (*Allium cepa*) to mitigate bitterweed toxicity. Onions contain high concentrations of naturally occurring thiols, thus offering the opportunity for the introduction of a diversionary substrate for the principle toxin, hymenoxyn, in a palatable feed-source. Weanling Rambouillet (n = 12; LS means ± SE = 22.7 kg ± 1.3 kg BW) and DBB (n = 12; LS means ± SE = 22.5 kg ± 0.8 kg BW) ram lambs were assigned randomly to one of four treatments: 0 percent (control), 25 percent, 50 percent, and 75 percent onions in diet (DM basis). The remainder of the

isonitrogenous diets consisted of alfalfa pellets to provide 32 g DM/kg BW per day. Animals were group-fed onions for a 10-d preconditioning period, then penned and fed individually for study. Individual onion feeding commenced on d 0 and continued through d 7. On d 3 of onion feeding, lambs were dosed with an aqueous slurry of dried bitterweed (0.25 percent of BW, DM-basis) daily through d 7. Blood samples were taken on d 3 and d 8 and analyzed for serum constituents indicating onion- or bitterweed-induced pathologies, respectively. Dry-matter intake and percentage of feed refusals were measured daily. In response to onion diets, serum urea nitrogen (SUN) decreased linearly ( $P < 0.001$ ). Concentrations of albumin, aspartate aminotransferase (AST), glucose, hematocrit and total serum protein (TSP) were greater in DBB lambs than in Rambouillet lambs ( $P < 0.01$ ), but remained clinically normal. The bitterweed challenge

elicited a breed effect ( $P < 0.05$ ) for serum measurements reflective of bitterweed toxicity; bilirubin, gamma-glutamyltransferase (GGT) and AST concentrations were greater ( $P \leq 0.001$ ) for DBBs than for Rambouillets. The AST, bilirubin, creatinine, GGT, and SUN were clinically high for all treatments, including controls, indicating acute toxicity. Feed refusals did not differ among treatments or by breed ( $P > 0.10$ ). In this study, dietary onions did not prevent pathological changes in serum chemistries following bitterweed dosing, and had no influence on feed refusals. Both breeds exhibited clinical signs of bitterweed toxicosis, with DBB sheep exhibiting greater abnormal deviation in serum chemistries than Rambouillets.

**Keywords:** Bitterweed, Onions, Hair Sheep, Dorper, Barbados Blackbelly, Rambouillet, Toxicosis

## Introduction

Noxious plants increase financial risks of ranching, especially on western rangelands. Bitterweed is a noxious weed common in the semiarid range areas from southwest Kansas through central and western Texas into Mexico, Arizona, New Mexico, and southeastern California (Kingsbury, 1964). In Texas, bitterweed has led to a loss in economic returns from sheep production in the Edwards Plateau and Trans-Pecos regions since the early 1900s and has contributed to a 50 percent reduction in sheep production in the region (Ueckert et al., 1980; Conner et al., 1988).

Historically, West Texas ranchers have used the Rambouillet breed of sheep for food and fiber production. In recent years, hair sheep have gained in popularity due to the development of a viable ethnic market for lighter weight lambs, and shortages of labor and skilled shearers.

While susceptibility of Rambouillet sheep to bitterweed has been studied extensively, the effect of bitterweed and other poisonous plants on hair sheep has yet to be evaluated scientifically. Given the increase in numbers of hair sheep in the United States, it is important to better understand their tolerance and/or susceptibility to poisonous-plant exposure.

Research aimed at preventing bitterweed toxicity in sheep has focused on supplementation with dietary components that can inactivate hymenoxon, a sesquiterpene lactone (Kim et al. 1975), the principle toxin in bitterweed. Some experimental success has been achieved with thiol-containing compounds, such as L-cysteine (Kim et al., 1974; Rowe et al., 1980), that provide a diversionary substrate for the toxic alkylating effects of hymenoxon. However, cysteine is unlikely to be used as a viable commercial antidote to bitterweed poisoning in sheep due to its cost and instability (Calhoun et al., 1989). Ethoxyquin (Kim et al., 1982) and activated charcoal (Dollahite et al., 1973; Poage et al., 2000) also mitigated bitterweed toxicity in research trials, due to antioxidant activity and non-selective binding, respectively. Field application potential of these compounds is limited due to

unpalatability (Calhoun et al., 1986) and particle chemistry (Poage et al., 2000).

Cull-onions are an inexpensive and commercially available food source for livestock (Frederickson et al., 1995), that may be adapted for ruminant veterinary therapies. Onions and other *Allium* species contain high concentrations of naturally occurring thiosulfonates, to which many medicinal properties have been attributed (Lanzotti, 2006). Feeding cull-onions to bitterweed-exposed sheep may provide the opportunity to successfully introduce therapeutic thiol compounds in a palatable feedsource.

The objective of this study was to evaluate hair and wool breed lambs' susceptibility to bitterweed. In addition, we investigated whether feeding onions at 25 percent, 50 percent and 75 percent of lambs' diets could reduce bitterweed toxicity and whether further research was warranted.

## Materials and Methods

All procedures involving animals were approved by the Texas A & M University Institutional Agricultural Animal Care and Use Committee under protocol, AUP 2006-179.

### Study site

The Barnhart Research Station is located (30 57'N, 101 07'W) on the northwest edge of the Edwards Plateau, Texas. The environment is semi-arid; mean-annual precipitation is 453 mm with peak precipitation during May and September. The study site is an open savanna dominated by a graminoid mosaic of tobosa grass [*Hilaria mutica* (Buckl.) Benth.] and the shortgrasses, curly mesquite [*Hilaria belangeri* (Steud.) Nash] and buffalograss [*Buchloe dactyloides* (Nutt.) Engelm.]. The scattered woody overstory consists of honey mesquite (*Prosopis glandulosa* Torr.; Mihlbachler, 1990). Major forb species include annual broomweed [*Xanthocephalum dracunculoides* (DC.) Nutt.], perennial broomweed [*Gutierrezia sarothae* (Prush) Britt. and Rugsby], Nuttall's milkvetch [*Astragalus nuttallianus* (DC.) Nutt.], and bitterweed [*Hymenoxys odorata* (DC.)].

## Animals

Weaned Rambouillet (n = 12; LS mean BW  $\pm$  SE = 22.7  $\pm$  1.3 kg) and Dorper x Barbados (DBB) ram lambs (n = 12; LS mean BW  $\pm$  SE = 22.5  $\pm$  0.8 kg) were used to evaluate if onions have potential to reduce bitterweed toxicity in sheep and if the breeds differed in susceptibility. Full-blooded wool sheep (Rambouillet) were selected, based upon 100 years of breeding for dual-purpose production and survival in West Texas. DBB lambs were selected as a viable alternative breed, suited for a low-input management environment. Lambs resulted from cross breeding Dorper rams, for meat characteristics (Burke et al., 2003), to Barbados Blackbelly ewes, for parasite resistance (Yazwinski et al., 1979, 1980) and fertility (Shelton, 1983; Wildeus, 1997). All lambs were pasture-born to ewes native to the site, removing the influence of behavioral conditioning on dietary preferences. All lambs were treated with an anthelmintic prior to the study.

### Feeding Treatments

Lambs were assigned randomly to 1-control and 3-treated groups: 0 percent, 25 percent, 50 percent, or 75 percent onions (percentage of diet calculated on a DM basis). There were three animals per breed fed on each treatment. Lambs were limit-fed to provide a baseline of 32 g DM/kg BW per day over the entire trial [100 percent of maintenance requirements and slight growth (NRC, 2007)]. Control animals received a diet of 100 percent alfalfa pellets. To provide an isonitrogenous diet across treatment groups, treated animals were supplemented with alfalfa pellets at a level calculated to provide total dietary protein (CP onion + CP alfalfa) isonitrogenous with the control diet. Lambs were pen-fed by diet group for a 10-d preconditioning period to incrementally adapt animals to the onions before the study began.

On August 8, lambs were placed in individual feeding pens and fed onions at their specific treatment percentage for 3 d to acclimate them to confinement. On August 11, d 0 of the study, data collection began. Animals were fed treatment percentages of onions d 0 to d 7. Lambs were weighed on d 0, d 3, and d 8. Bitter-

weed dosing began on d 3 and continued daily through d 7. Feed refusals were weighed d 1 to d 8 to determine intake.

Cull-onions were obtained from J&D Produce, Inc. (Deming, N.M.) and stored at 4° C to maintain freshness. Onions were fed fresh, and onion DM was determined by chopping and drying an aliquot of composite samples daily. Onion and alfalfa pellet DM were determined by air-drying samples and calculating percent moisture.

### Bitterweed Dose Preparation

Bitterweed samples were collected in Barnhart and Sonora, Texas in June 2006. Above-ground, whole-plant samples were dried 24 h at 60°C in a forced-draft oven, ground to pass through a 1-mm screen, and mixed to provide a homogenous substrate.

On d 3, lambs were weighed and at 0900 individually dosed with an aqueous slurry of bitterweed by oral gavage. The slurry provided a sub-acute dose of bitterweed at 0.25 percent of BW in 1.5 L of warm tap water, sufficient to elicit signs of toxicity without resulting in death as described by Calhoun et al. (1989).

### Blood Analysis

At 0800 on d 3 (prior to bitterweed dosing) and d 8, blood samples were obtained via jugular venipuncture into serum separator tubes. Blood samples were allowed to clot at 26°C for 30 min and serum was obtained by centrifugation at 2,000 × g for 15 min at 4°C. Serum samples were sent to the Texas Veterinary Medical Diagnostic Laboratory (TVMDL, College Station) for determination of blood-chemistry constituents. All blood chemical measures were analyzed on an Abbott Spectrum Spectrophotometer (Abbott Laboratories Diagnostics Division, Abbott Park, Ill.). Measures included albumin, aspartate transaminase (AST), creatine kinase (CK), creatinine, gamma-glutamyl transferase (GGT), glucose, serum urea nitrogen (SUN), total serum protein, and total bilirubin.

At the time of venipuncture, blood samples were collected for hematocrit measurement. Two heparinized, micro-hematocrit-capillary tubes per animal were filled with whole blood and hematocrit was measured after microcentrifugation for 5 minutes.

### Statistical Analysis

Days 1 to d 3, when lambs were fed onions, were considered period 1 for analysis. Days 4 to d 8, when lambs were fed onions and challenged with bitterweed, were considered period 2. Data were analyzed using PROC MIXED procedure of SAS (SAS Inst. Inc., Cary, N.C.). There was a period × breed × treatment interaction ( $P < 0.10$ ) and reduced models were analyzed by period (period 1 = onions; period 2 = onions and bitterweed dose) using a model that included breed, treatment, and breed × treatment; lamb was the experimental unit. Only main effects of breed and treatment are discussed for serum constituents, as there were no breed × treatment interactions ( $P > 0.10$ ). Data are reported as least squares means and standard errors. Blood samples and body weights were analyzed as one sample from each animal, and feed refusals and intake data were analyzed as the mean of daily samples from each time period. Treatment effects were tested using linear, quadratic, and cubic contrasts and only the highest order contrasts that were significant ( $P < 0.10$ ) were discussed.

## Results and Discussion

### Onion Diet

All lambs responded well to the onion diets. Serum chemistries remained in a clinically normal range (Texas Veterinary Medical Diagnostic Lab standards for ovine serum assay) with the exception of CK and SUN. Concentration of CK was clinically high in lambs at 0 percent-, 25 percent- and 75 percent-onion treatments (Table 1). Activity of CK can arise from minor muscle damage, due to such events as prolonged recumbency (Merck, 2010a) and stress due to physical exertion (Knowles and Warriss, 2000). One possibility is that concentrations of CK were elevated in lambs in this study as a result of confinement. All lambs were pasture raised in 324-hectare (800 ac) pastures, and despite acclimation to study pens, visibility of neighboring lambs, protection from heat, and low stress livestock handling, several still attempted escape during feeding and

serum collection periods during the study. As this effect was not treatment- ( $P > 0.10$ ) or breed- ( $P > 0.10$ ) related, it will not be discussed further.

As percentage of onions increased in Rambouillet and DBB lambs' diets, SUN decreased linearly ( $P < 0.001$ ; Table 1). This result agrees with other studies involving onion consumption by sheep (James and Binns, 1966; VanKampen et al. 1970; and Kirk and Bulgin, 1979). These authors did not include a discussion on the biochemical characteristics of onions that caused declining SUN. Elevated concentrations of SUN can indicate protein catabolism of body reserves to meet nutrient demands (Caldeira et al, 2007), but all values were clinically normal or below normal. It is unlikely that the lambs were in a catabolic state during period 1, as lambs at all treatments were fed at NRC (2007) dietary guidelines for maintenance and slight growth, and diets were isonitrogenous. In addition, other indicators of protein and nutritional status (TSP, glucose, and body weight) did not change ( $P > 0.10$ ).

Causes of low SUN relevant to this study include low dietary protein and liver failure (Merck, 2010b). We can rule out low protein and catabolism of body tissues for the reason mentioned in the previous paragraph. Liver failure as well can be ruled out as serum chemistries revealed normal liver function during onion consumption. These results are consistent with other studies; when James and Binns (1966) fed wild onions to bred ewes, in addition to marked decreases in plasma urea nitrogen, they reported no concurrent changes in liver function.

Hematocrit was not affected by dietary onions ( $P > 0.10$ ) and remained in the clinically normal range (Table 1). Previous diet studies with onions report a time-dependent anemia in onion-fed ewes after 14 d (Frederickson et al., 1995; Knight, 2000).

Sheep breeds responded differently to dietary onions, with DBB lambs exhibiting greater ( $P < 0.01$ ) albumin, AST, glucose, hematocrit, and TSP than Rambouillets (Table 2). Less water intake and decreased volume of extracellular-fluid may explain the greater albumin, glucose, hematocrit, and TSP seen in the DBB lambs. Authors of studies

**Table 1. Least square means of serum constituents and body weights for lambs fed four levels of onions and dosed with bitterweed.**

Measurement	Treatment <sup>1</sup>					P-value <sup>2</sup>				Reference Range <sup>3</sup>
	0	25	50	75	SEM <sup>4</sup>	Treatment	Linear	Quadratic	Cubic	
n	6	6	6	6						
Onion Diet (d 1 to 3)										
BW, kg	23.2	23.0	22.9	22.4	1.18	0.97	0.66	0.92	0.92	
Albumin, mg/dL	3.3	3.5	3.3	3.3	0.08	0.55	0.81	0.45	0.23	3.1 to 5.0 g/dL
AST, U/L	75.5	81.8	92.7	86.0	4.97	0.14	0.07	0.21	0.33	51 to 130 U/L
Bilirubin, mg/dL	0.1	0.1	0.2	0.1	0.04	0.30	0.15	0.46	0.29	< 0.3 mg/dL
CK, U/L	240.8	243.7	183.2	280.3	50.68	0.61	0.80	0.37	0.34	15 to 213 U/L
Creatinine, mg/dL	0.6	0.7	0.6	0.6	0.03	0.10	0.04	0.25	0.28	0.3 to 1.3 mg/dL
GGT, U/L	62.3	69.0	66.0	68.2	3.29	0.50	0.34	0.50	0.33	34 to 82 U/L
Glucose, mg/dL	70.0	71.8	64.8	73.8	2.59	0.13	0.70	0.19	0.05	58 to 109 mg/dL
Hematocrit, %	33.7	33.1	30.8	32.2	1.50	0.79	0.48	0.66	0.57	30.3 to 34.9 %
SUN, mg/dL	26.2	24.7	15.3	11.1	1.30	<0.001	<0.001	0.49	0.13	12 to 32 mg/dL
TSP, dL	6.3	6.2	6.2	6.0	0.12	0.43	0.13	1.0	0.53	6 to 8.6 dL
Bitterweed Challenge (d 4 to 8)										
BW, kg	22.2	22.8	22.1	22.8	1.09	0.95	0.82	0.97	0.60	
Albumin, mg/dL	2.9	3.0	3.1	2.9	0.10	0.28	0.65	0.08	0.54	3.1 to 5.0 g/dL
AST, U/L	465.5	568.0	617.0	476.5	150.33	0.87	0.90	0.43	0.84	51 to 130 U/L
Bilirubin, mg/dL	0.5	0.5	0.4	0.4	0.14	0.94	0.57	0.79	0.94	< 0.3 mg/dL
CK, U/L	149.3	392.3	197.8	813.0	167.10	0.05	0.03	0.28	0.11	15 to 213 U/L
Creatinine, mg/dL	1.2	1.3	1.2	1.7	0.28	0.62	0.31	0.55	0.58	0.3 to 1.3 mg/dL
GGT, U/L	390.7	283.3	257.0	234.3	86.90	0.60	0.22	0.63	0.84	34 to 82 U/L
Glucose, mg/dL	38.0	46.3	38.2	44.0	3.93	0.36	0.58	0.75	0.10	58 to 109 mg/dL
Hematocrit, %	36.9	33.8	31.9	29.4	1.77	0.05	< 0.01	0.88	0.83	30.3 to 34.9 %
SUN, mg/dL	33.4	41.1	39.6	45.6	7.56	0.72	0.31	0.91	0.63	12 to 32 mg/dL
TSP, dL	5.6	5.5	6.0	5.4	0.14	0.04	0.81	0.12	0.01	6 to 8.6 dL

<sup>1</sup> Treatments were 0%, 25%, 50%, or 75% onions. (as a percentage of daily DMI).

<sup>2</sup> Linear, quadratic and cubic contrasts of 0%, 25%, 50% and 75% onion diets.

<sup>3</sup> Abbreviations are as follows: aspartate transaminase (AST), serum urea nitrogen (SUN), creatine kinase (CK), gamma-glutamyl transferase (GGT), and total serum protein (TSP). Reference values = Texas A&M Veterinary Medical Diagnostic Laboratory (College Station).

<sup>4</sup> SEM = greatest standard error of the mean.

comparing hair and wool sheep uniformly reported lower water intake in hair sheep (Quick and Dehority, 1986; Horton et al., 1992). Though breed differences existed, all of these values were within the clinically normal range for lambs of this age. Concentrations of GGT were greater ( $P < 0.01$ ) in Rambouillets than DBB, but were clinically normal, and there was no treatment by breed effect ( $P > 0.10$ ). There were no treatment-by-breed interactions for serum constituents and body weights ( $P > 0.10$ ).

Onion intake did not differ by breed ( $P > 0.10$ ; Table 3). All lambs consumed their target amounts of onions and alfalfa. Onion intake did not affect

alfalfa consumption, as alfalfa refusals were consistent across treatments ( $P > 0.10$ ; Table 4), ranging from 0 percent to 6 percent (Table 4).

Body weights did not decline as a result of onion consumption ( $P > 0.10$ ; Table 1) indicating that lambs were receiving sufficient nutritional intake to maintain condition. Lamb body weights did not differ by breed or by treatment ( $P > 0.10$ ).

### Bitterweed Challenge

All lambs exhibited clinical signs of bitterweed intoxication (Hardy et al., 1932), including gradual loss of appetite and slight central nervous system depression and weakness, although excessive

salivation and vomiting did not occur. As in other studies, severity varied with individual animal susceptibility (Terry et al., 1981). One Rambouillet lamb (# 8339) in the 75 percent onion group died upon termination of the study. This lamb exhibited clinically high concentrations of AST (517 U/l), bilirubin (0.86 mg/dl), BUN (63.1 mg/dl), and CK (1692 U/L). Field necropsy results observed included gross lesions characteristic of bitterweed poisoning, most notably congestion of the lungs and petechial hemorrhages of the epicardium (Hardy et al., 1932).

Lamb-serum constituents responded to bitterweed dosing in a manner consistent with bitterweed toxicity (Terry et



**Table 2. Comparison of least square means of serum constituents and body weights of Dorper x Barbados Blackbelly (DBB) and Rambouillet lambs fed onions and dosed with bitterweed.**

Measurement (Reference Values) <sup>1</sup>	Breed		SEM <sup>2</sup>	P-value	Reference Range
	DBB	Rambouillet			
n	12	12			
Onion Diet (d 1 to 3)					
BW, kg	23.70	22.08	0.83	0.19	
Albumin, mg/dL	3.61	3.13	0.05	<0.001	3.1 to 5.0 g/dL
AST, U/L	92.08	75.92	3.51	0.005	51 to 130 U/L
Bilirubin, mg/dL	0.10	0.15	0.02	0.12	< 0.3 mg/dL
CK, U/L	201.33	272.67	35.84	0.18	15 to 213 U/L
Creatinine, mg/dL	0.65	0.61	0.02	0.234	0.3 to 1.3 mg/dL
GGT, U/L	61.25	71.50	2.33	0.007	34 to 82 U/L
Glucose, mg/dL	74.83	65.42	1.83	0.002	58 to 109 mg/dL
Hematocrit, %	37.71	27.19	1.50	<0.001	30.3 to 34.9 %
SUN, mg/dL	17.82	20.87	1.30	0.115	12 to 32 mg/dL
TSP, dL	6.37	6.01	0.08	0.007	6 to 8.6 dL
Bitterweed Challenge (d 4 to 8)					
BW, kg	23.25	21.77	0.78	0.20	
Albumin, mg/dL	3.28	2.74	0.07	<0.001	3.1 to 5.0 g/dL
AST, U/L	877.00	186.50	106.30	<0.001	51 to 130 U/L
Bilirubin, mg/dL	0.54	0.40	0.10	0.32	< 0.3 mg/dL
CK, U/L	399.42	376.83	118.16	0.89	15 to 213 U/L
Creatinine, mg/dL	1.46	1.27	0.20	0.52	0.3 to 1.3 mg/dL
GGT, U/L	465.58	117.08	61.44	<0.01	34 to 82 U/L
Glucose, mg/dL	40.17	43.08	2.78	0.47	58 to 109 mg/dL
Hematocrit, %	37.26	28.78	1.25	<0.001	30.3 to 34.9 %
SUN, mg/dL	37.92	41.99	5.35	0.60	12 to 32 mg/dL
TSP, dL	5.97	5.30	0.10	<0.001	6 to 8.6 dL

<sup>1</sup> Abbreviations are as follows: aspartate transaminase (AST), serum urea nitrogen (SUN), creatine kinase (CK), gamma-glutamyl transferase (GGT), and total serum protein (TSP). Reference values = Texas A&M Veterinary Medical Diagnostic Laboratory (College Station).

<sup>2</sup> SEM = greatest standard error of the mean

al., 1981, Calhoun et al., 1981, 1989). Clinically elevated AST, bilirubin, and GGT were representative of bitterweed-induced hepatotoxicity, and clinically elevated creatinine and SUN indicated nephrotoxicity (Table 1). Concentrations of glucose fell below reference ranges for both breeds, most likely as a response to declining feed intake (Calhoun et al., 1981).

The CK values were clinically elevated in the lambs on the 25 percent and 75 percent onion diets as compared to the other treatments, most notably in the lambs on 75 percent onions (LS mean  $\pm$  SE = 813.0 U/L  $\pm$  167.10 U/L). In addition, a treatment effect was observed; CK increased as onions increased ( $P < 0.05$ ). It is likely that the high mean value for the lambs on 75 percent onions was numerically influenced by # 8339 prior to morbidity. Variations

among other animals are attributable to minor muscle damage from confinement and dosing.

Albumin, TSP and hematocrit were the only serum constituents that varied in bitterweed-dosed lambs by dietary onion dosage (Table 1). Albumin tended to increase quadratically ( $P < 0.10$ ) as percentage of onions in the diet increased, but comparison with clinical reference ranges (TVMDL) indicated that animals at 0 percent, 25 percent and 75 percent were slightly hypoalbuminemic. Calhoun et al. (1981) reported decreases in albumin due to bitterweed and ruled out the influence of fasting and water deprivation, which increased albumin and serum protein.

Total serum protein increased cubically ( $P = 0.01$ ) as percentage of onions in the diet increased. Lambs at 50 percent onions had greater concentrations

of TSP than lambs in the other treatment groups. The reason for this is not understood.

Hematocrit decreased linearly ( $P < 0.01$ ) as percentage of onions in the diet increased in both breeds of lambs. N-propyl disulfide, a thiosulfinate present in cultivated and wild onions, is a causative agent in hemolytic anemias in ruminants (Knight and Walter, 2001). Frederickson et al. (1995) reported that across time, RBC hemolysis in lambs fed onion diets was evident by d 14, peaking by d 21, and diminished by d 28. Long-term feeding studies with sheep ( $> 60$  d) showed that stabilization of anemia occurred due to rumen microbial adaptation (Kirk and Bulgin, 1979; Frederickson et al., 1995; Knight et al., 2000).

A breed effect occurred similar to period 1; concentrations of albumin, hematocrit, and TSP, were greater in DBB

**Table 3. Effect of breed and onion diets on feed refusals and DMI of lambs challenged with bitterweed.**

Item	P-value <sup>1</sup>		
	Breed	Treatment	BxT <sup>2</sup>
Alfalfa Refusals, %			
Onion	0.30	0.48	0.44
Onion-Bitterweed	0.23	0.28	0.09
Onion DMI, g DM/kg BW			
Onion	0.53	<0.001	0.64
Onion-Bitterweed	0.38	<0.001	0.83
Total DMI, g DM/kg BW			
Onion	0.23	<0.001	0.40
Onion-Bitterweed	0.46	0.05	0.10

<sup>1</sup> Alfalfa refusals and DMI for each period were analyzed using Proc Mixed by period with 2 factors (breed: DBB vs. Rambouillet and treatment: 0 vs. 25% vs. 50% vs. 75% onions).

<sup>2</sup> B x T = breed by treatment interaction

compared to Rambouillets ( $P < 0.001$ ; Table 2). As this effect was present prior to bitterweed dosing, we attributed it to breed differences between hair and wooled sheep as previously reported (Horton and Burgher, 1992).

Breeds differed in serum-enzyme activity, indicative of liver impairment, with AST and GGT four times greater in DBB's than in Rambouillets ( $P > 0.05$ ). Variation among sheep in response to the toxic effect of bitterweed is a consistent observation in bitterweed research (Witzel, 1977; Calhoun et al., 1981), indicating a genetic component to tolerance. In a study evaluating the

effect of genetic origin and experiences early in life on bitterweed consumption in sheep, Frost et al. (2003) reported a significant sire effect on bitterweed intake. Pedigree records for Rambouillet and DBB lambs were not kept, however each maternal breeding group was exposed to four sires from different bloodlines per breed. There were no treatment-by-breed interactions for serum constituents and BW during the bitterweed challenge.

Onion DMI and total DMI did not differ among breeds ( $P > 0.10$ ; Table 3). All treatment groups consumed fewer onions after bitterweed dosing, and pre-

scribed intakes of onions were not met (Table 4). Alfalfa refusals did not vary by breed or treatment ( $P > 0.10$ ; Table 3). A slight breed-by-treatment interaction was present ( $P < 0.10$ ) indicating alfalfa refusals tended to vary slightly among breeds after bitterweed dosing. Feed refusals overall ranged from 0 percent to 75 percent with a high degree of variation among animals. Body weights for all lambs were similar across all dietary onion treatments ( $P > 0.10$ ; Table 2) and were within +/- 2.0 lbs from lamb body weights in period 1.

As one of the objectives was to evaluate whether feeding onions at a broad spectrum of dietary percentages could mitigate bitterweed toxicity, clinically high concentrations of AST, GGT, and bilirubin, indicated that onions did not prevent hepatocellular damage and toxicity. Although Calhoun et al. (1981) reported that the dose related depression in voluntary feed intake is a more sensitive indicator of bitterweed poisoning than are changes in serum constituents, feed refusal results reinforced the lack of a beneficial effect of onions.

Results indicated that DBB and Rambouillet lambs were susceptible to bitterweed poisoning. Although not representative of all hair sheep breeds, the degree of hepatic impairment in DBB lambs was greater than in Rambouillet lambs. DBBs are a recent breed combination, designed to improve the sustainability of sheep production in a low-input, highly variable environment. A

**Table 4. Effect of onion diet level on least square means of feed refusals and DMI of lambs dosed with bitterweed.**

Item	% Onion in the Diet <sup>1</sup>				SEM	P-value <sup>2</sup>			
	0	25	50	75		Treatment	Linear	Quadratic	Cubic
Alfalfa Refusals, %									
Onion	0.36	5.95	0.92	0.0	3.01	0.48	0.66	0.30	0.29
Bitterweed	44.26	47.86	44.84	24.12	9.20	0.28	0.14	0.20	0.79
Onion DMI, g DM/kg BW									
Onion	0.0	7.87	15.81	23.48	0.23				
Bitterweed	0.0	5.93	9.82	13.78	0.56				
Total DMI, g DM/kg BW									
Onion	33.65	35.91	41.44	45.35	0.90				
Bitterweed	19.07	22.88	24.09	30.63	2.72				

<sup>1</sup> Lambs were assigned randomly to 1 control and 3 treatment groups: 0%, 25%, 50%, or 75% onions (percentage of diet calculated on a DM basis).

<sup>2</sup> Alfalfa refusals and DMI for each period were analyzed using Proc Mixed by period with 2 factors (breed: DBB vs. Rambouillet and treatment: 0 vs. 25% vs. 50% vs. 75% onions).

century of historic selection for Rambouillet sheep in the region may have indirectly led to a beneficial genetic exploitation of resistance to bitterweed toxicity. Genetic selection for sheep tolerant of bitterweed (Frost et al., 2003) and development of bitterweed-resistant flocks of hair sheep, present an interesting direction for future research.

This study did not evaluate whether the greater susceptibility experienced by the DBB lambs in a forced-feeding situation translates to greater susceptibility in a free-choice, pasture environment. On semi-arid rangelands in Texas, Barbados Blackbelly sheep consumed more browse than Rambouillets (Warren et al., 1984). Bitterweed poisoning in livestock occurs primarily when range conditions and drought reduce the availability of other more palatable forages (Ueckert and Calhoun, 1988), so advantages for hair sheep due to dietary niche separation may not be expressed.

## Conclusion and Implications

A cost-effective and practical solution to bitterweed poisoning in sheep in the Western United States remains elusive. In this study dietary onions did not mitigate bitterweed toxicity in lambs. Pathological changes in serum chemistries of lambs consuming from 0 percent to 75 percent onions indicated toxic hepatitis and nephritis following bitterweed dosing. Both breeds exhibited clinical signs of bitterweed toxicosis, with greater deviations in serum chemistries in DBB lambs than in Rambouillets. The lack of a breed advantage in DBB lambs in this study reinforces the need for hair sheep producers to implement management practices, such as grazing management and supplemental feeding (Landers and Ueckert, 1981), reduced stocking rate (Taylor and Ralphs, 1992), and use of activated charcoal (Dollahite et al., 1973; Poage et al., 2000), currently practiced by Rambouillet producers to minimize losses due to bitterweed.

## Literature Cited

Amagase, H. A., B. L. Petesch, H. Matsuura, S. Kasuga, and Y. Itakura. 2001. Intake of garlic and its bioactive components. *J. Nutr.* 131:955S-62S.

- Burke, J. M., J. K. Apple, W. J. Roberts, C. B. Boger, and E. B. Kegley. 2003. Effect of breed-type on performance and carcass traits of intensively managed hair sheep. *Meat Science* 63:309-315.
- Caldeira, R. M., A. T. Belo, C. C. Santos, M. I. Vazques, and A. V. Portugal. 2007. The effect of long-term feed restriction and over-nutrition on body condition score, blood metabolites and hormonal profiles in ewes. *Small Rumin. Res.* 68:242-255.
- Calhoun, M. C., D. N. Ueckert, C. W. Livingston, Jr., and B. C. Baldwin. 1981. Effects of bitterweed (*Hymenoxys odorata*) on voluntary feed intake and serum constituents of sheep. *Am. J. Vet. Res.* 42:1713-1717.
- Calhoun, M. C., B. C. Baldwin, Jr., S. W. Kuhlman, and H. L. Kim. 1986. Bitterweed antidote research. *Tex. Agr. Exp. Stat. Rep.* 4382.
- Calhoun, M. C., B. C. Baldwin, Jr., S. W. Kuhlman, and H. L. Kim. 1989. Experimental prevention of Bitterweed (*Hymenoxys odorata*) poisoning of sheep. *Am. J. Vet. Res.* 50:1642-1646.
- Conner, J. R., J. L. Schuster, and E. M. Bailey, Fr. 1988. Impact of bitterweed on the economics of sheep production in the Texas Edwards Plateau. p. 145-151 in *The Ecology and Economic Impact of Poisonous Plants on Livestock Production*. L. F. James, M. H. Ralphs, and D. B. Nielsen, eds. Westview Press, Boulder, CO.
- Dollahite, J. W., L. D. Rowe, H. L. Kim, and B. J. Camp. 1973. Control of bitterweed (*Hymenoxys odorata*) poisoning in sheep. *Tex. Agr. Exp. Stat. Rep.* 3149.
- Frost, R. F., C. B. Scott, J. W. Walker, and F. S. Hartmann. 2003. Effects of origin, experiences early in life, and genetics on bitterweed consumption by sheep. *Appl. Anim. Behav. Sci.* 84:251-264.
- Fredrickson, E. L., R. E. Estell, K. M. Havstad, W. L. Shupe, and L. W. Murray. 1995. Potential toxicity and feed value of onions for sheep. *Livest. Prod. Sci.* 42:45-54.
- Hart, C. R., T. Garland, C. Barr, B. B. Carpenter, and J. C. Reagor. 2000. *Toxic Plants of Texas*. Extension publication B-6105.
- Hardy, W. T., V. L. Cory, H. Schmidt, and W. A. Dameron. 1932. Bitterweed poisoning in sheep. *Texas Agr. Exp. Sta. Bull.* 433:1-18.
- Horton, G. M. C., and C. C. Burgher. 1992. Physiological and carcass characteristics of hair and wool breeds of sheep. *Small Rumin. Res.* 7:51-60.
- James, L. F., and W. Binns. 1966. Effects of feeding wild onions (*Allium validum*) to bred ewes. *J. Am. Vet. Med. Assoc.* 149:512-514.
- Kim, H. L., M. Szabuniewicz, L. D. Rowe, B. J. Camp, J. W. Dollahite, and C. H. Bridges. 1974. L-Cysteine, an antagonist to the toxic effects of an alpha-methylene-gamma-lactone isolated from *Hymenoxys odorata* DC (Bitterweed). *Res. Comm. Chem. Pathol. Pharmacol.* 8:381-384.
- Kim, H.L., L.D. Rowe, and B.J. Camp. 1975. Hymenoxon, a poisonous sesquiterpene lactone from *Hymenoxys odorata* DC. (bitterweed). *Res. Commun. Chem. Pathol. Pharmacol.* 11:647-50.
- Kim, H.L., A.C. Anderson, B.W. Herrig, L.P. Jones, and M.C. Calhoun. 1982. Protective effects of antioxidants on bitterweed (*Hymenoxys odorata* DC) toxicity in sheep. *Am. J. Vet. Res.* 43:1945-50.
- Kingsbury, J. M. 1964. *Poisonous plants of the United States and Canada*. Prentice-Hall, Inc., Englewood Cliffs, NJ.
- Kirk, J. H. and M. S. Bulgin. 1979. Effects of feeding cull domestic onions (*Allium cepa*) to sheep. *Am. J. Vet. Res.* 40:387-399.
- Knight, A.P., D. Lassen, T. McBride, D. Marsh, C. Kimberling, M.G. Delgado, and D. Gould. 2000. Adaptation of pregnant ewes to an exclusive onion diet. *Vet. Hum. Toxicol.* 42:1-4.
- Knight, A. P., and R. G. Walter. 2001. *A Guide to Plant Poisoning of Animals in North America*. Teton New Media, Jackson, WY.

- Knowles, T. G., and P. D. Warriss. 2000. Stress physiology of animals during transport. p. 385-407. In: *Livestock Handling and Transport*. Ed. T. Grandin. CABI Publishing, New York, NY.
- Landers, R. Q, Jr., and D. N. Ueckert. 1981. Managing bitterweed to reduce sheep losses. *Tex. Agr. Ext. Serv. L-1845*.
- Lanzotti, V. 2006. The analysis of onions and garlic. *J. Chromatogr. A* 1112:3-22.
- Merck Manual. 2010a. Traumatic myopathies in cattle. [http://www.merckmanuals.com/vet/musculoskeletal\\_system/myopathies\\_in\\_ruminants\\_and\\_pigs](http://www.merckmanuals.com/vet/musculoskeletal_system/myopathies_in_ruminants_and_pigs). Accessed Oct. 10, 2014.
- Merck Manual. 2010b. Clinical biochemistry. [http://www.merckmanuals.com/vet/clinical\\_pathology\\_and\\_procedures/diagnostic\\_procedures\\_for\\_the\\_private\\_practice\\_lab\\_oratory/clinical\\_biochemistry.html](http://www.merckmanuals.com/vet/clinical_pathology_and_procedures/diagnostic_procedures_for_the_private_practice_lab_oratory/clinical_biochemistry.html). Accessed Oct. 15, 2014.
- NRC, 2007. *Nutrient Requirements of Small Ruminants: Sheep, Goats, Cervids, and New World Camelids*. Natl. Acad. Press, Washington, DC.
- Poage, G. W. III, C. B. Scott, M. G. Bisson, and S. F. Hartmann. 2000. Activated charcoal attenuates bitterweed toxicosis in sheep. *J. Range Manage.* 53:73-78.
- Quick, T. C., and B. A. Dehority. 1986. A comparative study of feeding behavior and digestive function in dairy goats, wool sheep, and hair sheep. *J. Anim. Sci.* 63:1516.
- Rowe, L. D., H. L. Kim, and B. J. Camp. 1980. The antagonistic effect of L-cysteine in experimental Hymenoxon intoxication in sheep. *A. J. Vet. Res.* 41:484-486.
- Shelton, M. 1983. Crossbreeding with "Barbado" breed for market lamb or wool production in the United States. In H. A. Fitzhugh, and B. E. Bradford Eds., *Hair Sheep of Western Africa and the Americas: a Genetic Resource for the Tropics* (p. 293-297). Boulder, Colorado: Westview Press.
- Taylor, C. A., Jr., and M. H. Ralphs. 1992. Reducing livestock losses from poisonous plants through grazing management. *J. Range Manage.* 45:9-12.
- Terry, M. K., H. L. Kim, D. E. Corrier, and E. M. Bailey, Jr. 1981. The acute oral toxicity of hymenoxon in sheep. *Res. Commun. Chem. Pathol. Pharmacol.* 31:181-184.
- Ueckert, D. N., C. J. Scifres, S. G. Whisenant, and J. L. Mutz. 1980. Control of bitterweed with herbicides. *J. Range Manage.* 33:465-469.
- Ueckert, D. N., and M. C. Calhoun. 1988. Ecology and toxicology of bitterweed (*Hymenoxys odorata*). p. 131-143. In: James, L. F., M. H. Ralphs, and D. B. Nielson. Ed. 1988. *The ecology and impact of poisonous plants on livestock production*. Westview Press, Boulder, Colo.
- Van Kampen, K. R. James, L. F. and Johnson, A. E. 1970. Hemolytic anemia in sheep fed wild onions (*Allium validum*). *J. Am. Vet. Med. Assoc.*, 156:328-332.
- Warren, L. E., D. N. Ueckert, and J. M. Shelton 1984. Comparative diets of Rambouillet, Barbado, and Karakul sheep and Spanish and Angora goats. *J. Range Manage.* 37:172.
- Wildeus S. 1997. Hair sheep genetic resources and their contribution to diversified small ruminant production in the United States. *J. Anim. Sci.* 75:630-40.
- Witzel, D. A., L. P. Jones, and G. W. Ivie. 1977. Pathology of subacute bitterweed poisoning in sheep. *Vet. Pathol.* 14:73-78.
- Yazwinski, T. A., L. Goode, D. J. Moncol, G. W. Morgan and A. C Linnerud. 1979. Parasite resistance in straightbred and crossbred Barbados Blackbelly sheep. *J. Anim. Sci.* 49:919-926.
- Yazwinski, T. A., L. Goode, D. J. Moncol, G. W. Morgan and A. C Linnerud. 1980. *Haemonchus contortus* resistance in straightbred and crossbred Barbados Blackbelly sheep. *J. Anim. Sci.* 51:279-284.