THE EFFECTS OF PROTEIN SUPPLEMENTATION ON BITTERWEED TOXICOSIS IN LAMBS

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ABSTRACT

This study determined if a supplemented protein diet high in escape protein or consisting of sulfur-containing amino acids would reduce the likelihood of bitterweed (*Hymenoxys odorata* DC) toxicosis. Forty recently weaned Rambouillet lambs were used with eight lambs randomly allocated to each treatment. They were placed in individual pens and allowed a seven-day adjustment period. Lambs were fed alfalfa pellets at 2.5% body weight, their respective treatment diet, and bitterweed. Treatments received either a (1) cottonseed meal (CSM), (2) CSM and Distiller's Dried Grain (DDG), (3) soybean meal (SBM), or (4) SBM and DDG-based supplement. Treatment 5 received only alfalfa pellets. All supplements were isonitrogenous (37%), and lambs were fed enough supplement and alfalfa to achieve 150 g of growth per day. Bitterweed was offered immediately after supplemental diets for one hour daily for 15 days. Intake of supplemental diets, bitterweed, and alfalfa were measured on an individual animal basis. Lambs fed the SBM-based supplement ate more (P < 0.05) bitterweed than lambs fed alfalfa alone. Other supplemental diets did not improve bitterweed intake. None of the lambs from any treatment exhibited signs of toxicosis. Producers should consider feeding a SBM-based supplement to reduce the likelihood of bitterweed toxicosis in sheep.

KEY WORDS: intake, hymenoxon, L-cysteine, DDG, amino acids

INTRODUCTION

In 1962, it was estimated that annual losses in livestock production from bitterweed (*Hymenoxys odorata* DC) toxicity were $3.6 million in the Edwards Plateau region of Texas alone (Witzel et al. 1974). At that time, Calhoun et al. (1981) suggested that bitterweed is the most serious toxic plant problem faced by sheep producers in Texas. It is a native, cool season, annual forb that is found in semi-arid regions from Kansas and southern Colorado to Texas and west all the way to southern California (Kingsbury 1964) and is particularly common in Central and West Texas.

The invasion of bitterweed can be attributed to several factors. Periodic droughts and overgrazing reduce cover of other grasses and forbs, thereby allowing room for bitterweed to invade (Hardy et al. 1931; Sperry 1949). Bitterweed is a drought-hardy plant

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in that it restricts growth and conserves the limited moisture it has during dry periods and resumes growth when moisture is again available (Sperry and Sultemeier 1965). More recently, increased oil and gas exploration, resulting in significant ground disturbance, has increased the amount of bitterweed in the Edwards Plateau and western Texas.

Bitterweed toxicosis is common because it is green and flowering in late fall and early winter when nutrient demands for livestock are at their highest (Ueckert and Calhoun 1988). This period coincides with warm season grasses entering into dormancy while nutrient demands increase because lambing and fluctuating ambient temperatures. Most sheep are reluctant to graze the plant because they associate its bitter taste with aversive postingestive feedback (Calhoun et al. 1981; Poage et al. 2000). However, during late fall and early winter, bitterweed may be the only green forage available (Rowe et al. 1973; Pfeiffer and Calhoun 1987). If there is a lack of other sufficient forage, hunger will eventually force sheep to graze the plant and suffer toxicosis (Ueckert and Calhoun 1988).

Hymenoxon, the toxic compound in bitterweed, is a sesquiterpene lactone with an exocyclic methylene group conjugated with the lactone carbonyl (Kim et al. 1975). This compound is also toxic to cattle and goats (Ueckert and Callhoun 1988), but they typically do not graze bitterweed in sufficient amounts to cause toxicosis. A lethal dose of hymenoxon in sheep causes symptoms such as bloating, Central Nervous System (CNS) depression, liver and kidney damage, and termination of rumen activity. It typically results in the death of the animal within 36 hours of ingestion (Rowe et al. 1973).

The amino acid cysteine contains sulfhydryl groups that can react and bind with this toxin in the rumen to form less toxic compounds (Kupchan et al. 1970). If detoxification does not occur in the rumen, toxic compounds are absorbed into the bloodstream, and travel to the liver for further detoxification in one of two reactions in the liver (Williams 1959). Phase I reactions alter existing functional groups or introduce a polar group into a non-polar compound to make it more hydrophilic and therefore, more easily excreted from the body (Bidlack et al. 1986). Phase II reactions are those that conjugate polar groups of foreign compounds with endogenous cofactors (Bidlack et al. 1986). As a result of these reactions, the xenobiotic compounds, like hymenoxon, become more hydrophilic and more easily excreted (Nebbia 2001).

Protein feeds high in glucogenic amino acids (e.g. distillers dried grains (DDG)) or feeds that contain higher levels of amino acids that escape digestion in the rumen (e.g., cotton seed meal) should aid in detoxification through Phase II reactions in the liver. These amino acids escape rumen digestion and are transported to the liver where they may be used for further conjugation with toxins.

Several studies have shown that protein supplementation can increase the consumption of some toxic plants by increasing their ability to tolerate the toxins. For example, goats increased redberry juniper (*Juniperus pinchotii* Sudw.) consumption while receiving a supplement containing cottonseed meal and DDG (George et al. 2010). Supplementation of lambs with barley and activated charcoal increased intake of the shrub big sagebrush (*Artemesia tridentata* Nutt.) (Banner et al. 2000). In addition, protein supplementation seems to reduce the likelihood of bitterweed toxicity (Calhoun et al. 1989).

Dosing sheep with the amino acid L-cysteine protects them from the acute effects of hymenoxon (Rowe et al. 1980; Calhoun et al. 1989). L-cysteine provides sulfhydryl groups for rumen degradation of toxins. Unfortunately, L-cysteine is unpalatable at levels necessary to prevent bitterweed toxicosis. Soybean meal is highly soluble in the rumen and

contains moderate levels of L-cysteine. Feeding supplements containing soybean meal could potentially alleviate some bitterweed toxicity problems.

Glucogenic amino acids found in cottonseed meal and DDG may provide the substrates for toxin conjugation and excretion in the liver (Freeland and Jansen 1974). Bitterweed is apparently partially detoxified in the liver. Terry et al. (1983) reported that hymenoxon was conjugated with gluconurides and excreted in the urine. This may explain why others (Calhoun et al. 1981) have reported reduced instances of bitterweed toxicity when sheep were fed supplements containing cottonseed meal.

Accordingly, this study was designed to determine if bitterweed toxicosis can be avoided or reduced by supplementing proteins containing glucogenic amino acids, sulfur-containing amino acids, or both to aid in detoxification of hymenoxon.

MATERIALS AND METHODS

This study was conducted at the Angelo State University Management Instruction and Research (MIR) Center, San Angelo, Texas. Forty recently weaned Rambouillet ewe lambs (28.3 kg, approximately five months of age) were used in this experiment with eight lambs used per treatment. Lambs were separated into individual pens (1 m X 1.5 m), and allowed seven days for a pen-adjustment period. During this adjustment period, they were fed their supplemental diets and alfalfa pellets. Alfalfa pellets were fed at 2.5% BW daily to meet or exceed their maintenance requirements throughout the trial (NRC 2007). Sheep also received fresh water and a calcium/phosphorus mineral with trace elements *ad libitum.*

The bitterweed plants used in this experiment were harvested in early to mid-spring at the Texas AgriLife Research Station near Barnhart, Texas and transported back to the MIR Center. Plants were then air dried for two weeks post-harvest. Bitterweed was hand chopped, composited, and thoroughly mixed before feeding.

Animals in each treatment received their respective supplements each day before bitterweed was fed. Ingredients and nutrient content of each supplement is listed in Table 1. Treatment 1 received a supplement with cottonseed meal as the protein source. Treatment 2 received a supplement with cottonseed meal and distiller's grain as the protein source. Treatment 3 received a supplement with soybean meal as the protein source, and Treatment 4 received a supplement with soybean meal and distiller's grain as the protein source. Treatment 5 served as the control group and received only alfalfa pellets. All supplemental diets were isonitrogenous at 37% crude protein. The amount of supplement for each lamb was based on providing 1.9 kg^{-1} BW to meet maintenance requirements, and in addition, 64 g of additional protein was fed each day to achieve 150 g of growth per day (NRC 2007). The amount of each supplement fed was based on requirements for maintenance and growth minus the number of grams of protein provided by alfalfa pellets.

Table 1. Ingredients and nutritional value of supplemented protein feeds.

Ingredients[a]	Ration/Treatment (%)			
	1	2	3	4
Cottonseed Meal	88.7	77.5	--	--
Soybean Meal	--	--	78.7	63.0
Digestible dried grains (DDG)	--	16.2	--	26.8
Cane Molasses	3.4	3.4	3.4	3.4
Rice bran with germ	7.5	2.5	17.5	6.5
Trace Mineral Premix	0.02	0.02	0.02	0.02
Vitamin ADE Premix	0.3	0.3	0.3	0.3
Nutrient Content				
TDN[b]	70.2	72.3	73.7	76.5
Crude Protein	37.3	36.0	39.6	37.3
MEt Energy	231.3 kcal/kg	77.1 kcal/kg	2498.8 kcal/kg	1767.5 kcal/kg

[a]All percentages based on one ton (909.1 kg)
[b]Total digestible nutrients

Lambs were randomly allocated to treatments and fed one of the four treatments daily for 15 days during the feeding trial. Protein supplementation was offered every day from 1300 hours to 1400 hours prior to feeding bitterweed. Bitterweed was then offered to all animals from 1400 to 1500 hours with intake recorded daily. Lambs were all offered 35 g of dried bitterweed to begin the trial. If any lamb consumed all the bitterweed offered for two consecutive days, the amount offered was increased to 50 g on the following day and by intervals of 25 g on days after that. Lambs were then offered alfalfa pellets at 2.5% BW to meet maintenance requirements from 1500 hours to 1700 hours. The amount of alfalfa fed to control animals was increased so that they received the same amount of protein each day. Intake of supplements, bitterweed, and alfalfa pellets were monitored daily by weighing refusals. Body weight changes were monitored during this experiment by weighing the animals before the feeding trial began and after it was over.

This study design was a completely randomized design. Differences between protein supplements (treatment means) were assessed using repeated measure analysis of variance. Individual lambs nested within treatments served as replications. Treatment means were analyzed as a fixed effect, individual animals as a random effect, and days of feeding as the repeated measure. Planned linear orthogonal contrasts were also used to compare each treatment to the control diet. Intake was adjusted on a body weight basis (g · kg^{-1} BW) to account for variations among animals. Means were separated using Least Significant Differences (LSD) where $P \leq 0.05$. Data were analyzed using the statistical package JMP (SAS Institute 2007).

RESULTS

Supplement and alfalfa intake were similar ($P > 0.05$) across treatments and across days in both the pretrial and trial. Lambs typically ate all of the alfalfa and supplement fed each day. The treatment and treatment X day interaction for bitterweed intake was similar

($P > 0.05$); however, bitterweed intake differed ($P < 0.05$) across days of feeding (Fig. 1). Initially, animals were reluctant to consume bitterweed (0.1 g/kg BW), however by day 20 had increased intake to (1.5 g/kg BW) (Fig. 1).
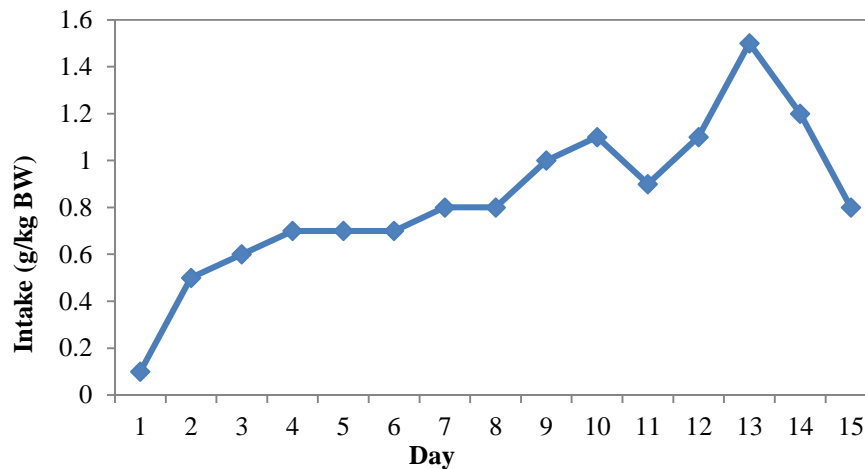

Figure 1. Average intake of bitterweed for the 15-day feeding trial.

When orthogonal contrasts were used to compare treatment means, one difference was evident. Lambs receiving the SBM supplement consumed more bitterweed than those receiving no supplement (Fig. 2). Bitterweed consumption of other groups did not differ statistically from the control group (Fig. 2).

Lambs in the CSM treatment lost weight during the trial. The control treatment gained an average of 1.4 ± 0.74 kg. The CSM/DDG treatment group gained an average of 1.7 ± 0.74 kg. The SBM treatment group gained an average of 1.74 ± 0.74 kg, and the SBM/DDG treatment group gained an average of 0.21 ± 0.74 kg. The CSM treatment group lost an average of 0.4 ± 0.74 kg (Fig. 3). No animals displayed any clinical symptoms of bitterweed toxicosis during the experiment.

DISCUSSION AND CONCLUSIONS

The results of this study indicate that lambs receiving a SBM protein supplement ate more bitterweed than lambs only receiving alfalfa pellets. When sheep are unable to meet their nutritional requirements (i.e., late fall, winter), bitterweed intake increases. In addition, most producers report higher incidences of bitterweed toxicosis in ewe lambs that are still growing and may not be able to meet their nutritional requirements from dormant, poor quality, warm season grasses. Supplementation with soybean meal should provide the substrates (L-cysteine) for rumen detoxification of hymenoxon and improve the likelihood of animals meeting their protein requirements.
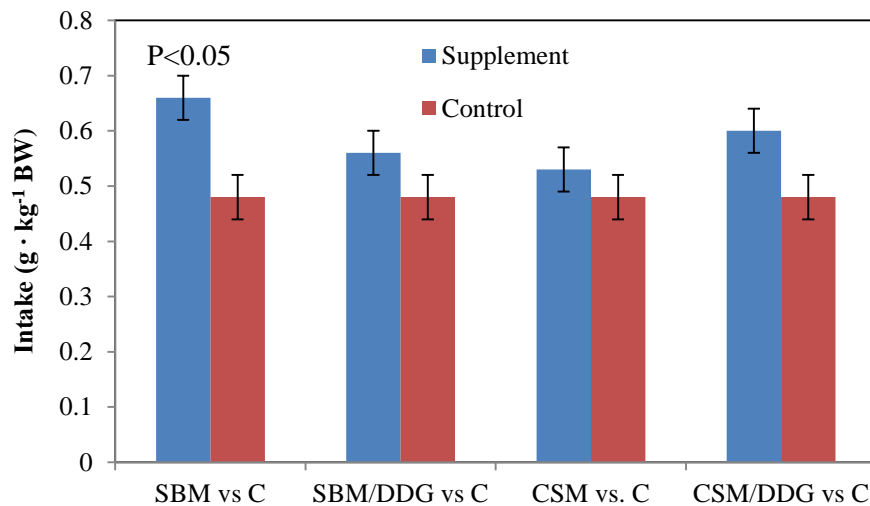
Figure 2. Comparison of average bitterweed intake between different treatments and control group for sheep fed bitterweed for one hour daily for 15 days.
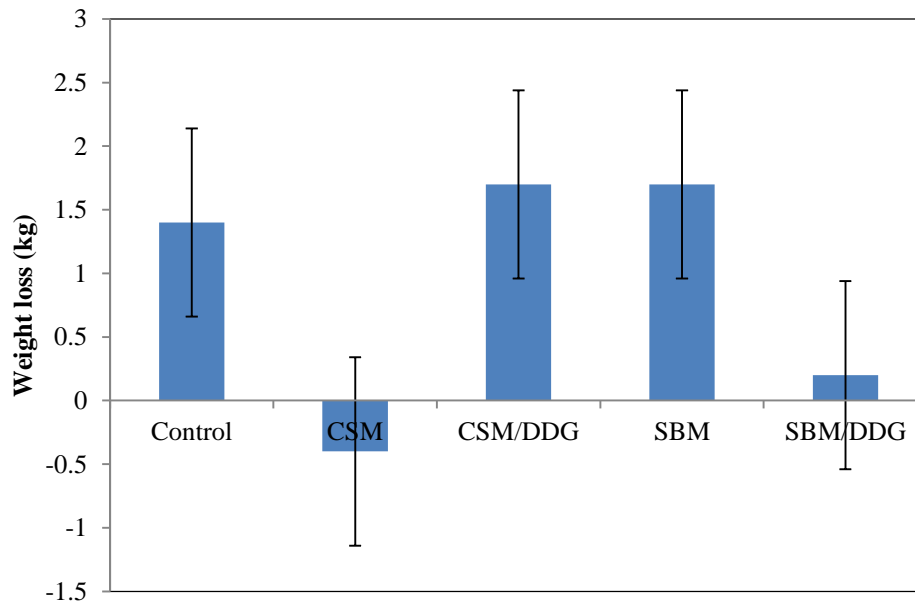

Figure 3. Average weight change of sheep after 22-day feeding trial.

The hypothesis that protein sources high in amino acids that escape rumen digestion would improve bitterweed intake was not confirmed despite that fact that other research illustrated that supplements containing both CSM and DDG improved intake of the toxic shrub redberry juniper (George et al. 2010). Given that both toxins are apparently altered and conjugated in the liver (Terry et al. 1983; Foley et al. 1995), we expected that

supplementation with CSM and DDG would increase bitterweed intake. In this study, supplementation for protein sources higher in amino acids that escape rumen degradation apparently had no impact on bitterweed intake.

Other research supports the importance of supplementation to reduce the likelihood of plant-induced toxicosis. Banner et al. (2000) showed that supplementation of lambs with barley and activated charcoal increased their consumption of big sagebrush. Supplemented lambs ate an average of 304 g of sagebrush versus control sheep that ate an average of 248 g. Similarly, supplemented sheep fed a 20% crude protein diet over a 10% crude protein diet had decreased signs of toxicosis from bitterweed (Calhoun et al. 1989).

None of the lambs in this experiment showed symptoms of bitterweed poisoning. Lambs were on an adequate plane of nutrition, and were meeting their requirements for maintenance and growth. If nutrient intake had been lower, lambs may have ingested sufficient levels of bitterweed to induce toxicosis.

During periods of nutritional stress, the body undergoes a depletion of glycogen stores, and increased glucogenesis from degraded amino acids and fatty acids being utilized for energy requirements. This response to starvation reduces the animal's ability to handle plant toxins (Bidlack 1982). Detoxification requires additional expenditures of amino acids and glucose to conjugate with toxins. Thus, feeding greater amounts of amino acids and high protein diets may provide a source of amino acids that can be used to synthesize glucose in the liver, in turn playing a role in conjugation of toxins to be excreted from the body (Illius and Jessop 1995).

Bitterweed is a toxic cool season annual forb that is green and growing in pastures in west central Texas at times when most other forages are dormant. This is also a time when the nutritional requirements of range animals are at their highest. Results of this study, as well as previous studies, have shown that protein supplementation can increase consumption of toxic plants (Banner et al. 2000; Campbell et al. 2007; George et al. 2010). Most pasture supplementation in west central Texas begins around mid to late November when warm season forages enter dormancy and bitterweed plants are growing. Producers that have bitterweed present should consider feeding a soybean meal-based range supplement to their animals. This can be used as a method of protecting sheep against hymenoxon poisoning by providing the sulfur containing amino acids that apparently bind with the toxin and aid in meeting nutritional requirements, thereby reducing the likelihood of bitterweed ingestion.

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