

Intake of Bitterweed by Dorper-cross and Rambouillet Sheep Both With and Without Supplementation¹

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ABSTRACT

Bitterweed (*Hymenoxys odorata*, DC.) toxicity continues to threaten sheep production in west-central Texas. Strategic protein supplementation, by providing higher levels of certain amino acids, may provide some protection from toxicity. For instance, the amino acid L-cysteine, which is found in onions (*Allium cepa*, L.) and other protein sources, may reduce the likelihood of bitterweed poisoning. The objective of this study was to determine if the supplementation of onions or soybean meal would increase the consumption of bitterweed without increasing the incidence of toxicity. Trial 1 used Dorper-cross rams and Trial 2 used Rambouillet lambs. Sheep were randomly allocated into three treatments. Treatment 1 was supplemented with onions, Treatment 2 was fed a protein supplement containing soybean meal, while Treatment 3 received alfalfa pellets. Sheep in all treatments in both trials received alfalfa pellets (2% body weight), fresh water, and a calcium/phosphorus mineral supplement to meet maintenance requirements. Intake of onions, supplement, alfalfa, and bitterweed were measured daily. Bitterweed intake was higher ($P < 0.05$) for Dorper-cross sheep fed onions but was similar ($P > 0.05$) among treatments with Rambouillet wethers. The hypotheses that supplementation with onions or with a supplement containing soybean meal would improve bitterweed intake were not confirmed.

KEY WORDS: onions, soybean meal, intake, toxicity

INTRODUCTION

The toxic plant bitterweed (*Hymenoxys odorata*, DC.) causes acute, subacute, and chronic toxicosis, primarily affecting sheep. Hymenoxon, a sesquiterpene lactone, is the principle compound in bitterweed (Ueckert and Calhoun 1988). This toxin can cause such symptoms as bloating, central nervous system recession, termination of rumen activity, and eventual death (Rowe et al. 1973).

All species of livestock are susceptible to bitterweed toxicity, but sheep seem to be the most likely of livestock species to consume bitterweed because of their affinity for forbs. This is particularly evident during winter months when bitterweed may be the only

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forb species readily available. The likelihood of bitterweed toxicity, predation issues, and volatile wool and lamb markets have combined to dramatically reduce sheep numbers in the western Edwards Plateau of Texas.

Considerable efforts have attempted to reduce susceptibility to bitterweed toxicity in sheep. Some resolutions have included supplementation with activated charcoal, supplementation with the antioxidant Santoquin, and the use of herbicides. Activated charcoal adsorbs hymenoxon in bitterweed, but is very hard to supplement because of its fine particle size (Poage et al. 2000). The antioxidant Santoquin reduces the likelihood of bitterweed toxicosis, but it is a very unpalatable supplement and may be ineffective because of low intake (Kim et al. 1982; Calhoun et al. 1989; Ueckert and Calhoun 1988). Spraying with herbicides reduces bitterweed cover (Bunting and Wright 1974); however, spraying may be cost-prohibitive because of the cost of application.

Sheep dosed with the amino acid L-cysteine increased survival rate up to 12-fold over sheep that were not dosed (Rowe et al. 1980), suggesting that L-cysteine can be an affective antidote for hymenoxon poisoning. Unfortunately, L-cysteine is unpalatable, and sheep refuse to consume sufficient amounts to avoid toxicity. Onions (*Allium cepa*, L.) are high in L-cysteine. Sheep will readily consume onions and, when on a 100% onion diet, will perform as well as sheep fed grain diets (Knight et al. 2000). In addition, Coffman et al. (2014) reported that supplementation with soybean meal, which is moderately high in cysteine, increased bitterweed intake without signs of toxicosis.

The Rambouillet breed of sheep has been utilized in the Edwards Plateau region of Texas since livestock were introduced into the region. Given the prevalence of bitterweed, it seems likely that Rambouillet sheep may have a higher tolerance to bitterweed through selection of top performing individuals by ranchers. Hair sheep breeds, such as the Dorper, are gaining popularity among ranchers in west-central Texas because of their durability, adaptability, and reduced cost of shearing. However, little is known about the Dorper's susceptibility to hymenoxon and whether they will be able to survive on bitterweed-infested rangelands.

MATERIALS AND METHODS

This study consisted of two trials. For the first trial, 27 Dorper-cross rams (nine sheep/treatment) approximately one year of age (weight: 71 ± 4.7 kg) were used. Initially, 30 rams were allocated to Trial 1, but three died from natural causes prior to initiation of the study. Rams were separated into individual pens (1.5 X 1.5 m) and allotted seven days for pen adjustment. Alfalfa pellets were fed at 2% body weight (BW) to meet the animal's maintenance requirement (NRC 2007). Rams were offered fresh water and a calcium/phosphorus mineral *ad libitum* throughout the trial.

After the adjustment period, rams were randomly allocated to one of three supplement treatments. The first treatment was supplemented with raw onions, the second supplemented with a soybean meal-based supplement (Table 1), and the third group (control) was fed only the alfalfa pellets. Supplements were fed one hour each day for 14 days. Immediately after feeding supplemental diets, bitterweed was fed for 30 minutes followed by feeding alfalfa pellets for two hours. Intake of each was measured daily.

Table 1. Ingredients and nutritional value of soybean meal (SBM) supplemented protein feed.

| Ingredients | Percent (%) in feed |
|----------------------|---------------------|
| Soybean Meal | 78.7 |
| Cane Molasses | 3.4 |
| Rice bran with germ | 17.5 |
| Trace Mineral Premix | 0.02 |
| Vitamin ADE Premix | 0.3 |
| | |
| TDN (Ruminants) | 73.7 |
| Protein | 39.6 |
| Met Energy | 2496.8 kcal/kg |

¹ All percentages based on one ton (909.1 kg)

The amount of diets fed was adjusted so that all diets were iso-nitrogenous. Assuming an average weight of 73 ± 4.7 kg for rams used in this study, rams would receive 1,460 g of alfalfa (2% BW) to meet maintenance requirements. When 75% of the alfalfa was replaced with onions, rams received approximately 1,095 g of onions, providing 165 g of protein. For an equivalent protein intake, rams supplemented with a soybean meal supplement (CP 37.6%), received approximately 417 g of supplement. For the control group, the amount of alfalfa fed was increased so that all treatment diets were iso-nitrogenous.

Over-ingestion of onions can cause anemia and lead to death of livestock (Cheeke 1998). However, cattle and sheep will readily consume onions and avoid problems with anemia if intake is increased slowly over several days. Campbell et al. (unpublished data) fed sheep diets consisting of 0, 25, 50, and 75% onions with no signs of toxicosis. The preconditioning phased was designed to increase the amount of onions fed slowly over a 28-day period until sheep were consuming the desired amount. This step-up ration approach began with $25 \text{ g} \cdot \text{hd}^{-1} \cdot \text{day}^{-1}$. For this trial, rams were reluctant to consume onions, requiring an additional 31 days added to the preconditioning phase to ensure sheep were consistently consuming the desired amount. The amount of onions fed was increased until onions made up 75% of the diet. An equivalent amount of a soybean meal (SBM) supplement was fed daily to another treatment so that diets were iso-nitrogenous. Cave et al. (2014) estimated the crude protein content of the same type of onions used in this study at 12%.

Immediately after onion or protein supplementation, 50 g of dried bitterweed, excluding roots and stems, were offered daily for 30 min. If sheep consumed all 50 g for two consecutive days, an additional 25 g were offered the following day.

Bitterweed was harvested from November through February at the Texas AgriLife Range Station in Barnhart, Texas, and then air dried. Hymenoxon levels vary among collection periods (Pfeiffer and Calhoun 1987). Hymenoxon levels were not measured in this study. To insure consistency of hymenoxon, bitterweed was chopped, composited, and thoroughly mixed prior to feeding.

Any weight changes that occurred were recorded. Individual weights (kg) were taken at the beginning and the end of the trial. Blood serum levels were also measured to detect tissue damage caused by toxicity. Serum metabolite levels were monitored at day 0, 7, and 14 to assess physiological status. In previous studies, dosing sheep with bitterweed

affected serum constituent levels (serum aspartate transaminase (AST), blood urea nitrogen (BUN), gamma glutamyltransferase (GGT), creatinine, and bilirubin. Changes in serum levels are indicative of toxicosis (Cornelius 1989; Cheeke 1998). It appears that a minor hepatic insult occurs when sheep are exposed to bitterweed and may approach pathological levels. Blood was collected via jugular vein puncture and serum was extracted by centrifugation, frozen, and sent to the Texas Veterinary Medical Diagnostic Lab in College Station, TX, USA for chemical analysis.

The second trial was similar to Trial 1. Intake of onions, supplement, and alfalfa were measured. Dorper-cross rams were replaced with castrated Rambouillet lambs. The second trial consisted of 30 Rambouillet lambs, approximately three months old with an average beginning weight of 28 ± 2.3 kg. Lambs were randomly allocated to the same treatments and the same variables were measured as in the first trial.

Serum levels were not measured in Trial 2, but packed cell volume (PCV), which is a measure of anemia, was measured. Whole blood samples were collected to measure PCV levels. Packed cell volume was measured as a percent of red blood cells (RBC) by transferring fresh blood in capillary tubes 30 min after collection, then centrifuging the tubes for 15 min and measuring the red blood cells with the scale affixed to the centrifuge.

In both trials, intake ($\text{g} \cdot \text{kg}^{-1}$ BW), weight change (kg), and serum levels (Trial 1) were compared among supplement types using repeated measures analysis of variance. Animals were the experimental unit while day of observation was the repeated measure. In Trial 2, PCV values were compared between treatments using a one-way analysis of variance. Comparisons were not made between breeds because breed effect was confounded with trials (Trial 1: Dorper-cross, Trial 2: Rambouillet). Means were separated using Tukey's LSD test when $P \leq 0.05$. Data were analyzed using the statistical package JMP (SAS Institute 2007).

RESULTS

Initially, a 14-day preconditioning phase was planned prior to feeding bitterweed to allow rams to adapt to a diet consisting of onions or the SBM supplement. After 14 days of feeding onions, most of the rams refused to consume the entire amount offered ($3.17 \pm 0.13 \text{ g} \cdot \text{kg}^{-1}$ BW) (Fig. 1). The preconditioning phase continued for an additional 31 days (45 days total) before rams would consume enough onions to begin feeding bitterweed. Once the trial began, sheep generally consumed most of the onions offered (Fig. 1). In addition, rams ate all of the SBM supplement and alfalfa offered daily throughout the trial beginning during the preconditioning phase.

Bitterweed intake differed ($P < 0.05$) among treatments for Trial 1. Dorper-cross rams receiving the onions as a supplement consumed more bitterweed on a daily basis than sheep receiving SBM as a supplement or no supplement at all. Treatment means for bitterweed intake varied from $0.34 \pm 0.05 \text{ g} \cdot \text{kg}^{-1}$ BW for rams fed a supplement containing SBM, $0.36 \pm 0.05 \text{ g} \cdot \text{kg}^{-1}$ BW for the control, and $0.71 \pm 0.05 \text{ g} \cdot \text{kg}^{-1}$ BW for rams supplemented with onions. The treatment by day interaction for bitterweed intake was also significant ($P < 0.05$) (Fig. 2). Initially, sheep were hesitant to consume bitterweed ($0.03 \pm 0.09 \text{ g} \cdot \text{kg}^{-1}$ BW, SBM, and alfalfa; $0.06 \pm 0.09 \text{ g} \cdot \text{kg}^{-1}$ BW, onions); however, by day 10, bitterweed intake had increased, especially with sheep given onions ($0.05 \pm 0.09 \text{ g} \cdot \text{kg}^{-1}$ BW, SBM and alfalfa; $0.97 \pm 0.09 \text{ g} \cdot \text{kg}^{-1}$ BW, onions).

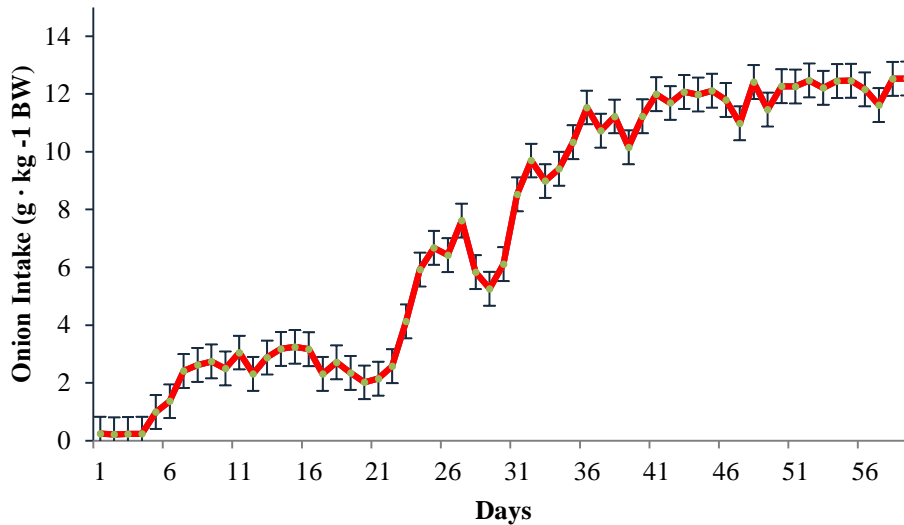


Figure 1. Average daily onion intake (g · kg⁻¹ BW) of Dorper-cross rams.

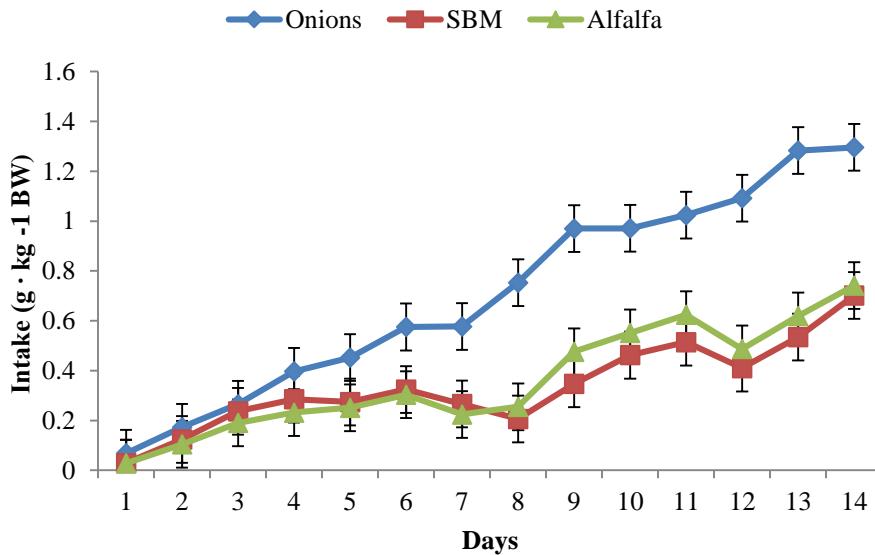


Figure 2. Bitterweed intake (g · kg⁻¹ BW) of Dorper-cross rams when supplemented with onions, soybean meal (SBM), or no supplement (alfalfa only) immediately prior to being fed bitterweed for 14 days. Treatment by day interaction differed ($P < 0.05$).

No soft tissue damage was detected from consuming bitterweed. Serum constituents of BUN, creatinine, bilirubin, AST, and GGT were similar among all treatments ($P > 0.05$) and remained within the range for healthy sheep (Table 2).

In Trial 2, Rambouillet lambs consumed the entire alfalfa and SBM supplement that was offered each day in both the preconditioning phase and during the feeding trial when bitterweed was fed. Once again, lambs were reluctant to consume onions prior to feeding bitterweed and during the feeding trial when bitterweed was offered (Fig. 3). Most refused to eat all of the onions throughout preconditioning and the feeding trial, even after feeding onions for 27 days.

Table 2. Serum metabolite levels of Dorper-cross rams when supplemented with onion, soybean meal (SBM), or no supplement (alfalfa only) immediately prior to being fed bitterweed for 14 days. Means were similar ($P > 0.05$) among treatments.

| | Treatment | | | Standard Error of the Mean (SEM)* | Normal Range ¹ |
|--------------------|-----------|------|----------|-----------------------------------|---------------------------|
| | Onions* | SBM* | Control* | | |
| BUN (mg/dl) | 20.1 | 30.2 | 23.9 | 1.4 | 12-32 |
| Creatinine (mg/dl) | 1.33 | 1.05 | 1.12 | 0.1 | 0.3-1.3 |
| Bilirubin (mg/dl) | 0.17 | 0.13 | 0.13 | 0.1 | < 0.3 |
| AST (U/l) | 76.1 | 80.2 | 79.7 | 4.6 | 51-130 |
| GGT (U/l) | 57.8 | 59.5 | 56.7 | 2.6 | 34-82 |

*All were within the normal ranges for sheep.

¹Normal ranges based on reported values from the Texas Veterinary Medical Diagnostics Lab, College Station, Texas.

Bitterweed intake was similar ($P > 0.05$) among all treatments. Much like the Dorper-cross sheep, the Rambouillet sheep were hesitant to consume bitterweed ($0.06 \pm 0.15 \text{ g} \cdot \text{kg}^{-1} \text{ BW}$ across all treatments). Treatment means for bitterweed intake varied from $0.72 \pm 0.1 \text{ g} \cdot \text{kg}^{-1} \text{ BW}$ for the control, $0.94 \pm 0.1 \text{ g} \cdot \text{kg}^{-1} \text{ BW}$ for the sheep supplemented with onions and $0.95 \pm 0.1 \text{ g} \cdot \text{kg}^{-1} \text{ BW}$ for sheep receiving a SBM supplement. Bitterweed intake steadily increased throughout the feeding trial (Fig. 4) averaging $1.5 \pm \text{g} \cdot \text{kg}^{-1} \text{ BW}$ by the end of the trial. There was a significant difference in the treatment by day interaction ($P < 0.05$). Intake increased steadily throughout the feeding trial across treatments; however, sheep supplemented with onions tended to eat more bitterweed toward the end of the study (Fig. 4).

Packed cell volumes were similar ($P > 0.05$) among treatments. Packed cell volume levels were $30.2 \pm 1.4 \%$ for Dorper-cross rams supplemented onions, $31.9 \pm 1.4 \%$ for those supplemented with SBM, and $32.1 \pm 1.4 \%$ for those in the control group (Table 3).

Serum constituents were not measured in Trial 2 because of a lack of difference in Trial 1. Sheep that were supplemented with onions lost more ($P < 0.05$) weight than lambs supplemented with a SBM-based supplement or fed alfalfa alone (Table 4). Those individuals that were supplemented with SBM or no supplement had little ($P > 0.05$) change in weight.

Data were pooled across breeds and stratified based on average intake of onions. Sheep were assigned to three different groups; those that ate 25% of their diet in onions, 50% of their diet in onions, or 75% of their diet in onions. There was no significant difference ($P > 0.05$) in bitterweed intake among groups (Fig. 5).

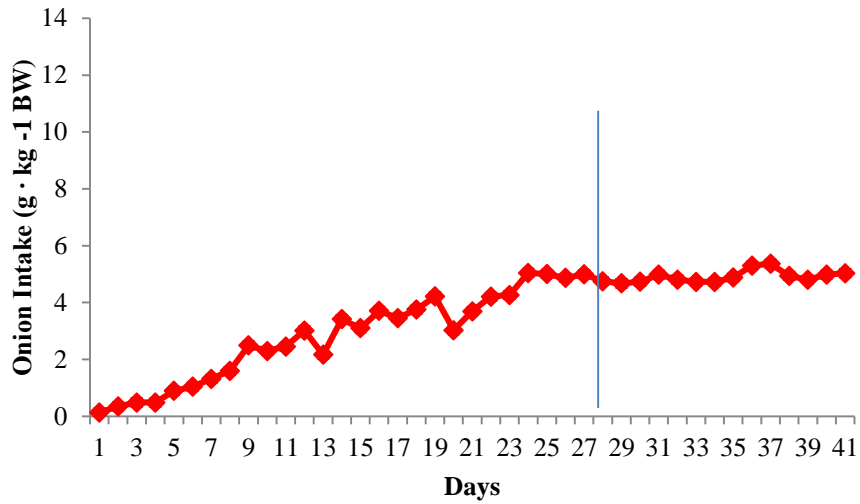


Figure 3. Average daily onion intake (g · kg⁻¹ BW) of Rambouillet lambs.

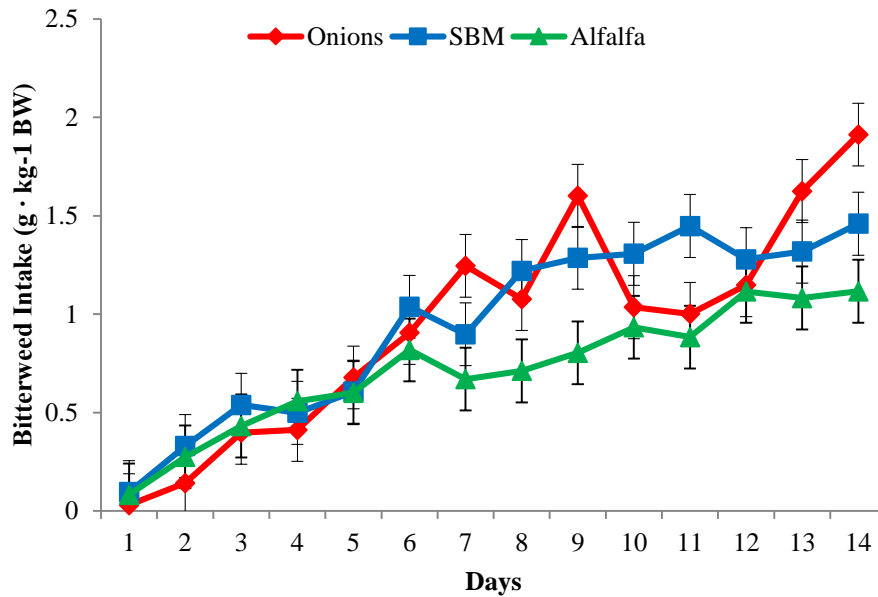


Figure 4. Bitterweed intake (g · kg⁻¹ BW) of Rambouillet lambs when supplemented. Treatment by day interaction differed (P < 0.05).

Table 3. Pack cell volume (PCV) for Rambouillet lambs supplemented with either onions, soybean meal (SBM), or no supplement (alfalfa only) immediately prior to being fed bitterweed. Means were similar ($P > 0.05$) among treatments.

| Treatment | Pack Cell Volume (%) | SEM |
|-----------|----------------------|-----|
| Onions | 30.2 | 1.4 |
| SBM | 31.9 | 1.4 |
| Alfalfa | 32.1 | 1.4 |

¹All percentages were in the normal range for sheep.

Table 4. Average weight loss (kg) of Dorper-cross rams and Rambouillet lambs supplemented with onions, soybean meal (SBM), or no supplement (alfalfa only) throughout preconditioning and the 14-day feeding trial.

| Treatment | Dorper-cross | Rambouillet | SEM |
|-----------|------------------|------------------|-----|
| Onions | 9.6 ^a | 8.0 ^a | 1.3 |
| SBM | 0.3 ^b | 2.8 ^b | 1.3 |
| Alfalfa | 2.2 ^b | 3.0 ^b | 1.3 |

^{a-b}Means within columns with different superscripts differ ($P < 0.05$), was true for both.

DISCUSSION AND CONCLUSIONS

Preliminary results suggested that supplementing onions (E. Campbell et al. unpublished data) or a supplement containing soybean meal (Coffman et al. 2015) would increase bitterweed intake. Onions unsuitable for human consumption are sometimes used as supplemental feeds in other regions of the U.S. (McBride 2004). Sheep supplemented with onions in the first trial consumed more bitterweed but not in the second trial. The hypothesis that supplementation with onions would increase bitterweed intake was not confirmed. In addition, supplementation with SBM apparently had no effect on bitterweed intake in this study. Both onions and SBM are relatively high in L-cysteine. Other studies have clearly illustrated that supplementation with L-cysteine increased bitterweed intake (Rowe et al. 1980; Calhoun et al. 1989). In addition, Coffman et al. (2015) reported that lambs supplemented with a SBM-based supplement ate more bitterweed than lambs receiving alfalfa alone. Results from this study do not support this observation. Reasons for contradictory results remain unclear. However, in both studies, intake of bitterweed remained relatively low, which may have affected the results.

Other protein sources may have resulted in different results. Supplements containing cottonseed meal have been shown to reduce the prevalence of bitterweed toxicity (Ueckert and Calhoun 1988). However, Coffman et al. (2014) failed to confirm this observation; supplementing lambs with a supplement containing cottonseed meal and digestible dried grains did not improve bitterweed intake. Conversely, supplementation with the same supplement did improve intake of the toxic shrub redberry juniper (*Juniperus pinchotii* Sudw) (George et al. 2010).

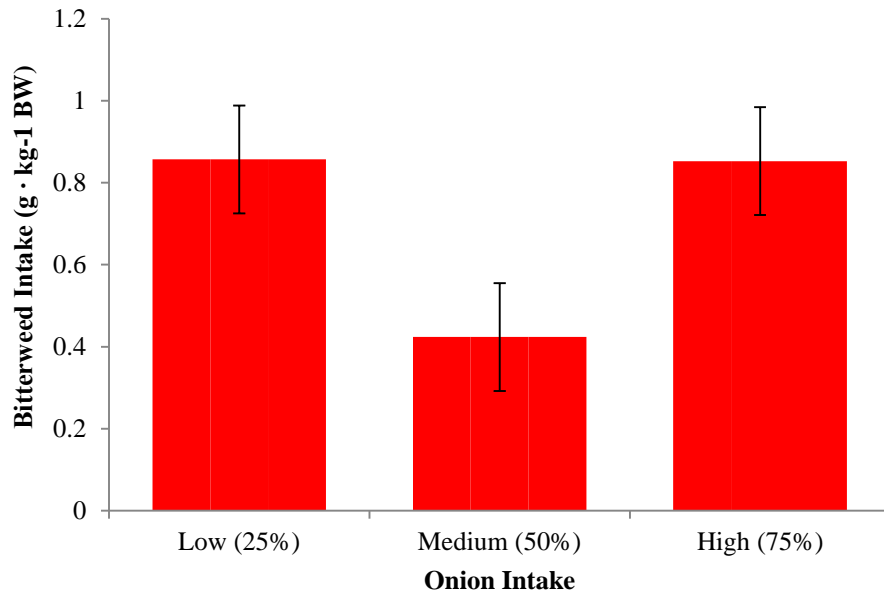


Figure 5. Level of onion intake ($\text{g} \cdot \text{kg}^{-1}$ BW) effect on bitterweed intake of both Dorper-cross rams and Rambouillet lambs.

Alfalfa intake was reduced for the treatment supplemented with onion (75% onions and 25% alfalfa). Given the reduced alfalfa fed and the reluctance of both Dorper-cross rams and Rambouillet lambs to consume onions, sheep may have increased intake of bitterweed simply to meet nutritional requirements. Toxins such as hymenoxon cause a decrease in preference for food but do not necessarily prevent ruminants from eating a food, especially if the food contains needed nutrients (Wang and Provenza 1996).

Similarly, ruminants will avoid levels inducing toxicity by reducing intake once aversive post-ingestive feedback is experienced (Provenza 1996). There were no differences in serum metabolite levels among treatment groups in the first trial. Likewise, there were no differences in PCV levels among treatments in the second trial. The lack of differences in both trials may be the result of animals maintaining intake of bitterweed and onions below toxic levels. Both Dorper-cross and Rambouillet lambs lost more weight when supplemented with onions. Their reluctance to consume all of the onions offered may have reduced total nutrient intake, resulting in weight loss.

When sheep from both trials were stratified based on onion consumption (25, 50, 75% onions consumed in the diet), there were no differences in bitterweed intake among groups. If supplementation with onions had affected bitterweed intake, a linear response should have occurred ($25\% < 50\% < 75\%$). However, those that consumed 50% onions consumed less bitterweed than those that consumed either 25% or 75% onions.

Unlike Rambouillet sheep, Dorper and other hair sheep do not require shearing and tend to be more parasite resistant (Vanimisetti et al. 2004; Burke and Miller 2002, 2004). Generally, the hair sheep breeds have evolved in a tropic environment and are considered to be better adapted to more stressful production conditions than the wool breeds, such as the Rambouillet (Wildeus 1997). Dorpers have been reported to utilize a

larger number of different plant species than wool breeds, and may be willing to select lower quality forages than wool breeds when nutrients are limited (Brand 2000).

Because the two breeds used in this study were fed in different trials, statistical comparisons cannot be made between breeds. However, Rambouillet lambs ate almost twice as much bitterweed (0.86 vs 0.47 g · kg⁻¹ BW) as Dorper-cross ram lambs. The Rambouillet breed may have become more tolerant of this specific plant through generations of selection on bitterweed-infested rangelands.

Based on the results of this study, further investigation is needed before recommending onions as a viable supplement to reduce bitterweed toxicity. One fact that does remain clear from this study and others (Poage et al. 2000; Frost et al. 2003) is that sheep are reluctant to eat bitterweed. Thus, providing nutritious alternative forages, particularly when bitterweed is readily available, should reduce the incidence of bitterweed toxicosis (Ueckert and Calhoun 1988). In addition, most forages are dormant and characterized by low protein content when bitterweed is readily available during winter. Producers should consider feeding some type of protein supplement during winter to all sheep to meet their protein requirements. As long as nutrient requirements are met, sheep should avoid eating bitterweed, reducing the likelihood of suffering from bitterweed toxicosis.

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