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Publication No. R-13 | Spring 2012

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The MIR Center serves three primary objectives. The first objective is to conduct research to improve animal production, food technology, and range and wildlife management. These include studies related to improving the reproductive and nutritional efficiency of sheep, cattle, and goats in conjunction with projects designed to improve range and wildlife management practices. Some research projects are very basic in nature and designed to better understand mechanisms controlling animal and plant production. Others are applied studies with immediate implications for management.

The second objective is to serve as a demonstration site for Agricultural industry. This objective is accomplished by managing rangelands for livestock, wildlife, and food production. The development of sustainable, economically efficient management systems is a primary a focus of the MIR Center. All experiments, investigations, and research projects sponsored through the MIR Center are open for review by the public. Personnel at the Center are available to assist ranchers, farmers, and others in the region with management issues associated with their operations. Where appropriate, results of research and management demonstrations are published in area newspapers, magazines, and agricultural journals. The Center's facilities are also utilized by various agricultural groups for meetings throughout the year which include a 4-H/FFA livestock judging contest, wool and mohair judging contest, meat judging contest, and wildlife contests.

The third objective of the MIR Center is to serve as a laboratory and outdoor classroom for the undergraduate and graduate programs in Agriculture. Laboratory classes are taught weekly at the MIR Center for Agricultural Science and Leadership, Animal Science, Food Science, and Range Management courses. Undoubtedly, these "hands-on" experiences have been essential in shaping the knowledge of the department's 1,056 graduates since 1974.

The MIR Center is crucial for the Master of Science (M.S.) program in Animal Science. Since 1978, the department has produced 189 M.S. graduates. Several of those have continued their education at other universities by pursuing a Ph.D.

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# JUNIPER CONSUMPTION DOES NOT ADVERSELY AFFECT MEAT QUALITY IN BOER-CROSS GOATS

*Matthew W. Menchaca, Cody B. Scott, Kirk W. Braden, Corey J. Owens and Loree A. Branham*

## ABSTRACT

Goat browsing can be used as an alternative brush management option for redberry (*Juniperus pinchotii* ) and ashe (*Juniperus asheii*) juniper instead of more expensive and invasive brush control methods, assuming consumption of juniper does not adversely affect the marketability of offspring. Some wildlife species reportedly retain juniper flavor when consumed. We determined if juniper consumption affected meat quality or flavoring of Boer-cross kid carcasses. Twenty recently weaned, Boer-cross wethers were randomly assigned to 1 of 4 treatments with treatments fed different amounts of juniper (0-10-20-30% juniper in the diet). All goats were fed juniper for 28 days at the Angelo State University (ASU) Management, Instruction, and Research Center. All goats were also fed a feedlot ration to meet maintenance requirements (2% BW). Juniper intake varied ($P<0.05$) between all treatments (0-10-20-30%) primarily because treatments were fed different amounts of juniper. Following a 28 day trial, goats were harvested at ASU Food Safety and Product Development Laboratory. Carcass characteristics including live weight, hot carcass weight, dressing percentage, loineye area, body wall fat thickness, and leg circumference were similar ($P>0.05$) among treatments. Sensory characteristics including tenderness, juiciness, flavor intensity, off flavor, and overall acceptability were also similar ($P>0.05$) among treatments. Landowners can utilize goats as a biological management tool without adversely affecting goat meat quality or flavoring.

## INTRODUCTION

The number of meat goats has increased throughout the United States since the 1990's (Spencer 2008). In 2008, the USDA reported 3,150,000 goats in the U.S.; yet producers in the U.S. are still unable to meet demand for goat meat (Spencer 2008). The result has been that the average price per pound for meat goat products is usually higher than other red meat species (Glimp 1995). In addition, goats complement beef cattle operations because of differences in forage preferences (Bryant et al. 1979; Taylor et al. 1980; Morrow 1998). Both Spanish and Boer breeds of goats are highly adaptable to a wide variety of environmental conditions and are common throughout the southwestern U.S.

Two species of juniper, redberry (*Juniperus pinchotii*) and ashe (*Juniperus asheii*) juniper, are problematic species invading millions of hectares of land in Oklahoma, New Mexico, and Texas (Ansley et al. 1995; Smeins and Fuhlendorf 1997). Increased amounts of juniper cover on rangelands are attributed to overgrazing, the lack of fire, and drought (Ellis and Schuster 1968).

Juniper contains monoterpenoid oils which are comprised of five carbon rings with hydrogen, alcohol, and ketone side groups (Owens et al. 1998; Campbell and Taylor 2007). Juniper is considered unpalatable because monoterpenoids cause aversive postingestive feedback (Riddle et al. 1996; Pritz et al. 1997). Fortunately, goats will consume juniper, after exposure to the plant at weaning (Bisson et al. 2001; Ellis et al. 2005; Dunson et al. 2007) and continue to consume juniper after returning to a rangeland situation (Dietz et al. 2010). Despite the interest in using goats to control juniper, the effect of juniper consumption on goat meat quality remains unknown. If juniper consumption does affect meat flavor or quality, goats may not be a viable choice for biological control of juniper in all situations.

Several ingredients including fish meal, raw soybeans, canola oil and meal, and some grasses cause undesirable flavors in red meat because compounds are deposited in lipids or lean

muscle (Melton 1990). The lipid fraction of meat, such as marbling and other adipose tissue, is partially responsible for characteristic flavors of meat products (Melton 1990). Many hunters argue that in some wildlife species the strong salient flavor of juniper is very prevalent, especially in years of drought when nutritive intake can be limited by lack of food. Indeed, the observation may be warranted; some toxins are partially metabolized and deposited in adipose tissue throughout the body thereby limiting their effect in a centralized location in the body like the liver (Bidlack 1982; Cheeke 1998). Many of these toxins remain in the body for extended periods of time. For example, drug addicts often experience hallucinations years after consuming *phencyclidine* (PCP). A possible explanation for the flashbacks is the drug has been deposited in fat tissue throughout the body, and when the tissue is mobilized, for example during exercise, the stored PCP is released (Ruiz et al. 2007). Once fat tissue is metabolized, the drug is released in the body resulting in hallucinations. Depositions of toxins in fat tissue could also affect meat quality because compounds could be released during cooking affecting meat quality, especially if the toxin had a strong salient flavor like the monoterpenoid oils found in juniper.

It appears that most of the toxins in juniper are partially oxidized in the liver and conjugated with gluconurides in the liver and excreted (Foley et al. 1995; Dunson et al. 2007; George et al. 2010). However, redberry juniper contains 16 identified monterpenoids and ashe juniper contains 17 identified monoterpenoids (Owens et al. 1998; Dietz et al. 2010). Because of the large variety of monoterpenoids found in juniper, it is unclear how the body handles each individual toxin (E. Campbell, pers. comm.). Theoretically, some of the monoterpenoids in juniper may be sequestered within the body in muscle or adipose tissue. Compared to other species, goats deposit little fat in or around muscle fibers. With that in mind, juniper consumption may have little impact on goat meat flavor. The objective of this study was to determine if juniper consumption has an effect on goat meat quality (tenderness and juiciness) and flavor (characteristic goat flavor, off-flavor).

## MATERIALS AND METHODS

For this experiment, 20 recently weaned, multiple sire, Boer-cross wethers (*Capra hircus*) (43 kg, approximately 6 months of age) were randomly assigned to one of four treatments (5 goats/treatment) in August 2008. All wethers were weaned at 4 months of age and fed a feedlot ration until reaching a target weight of 45 kg. Animals were fed in one large group at the Angelo State University Management, Instruction, and Research (MIR) Center (Lat: 31.38, Long: 100.5). The feedlot ration used consisted of sorghum (45.0%), cottonseed meal (10.0%), soybean hulls (22.5%), alfalfa pellets (17.0%), cane molasses (3.5%), and a vitamin/mineral premix (2.0%). Goats had been de-wormed using an Ivermectin sheep drench and vaccinated for Enterotoxemia prior to being placed on feed. After reaching the target slaughter weight, animals were separated into individual pens (1.5 m by 1.5 m) allowing 7 days for pen adjustment. Goats were fed the basal ration at 2% BW (approximately 900 kg/day) during the feeding trial (described below) to maintain body condition (NRC 2007). The goats also had access to fresh water *ad libitum* and excreta were removed weekly.

After the 7-day pen adjustment period, goats were assigned juniper treatments with different amounts of juniper for each treatment. Juniper and the basal diet were fed separately to monitor intake of both. Treatments included: 1) the basal diet alone (control), 2) 10% of the basal diet replaced with redberry juniper, 3) 20% of the basal diet replaced with redberry juniper, or 4) 30% of the basal diet replaced with redberry juniper. Juniper offered in the 10, 20, and 30% treatment groups was gradually increased over 14 days during the 28 day trial period to avoid adverse physiological effects and the formation of conditioned food aversions from the toxic monoterpenoids found in juniper. Initially, 50 g of juniper was offered to each goat. If an

individual goat consumed all juniper offered, the amount fed was increased daily until the target percentage of the diet was reached (10, 20, or 30%).

Redberry juniper was fed once daily at 0800 for 1 hour. Redberry juniper was harvested from randomly selected trees at the Texas AgriLife Research Center, Sonora, Texas (Lat: 30.58 Long: 100.65). Leaves were stripped from the stems before feeding and composited to insure consistency of juniper offered. Juniper was collected two days before initiation of the study and stored at 4 °C until completion of the study (Utsumi et al. 2006). Juniper refusals were collected and weighed to measure intake. The basal diet was offered from 0900 to 1700 hours, after which refusals were collected and weighed to measure intake.

Within a 24 hour period following the 28$^{th}$ day of the trial, animals were harvested at the Angelo State University Food Safety and Product Development Laboratory. Each carcass was surface sprayed with a 2.5% organic acid to reduce microbial contamination of carcasses (ASU 2007). The carcasses were then chilled for a minimum of 24 hr in order to reach an internal target temperature at the thickest portion of less than 7.2° C. The carcasses were fabricated with an emphasis on the right longissimus muscle and shoulder, which was used for the experiment. The loin, which contains the 13$^{th}$ rib, was cut from the point between the 12$^{th}$ and 13$^{th}$ rib to a point approximately anterior to the hip bone. It was then split along the back bone with the flank and 13$^{th}$ rib removed at approximately 2.54 cm from the loin muscle. In order to provide a similar sample unit, loin chops from the anterior end were cut at 2.54 cm, and 4 rib chops from the posterior end were cut providing a 10.16 cm sample of the loin muscle. The samples were then vacuum packaged and frozen at a temperature < 0° C. The right side square cut shoulder was deboned and frozen whole at a temperature < 0° C.

Chops and shoulders were thawed at 4° C 24 hours prior to assessment of meat flavor, quality, and tenderness. Shoulders were ground through a hamburger grinding plate and made into 114 g patties shaped and formed by a patty press. Temperature and weight were taken both prior to and post preparation of the samples. The chops and patties were cooked using clamshell grills (Kerth et al. 2003) to 71° C, a medium degree of doneness.

A trained sensory panel, consisting of 2 faculty and 4 students, was utilized for sensory evaluation (AMSA 1995). All sensory evaluation panel members were trained to recognize and identify sensory attributes (Cross et al. 1978). Each member tasted a patty and chop from each animal. Trained panelists were asked to evaluate initial and sustained juiciness, initial and sustained tenderness, flavor intensity, and overall acceptability on a scale from 1 to 8 (8=extremely juicy, tender, intense, acceptable and 1=extremely dry, tough, bland, prominent off flavor, unacceptable), and off flavor was evaluated on a 4 point scale (4= no off flavor and 1= extremely prominent off flavor). This research complies with the guidelines set by the Institutional Review Board for human subject use.

Tenderness was also estimated utilizing Warner-Bratzler shear (AMSA 1995). Warner-Bratzler shear force measurements were taken by a calibrated Warner-Bratzler shear force machine through a 1 cm round core sample of the desired cut (*Longissimus dorsi* muscle) cooked to a medium degree of doneness. Sample analysis and data collection followed the guidelines set by the American Meat Science Association and National Livestock and Meat Board research guidelines manual (1995).

Treatment means for juniper and the basal ration intake were compared among treatments using repeated measures analysis of variance with individual goats as the experimental unit and day of observation as the repeated measure. Meat quality, tenderness, and meat flavor were compared among treatments using the same model but without a day effect. Difference between means were assessed using Tukey's LSD test when $P<0.05$. Data was analyzed using the statistical package JMP (SAS 2007).

## RESULTS

Juniper intake differed ($P<0.05$) among treatments but did not reach the targeted levels of 10% (2 g $kg^{-1}$), 20% (4 g $kg^{-1}$), or 30% (6 g $kg^{-1}$) of the diet. The treatment fed a diet of 30% juniper ate more juniper (3.5 g $kg^{-1}$) than goats fed a diet consisting of 20% juniper (2.2 g $kg^{-1}$), which ate more than goats fed a diet consisting of 10% juniper (1.2 g $kg^{-1}$). Initially, goats were reluctant to consume the total amount offered. After day 7, goats increased juniper consumption, but intake continued to fluctuate throughout the study ($P<0.05$ treatment X day interaction) (Fig 1). During the last 7 days of the study, goats fed a diet consisting of 30% juniper ate 4.4 g $kg^{-1}$ of juniper, goats receiving a diet containing 20% juniper ate 3.0 g $kg^{-1}$ of juniper, while goats receiving a diet of 10% juniper at 1.6 g $kg^{-1}$ of juniper. Goats readily accepted the basal diet during a 7-14 day pen adjustment period and consumed the entire amount offered throughout the 28 day trial.

Carcass characteristics, including hot carcass weight, dressing percent, loineye area, body wall fat thickness, and leg circumference were similar ($P>0.05$) among treatments (Table 1). Tenderness measurements taken from the loin with a Warner-Bratzler shear force machine were also similar ($P>0.05$) among treatments (Table 1). Sensory data for loin chops and ground shoulder patties, such as tenderness, juiciness, flavor intensity, off flavor or overall acceptability were also similar ($P>0.05$) among treatments (Table 2).

## DISCUSSION

Results of the study suggest that goats consuming a diet consisting of up to 30% juniper in their diet had no affect on carcass quality or meat flavor. Dietz et al. (2010) showed that goats will consume juniper at levels up to 30% of their diet on pasture, when exposed to the forage early in life. Prior to this study, there was no data available on how juniper consumption would affect goat meat quality. Even though the targeted levels of 10, 20, and 30% of the diet were not reached, goats consumed juniper throughout the 28-day trial and intake differed among treatments. Thus, consumption of juniper at levels and duration observed in this study did not affect quality or carcass characteristics.

Juniper intake fluctuated daily (Fig 1). Intake of toxic plants typically cycles with intake increasing until aversive postingestive feedback is experienced followed by a decline in intake on subsequent days (Provenza 1995). Intake fluctuations observed in this study were probably in response to aversive postingestive feedback as intake approached 30%.

It is possible juniper consumption for longer periods of time (e.g., year-long consumption) could affect meat quality or flavor. However, it seems unlikely that monoterpenoids in juniper would affect meat flavor if most of the toxins are indeed metabolized and excreted through urination (Foley et al. 1995). In addition, most goats harvested for meat production are harvested at a relatively young age (< 6 months). Arguably, if intake had exceeded 30% of the diet for longer periods of time, juniper may have affected meat quality or flavor. In previous studies, juniper intake typically did not exceed 30% of the diet for any length of time (Bisson et al. 2001; Ellis et al. 2005; Dunson et al. 2007) apparently because rumen microbial death occurs when intake exceeds 30% of the diet (Straka et al. 2004).
Feed source is the most important environmental factor in meat flavor (Shahidi and Rubin 1986). For instance, excessive levels of fish meal to swine diets may be responsible for a fishy flavor in pork (Castell and Falk 1980). Soybean meal, when fed to lambs, is associated with less desirable lamb meat flavor (Crouse et al. 1983). Steers fed grain have different meat flavor than steers fed grass (Larick et al. 1987). Fatty acid composition can be changed with the addition of grain to the diet (Kemp et al. 1981). Fat color, an important eye appeal aspect, is changed from yellow to white when grain is fed as the diet.

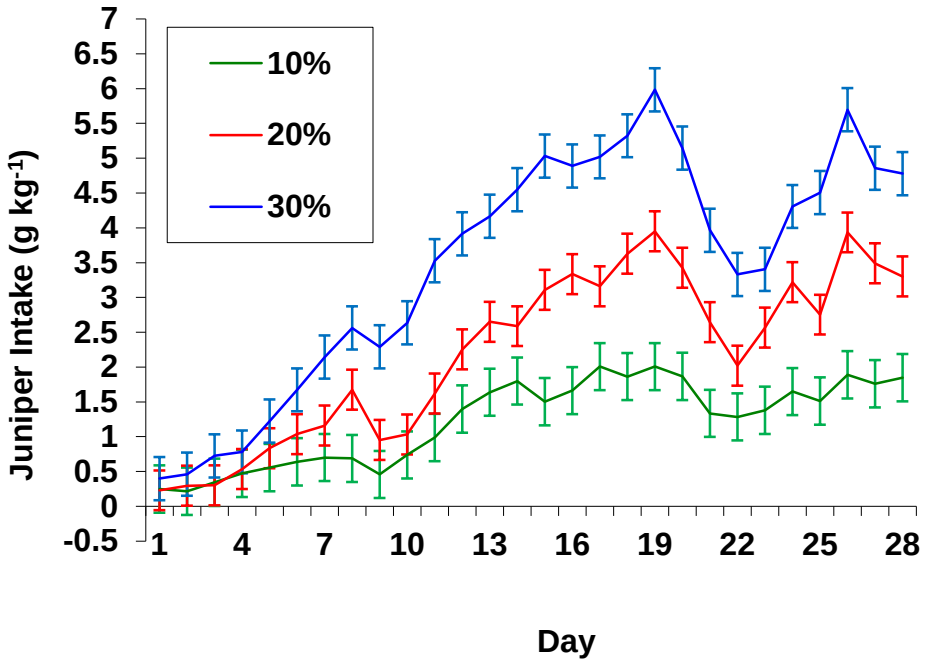


Figure 1. Daily juniper intake (means and standard errors) among treatments (5 goats/treatment) across the 28 days when goats were fed a diet consisting of 10, 20, or 30% juniper

Table 1. Carcass characteristics and shear force means ± SEM among treatments for goats fed different concentrations of juniper (0, 10, 20, or 30% of the diet).

| Characteristic | Percent Juniper in the Diet | | | | SEM |
|---|---|---|---|---|---|
| | 0 | 10 | 20 | 30 | |
| Live weight (kg) | 44.7 | 37.0 | 39.9 | 39.2 | 2.3 |
| Hot carcass weight (kg) | 23.4 | 20.0 | 21.8 | 21.0 | 1.3 |
| Dressing percentage (%) | 52.3 | 54.0 | 54.4 | 54.0 | 1.4 |
| Loineye area (cm) | 4.8 | 4.7 | 4.7 | 4.7 | 0.2 |
| Body fat thickness (cm) | 1.9 | 1.6 | 1.7 | 1.6 | 0.1 |
| Leg circumference (cm) | 57.4 | 55.2 | 56.7 | 56.3 | 1.1 |
| Shear force[1] (kg) | 2.1 | 2.2 | 2.9 | 2.4 | 0.3 |

[1] Shear force taken by Warner-Bratzler shear force machine measured in kg

Table 2. Loin chop and ground shoulder patty trained sensory means ± SEM for goats fed different concentrations of juniper (0, 10, 20, or 30% of the diet). All sensory attributes were assessed on a scale of 1(poor) to 8 (excellent).

| Sensory Attribute | Percent Juniper in the Diet | | | | |
|---|---|---|---|---|---|
| | 0 | 10 | 20 | 30 | Sem |
| **Loin Chop** | | | | | |
| Cooking loss (g) | 25.9 | 29.3 | 29.9 | 22.5 | 2.1 |
| Initial juiciness | 4.6 | 3.8 | 4.4 | 4.7 | 1.3 |
| Sustained juiciness | 4.8 | 4.2 | 4.8 | 4.9 | 1.4 |
| Initial tenderness | 5.0 | 4.3 | 4.5 | 5.0 | 0.3 |
| Sustained tenderness | 5.4 | 4.6 | 5.0 | 5.3 | 0.3 |
| Flavor intensity | 4.9 | 4.8 | 4.9 | 4.7 | 0.1 |
| Off flavor | 3.9 | 3.7 | 3.9 | 3.9 | 0.1 |
| Overall acceptability | 5.2 | 4.2 | 4.8 | 5.1 | 0.2 |
| **Ground shoulder patty** | | | | | |
| Cooking loss (g) | 36.6 | 32.9 | 29.9 | 28.4 | 1.5 |
| Initial juiciness | 4.6 | 5.2 | 5.4 | 5.5 | 0.2 |
| Sustained juiciness | 5.1 | 5.5 | 5.8 | 5.9 | 0.2 |
| Initial tenderness | 5.5 | 5.6 | 5.9 | 6.1 | 0.1 |
| Sustained tenderness | 5.9 | 5.9 | 6.2 | 6.4 | 0.2 |
| Flavor intensity | 5.2 | 5.2 | 5.5 | 5.0 | 0.1 |
| Off flavor | 3.8 | 3.8 | 3.7 | 4.0 | 0.1 |
| Overall acceptability | 5.4 | 5.9 | 5.6 | 5.8 | 0.1 |

It is unknown why juniper intake apparently affects the flavor of some wildlife species but not goats. Different species may handle toxic compounds differently. Some, like the wild turkey, may sequester monoterpenoid oils in adipose tissue thereby affecting meat flavor. In addition, turkeys and other wildlife species may rely on consumption of juniper fruit more so than the foliage. For this study, goats were fed primarily the foliage.

## IMPLICATIONS

Using juniper as forage does not appear to adversely affect carcass characteristics or goat meat quality or flavor within the time frame of this study. Other research has also shown that juniper consumption does not adversely affect production or reproduction (Owens et al. 2010). During a year-long grazing trial goats that selected relatively large amounts of juniper (30%) did not suffer from any other observable adverse effects from juniper consumption (Dietz et al. 2010). Ranchers may use goats as a juniper management tool apparently without damaging the goat flock or the products made from goat meat. Therefore, goats should be used as a tool in managing juniper on juniper-dominated rangelands and continue to be a valuable part of the food supply.

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## JUNIPER CONSUMPTION BY THREE DIFFERENT BREEDS OF SHEEP

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### ABSTRACT

In the Southwestern United States, redberry (*Juniperus pinchotii*) and ashe (*Juniperus ashei*) juniper are two invasive species that dominate some rangelands. Control options such as mechanical grubbing, prescribed fire, and herbicides are effective means of removal of juniper but can be very expensive. Biological control of juniper is relatively effective with goats. If goats are conditioned to juniper at weaning, they will consume up to 30% of their diet in juniper in pasture situations. The objective of this study was to determine if sheep can be conditioned to consume juniper and compare intake among different breeds. Three breeds of sheep, Rambouillet (n=10), Suffolk (n=10), and Dorper-cross (n=10) were randomly placed in individual pens for 32 days of testing. A basal diet of 2.5% BW alfalfa pellets and juniper were fed. Following the first 18 days of feeding juniper, the basal diet was reduced to 2% BW for 7 days and then reduced to 1.5% BW for the final 7 days. Intake of juniper and alfalfa were measured daily. Serum metabolite levels and live body weight were measured to assess any adverse physiological effects from juniper consumption. Intake of juniper was similar ($P>0.05$) among breeds of sheep. Lambs readily consumed juniper and increased ($P<0.05$) intake of juniper as the amount of alfalfa (basal diet fed) was reduced. Weight change and serum metabolite levels were also similar ($P>0.05$) among treatments. We contend that sheep will consume juniper and may be an effective biological control mechanism after conditioning an acceptance of the plant in individual pens.

### INTRODUCTION

The rapid expansion of two juniper species, redberry (*Juniperus pinchotii)* and ashe (*Juniperus ashei*) juniper are a major problem in the Southwestern United States (Ansley et al. 1995; Smeins and Fuhlendorf 1997). Both species are rapidly increasing and resulting in a loss of plant diversity, reduced herbaceous production, and decreased water yield (Dye et al. 1995; Wilcox et al. 2002, 2005). Livestock and wildlife avoid both species of juniper because both species of juniper contain monoterpenoids that cause aversive post-ingestive feedback and the formation of conditioned food aversions (Riddle et al. 1996; Pritz et al. 1997). Control options are available but are cost-prohibitive in most situations (Johnson et al. 1999). Chemical treatment is effective but is costly and only cost-effective on small junipers given the volume of spray required for tree mortality. Prescribed burning can be used to control ashe juniper, but redberry juniper resprouts after topkill (Steuter and Britton 1983; Wink and Wright 1973).

Goats will consume juniper on pasture after conditioning a preference for the plant in individual pens (Dietz et al. 2010). Goats increase intake of juniper and apparently avoid aversive feedback (i.e., toxicosis) after feeding the plant 14 days after weaning (Bisson et al. 2001; Ellis et al. 2005; Dunson et al. 2007). During a year-long grazing trial, goat browsing reduced the number of juniper seedlings (Taylor et al. unpubl. data) and caused the formation of browse lines on mature juniper trees (Dietz et al. 2010).

Some livestock producers prefer to stock sheep rather than goats or may stock both species on the same rangelands. At this time, there is little available evidence regarding juniper acceptance by sheep. In addition, the hair sheep breeds like the Dorper breed are gaining popularity across the southwestern U.S. because of their reduced susceptibility to internal parasites, because they do not require annual shearing, and because they may browse more than other breeds of sheep like the Rambouillet (Salisbury et al. unpubl. data). Unfortunately, it is not known if conditioning (i.e., feeding juniper at weaning) could be used to increase

acceptance of the plant by sheep. The purpose of this project was to determine if sheep can be conditioned to consume juniper and if there were any differences among three common breeds' acceptance of juniper. Arguably, sheep may be reluctant to consume juniper unless the basal diet is reduced. This project also assessed the effect of the basal diet restriction on juniper consumption by sheep.

## MATERIALS AND METHODS

This study was held at Angelo State University's Management, Instruction, and Research Center in San Angelo, Texas (Lat: 31.38, Lon: 100.5). To determine if breeds differ in their willingness to consume juniper, 30 freshly weaned 7 month old ewe lambs with an average weight of 31.8 kg were offered juniper daily immediately after weaning.

Treatments consisted of three breeds of lambs (i.e., Rambouillet, Suffolk, and Dorper-cross) each breed consisting of 10 ewe lambs. Prior to initiation of the study, lambs were weighed and randomly placed in individual pens (1 m X 1.5 m). All animals were provided fresh water and trace mineral blocks *ad libitum* throughout the study, and excreta were removed weekly. In addition, all lambs were offered juniper daily. Fresh redberry juniper was collected at the Texas AgriLife Research Station, Sonora, Texas (Lat: 30.58 Lon: 100.65). Leaves were hand-stripped from randomly selected trees and stored at 4$^{o}$C until feeding (Utsumi et al. 2006). Immediately prior to feeding, samples were composited and hand-mixed to insure consistency.

All sheep were given a 7-day adjustment period and fed alfalfa pellets (2.5% BW) in order to meet nutritional requirements (NRC 2007). Immediately following the adjustment period, lambs were offered 50 g of redberry juniper daily for 17 days along with alfalfa pellets (2.5% BW). Juniper was offered from 0800 to 0830 each day. Thereafter, alfalfa was fed from 0900 until 0800 the following day. The amount of juniper and alfalfa consumed was recorded daily for individual lambs. If the entire amount of juniper offered was consumed, the amount of juniper fed was increased until refusals were noted.

On day 18 through 24, each animal's basal diet of alfalfa pellets was decreased to 2% BW. On day 25 through 31 the basal diet was decreased to 1.5% BW with juniper intake monitored throughout both periods. Immediately following day 31, all animals were weighed. Previous live weights recorded were compared to final live weights to identify weight gain/loss.

Serum levels that are indicative of soft tissue damage from toxicosis (Cornelius 1989) were taken throughout the study. Blood samples were taken via jugular venipuncture on the first day the animals were exposed to juniper, midway through the study, and at the conclusion of the study to identify any indications of toxicity caused by juniper consumption. These samples were placed in a centrifuge to separate the serum, and then stored in a freezer at -80° C. Following the completion of the study, the samples were sent to the Texas Veterinary Medical Diagnostic Laboratory in College Station, Texas to be analyzed. These samples were analyzed particularly for serum aspartate transaminase (AST), gamma glutamyltransferase (GGT), blood urea nitrogen (BUN), creatinine and bilirubin.

Intake, weight change, and serum metabolite data were compared among treatments and feeding periods using repeated measures analysis of variance. Individual lambs nested within treatments and feeding periods served as replications (random effect). Day of observation served as a repeated measure. Means that differed were separated using Tukey's LSD when $P<0.05$. Data was analyzed using the statistical package JMP (SAS 2007).

## RESULTS

Intake of juniper and alfalfa was similar among breeds of sheep (i.e. treatments) throughout the study. Dorpers consumed 3.8 g kg$^{-1}$, Rambouillets consumed 2.8 g kg$^{-1}$, while Suffolks consumed 3.7 g kg$^{-1}$ (SEM = 0.5). Intake of juniper increased ($P<0.05$) across the 31 days

of feeding when data was combined across 3 treatments (treatment x day interaction was not significant P= 0.99) (Fig. 1). All three breeds of sheep increased intake of juniper in a similar manner from Period 1 to Period 3. Sheep were initially hesitant to consume juniper; however, intake increased daily throughout the trial. By the end of the trial, sheep were consuming over 6 g $kg^{-1}$ (24%) body weight on average.

When juniper and alfalfa intake (g $kg^{-1}$) were compared across the three periods of the study, juniper intake increased. Similarly, alfalfa intake declined as the amount of alfalfa offered daily declined (Table 1). During period 1, juniper made up 4.6% of diet, 15.3% in period 2, and 24.1% in period 3 (Table 1).Weight loss was similar (P>0.05) among all breeds of sheep (Table 2). All breeds lost weight from the beginning to the end of the study.

Serum metabolite levels were similar among breeds (Table 3) but differed by day of collection (Table 4). Blood urea nitrogen levels were lower at the end of the study. Bilirubin levels were higher initially and declined as the study progressed. Creatinine levels increased as the study progressed. Serum aspartate transaminase (AST) levels were similar across all 4 collection periods. Gamma glutamyltransferase (GGT) levels also decreased as the study progressed. All serum metabolite levels remained within normal range for healthy individuals. In addition, most serum metabolite levels declined the longer sheep were fed juniper. Toxicosis resulting in tissue damage typically elevates serum metabolite levels that are indicative of soft tissue damage.

## DISCUSSION

Previous studies have shown that juniper intake differs among breeds of goats. Spanish goats consume more juniper than Boer or Angora goats; while Boer goats consume more juniper than Angora goats (Pritz et al. 1997, C.A. Taylor, Jr. pers. comm.). In addition, there is evidence that goats can be genetically selected for juniper consumption (Campbell et al. 2007). Thus, it seems likely that breeds of sheep that have been selected for different production characteristics (i.e. wool production vs. meat production) could differ in their ability to consume juniper. Results from this study suggest that intake of juniper by sheep was similar among the Rambouillet, Suffolk, and Dorper-cross breeds in this study.

All breeds of sheep used in this study increased intake of juniper over days of exposure. These results are consistent with the results with goats; feeding juniper in individual pen increased intake over time (Bisson et al. 2001, Ellis et al. 2005, Dunson et al. 2007). In addition, goats continue to consume juniper when released on pasture (Dietz et al. 2010). Unfortunately, it is unknown if the results observed in pen situation with sheep will continue on pasture**.** Sheep were fed juniper at weaning for this study. Both ruminant and non-ruminant animals form food preferences early in life (Provenza 1995, 1996). In some cases, animals that are exposed to toxic plants early in life continue to consume poisonous plants later in life and may avoid toxicosis because of physiological adaptations allowing for detoxification of phytotoxins (Distel and Provenza 1991; Walker et al. 1992; Olson et al. 1996). The neurological, physiological, and morphological processes are susceptible to change in immature animals and can be manipulated to enhance their foraging skills and preferences (Provenza 1995). The sheep in these experiments were introduced to juniper after weaning, which is an important time in the development of feeding preferences because of the loss of maternal influences (Hinch et al. 1987). There is some evidence that digestive physiology can be altered through exposure to poisonous plants early in life to the point that ruminants can avoid aversive feedback and tissue damage from toxicosis. This observation was illustrated by Distel and Provenza (1991). Goats at 6 weeks of age were fed blackbrush (*Coleogyne ramosissima* Torr.) daily. Blackbrush contains condensed tannins that are toxic to ruminants. Goats introduced to blackbrush early in life consumed 95% (P<0.01) more blackbrush than naïve goats, were more efficient at digesting

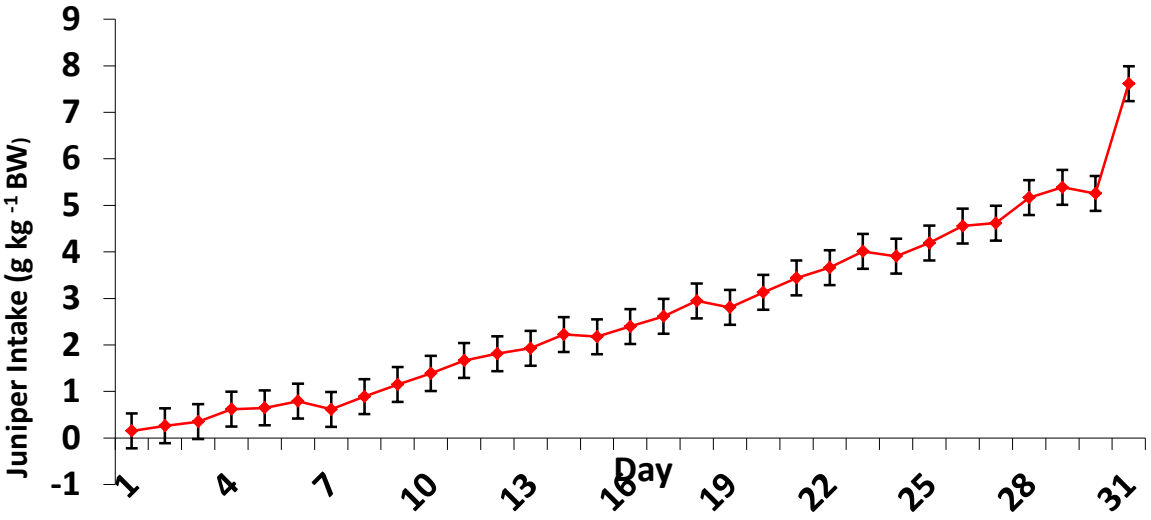


Figure 1. Intake (g kg $^{-1}$) of redberry juniper combined across the three breeds (treatment X day interaction did not differ) of sheep over 31 days of exposure. Intake differed (P<0.05) across days of feeding

Table 1. Redberry juniper and alfalfa intake (g kg $^{-1}$ and %) diet across three periods. The basal diet of alfalfa was decreased from 2.5% BW (Period 1) to 2.0% BW (Period 2) to 1.5% BW in Period 3.

| | Period | | |
|---|---|---|---|
| Forage | 1 | 2 | 3 |
| | | ------g kg$^{-1}$------- | |
| Alfalfa | 24.9 | 19.0 | 15.3 |
| Juniper | 1.3 | 3.4 | 5.6 |
| | | ------% ------ | |
| Alfalfa (% BW) | 2.5 | 2.0 | 1.5 |
| Juniper (% of diet) | 4.6 | 15.3 | 24.1 |

Table 2. Weight change among 3 breeds of sheep. All animals lost weight, however, no statistical difference was found among the 3 breeds.

| Breed | Weight (kg) | | Loss |
|---|---|---|---|
| | Initial | Final | |
| Rambouillet | 35.0 | 28.3 | 6.6 |
| Suffolk | 29.7 | 26.8 | 2.9 |
| Dorper | 30.7 | 27.0 | 3.7 |

Table 3. Serum metabolite levels compared across Rambouillet, Suffolk, and Dorper breeds when sheep were fed juniper for 31 days.

| Serum Metabolite | Breeds | | | |
|---|---|---|---|---|
| | Rambouillet | Suffolk | Dorper | Normal Range |
| BUN | 22.6 | 22.9 | 21.9 | 12-32 |
| Bilirubin | 0.1 | 0.1 | 0.1 | <0.3 |
| Creatinine | 0.8 | 0.9 | 0.8 | 0.3-1.3 |
| AST | 82.9 | 81.2 | 86.8 | 51-130 |
| GGT | 52.0 | 53.3 | 60.5 | 34-82 |

Table 4. Serum metabolite levels pooled across three breeds of sheep that were fed juniper for 31 days. Serum metabolite levels were similar among breeds.

| | Collection Periods | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | Normal Range |
| BUN | 21.7[b] | 24.9[a] | 24.4[a] | 18.8[c] | 12-32 |
| Bilirubin | 0.1[a] | 0.1[ab] | 0.1[bc] | 0.1[c] | < 0.3 |
| Creatinine | 0.7[c] | 0.8[b] | 0.9[b] | 1.0[a] | 0.3-1.3 |
| AST | 82.0 | 80.6 | 84.2 | 88.0 | 51-130 |
| GGT | 63.0[a] | 57.8[b] | 51.1[c] | 49.1[c] | 34-82 |

[abc] Indicate significant differences (P <0.05) across collection periods.

blackbrush, and excreted more uronic acid apparently because of an increased ability to detoxify the tannins in blackbrush.

Recent research with redberry juniper has also speculated that goats may be adapting to the monoterpenoids in the plant (Bisson et al. 2001; Ellis et al. 2005). Dunson et al. (2007) illustrated that goats did not adapt to juniper through changes to the rumen environment. Hepatic involvement seems more likely. Monoterpenoids are converted from lipophilic compounds to hydrophilic conjugated compounds by phase 1 and phase 2 detoxification enzymes in the liver before urinary excretion (Foley et al. 1995). Moderate doses of juniper oil at levels nearing exposure levels seen at maximal intake levels of free ranging goats (0.18 g oil $kg^{-1}$) resulted in mild hepatic injury in the form of lipid vacuolization. At higher dose levels (0.36 g oil $kg^{-1}$), cellular necrosis and lobular encapsulation were evident (Straka et al. 2004).

Sheep were reluctant to consume juniper at day 1 but after 31 days of testing, the three treatment breeds consumed over 24% body weight of juniper. When goats consume over 30% BW in juniper, loss of rumen bacteria is evident (Straka et al. 2004). Sheep would probably suffer from the same adverse effects if intake exceeded 30% of the diet. Monoterpenoid levels in juniper vary monthly (Owens et al. 1998) and probably vary from year to year depending on ambient conditions. The redberry juniper fed in this study may have contained higher monoterpenoid levels than juniper fed in previous years.

Throughout this study, there was no indication of sheep that showed signs toxicosis. Serum metabolite levels collected differed across four periods, however all metabolite levels remained within normal range for sheep and did not increase or decrease in a linear manner. All animals did lose weight. However, it remains unclear why all animals lost weight. Given the decrease in the basal diet over the course of study, sheep may have lost weight because they were unable to meet their nutritional requirements.

Juniper intake data from Period 1 illustrates that sheep will consume juniper in a pen situation even though their basal diet (2.5% BW) meets or exceeds nutritional requirements.

When the basal diet was reduced to 2% BW, intake increased. Intake again increased during period 3 when the basal diet was reduced to 1.5% BW. Thus, it is probable that (1) sheep will consume juniper even when alternate forages are available and (2) sheep will increase consumption as forage quantity declines.

In summary**,** the results of this study suggest that sheep will consume juniper and that there is no difference in the three breeds most common to West Central Texas. Future research should determine if sheep will continue to consume juniper when released into pasture after weaning. In addition, recent research with goats has suggested that supplements formulated with protein sources that partially escape digestion in the rumen may further improve juniper consumption (George et al. 2010). Future efforts should also investigate the possibility of using protein supplementation to improve juniper consumption by sheep.

## IMPLICATIONS

Based on the results of this study, sheep can be conditioned to consume redberry juniper in a pen situation by feeding the plant at weaning. Thus, producers can utilize sheep as a biological control of juniper regardless of the breed of sheep being raised. Producers typically select replacement ewes at weaning and place those individuals in a separate pen or pasture until breeding. During weaning, ruminants typically broaden their dietary habits through exposure to new plant species. Producers should hand-feed juniper to replacements for 14-30 days during weaning to increase the likelihood of consumption on pasture.

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## WILL SUPER JUNIPER-EATING SIRES PRODUCE SUPER JUNIPER-EATING OFFSPRING?

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**ABSTRACT**

When preconditioned in pens, goats develop a preference for juniper on pasture. The objective of this study was to see if sires selectively bred for high juniper consumption produce offspring that consume more juniper than offspring from sires chosen for production characteristics. We took five sires chosen for high juniper consumption and five sires chosen for other production characteristics and bred each to 7 does (n= 70). Kids were weaned at 90 days of age and placed in individual pens for feeding trials. Consumption of juniper was measured and compared among sire groups. Body condition scores and weights were taken and compared among sire groups after goats were on feed for 30 days following each feeding trial. There were no differences in juniper consumption, body condition scores, and weights among treatments. Goats increased juniper consumption daily in individual pens. There was no influence on juniper consumption by sires.

**INTRODUCTION**

Redberry (*Juniperus pinchotii*) and ashe (*Juniperus asheii*) juniper are invasive woody species found throughout west central Texas (Ansley et al. 1995; Owens et al. 2010; Smeins et al. 1997). Both redberry and ashe juniper are evergreens that were historically found on rocky outcrops and north-facing slopes where both were protected from fires (Ellis and Schuster 1968). During the past 100 years, juniper has encroached onto grasslands reducing the amount of available forage for grazing animals (Dye et al. 1995; Ueckert et al. 2001). Juniper dominates rangelands throughout western and central Texas because it is avoided while other forbs, shrubs, and grasses are consumed (Archer 1994).

Monoterpenoids, a form of terpenes contained in *Juniperus* sp., cause aversive postingestive feedback and the formation of conditioned food aversions (Riddle et al. 1996; Pritz et al. 1997). Goats consume juniper, but intake is limited because monoterpenoids kill rumen bacteria when intake exceeds 30% of the diet (Straka et al. 2003). It appears that goats are able to acclimate to the monoterpenoids in the juniper if exposed to the plant slowly over several days (Bisson et al. 2001). Two studies have confirmed this observation by feeding juniper to goats in individual pens for 10-14 days (Ellis et al. 2005; Dunson et al. 2007). In addition, goats will continue to consume juniper on pasture at levels up to 30% when preconditioned for 14 days at weaning (Dietz et al. 2010). When preconditioned goats were placed on pastures for 1 year, browse lines became apparent on mature junipers and several immature junipers were defoliated (Dietz et al. 2010).

Genetic selection has led to the development of different breeds for production characteristics (milk, meat, and fiber), behavior, color, size, and resistance to disease, pests, or environmental extremes (Lasley 1987). Selection and breeding of animals with specific diet characteristics could be used to develop livestock for vegetation management such as weed control or improved forage utilization (Snowder et al. 2001). Undoubtedly, genes play an important role in physiological mechanisms affecting food preference and detoxification of chemicals (Walker 1995). Recent research conducted by the Texas AgriLife Research Station has selected both sires and dams for their ability to consume juniper. Over the past several generations, these efforts have increased juniper intake in the lineage of goats selected for the willingness to consume juniper (Campbell et al. 2007, Waldron et al. 2009). The objective of this study was to determine if sires selected for their willingness to consume juniper will produce offspring that consume more juniper than others selected for production characteristics.

## MATERIALS AND METHODS

This study was conducted at the Angelo State University Management, Instruction, and Research (MIR) Center. During October, five sires genetically chosen for high juniper consumption, while 5 unrelated sires selected for other production characteristics were acquired. Each of the 10 sires was placed in an individual research pen (3 X 9 m) with a breeding group of 7 randomly selected does selected for production characteristics. Breeding occurred in December of 2009. Prior to breeding, does received two injections of lutelyse to synchronize estrus. Each individual breeding group of one sire and seven does was fed a ration of Ram 20 (Table 1) to meet their maintenance requirements. Does were maintained on a wheat pasture throughout gestation.

When kidding occurred in the spring (March-April), kids were ear tagged according to sire. Kids and does were housed on haygrazer fields prior to weaning. All kids were weaned at 90 days. After weaning, kids from each sire group were assigned to 3 separate feeding trials and placed in individual pens. The facilities at the MIR Center will house 46 kids at one time in individual pens. For Trial 1, the 46 largest kids were randomly assigned to individual pens. For the second trial, 46 of the remaining kids were randomly assigned to individual pens. For the last trial, the remaining 11 kids were randomly assigned to individual pens.

Once a feeding trial began, the kids were allowed a seven day adjustment period to adjust to the basal diet and environment change. Each kid was fed a basal diet of alfalfa pellets at 2.5% body weight for maintenance requirements as well as the treatment diet (NRC 2007). The basal diet and fresh redberry juniper was fed for 14 days. Initially, 50 g of juniper was fed daily for 30 minutes. Once an individual goat consumed all of the juniper offered on 2 consecutive days, the amount offered was increased by 25 grams until refusals were noted. Juniper and alfalfa consumption was measured by weighing the amount offered then weighing back and subtracting the refusals. Intake of both alfalfa and juniper were recorded daily.

The kids waiting to enter the feeding trial were housed separately and fed Ram 20, a feedlot ration used on the MIR Center. A week before they entered the feeding trial they were fed alfalfa pellets *ad libitum* to acclimate them to the basal diet used in each trial. Intake of juniper by kids from the two different sets of sires was compared to determine if kids from the sires genetically chosen for juniper consumption consumed more juniper than the kids from the sires chosen for production characteristics.

Alfalfa pellet and juniper intake (g kg $BW^{-1}$) from the pen feeding trial of the study was analyzed using repeated measures analysis of variance. Individual goats were the experimental unit. Day of observation served as the repeated measure. Means were separated using Tukey's LSD test when $P \leq 0.05$. Offspring intake of juniper was also compared among individual sires using the same statistical model. Data was analyzed using the statistical package JMP (SAS 2007).

## RESULTS

Fifty kids were reared out of the 35 nannies bred to sires selected for juniper consumption (A&M sires). Fifty three kids were reared out of the 35 nannies that were bred to sires selected for other production characteristics (other sires).

Juniper intake differed ($P<0.05$) among trials (Table 2). Kids in the second trial consumed more juniper than kids in the first or third trials. Alfalfa intake was similar ($P>0.05$) across the three trials (Table 2).

Table 1. Ingredients and nutrient contents of RAM 20 ration.

| Ingredients/Nutrients | As fed (%) |
|---|---|
| Alfalfa Pellets | 10 |
| Cotton Seed Meal | 12.5 |
| Soybean hulls | 31.5 |
| Cane Molasses | 3.5 |
| Premix | 2.5 |
| Sorghum Grain (milo) | 40 |
| DE | 2.597 Mcal/kg |
| TDN | 58.963 |
| Crude Protein | 14.512 |
| Crude Fiber | 14.181 |

Table 2. Average intake (g kg $BW^{-1}$) of redberry juniper and alfalfa pellets across the the trial of this study.

| **Feed** | **Trial** | | |
|---|---|---|---|
| | **1** | **2** | **3** |
| Juniper | $2.5^{b}$±.09 | $4.1^{a}$ ±.09 | $2.8^{b}$±.17 |
| Alfalfa | 23.4±.46 | 24.5 ± .46 | 24.6±.73 |

$^{a-b}$Means within rows with different superscripts differ (P<0.05).

Juniper intake was similar between treatments (Table 3). Likewise, there were no differences among sire groups (Table 4). The hypothesis sire selection for juniper consumption would increase intake of juniper by their offspring was rejected; kids from the genetically chosen sires (i.e. A&M sires) did not consume more juniper than kids from sires chosen for production characteristics. Alfalfa intake was also similar among treatments but differed across sire groups (Table 3). Kids out of sires 4799 and K2 consumed more alfalfa than kids out of sire K1. There were no differences among the other sires.

Goats were reluctant to consume the juniper on the first day of feeding but soon began consuming juniper by days two and three. Juniper consumption by goats in both treatments steadily increased throughout the trials from .35 ± .33 $g \cdot kg^{-1}$ BW on the first day to 6.33 ± .33 $g \cdot kg^{-1}$ BW on the last day by kids from the genetically chosen sires and .32 ± .33 $g \cdot kg^{-1}$ BW on the first day to 5.69 ± .33 $g \cdot kg^{-1}$ BW on the last day by the kids from the sires chosen for production characteristics. The day effect in the model differed for juniper consumption but the treatment X interaction was similar (Fig. 1). The hypothesis that exposure to juniper at weaning would increase consumption of juniper regardless of genetic background was confirmed. Alfalfa consumption varied across days of feeding (P<0.05) while the treatment X day interaction was similar. Intake stayed consistent from day eight to day seventeen with the introduction and consumption of juniper going from 22.69 ± .66 $g \cdot kg^{-1}$ BW on day eight to 24.71 ± .66 $g \cdot kg^{-1}$ BW on day seventeen by kids from the genetically chosen sires and from 23.90 ± .78 $g \cdot kg^{-1}$ BW on day eight to 24.50 ± .65 $g \cdot kg^{-1}$ BW on day seventeen by kids from the sires chosen for production characteristics.

Body condition scores were similar among treatments (Table 5). Final weights taken 30 days after completion of each feeding trial were also similar among treatments (Table 5).

Table 3. Average intake (g kg $BW^{-1}$) of redberry juniper and alfalfa pellets for kids out of sires selected for juniper consumption (A&M) or sires selected for other production characteristics.

| Feed | Treatment | |
|---|---|---|
| | A&M | Other |
| Juniper | 3.5 ± .26 | 3.0 ± .25 |
| Alfalfa | 24.0 ± .48 | 23.8 ± .48 |

Table 4. Average intake (g kg $BW^{-1}$) of redberry juniper and alfalfa pellets for kids out of each sire selected for juniper consumption (A&M) or sires selected for other production characteristics.

| Sires | Feed | |
|---|---|---|
| | Juniper | Alfalfa |
| **A&M** | | |
| 4332 | 3.3 ± .73 | $24.3^{ab}$ ± .57 |
| 4433 | 3.5 ± .49 | $23.7^{ab}$ ± .62 |
| 4571 | 3.0 ± .57 | $23.4^{ab}$ ± .72 |
| 4602 | 4.2 ±.52 | $24.1^{ab}$ ± .65 |
| 4799 | 3.6 ± .47 | $24.7^{a}$ ± .59 |
| **Other** | | |
| 305 | 2.6 ± .45 | $24.5^{ab}$ ± .57 |
| 716 | 2.7 ± .49 | $24.3^{ab}$ ± .62 |
| K1 | 3.0 ± .58 | $20.7^{b}$ ± .73 |
| K2 | 3.7 ± .55 | $25.6^{a}$ ± .69 |
| VICK'S | 3.2 ± .51 | $23.9^{ab}$ ± .65 |

[a-b]Means within columns with different superscripts differ (P<0.05).

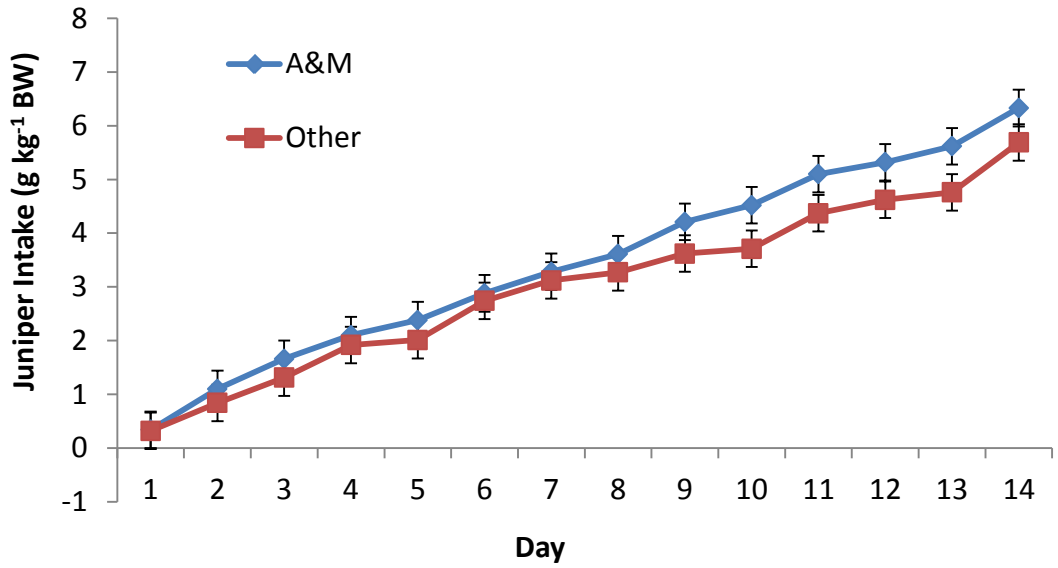


Fig. 1. Average daily juniper intake (g kg $BW^{-1}$) for kids out of sires either selected for juniper consumption or for sires selected for other production characteristics.

Table 5. Body condition scores (1-5) and final weights for goats taken 30 days after the completion of each trial. Two observers independently assigned a score to each goat, where 1=perfect structure and conformation and 5=poor structure and conformation.

| Production Characteristic | Treatment | |
|---|---|---|
| | A&M | Other |
| Body Condition | 2.9±2.9 | 2.7±2.7 |
| Final Weight (kg) | 22.7±0.4 | 22.4±0.4 |

## DISCUSSION

Results from this study support the observation that goats will increase intake of redberry juniper at weaning when offered the plant in individual pens for 14 days (Bisson et al. 2001; Ellis et al. 2005; Dunson et al. 2007). Once released on pasture, conditioned goats continued to consume juniper throughout the year, with juniper accounting for 30% of their diet (Dietz et al. 2010). If sire selection had influenced juniper consumption, kids from the A&M sires should have either (1) began consuming juniper faster, or (2) consumed more juniper than kids from sires selected for other production characteristics. Both groups increased consumption of juniper over days of feeding at the same rate and consumed similar amounts of juniper. When sires are bred to a group of randomly selected doe, sire selection appears to have little impact on juniper consumption of offspring.

The sires in the A&M treatment in this study came from a flock that had been selectively bred for six years; both sires and dams had been selected for their willingness to consume juniper. The other five sires obtained made up the other group of sires chosen for production characteristics. The does used in this study were either (1) purchased from a commercial breeder with no knowledge of their willingness to consume juniper, or (2) originated from a flock of goats that consumed an average amount of juniper; dams were classified as neither consuming high nor low amounts of juniper. The results of this study showed no influence on juniper consumption of offspring from sires selected for their willingness to consume juniper. Ellis et al. (2005) reported that heritability of redberry juniper was low for half-siblings. Waldron et al. (2009) reported a heritability index of 0.13 for juniper consumption after selection of both dams and sires for juniper consumption. Indeed the authors argued that improvements in juniper consumption by genetic selection would be slow. Conversely, Snowder et al. (2001) reported that mountain big sage brush consumption heritability was moderately high ($H^2$ = .28) while Warren et al. (1983) reported the heritability of nonpreferred species of vegetation was $H^2$ = .30. Both mountain sagebrush and redberry juniper are considered nonpreferred shrubs because both contain similar toxins (monoterpenoids). It is unclear why genetic selection improves consumption of some nonpreferred species with similar toxins. Apparently, the metabolism of the toxic compounds in juniper is not influenced by genetic selection in the same manner.

## IMPLICATIONS

Based on the results of this study goats will increase juniper consumption when fed juniper in individual pens at weaning. The selection of sires for willingness to consume juniper had no influence on juniper consumption of their offspring. With this, producers can precondition goats at weaning for juniper consumption and use them as a source of biological control of juniper. Instead of selecting sires for willingness to consume juniper, producers can make selections for production characteristics that best suit the goals they have for their operation. In the results of the study of done by (Dietz et al. 2010) female goats will select juniper at levels up to 40% of total bite taken on pasture when preconditioned for 14 days at

weaning. Producers normally select replacement does at weaning and separate them from the rest of the herd at that time. During this period, they can then precondition the replacement does to consume juniper by feeding juniper stripped from limbs or simply cutting limbs from trees and placing them in pens with the replacement does. Producers will have conditioned their goat herds for consumption of juniper and also, allowed a good opportunity for social facilitation of juniper consumption to take place. Ranchers with the problem of juniper encroachment can improve management of this problem with biological control through goats accompanied with chemical and mechanical means of control.

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## SELECTING ANGORA GOATS TO CONSUME MORE JUNIPER

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### ABSTRACT

This research project was initiated in 2003 to develop a more effective tool for biological management of invading juniper species on rangelands through herbivory by Angora goats. After we had established that juniper consumption in free-ranging goats has a genetic component (heritability = 13%), male and female goats were bred selectively for above- (high) and below-average (low) juniper consumption that was estimated by fecal near-infrared reflectance spectroscopy. Divergent lines are being produced to facilitate the identification of physiological mechanisms that permit some goats to consume considerably more juniper than others as a regular component of their diet. Because diet is known to affect growth and fiber production, another objective of the project is to establish the effects of the selection protocol on body weights, fleece weights, and fiber characteristics. Mature females (age > 1.5 yr) and kids were maintained on rangeland and shorn twice a year. Extreme high- and low-consuming yearling males (10 of each per year) were evaluated annually in a central performance test. The selection protocol resulted in average EBV for percentage juniper consumption of 3.9 and -0.4 ($P < 0.0001$) respectively for the 2006-born high- and low-consuming yearlings. A physiological difference in bioavailability of monoterpenes between high and low consumers was recently detected. Fiber data for 2006-born 12-mo-old kid goats indicated no significant differences ($P > 0.1$) in body weight, mohair production and properties between high and low consumers. However, the adult data for the extreme males indicated that high consuming males have lower body weights than low consumers (53.8 vs. 57.9 kg, $P = 0.01$). Differences in body weight and several mohair production and quality traits have also been detected in the mature females but at this early stage of the selection program, no substantial differences have been observed and certainly none that would have an economic impact for producers. Ultimately, we expect to demonstrate that the high-consuming line controls juniper more effectively than either the low-consuming line or unselected Angora goats. Subsequently, we plan to release high juniper-consuming genetics to commercial breeders for use in range management.

### INTRODUCTION

Juniper encroachment of southwestern U.S. rangelands is having a negative impact on livestock production, water availability and quality, and wildlife habitats. The cost of conventional control methods (mechanical and chemical) is prohibitively high. Goats are known to consume juniper despite its high terpenoid content. Research conducted at the Texas AgriLife Research Station (formerly the Texas Agricultural Experiment Station) at Sonora has shown that animals vary in their consumption of juniper, indicating they may also vary in their ability to detoxify the terpenoids (Campbell et al., 2007). Research was initiated in 2001 to investigate the use of selective breeding to increase the preference of Boer x Spanish goats for juniper. Near-infrared reflectance spectroscopy of fecal material (fecal NIRS) was used to predict the percentage of juniper in diets of penned and free-ranging goats (Whitworth, 2002). Taylor et al. (2003) reported that heritability of juniper consumption in this meat goat population was 31%, indicating substantial scope for improvement by genetic selection. In May 2003, a flock that included 272 mature Angora females was added to the project to determine the juniper consuming potential of this breed. The 4 major objectives of the project to date have been: 1) improve the accuracy and precision of fecal NIRS equations for predicting juniper consumption in free-ranging goats, 2) use selective breeding to produce divergent lines of high and low

juniper-consuming goats, 3) identify physiological and nutritional mechanisms of secondary chemical metabolism in high juniper-consuming goats and evaluate their capacity to consume other noxious brush species, and 4) monitor mohair production and quality in the high and low juniper-consuming selection lines. By selecting goats that exhibit increased preference for juniper, we are selecting animals with an unspecified higher physiological capacity to avoid pathological effects of terpenoids that could be correlated with production traits. Metabolic fate of the terpenoids involves hepatic biotransformation and subsequent excretion in urine (Sheline, 1991). This short communication summarizes progress in the 4 objectives but will focus primarily on the effects of selection on mohair production.

## MATERIALS AND METHODS

Mature Angora females (n = 272) were purchased from 5 breeders in the summer of 2003 and maintained on rangeland at the Read Ranch, Crockett County, Texas (300° 32' 53" N, 101° 3' 27" W). In October 2003 and 2004, the nannies were mated to performance-tested males (one exception) purchased from 7 different breeders with the primary objective of increasing genetic diversity in the kids (Table 1). Parentage of the first two kid crops was established by DNA analysis of blood. In subsequent years, conventional methods (single-sire matings, pairing kids and nannies) were used to establish parentage of offspring. Percentage of juniper consumed by free-ranging nannies and kids was estimated using fecal NIRS (Walker et al., 2007) at 4 separate times throughout the first production year. In subsequent years, these estimates were made from fecal samples collected in mid-winter. Estimated juniper consumption was used as a phenotypic trait in the calculation of Expected Breeding Values (EBV; Henderson,1984) and an animal's EBV was used to assign males and females to high and low juniper-consuming mating groups. The rationale was that by selecting for extreme consumers, we are more likely to discover (in future experiments) physiological reasons for the differences in juniper consumption. Since 2005, the 10 highest and 10 lowest juniper-consuming male yearlings were also evaluated in a central performance test (Waldron and Lupton, 2005). The males used to breed were selected primarily on their EBV for juniper consumption, but their overall performance in this test was also taken into account (one exception). In the fall of 2005, the high and low mature female population was reduced to 75 goats per selection line and the intermediate consumers were sold. Subsequently, as selected yearling nannies were included into the breeding flock, older and unsound goats were removed in order to maintain a total of about 150 breeding females. Because selection for increased (or decreased) juniper consumption could affect body weight, mohair production, and fiber properties, animals and fleeces were weighed after each biannual shearing (in February and August) and individual fleeces were analyzed (August 2005 to August 2007) for clean yield (ASTM, 2004a), fiber diameter (ASTM, 2001), staple length (ASTM, 2004b), opacity and medullation (IWTO, 1998 and ASTM, 2005), and fiber curvature (van Rensburg, 2000).

The GLM procedure of SAS (SAS Institute Inc., Cary, NC) was used to identify differences between high and low juniper-consuming goats for each of the measured or calculated traits. Separate analyses were performed for mature females (age = 18 months and older), performance-tested males, and current sires. Because sire was unknown for two thirds of the mature females, sire was not used in the model. Therefore, significance levels are not exact. The MIXED procedure of SAS was used to analyze data from the 2006-born kids at 12 months of age. The model used selection line and sex as fixed effects and sire and residual as random effects.

## RESULTS AND DISCUSSION

*Objective 1.* NIRS calibration equations are normally developed and checked for accuracy in conjunction with reference samples and methods using standard laboratory procedures. In the case of fecal NIRS with goats, reference samples are not available and the accuracy of

Table 1. Number of male and female Angora goats bred by year

| Year | Number of females exposed | Number of sires | Method of exposure |
|---|---|---|---|
| 2003 | 272 | 7 | Single sire |
| 2004 | 388 | 6 | Group mated (high, low) |
| 2005 | 150 | 7 | Single sire |
| 2006 | 161 | 8 | Single sire |
| 2007 | 147 | 8 | Single sire |

microhistological analysis of goat feces is not accurate or precise (Whitworth, 2002). We have concluded that fecal NIRS predictions of juniper consumption represent an interval scale of measurement (i.e., treatments can be ranked and the differences have meaning and are equal across the range of measurements). However, a true zero point is not known. Consequently, we have made no claims concerning the absolute quantities of juniper consumed by high and low groups. But we are confident that reported differences are correct. We have also shown that most non-treatment factors such as sex can bias predictions and comparisons (Walker et al., 2007).

To determine the appropriate season and sampling intervals to accurately estimate the true percentage of juniper in the goats' diet, data from a 2-year study on juniper intake were analyzed to test for the presence of long-term, short-term, and episodic periodicities. A model containing temperature, precipitation, and two autoregressive terms accounted for 71% of the variability of juniper in the diet. Inclusion of wind direction and barometric pressure into the model produced a small improvement in $R^2$ (to 0.75). A trend for high-consuming Angoras to consume greater percentages of juniper in their diet was observed for every month of this study.

*Objective 2.* Divergent selection within the Angora flock has been conducted for only 4 years. Nevertheless, divergence (as measured by EBV for % juniper consumption) has been observed between the high and low 2006-born kids (Table 3, 3.9 and -0.4, respectively) and a larger difference is present between the extreme high and low males being used to breed in 2007 (Table 5, 6.2 and -2.4, respectively).

*Objective 3.* A pharmacokinetic study identified conclusive evidence of a physiological difference in bioavailability of monoterpenes between extreme high and low juniper consuming goats. This result represented the first independent verification of the breeding program through identification of differences in physiological mechanism by which goats process juniper terpenoids (Taylor et al., 2007). In another study, protein supplementation of high consuming goats with soybean meal increased juniper in the diet by 5% (Campbell et al., 2007).

*Objective 4.* Tables 2-5 summarize EBV for juniper consumption, body weight, mohair production, and mohair quality traits for mature females (Table 2), 2006-born kids (12-month clip, Table 3), extreme male yearlings (Table 4), and extreme males used for breeding in 2007 (Table 5). Selection of mature females has resulted in the high consuming goats having smaller body weights (28.0 vs. 30.9 kg, $P = 0.005$), higher mohair production per unit of body weight (62.2 vs. 54.8 g/kg, $P = 0.009$), finer fiber (33.3 vs. 34.9 µm, $P = 0.003$), less flat fibers (19.7 vs. 28.0 per 10,000 fibers), and higher fiber curvature (15.5 vs. 14.6 deg/mm, $P = 0.036$). These differences were not present in the 2006-born kids (Table 3). Except for EBV means, no differences were detected between the high- and low-consuming kids measured at 12 months of age. The lack of differences in the kids suggests that the significant differences in the mature females should be interpreted with caution.

Perhaps the best indicators of future trends are the data for extreme males and particularly that of the 6 goats that were used to breed in 2007. Data collected during the

Table 2. Least squares means of expected breeding values, body weights, fleece weights, and fiber properties for high and low juniper-consuming mature (18 mo and older) Angora females at Fall 2007 shearing

| Item | High (83) | Low (95) | SE | *P* |
|---|---|---|---|---|
| Expected breeding value (% juniper consumption) | 2.8[a] | -0.9[b] | 0.2 | < 0.0001 |
| Body weight (kg) | 28.0[b] | 30.9[a] | 0.7 | 0.0050 |
| Grease fleece weight (kg, adjusted to 182.5 d) | 2.0 | 1.9 | 0.05 | 0.3435 |
| Clean yield (%) | 83.1 | 83.1 | 0.5 | 0.9743 |
| Clean fleece weight (kg, adjusted to 182.5 d) | 1.6 | 1.6 | 0.04 | 0.3350 |
| Clean mohair per unit BW (g/kg) | 62.2[a] | 54.8[b] | 2.0 | 0.0089 |
| Lock length (cm, adjusted to 182.5 d) | 12.5 | 12.5 | 0.2 | 0.9528 |
| Average fiber diameter (µm) | 33.3[b] | 34.9[a] | 0.4 | 0.0025 |
| Opacity (%) | 51.8 | 51.5 | 0.2 | 0.3627 |
| Total medullation (per 10,000 fibers) | 69.9 | 80.3 | 3.9 | 0.0513 |
| Objectionable fibers (per 10,000 fibers) | 13.6 | 15.4 | 1.2 | 0.2781 |
| Flat fibers (per 10,000 fibers) | 19.7[b] | 28.0[a] | 2.3 | 0.0086 |
| Average fiber curvature (deg/mm) | 15.5[a] | 14.6[b] | 0.3 | 0.0356 |

[a,b] Means in the same row with different superscripts differ ($P < 0.05$).

Table 3. Least squares means of expected breeding values, body weights, fleece weights, and fiber properties for high and low juniper-consuming 2006-born female and male Angora kids (approximately 12 months of age at time of shearing)

| Item | High (33) | Low (43) | SED | *P* |
|---|---|---|---|---|
| Expected breeding value (% juniper consumption) | 3.9[a] | -0.4[b] | 0.5 | < 0.0001 |
| Body weight (kg) | 21.8 | 22.7 | 0.9 | 0.3764 |
| Grease fleece weight (kg, adjusted to 182.5 d) | 2.2 | 2.1 | 0.2 | 0.7054 |
| Clean yield (%) | 82.0 | 80.2 | 1.0 | 0.1279 |
| Clean fleece weight (kg, adjusted to 182.5 d) | 1.8 | 1.7 | 0.1 | 0.5110 |
| Clean mohair per unit BW (g/kg) | 86.2 | 77.6 | 7.8 | 0.3085 |
| Lock length (cm, adjusted to 182.5 d) | 14.2 | 14.1 | 0.6 | 0.9070 |
| Average fiber diameter (µm) | 29.8 | 29.0 | 0.6 | 0.2389 |
| Opacity (%) | 51.2 | 51.2 | 0.4 | 0.8926 |
| Total medullation (per 10,000 fibers) | 91.2 | 101.4 | 8.6 | 0.2729 |
| Objectionable fibers (per 10,000 fibers) | 12.8 | 18.8 | 3.4 | 0.1261 |
| Flat fibers (per 10,000 fibers) | 42.9 | 41.0 | 4.4 | 0.6827 |
| Average fiber curvature (deg/mm) | 17.7 | 18.4 | 0.6 | 0.2757 |

[a,b] Means in the same row with different superscripts differ ($P < 0.05$).

Table 4. Least squares means for extreme high and low juniper-consuming male yearlings completing an annual central performance test concluding in 2005 through 2007

| Item | High (33) | Low (32) | *P* |
|---|---|---|---|
| Expected breeding value (% juniper consumption) | 3.4[a] | -1.7[b] | < 0.0001 |
| Initial weight (kg) | 25.5[b] | 27.9[a] | 0.0028 |
| Final weight (kg) | 53.8[b] | 57.9[a] | 0.0107 |
| Body average daily gain (kg/d) | 0.2 | 0.2 | 0.5904 |
| Grease fleece weight (kg, adjusted to 182.5 d) | 4.9[b] | 5.4[a] | 0.0232 |
| Clean yield (%) | 74.1[a] | 71.1[b] | 0.0048 |
| Clean fleece weight (kg, adjusted to 182.5 d) | 3.7 | 3.8 | 0.2626 |
| Average lock length (cm, adjusted to 182.5 d) | 17.3 | 17.3 | 0.9221 |
| Average fiber diameter (µm) | 36.5 | 37.5 | 0.1366 |
| Med content (%) | 0.7 | 0.9 | 0.0528 |
| Kemp content (%) | 0.1 | 0.2 | 0.0775 |
| Scrotal circumference (cm) | 27.8[b] | 28.9[a] | 0.0473 |

[a,b] Means in the same row with different superscripts differ ($P < 0.05$).

central performance tests (Table 4) indicate the extreme high males are smaller, higher yielding, and grow similar amounts of clean mohair compared to the low consumers. The same trends are present for the 2007 stud billies (Table 5) although conclusions should not be drawn from such small samples (n=3 per line). At this point during the selection experiment (still early days), the only real cause for concern is the lower body weights of the high-consuming goats. Clean mohair production was not reduced and no major differences were present in the mohair quality traits. To date, much of the genetic progress to increase juniper consumption can be traced to one sire present in the 2003 source population. Therefore, much of the line effect may be due to this sire. Because creation of increasingly divergent lines was our primary goal, little attention to date has been paid to the consequences of inbreeding. Nevertheless, it is a concern and we will be maintaining our search for alternative genetic sources of high juniper-consuming Angora males.

## CONCLUSIONS

Fecal sampling procedures and fecal NIRS equations have been developed that have permitted us to clearly distinguish high and low juniper-consuming Angora goats and accurately estimate the relative amounts of juniper in their diets. A recently concluded study provided evidence of a physiological difference in bioavailability of monoterpenes between high and low juniper-consuming goats. Once the mechanism of action is understood, we will attempt to identify a genetic marker associated with the trait that will aid in identification of high juniper-consuming goats in the flocks of other breeders. At this early stage of the selection program, no substantial differences in mohair production or quality have been observed and certainly none that would have an economic impact for producers. Ultimately, we expect to demonstrate that the high-consuming line controls juniper more effectively than either the low line or unselected Angora goats. Subsequently, we plan to release high juniper-consuming genetics to commercial breeders for use in range management. These goats will benefit landowners not only through brush control but also through their conversion of unutilized and undesirable brush species to meat and fiber.

Table 5. Mean values for extreme high and low juniper-consuming males used as primary stud goats in the 2007 breeding season measured during a central performance test

| Item | High (3) | Low (3) | *P* |
|---|---|---|---|
| Expected breeding value (% juniper consumption) | 6.2[a] | -2.4[b] | < 0.0001 |
| Initial weight (kg) | 21.0[b] | 30.1[a] | 0.0280 |
| Final weight (kg) | 48.7 | 54.9 | 0.1267 |
| Body average daily gain (kg/d) | 0.2 | 0.2 | 0.5965 |
| Grease fleece weight (kg, adjusted to 182.5 d) | 4.8 | 5.1 | 0.4305 |
| Clean yield (%) | 73.5[a] | 66.1[b] | 0.0422 |
| Clean fleece weight (kg, adjusted to 182.5 d) | 3.5 | 3.4 | 0.4568 |
| Average lock length (cm, adjusted to 182.5 d) | 16.1 | 16.3 | 0.6670 |
| Average fiber diameter (µm) | 34.1 | 37.2 | 0.2569 |
| Med content (%) | 0.6 | 1.2 | 0.0781 |
| Kemp content (%) | 0.2 | 0.2 | 0.5777 |
| Scrotal circumference (cm) | 29.0 | 29.4 | 0.7995 |

[a,b] Means in the same row with different superscripts differ (*P* < 0.05*).*

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## USING REDBERRY JUNIPER FORAGE MATERIAL TO REDUCE *HAEMONCHUS CONTORTUS IN VITRO* VIABILITY AND INCREASE IVERMECTIN EFFICACY

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### ABSTRACT

A modified larvae migration inhibition assay was used to determine if redberry juniper (*Juniperus pinchotii* Sudw.) can reduce *Haemonchus contortus in vitro* viability and increase ivermectin efficacy. Rumen fluid (RF) was mixed with buffer solution and either no material (CNTL) or dry juniper (DRY), fresh juniper (FRE), or distilled juniper terpenoid oil (OIL) and anaerobically incubated for 16 h. During Trial 1, larvae were incubated in CNTL, DRY, FRE, or OIL for 16 h (Trial 1). Trials 2 (CNTL or OIL; OIL) and 3 (CNTL, DRY or FRE) evaluated larvae after incubation in treatment solution for 2 hr, then incubated an additional 2 hr in multiple IVM doses (0, 0.1, 1, 3, and 6 µg/mL IVM) and placed onto a screen. Larvae that passed through the 20-µm screen within a 96-well plate were considered viable. Adding DRY, FRE, or OIL to a RF-buffer solution, reduced ($P < 0.001$; Trial 1) larvae viability as compared to CNTL. A treatment × ivermectin dose interaction ($P = 0.02$) was observed during Trial 2, due to OIL unexpectedly decreasing IMV efficacy at IVM concentrations of 1 ($P = 0.07$), 3, and 6 ($P < 0.002$) µg/mL. No treatment × ivermectin dose interaction ($P = 0.57$) was observed during Trial 3, but larvae incubated in DRY had less ($P < 0.004$) total viability than larvae incubated in CNTL or FRE. Juniper forage material reduced *H. contortus* viability, but IVM efficacy was increased only by initially incubating larvae in DRY.

### INTRODUCTION

Gastrointestinal control programs based on synthetic dewormers are failing because of widespread dewormer resistance (Craig and Miller, 1990; Prichard, 2001); thus, alternative strategies are necessary. Plants and their extracts, i.e. condensed tannins (CT), can directly or indirectly decrease larvae viability (Min and Hart, 2003; Molan et al., 2004) and total worm burden (Niezen et al., 1995) and essential oils can be bacteriocidic (Chao et al., 2000) and toxic to nematodes (Lei et al., 2010). Redberry juniper (*Juniperus pinchotii* Sudw.) contains CT and terpenoids and is consumed by sheep and goats (Riddle et al., 1996; Whitney and Muir, 2010) but its effects on *Haemonchus contortus* is unknown.

Over 20 million acres of Texas rangelands are infested with juniper (Ansley et al., 1995) and managing its encroachment by mechanical and chemical methods is not always cost effective (Lee et al., 2001). Increasing the "value" of juniper as a biological anthelmintic could potentially increase mechanical harvesting of this invasive plant, thereby increasing top-growth biomass for livestock consumption, and reducing its encroachment. Objectives were to use a modified larvae migration inhibition (LMI) assay to determine if redberry juniper can reduce *Haemonchus contortus in vitro* viability and increase ivermectin efficacy.

### MATERIALS AND METHODS

*Juniper forage, juniper terpenoid oil, and other test materials*

Fresh juniper material (FRE; stems and leaves) was harvested, immediately placed on dry ice, and chopped into approximately 3-cm pieces. A subsample of FRE was immediately lyophilized, stored at -80 ºC, and used in the LMI assays within 2 days; other subsamples were collected (June 9 and 22, 2009) and immediately transferred on dry ice to another laboratory for to determine total oil concentrations. To collect juniper terpenoid oil (OIL), fresh juniper branches with leaves were chopped and distilled in water; OIL was stored in glass vials at -80 ºC. For dry juniper (DRY), randomly selected branches from multiple trees were collected (April 25, 2009), chopped, dried at 55 ºC in a forced-air oven for 96 h to reduce volatile terpenoid concentrations, and stored at -80 ºC.

The CT extract used in this study originated from the Quebracho Colorado tree (*Schinopsis lorentzii* Griseb. Engl.; UNITAN S.A.I.C.A, Argentina), which contains approximately 73% CT and 19% simple phenolics and is 100% water soluble at 40-45 ºC (Athanasiadou et al., 2001). Ivermectin (IVM; 0.08% solution; Ivomec, Merial Limited, Iselm, NJ) and dimethyl sulfoxide (DMSO; anhydrous, 99.9%; Sigma Aldrich, St. Louis, MO) were also used in the current study.

*In vitro rumen system*

Rumen fluid (RF) was collected from 3 fistulated goats fed alfalfa hay and pellets (2% of BW) at 0800. Each trial was replicated using fresh RF that was never collected more than once every 2 days. To dislodge some of the particle-associated bacteria, approximately 150 g of rumen material was also added to each pre-warmed thermos, which was vigorously shaken for 1 min. The RF was pooled, strained through 4 layers of cheesecloth, and flushed with $CO_2$ to ensure an anaerobic environment. Strained RF was mixed with McDougal's buffer solution at a ratio of 1:4, but only using 25% of suggested urea because nitrogen concentration can affect *H. contortus in vitro* motility (Howell et al., 1999) and nutrition and parasite interactions can occur (Coop and Holmes, 1996). Containers were subsequently flushed with $CO_2$ whenever opened. Initial pH of the RF-buffer mixture remained between 6.8 and 6.9 as it was transferred to incubation jars and placed into a Daisy$^{II}$ Incubator (Ankom Technology, Fairport, NY) for 16 h at 37 ºC. Specific details of the Daisy$^{II}$ incubator have been previously reported (Robinson et al., 1999).

Test material was thoroughly mixed with the RF-buffer mixture and amount of test material placed into the jar represented a 55-kg sheep consuming 2.5% of BW (DM basis) and the following were considered: 1) juniper consumed at 40% of diet DM; 2) DRY and FRE contained 95% and 60% DM, respectively; 3) juniper material contained 4% terpenoid oil (DM basis); and 4) 1 mL OIL = 0.825 g of oil. A conversion factor of 0.125 (1:8 ratio) was used to adjust the quantity of test material placed into the jar, considering that the incubation jar was 1 L and assuming an 8-L rumen (Purser and Moir, 1966). After incubating, RF-buffer solution was centrifuged at 3,000 rpm for 5 min to remove debris from the buoyant top layer. Aliquots (5 mL) of the middle supernate layer were transferred to 2 specimen cups; 1 cup was used to incubate ensheathed *H. contortus* larvae and 1 was used for treatment solution only. Approximately 1125 larvae (0.75 mL) were then added to the cups resulting in a final volume of 5.75 mL (approximately 195 larva/mL). Cups were flushed with $CO_2$, sealed, and incubated at 37 ºC with the treatment fluid for 16 h (Trial 1) or 2 hr (Trials 2 and 3).

*Laboratory analysis of juniper material and oil and rumen fluid*

Nitrogen was analyzed by a standard combustion method (990.03; AOAC, 2006) and CP calculated as 6.25 × N. Neutral detergent fiber and ADF were analyzed with Van Soest et al. (1991) procedures modified for an Ankom 2000 Fiber Analyzer (Ankom Technol. Corp., Fairport, NY) without correcting for residual ash, using α-amylase, and omitting Na sulfite. Ash was analyzed by a standard method (942.05; AOAC, 2006). Part of each sample was dried in a forced-air oven at 103 ºC for 3 h to determine DM concentration. Condensed tannins in freeze-dried, fresh redberry juniper leaves were assayed for soluble, protein-bound, and fiber-bound fractions by methods described by Terrill et al. (1992). Samples were oven-dried and standards were prepared from redberry juniper as recommended by Wolfe et al. (2008). The DRY and FRE were analyzed for percentage of terpenoid oil concentration using procedures described by Tellez et al. (1997).

*Preparation of L3 larvae for LMI assay*

Fecal samples were collected from a goat and lamb. After culturing fecal samples at room temperature, a Baerman apparatus (Dinaburg, 1942) was used to collect the infective L3 *Haemonchus contortus* larvae, which were IVM-resistant and ensheathed. Larvae were stored (< 2.5 mo) in tissue culture flasks containing 3 mm of room-temperature water and 5 µg/mL of fungicide

(Fungizone, 250 µg/mL stock solution; Omega Scientific Inc., Torzana, CA USA). Larvae were not artificially exsheathed because this study attempted to represent a true *in vitro* rumen environment.

*Larval migration inhibition bioassay*

The larvae migration inhibition assay used in the current study was similar to procedures reported by Kotze et al. (2006) who modified procedures developed by Wagland et al. (1992) by using a 96-well MultiScreen filter plate (Millipore Corp., Bedford, MA USA). The plate consists of a plastic underdrain with 96 tear-drop shaped wells and a removable 20-µm screen plate, which has been reported to ensure active L3 migration while preventing L3 from falling through the screen (Rabel et al., 1994). Wells of the plastic underdrain were filled with 150 µL of test solution immediately prior to the screen being placed into the solution, making sure no air bubbles were present.

After larvae incubated in treatment solution, 20-µL droplets of larvae-solution were pipetted from the cup onto a microscope slide. All larvae (dead and alive) were counted using 100 magnifications until 15 to 40 larvae were present. Larvae were then transferred onto the individual screens in the MultiScreen filter plate. Larvae that did not get transferred from each droplet on the microscope slide were subtracted from the total number of larvae placed onto the screen. After at least 15 larvae were placed onto the screen, the total well volume was brought up to 250 µL with test solution as needed. MultiScreen plates were gassed with $CO_2$ and incubated for 16 hrs at 37 ºC. After the plates incubated, the entire solution of each well of the tear-drop plate was transferred by pipette onto a microscope slide and all larvae were counted using 100 × power.

*Experimental design*

Trial 1 was used to determine if DRY, OIL, or FRE treatments reduced LV. The CNTL (no material), DRY (7.24% w/v), OIL (0.334% v/v; mixed with 4% DMSO), or FRE (11.46% w/v) were incubated in 800 mL of buffer and 200 mL of RF for 16 hr. The LMI was evaluated after larvae were incubated in this solution for 16 h. A positive control (quebracho tannin; 2.5% w/v) was similarly evaluated and LV remained less than 5%; QT concentration was determined according to Athanasiadou et al. (2001). Twenty seven replicates per treatment were evaluated using 3 runs (3 cups/run and 3 wells/cup).

Trial 2 used a 2 × 5 factorial design (treatment RF and IVM concentration) to determine if larvae initially incubated in OIL would be more susceptible to IVM. The CNTL or OIL (0.334% v/v; mixed with 1% DMSO) treatments were incubated in 400 mL of buffer and 100 mL of RF for 16 hr. Larvae were then incubated in this solution for an additional 2 hr. Multiple IVM concentrations (0, 0.1, 1, 3, and 6 µg/mL; mixed with 1% DMSO), were then added to these larvae, which incubated an additional 2 hr before LV was evaluated. An IVM concentration of 45 µg/mL mixed with 1% DMSO (positive control) was similarly evaluated and LV remained less than 4%). Six replicates per treatment were evaluated using 2 runs (1 cup/run and 3 wells/cup). Trial 3 was identical to Trial 2, but used a 3 × 5 factorial design (CNTL, DRY, and FRE treatments and 0, 0.1, 1, 3, and 6 µg/mL IVM) and added DMSO only to IVM. The positive control (45 µg/mL ivermectin in 1% DMSO) resulted in < 0.6% LV. Nine replicates per treatment were evaluated using 3 runs (1 cup/run and 3 wells/cup).

*Statistical analyses*

Data were analyzed using the MIXED procedure (SAS Inst. Inc., Cary, NC). Trial 1 used a model that included treatment with run as the repeated measure and well within cup as the subject. Treatment effects were tested using the following single degree of freedom non-orthogonal contrasts: 1) control vs. average of dry juniper, fresh juniper, and oil; 2) control vs. average of dry and fresh juniper; and 3) dry juniper vs. fresh juniper. Trials 2 and 3 were analyzed using a model that included treatment, IVM dose, and treatment × IVM dose interaction. The slice option of SAS was used when treatment × IVM dose interaction was observed, and linear slopes were compared

between treatments according to Littell et al. (2006). Data are reported as least squares means with standard errors.

## RESULTS AND DISCUSSION

The DM and CP concentrations in fresh and dry juniper were 66.1% and 93.8% and 7.18% and 6.6%, respectively. The NDF and ash concentrations of fresh and dry juniper were 43.8% and 39.3%, and 3.5% and 3.2%, respectively. The ADF concentrations of fresh and dry juniper were similar to NDF concentrations: 42.2% and 38.3%, respectively. Dry juniper had less total distilled oil than fresh juniper that was harvested on 6/9/2009 or 6/22/2009 (25.3, 36.8, and 46.7 mg oil/g DM, respectively). Total CT concentration in fresh juniper leaf and stem material was 7.3% and fractions consisted of 6.1%, 1.1%, and 0.1% extractable, protein-bound, and fiber-bound, respectively. Barry and Manley (1986) suggested that extractable CT are more reactive than bound CT; thus, the extractable CT concentration in juniper may increase its anthelmintic properties.

Adding DRY, FRE, or OIL to RF-buffer solution reduced ($P < 0.001$; Trial 1; Figure 1) average LV as compared to larvae incubated in CNTL. Average viability of larvae incubated in DRY or FRE was less ($P < 0.001$) than larvae incubated in CNTL. The reduction in LV can be attributed mostly to the juniper CT concentration. Min and Hart (2003) found that CT extracted from various forages decreased LV of several nematodes including *H. contortus*. Larvae incubated in OIL and RF-buffer solution had less viability than larvae incubated in CNTL, but results could be due to DMSO concentration vs. a true treatment effect. In our lab (unpublished data), LV was approximately 80% less when 4% DMSO was used vs. 1% DMSO. In addition, larvae incubated in DRY or FRE had similar ($P = 0.44$) viability, which further supports the fact that CT affected viability and not terpenoids. If terpenoids negatively affected larvae, then larvae incubated in FRE would be expected to have less viability than larvae incubated in DRY due to synergistic effects of CT and terpenoids. Another, less likely, scenario is that terpenoids in FRE did not mix as well as OIL because DMSO was only used in OIL treatment.

During Trial 2, a treatment × ivermectin dose interaction ($P = 0.02$; CNTL and OIL; Figure 2) was observed due to OIL unexpectedly decreasing IMV efficacy at IVM concentrations of 1 ($P = 0.07$), 3, and 6 ($P < 0.002$) µg/mL. In addition, as IVM dose increased, viability of larvae incubated in CNTL or OIL linearly decreased at different rates ($P < 0.001$). Lei et al. (2010) reported that specific monoterpenoids were toxic to *Caenorhabditis elegans* and *Ascaris suum* nematodes. Results of the current study were probably not related to OIL being hydrophobic because DMSO was used in the OIL treatment and each IVM dose.

No treatment × ivermectin dose interaction ($P = 0.57$; CNTL, DRY, and FRE; Figure 3) was observed during Trial 3, and LV linearly decreased at similar ($P = 0.54$) rates among treatments. However, larvae incubated in DRY RF-buffer solution had less ($P < 0.004$) average viability than larvae incubated in CNTL or FRE RF-buffer solution. Results suggest that incubating larvae in DRY increased *in vitro* IVM efficacy.

## CONCLUSIONS

Dry and fresh juniper material reduced LV, but only dry juniper increased IVM efficacy. The reduction in IVM efficacy due to larvae initially being incubated in OIL was exactly opposite of what was expected and is currently unexplainable. Further *in vivo* research is warranted to determine if feeding dry juniper in mixed feeds to sheep and goats can reduce *in vivo H. contortus* viability, and fecal egg shedding, and increase IVM efficacy. Numerous benefits such as re-instating non-effective synthetic anthelmintics by increasing their efficacy would be realized if feeding sheep and goats dry juniper can weaken larvae in the host prior to drenching.

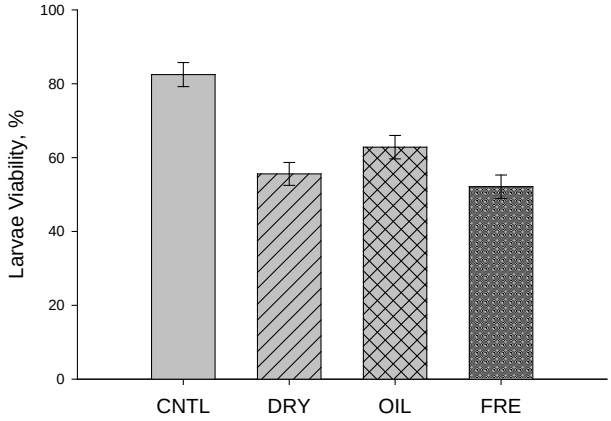


**Figure 1.** Percent larval viability of *H. contortus* incubated with no forage material (CNTL), dry juniper (DRY; 7.24% w/v), monoterpenoids (OIL; 0.334% v/v), or fresh juniper (FRE; 11.46% w/v). Average LV of DRY, OIL, and FRESH and average LV of DRY and FRE were less ($P < 0.001$) than CNTL; LV of DRY and FRE were similar ($P = 0.44$).

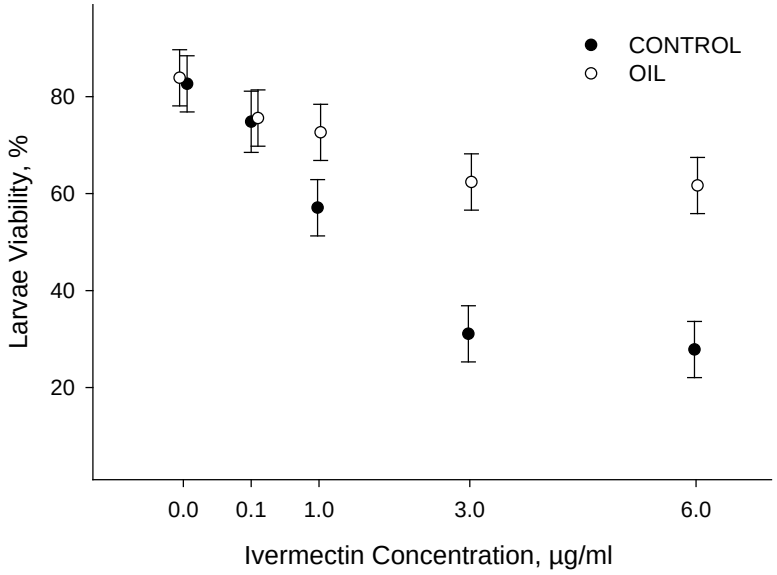


**Figure 2.** Percent larval viability of *H. contortus* incubated with no forage material (control) or monoterpenoid oil (OIL) and then dosed with ivermectin. A treatment × ivermectin dose interaction ($P = 0.02$) was observed due to OIL unexpectedly decreasing IMV efficacy at IVM concentrations of 1 ($P = 0.07$), 3, and 6 ($P < 0.002$) µg/mL.

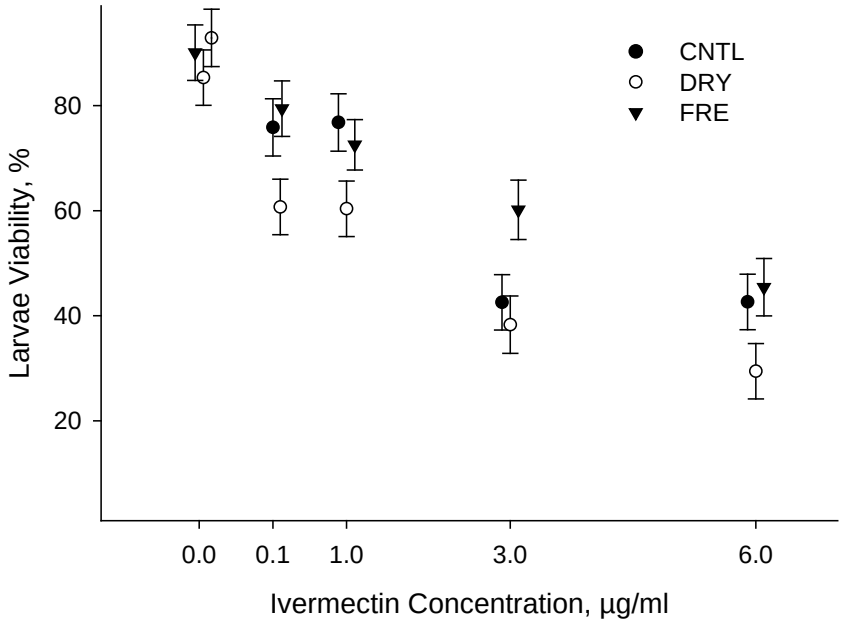


**Figure 3.** Percent larval viability of *H. contortus* incubated with no forage material (control), dry juniper (DRY), or fresh juniper (FRESH) and then dosed with ivermectin. No treatment × ivermectin dose interaction ($P = 0.57$) was observed, but larvae incubate in DRY had less ($P < 0.004$) average viability than larvae incubated in CNTL or FRE.

**ACKNOWLEDGEMENTS**

This study was funded in part by the Food and Fibers Research Grant Program administered by the Texas Department of Agriculture. Appreciation is expressed to Rick Estell (USDA-ARS Jornada Experimental Range, Las Cruces, NM) for analyzing samples for percentage of terpenoids. Some of the L3 *Haemonchus contortus* larvae were kindly donated by Dr. Anne Zajac, Virginia-Maryland Regional College of Veterinary Medicine at Virginia Tech, Blacksburg.

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## A MODIFIED *IN VITRO* LARVAE MIGRATION INHIBITION ASSAY USING RUMEN FLUID TO EVALUATE *HAEMONCHUS CONTORTUS* VIABILITY

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**ABSTRACT**

Anthelmintic effects of plant secondary compounds may be occurring in the rumen, but *in vitro* larvae migration inhibition (LMI) methods using rumen fluid and forage material have not been widely used. Forage material added to an *in vitro* system can affect rumen pH, ammonia N, and volatile fatty acids, which may affect larvae viability (LV). Validating a LMI assay using rumen fluid and a known anthelmintic drug (Ivermectin) and a known anthelmintic plant extract (Quebracho tannins; QT) is important. Rumen fluid was collected and pooled from 3 goats, mixed with buffer solution and a treatment (1 jar/treatment), and placed into an anaerobic incubator for 16 h. Ensheathed larvae (< 3 months old) were then anaerobically incubated with treatment rumen fluid for 2, 4, or 16 h depending on the trial. Larvae (n = 15 to 45) were then transferred onto a screen (n = 4 to 6 wells/treatment) within a multi-screen 96-well plate that contained treatment rumen fluid. Larvae were incubated overnight and those that passed through the 20-µm screen were considered viable. Adding dry or fresh juniper material reduced ($P < 0.05$) pH, ammonia N, and isobutyric, butyric, isovaleric, and valeric acids, and increased ($P < 0.001$) acetic, propionic, and total VFA. Including 4.5% w/v polyethylene glycol (PEG) in rumen fluid mixture with or without forage material reduced ($P < 0.01$) LV. However, LV was similar at all PEG concentrations tested (0 to 2% w/v; 89.4, 78.9, 76.5, 75.5, and 77.5% viable). Quebracho tannin concentrations from 0 to 1.2% w/v quadratically reduced ($P < 0.001$) LV; 89.4, 65.5, 22.8, and 9.2%. Ivermectin concentrations from 0, to 15 µg/mL quadratically reduced ($P < 0.001$) LV; 90.2, 82.6, 73.6, 66.3, 51.9, 56.5, 43.5, 41.9, 29.3, and 19.9% viable, respectively. Effects of altering *in vitro* rumen fluid pH, ammonia N, and VFA and using PEG when evaluating LV need to be further investigated. *In vitro* rumen fluid assays using QT and Ivermectin resulted in decreased LV, validating the efficacy of this technique for measuring *H. contortus* larval viability.

**INTRODUCTION**

Increased resistance of gastrointestinal parasites, especially *Haemonchus contortus*, to synthetic anthelmintics (Kaplan, 2004; Wolstenholme et al., 2004) has increased the need to evaluate non-synthetic compounds that can replace or enhance the efficacy of current products. Numerous plant species and extracts have been evaluated and research efforts have mainly focused on condensed tannins (CT; Hoste et al., 2006; Alonso-Diaz et al., 2008). Due to the quantity of the different types of secondary compounds that exist in plants that could potentially be used as anthelmintics, rapid and cost-effective *in vitro* screening is necessary (Niezen et al., 1995; Molan et al., 2000).

Problems associated with subjective assessment of larvae motility led to an *in vitro* larvae migration inhibition (LMI) assay based on L3 larvae migration through a 20-µm screen (Wagland et al., 1992). Rabel et al. (1994) modified this assay and Kotze et al. (2006) enhanced its effectiveness by using a 96-well screen plate and agar. Even though agar was successfully used by Kotze et al. (2006), they reported that agar concentration affected migration. Rabel et al. (1994) suggested that the use of an agar layer on top of the screen can result in problems related to agar concentration and temperature.

The rumen is the first location in the animal that can be manipulated to reduce larvae viability (LV), exsheathment, and establishment. The rumen environment and the chemical nature

of the consumed material can be altered as forages and their biological compounds are digested (Rogosic et al., 2003; Patra and Saxena, 2009), which can potentially affect ingested larvae (Howell et al., 1999; DeRosa et al., 2005; Brunet et al., 2007). Therefore, creating an *in vitro* rumen environment where quantity of forage material representing true *in vivo* conditions can be included, and solution from the digested material can be used to rapidly evaluate larvae motility is important. Reducing time and variability associated with counting larvae is also important. Wagland et al. (1992) reported a coefficient of variation (CV) greater than 10% if there were less than 150 larvae per tube. Rabel et al. (1994) reported a CV between 12 to 14% when counting 140 to 200 larvae and a CV greater than 20% when numbers of larvae were outside of this range. The current study slightly modifies the LMI assay described by Kotze et al. (2006) to reduce evaluation time and variability while determining concentrations of common LMI assay additives needed to effectively reduce LV in an *in vitro* rumen environment.

## MATERIALS AND METHODS

*Juniper forage, juniper monoterpenoid oil, and other test materials*

Branches with leaves from randomly selected mature redberry juniper (*Juniperus pinchotii* Sudw.) trees (n = 8) were cut at a nearby ranch in 1 general location to reduce variability in leaf quality as reported by Riddle et al. (1996). New growth from previously cut trees was never re-cut. For fresh juniper material (FRE), branches were immediately placed on dry ice, transported to the laboratory, and chopped into approximately 3-cm pieces using hand shearers. A subsample of FRE was immediately lyophilized, stored at -80 ºC, and used in the LMI bioassays within 2 days; other subsamples were collected (June 9 and 22) immediately transferred on dry ice to another laboratory to be analyzed for individual and total monoterpenoid concentrations. To collect juniper monoterpenoid oil (OIL), multiple fresh juniper branches with leaves were randomly collected, chopped, mixed with water, and distilled; juniper material was discarded and the OIL was decanted and stored in glass vials at -80 ºC. For dry juniper (DRY), randomly selected branches from multiple trees were collected (April 25, 2009), chopped, dried at 55 ºC in a forced-air oven for 96 h to reduce volatile monoterpenoid concentrations, and stored at -80 ºC.

The Quebracho tannin (QT) used in this study originated from the Quebracho Colorado tree (*Schinopsis lorentzii* Griseb. Engl.; UNITAN S.A.I.C.A Monasterio, Argentina) and contains approximately 73% CT and 19% simple phenolics and is 100% water soluble at 40-45 ºC (Athanasiadou et al., 2001). Polyethylene glycol 6000 (PEG; Alfa Aesar, Ward Hill, MA USA), ivermectin (IVM; 0.08% solution; Ivomec, Merial Limited, Iselm, NJ), and DMSO (anhydrous, 99.9%; Sigma Aldrich, St. Louis, MO) were also evaluated.

*In vitro rumen system*

Rumen fluid (RF) was collected at 1100 through 1 layer of cheesecloth into warmed thermoses from 3 fistulated goats fed alfalfa hay and pellets (2.0% of BW) at 0800. Each trial was replicated multiple times, using fresh RF that was never collected more than once every 2 days. Chemical (Merry and McAllan, 1983; Legay-Carmier and Bauchart, 1989) and metabolic (Latham, 1980; Williams and Strachan, 1984) differences exist between particle- and liquid-associated bacteria; thus, to dislodge some of the particle-associated bacteria, approximately 150 g of rumen material was also added to each thermos, which was vigorously shaken for 1 min using a wrist shaker (Labline, Melrose Park, IL USA). The RF was pooled, strained through 4 layers of cheesecloth into per-warmed beakers, and flushed with $CO_2$ for 45 sec to ensure an anaerobic environment. Strained RF was mixed with McDougal's buffer solution at a ratio of 1:4 (McDougal, 1948), but only using 25% of suggested urea because nitrogen concentration can affect *H. contortus in vitro* motility (Howell et al., 1999) and nutrition and parasite interactions can occur (Coop and Holmes, 1996). Containers were subsequently flushed with $CO_2$ whenever opened. Initial pH of the RF-buffer

mixture (total volume = 500 or 1,000 mL depending on trial) remained between 6.8 and 6.9 as it was transferred to incubation jars and placed into a Daisy$^{II}$ Incubator (Ankom Technology, Fairport, NY) for 22 h at 37 ºC. Specific details of the Daisy$^{II}$ incubator have been previously reported (Robinson et al., 1999).

When test material was included in the incubation jar, it was thoroughly mixed with the RF-buffer mixture. Amount of test material that was placed into the jar represented a 55-kg sheep consuming 2.5% of BW (DM basis) and the following were considered: 1) juniper at 40% of diet DM; 2) DRY and FRE contained 95% and 60% DM, respectively; 3) juniper material contained 4% monoterpenoid oil (DM basis); 4) 1 mL OIL = 0.825 g of oil; and 5) PEG used at a 2:1 ratio to effectively bind and reverse the effects of CT (Yu et al., 1995; Molan et al., 2003). A conversion factor of 0.125 (1:8 ratio) was used to adjust the quantity of test material placed into the jar, considering that the incubation jar was 1 L and assuming an 8-L rumen (Purser and Moir, 1966).

After incubating, RF-buffer solution was centrifuged (Sorvall RC-5C, DuPont Company, Wilmington, Delaware) in multiple 25-mL tubes at 3,000 rpm for 5 min at room temperature to remove debris. After the top layer, consisting of buoyant material, was discarded, two 5-mL aliquots of the middle supernate layer were transferred to two 40-mL specimen cups; 1 cup used to incubate *H. contortus* larvae and 1 used for treatment solution only. Approximately 1,125 larvae (0.75 mL) were then added to the cups, resulting in a final volume of 5.75 mL (approximately 195 larvae/mL). Cups were flushed with $CO_2$, sealed, and incubated at 37 ºC for various periods of time depending on the trial. Initial pH was recorded and two, 1-mL aliquots/treatment were acidified with 4 mL of 0.1 *N* HCl (Farmer et al., 2004) and stored at -80 ºC for ammonia N and VFA analysis.

*Laboratory analysis of juniper material and oil and rumen fluid*

Nitrogen was analyzed by a standard combustion method (990.03; AOAC, 2006) and CP calculated as 6.25 × N. Neutral detergent fiber and ADF were analyzed with Van Soest et al. (1991) procedures modified for an Ankom 2000 Fiber Analyzer (Ankom Technol. Corp., Fairport, NY) without correcting for residual ash, using α-amylase, and omitting Na sulfite. Ash was analyzed by a standard method (942.05; AOAC, 2006). In addition, part of each sample was dried in a forced-air oven at 103 ºC until weight was constant to determine DM concentration. Condensed tannins in freeze-dried, fresh redberry juniper leaves were assayed for soluble, protein-bound, and fiber-bound fractions by methods described by Terrill et al. (1992). Samples were oven-dried and standards were prepared from redberry juniper as recommended by Wolfe et al. (2008). The OIL, DRY, and FRE were analyzed for individual monoterpenoids using procedures described by Tellez et al. (1997).

*Preparation of L3 larvae for LMI assay*

Two strains of *H. contortus* L3, IVM-resistant larvae were copricultured from fecal material collected from pygmy-Boer cross goats and lambs. Larvae from the goats were confirmed to be resistant according to the DrenchRite assay (Microbial Screening Technologies, Armidale, New South Wales, Australia); larvae from the lambs were confirmed to be resistant by fecal development assays and fecal egg reduction tests. Larvae viability was similar between both strains incubated in a control RF-buffer solution and in various IVM concentrations (evaluated across studies; data not shown). A Baerman apparatus (Dinaburg, 1942) was used to collect infectious L3 larvae, which were considered to be from ivermectin-resistant strains and used within 2.5 months. *Haemonchus contortus* was the only larvae species identified. Approximately 1,000 to 1,500 larvae/mL water were stored horizontally in tissue culture flasks with canted necks and a vented caps (Nunclan cell culture flask, Thermo Scientific, Rochester, NY) containing 3 mm of room-temperature water and 5 µg/mL of fungicide (Fungizone, 250 µg/mL stock solution; Omega Scientific Inc., Torzana, CA USA). During this study, percentages of viable and ensheathed larvae stored in the flasks remained greater

than 92% and 99%, respectively. Larvae were not artificially exsheathed because exsheathment was a dependant variable and this study attempted to represent a true *in vitro* rumen environment.

*Larval migration inhibition bioassay*

This study modified the LMI assay procedures developed by Wagland et al. (1992) and refined by Rabel et al. (1994). Kotze et al. (2006) enhanced the assay by using a 96-well MultiScreen filter plate (Millipore Corp., Bedford, MA USA) and described a schematic diagram of the filter plate system. The plate consisted of a plastic underdrain with 96 tear-drop shaped wells and a removable 20-µm screen plate, which has been reported to ensure active L3 migration while preventing L3 from falling through the screen (Rabel et al., 1994). Rabel et al. (1994) used 275 µL total maximum volume for each well when the screen was inserted, but 250 µL was used in the current study. Wells of the plastic underdrain were filled with 150 µL immediately prior to the screen being placed into the solution, making sure no air bubbles were present. The plate was placed on a 37 ºC surface as larvae were added to each corresponding well.

Each cup contained approximately 195 larvae/mL; if larvae were evenly dispersed through agitation, then each 20-µL droplet contained approximately 4 larvae, which is not sufficient for this assay. Thus, cups were not agitated and placing cups at an angle to concentrate the larvae in 1 general location greatly increased counting efficiency. After cups had incubated, 50-µL aliquots of solution (n = 2) containing larvae were removed and placed onto a microscope slide and the first 15 to 20 randomly selected larvae were evaluated for initial percentages of viable exsheathed larvae using 100 × power. Larvae were considered dead if they were straight and non-motile, or slightly curled but non-motile after gently tapping the slide 5 times as described by Howell et al. (1999).

Next, numerous 20-µL droplets were pipetted from the cup containing larvae and placed onto a dissecting microscope (40× power) slide to estimate larvae numbers. If a droplet contained a sufficient quantity of larvae (n = 15 to 45), it was transferred to another slide. Precisely counting larvae was impossible if the droplet contained > 50 larvae; thus, a droplet was split if it contained > 45 larvae. All larvae (dead and alive; sheathed and exsheathed) were counted in these droplets until 15 to 40 larvae were present; total number droplet-volume never exceeded 100 µL. Droplets containing the counted larvae were transferred onto the screen in the MultiScreen well plate. Larvae that did not get transferred from each droplet on the microscope slide were subtracted from the total number of larvae placed onto the screen; this ensured that the exact quantity of larvae was recorded.

At times, over half of the larvae in a droplet did not get transferred; thus, an additional 10 or 20 µL of test solution was immediately used to rinse the droplet and was included on top of the corresponding screen; the process of counting the remaining larvae on the microscope slide was repeated. After at least 15 larvae were placed onto the screen, the total well volume was brought up to 250 µL with test solution as needed. MultiScreen plates were gassed with $CO_2$ for 30 sec, wrapped in parafilm, placed into clear plastic bag, gassed with $CO_2$ for 15 sec, sealed, and incubated under a heat lamp for 16 h at 37 ºC.

After incubation, each screen plate was gently tapped as it was removed from the tear drop plate. The entire solution (250 µL) of each well of the tear-drop plate was transferred by pipette onto a microscope slide and all larvae (dead and viable) were counted through a microscope (100 × power). To ensure that no larvae remained in the teardrop well, each well was rinsed and remaining larvae evaluated; no residual larvae were recovered for the first 6 plates, thus rinsing was discontinued. To wash the MultiScreen plates, each teardrop well plate and the screen plate was flushed 10 times with warm water and once with reverse osmosis water. Larvae were effectively evaluated without using Lugols iodine.

*Experimental design*

*Trials 1a and 1b*

Trial 1a determined if rumen volatile fatty acids (VFA), pH, and ammonia N were affected by not adding any material (CNTL), or by adding QT, OIL, DRY, or FRE (2.5% w/v, 0.33% v/v, 7.24% w/v, 11.46% w/v; as-fed basis, respectively) to a RF-buffer solution, which incubated for 16 h. Rumen fluid-buffer solution from each treatment was evaluated because the manufacturer suggested that 12.5 g of forage material is the maximum quantity that should be used in the Daisy incubation jar (placed into multiple fiber digestion bags); thus, the use of approximately 6 times this quantity in the current study had potential to alter *in vitro* rumen parameters and affect larvae. Four replicate samples were analyzed for each treatment using 2 runs. Trial 1b was identical to Trial 1a, except that CNTL, DRY, or FRE was added to RF-buffer with or without 4.5% w/v PEG.

*Trials 2a and 2b*

Trials 2a and 2b determined if PEG added to RF-buffer affected LMI. For Trial 2a, LMI was evaluated after larvae were incubated for 16 h in the treatment solutions described for Trial 1b. A total of 27 replicate samples were analyzed for each treatment using 3 runs (3 cups/run and 3 wells/cup). Trial 2b was similar to Trial 2a, except that multiple PEG concentrations (0, 0.4, 0.8, 1.4, and 2% w/v) were incubated for 16 h in treatment solutions; larvae were then added to this solution and incubated an additional 4 h and LMI evaluated. Six replicate samples were evaluated per treatment using 2 runs (1 cup/run and 3 wells/cup).

*Trials 3 and 4*

Trials 3 and 4 evaluated effects of multiple QT (Trial 3) or IVM (Trial 4) concentrations on LMI. For Trial 3, QT concentrations (0, 0.15, 0.6, and 1.2% w/v) were incubated for 16 h with RF-buffer solution; LMI was then evaluated after larvae were incubated in this solution for 4 h. For Trial 4, CNTL was incubated for 16 h in RF-buffer and larvae were then incubated in this solution for 2 h with multiple IVM concentrations (0, 0.05, 0.1, 0.5, 2.0, 3.0, 6.0 and 15.0% w/v). Trials 3 and 4 each used six replicate samples per treatment using 2 runs (1 cup/run and 3 wells/cup).

*Calculation of data and statistical analyses*

Data were analyzed using the MIXED procedure (SAS Inst. Inc., Cary, NC). Trial 1a used a model that included treatment, time (just prior to or after larvae incubation), and treatment × time interaction with run as the random error and cup as the experimental unit. Treatment effects were tested using the following single degree of freedom orthogonal contrasts: 1) control vs. dry and fresh juniper; 2) QT vs. dry and fresh juniper; 3) oil vs. dry and fresh juniper; and 4) dry vs. fresh juniper. Trial 1b used the same model as Trial 1a, but treatment effects were tested using the following single degree of freedom non-orthogonal contrasts: 1) control vs. control with PEG; 2) dry vs. dry with PEG; 3) fresh vs. fresh with PEG; and 4) average of treatments with PEG vs. average of treatments without PEG. For Trials 1a and 1b, even if treatment × time interaction was not significant ($P < 0.05$), the model was analyzed by time. Trials 2a, 2b, 3, and 4 used a model that included treatment with run as the repeated measure and well within cup as the subject. Initially, Trial 2a used the same contrasts as Trial 1b, but PEG was toxic to all larvae; thus, only treatments without PEG were compared using least squares means. Trials 2b, 3, and 4 used single degree of freedom linear and quadratic orthogonal polynomial contrasts to compare treatment means and only the highest order contrast that was significant ($P < 0.05$) was discussed. Coefficients for the linear and quadratic contrasts with unequal spacing were generated using the IML procedure of SAS. Data are reported as least squares means with standard errors.

## RESULTS AND DISCUSSION

*Chemical composition of juniper material and distilled monoterpenoid oil*

Chemical composition of juniper material was not statistically analyzed, but differences were observed. For example, DM and CP concentrations in fresh and dry juniper were 66.1% and 93.8% and 7.18% and 6.6%, respectively. The NDF and ash concentrations of fresh and dry juniper were 43.8% and 39.3%, and 3.5% and 3.2%, respectively. The ADF concentrations of fresh and dry juniper were similar to NDF concentrations; 42.2% and 38.3%, respectively.

Composition of individual monoterpenoids in distilled juniper oil and dry (harvested 4/25/09) and fresh (harvested 6/9/09 and 6/22/09) juniper forage were not statistically analyzed (Table 1). Percentages of certain individual monoterpenoids in the total amount of distilled oil were different

from dry and fresh juniper. Dry juniper had less total monoterpenoids than fresh juniper, which was expected due to being dried at 55 ºC for 96 h. Certain monoterpenoids are volatile and can be affected by temperature (Animut et al. 2004; Utsumi et al., 2006). Even though drying reduced monoterpenoid concentrations, dried juniper still contained greater concentrations of monoterpenoids than reported for fresh one-seed juniper leaves (*Juniperus monosperma* [Engelm.] Sarg.; 2.17%, Utsumi et al., 2006) and fresh ashe juniper leaves and small stems (*Juniperus ashei* Buchholtz; approximately 1.8%, Owens et al., 1998). Differences in total and individual monoterpenoid concentrations between the fresh redberry juniper samples harvested 13 days apart could have been due to a season effect as previously reported (Adams; 1970; Riddle et al., 1996; Owens et al., 1998).

Total CT concentration in fresh juniper leaf and stem material was 7.3% and fractions consisted of 6.1%, 1.1%, and 0.1% extractable, protein-bound, and fiber-bound, respectively. Whitney and Muir (2010) reported 5.5% CT (4.12%, 0.96, and 0.38 extractable, protein-bound, and fiber bound, respectively) in dry redberry juniper leaves. Thus, the addition of stem material (increasing CT) may be a means by which CT concentrations in mixed feed can be controlled. Barry and Manley (1986) suggested that extractable CT are more reactive than bound CT; thus, the extractable CT concentration in juniper may increase its anthelmintic properties. However, pelleting Sericea lespedeza (*Lespedeza cuneata* [Dum-Cours.] G.Don.) hay enhanced its anthelmintic properties compared to ground Sericea lespedeza hay, even though pelleting resulted in greater protein-bound CT at the expense of extractable-CT (Terrill et al., 2007). Furthermore, Sericea lespedeza hay negatively affected gastrointestinal nematodes (Shaik et al., 2006; Terrill et al., 2007) and has been reported to contain up to 22.4% CT with 3.6%, 13.4%, and 5.4% extractable, protein-bound, and fiber-bound fractions, respectively.

*Trials 1a and 1b*

The *in vitro* rumen system used for this LMI assay affected VFA, pH, and ammonia N (Trial 1a; Table 2). Concentrations of acetate, propionate, and total VFA in the RF-buffer solutions were less ($P < 0.004$) and concentrations of isobutyric, butyric, isovaleric, valeric, pH, and ammonia N of the RF-buffer solutions were greater ($P < 0.05$) when no material was added (CNTL) compared to when DRY or FRE (averaged) was added to the solution. Concentrations of acetate, propionate, butyric, total VFA were less ($P < 0.04$) and pH and ammonia N were greater ($P < 0.001$) when QT or OIL was added to the solution compared to when DRY or FRE (averaged) was added to the solution. These results could have been related to CNTL, QT, and OIL solutions not containing any forage material vs. DRY and FRE being added to the RF-buffer, or to CT reducing microbial activity (Scalbert, 1991; Jones et al., 1994), propionate, ammonia N, and total VFA, and increasing acetate (Scharenberg et al., 2008). Essential oils such as monoterpenoids also have antimicrobial properties (Cowan, 1999; Burt,

**Table 1**

Composition of individual monoterpenoids in monoterpenoid oil and dried and fresh redberry juniper leaf and stem material[1].

| | Juniper Oil | Dry Juniper, 4/25/09 | | Fresh Juniper, 6/9/09 | | Fresh Juniper, 6/22/09 | |
|---|---|---|---|---|---|---|---|
| Compound | % of total | mg oil/g DM | % of total | mg oil/g DM | % of total | mg oil/g DM | % of total |
| Tricyclene | 0.91 | 0.21 | 0.82 | 0.19 | 0.52 | 0.22 | 0.47 |
| alpha-Thujene | 2.03 | 0.41 | 1.63 | 0.80 | 2.18 | 0.84 | 1.83 |
| alph-Pinene | 3.77 | 0.50 | 1.97 | 0.96 | 2.61 | 0.84 | 1.82 |
| Camphene | 1.04 | 0.24 | 0.94 | 0.28 | 0.76 | 0.27 | 0.59 |
| Sabinene | 31.54 | 6.60 | 26.03 | 8.74 | 23.78 | 9.84 | 21.31 |
| beta-Pinene | 0.20 | 0.03 | 4.29 | 0.06 | 0.16 | 0.06 | 4.12 |
| Myrcene | 5.71 | 1.09 | 4.30 | 1.61 | 4.38 | 1.90 | 4.12 |
| alpha-Phellandrene | 0.19 | 0.03 | 0.12 | 0.05 | 0.14 | 0.07 | 0.15 |
| 3-Carene | 0.98 | 0.07 | 0.27 | 1.44 | 3.92 | 0.17 | 0.37 |
| alpha-Terpinene | 3.87 | 0.67 | 2.66 | 1.42 | 3.86 | 2.14 | 4.62 |
| p-Cymene | 0.27 | 0.03 | 0.12 | 0.04 | 0.11 | 0.04 | 0.09 |
| Limonene | 10.21 | 1.61 | 6.34 | 2.15 | 5.85 | 2.74 | 5.93 |
| gamma-Terpinene | 5.81 | 1.10 | 4.33 | 2.25 | 6.12 | 3.12 | 6.76 |
| cis-Sabinene_hydrate | 0.36 | 0.17 | 0.66 | 0.19 | 0.52 | 0.30 | 0.65 |
| Terpinolene | 2.62 | 1.02 | 1.04 | 0.94 | 2.56 | 1.07 | 2.32 |
| trans-Sabinene_hydrate | 0.21 | 0.18 | 0.69 | 0.15 | 0.41 | 0.32 | 0.70 |
| cis-para-Menth-2-en-1-ol | 0.33 | 0.21 | 0.83 | 0.32 | 0.87 | 0.52 | 1.13 |
| trans-para-Menth-2-en-1-ol | 0.21 | 0.12 | 0.47 | 0.21 | 0.57 | 0.30 | 0.65 |
| Camphor | 9.03 | 4.68 | 18.48 | 5.17 | 14.07 | 6.82 | 14.78 |
| Camphene_hydrate | 0.26 | 0.17 | 0.69 | 0.16 | 0.44 | 0.20 | 0.43 |
| Citronellal | 0.85 | 0.12 | 0.47 | 0.29 | 0.79 | 0.21 | 0.45 |
| Borneol | 0.17 | 0.19 | 0.73 | 0.02 | 0.05 | 0.12 | 0.26 |
| Terpin-4-ol | 7.14 | 3.09 | 12.20 | 5.90 | 16.05 | 8.66 | 18.77 |
| alpha-Terpineol | 0.36 | 0.14 | 0.55 | 0.24 | 0.65 | 0.36 | 0.78 |
| Citronellol | 2.99 | 0.32 | 1.24 | 0.34 | 0.93 | 0.41 | 0.88 |
| Bornyl_acetate | 2.97 | 0.63 | 2.50 | 0.82 | 2.23 | 0.80 | 1.73 |
| E-Caryophyllene | 0.21 | 0.02 | 0.08 | 0.01 | 0.03 | 0.02 | 0.04 |
| alpha-Humulene | 0.25 | 0.01 | 0.04 | 0.01 | 0.03 | 0.01 | 0.02 |
| Elemol | 3.73 | 1.21 | 4.79 | 1.19 | 3.24 | 2.42 | 5.24 |
| Germacrene_B | 0.23 | 0.02 | 0.08 | 0.01 | 0.03 | 0.04 | 0.09 |
| gamma-Eudesmol | 0.35 | 0.12 | 0.47 | 0.25 | 0.68 | 0.43 | 0.93 |
| beta-Eudesmol | 0.47 | 0.15 | 0.59 | 0.21 | 0.57 | 0.39 | 0.84 |
| alpha-Eudesmol | 0.54 | 0.17 | 0.67 | 0.27 | 0.73 | 0.46 | 1.00 |
| **Total Oil** | | 25.34 | | 36.75 | | 46.17 | |

[1]Dry juniper = fresh juniper harvested at the beginning of the study, dried at 60 °C for 96 h and stored at -80 °C. Fresh juniper was harvested, placed on dry ice, and stored at -80 °C.

**Table 2**

Effects of including quebracho tannin (QT), monoterpenoid oil (OIL), or dry or fresh juniper material to goat rumen fluid and buffer mixture in an *in vitro* system.

| | Treatment[1] | | | | | | *P*-value[2] | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Item[3] | *CNTL* | *QT* | *OIL* | *DRY* | *FRESH* | *SEM* | *1* | *2* | *3* | *4* |
| VFA, mMol | | | | | | | | | | |
| Acetate | 15.73 | 14.16 | 14.29 | 25.12 | 23.83 | 0.89 | <0.001 | <0.001 | <0.001 | 0.10 |
| Propionate | 11.97 | 8.58 | 15.82 | 25.56 | 21.69 | 1.31 | <0.001 | <0.001 | <0.001 | 0.01 |
| Isobutyric | 0.95 | 0.52 | 0.35 | 0.69 | 0.69 | 0.12 | 0.003 | 0.003 | 0.07 | 0.93 |
| Butyric | 7.43 | 3.81 | 4.67 | 6.22 | 6.42 | 0.55 | 0.04 | 0.003 | 0.03 | 0.72 |
| Isovaleric | 1.90 | 0.27 | 0.81 | 0.65 | 0.72 | 0.30 | <0.001 | 0.03 | 0.43 | 0.59 |
| Valeric | 2.51 | 0.56 | 1.39 | 1.69 | 1.78 | 0.33 | 0.005 | 0.003 | 0.25 | 0.72 |
| Total VFA | 40.49 | 27.22 | 37.00 | 59.93 | 55.12 | 2.42 | <0.001 | <0.001 | <0.001 | 0.02 |
| pH | 7.30 | 7.36 | 7.31 | 6.90 | 6.97 | 0.06 | <0.001 | <0.001 | <0.001 | 0.16 |
| Ammonia N, mg/dL | 5.87 | 2.95 | 4.95 | 2.32 | 2.23 | 0.41 | <0.001 | 0.15 | <0.001 | 0.82 |

[1]CNTL = no material added; QT = 2.5% w/v; OIL = 0.33% w/v; DRY = 7.24% w/v; FRESH = 11.46% w/v.
[2]Non-orthogonal contrasts: 1) CNTL vs. DRY FRESH, 2) QT vs. DRY FRESH, 3) OIL vs. DRY FRESH, 4) DRY vs. FRESH
[3]Rumen fluid-buffer solution evaluated after incubating for 16 h; just prior to larvae incubation.

2004) and can reduce rumen ammonia (Cardozo et al., 2005) and microbial attachment to starch-rich grains and protein (McEwan et al., 2002). Rumen pH is another important consideration during *in vitro* LMI assays evaluating CT, because CT-protein bonds can dissociate at pH below 5 or above 7 (Mueller-Harvey and McAllan, 1992).

Adding DRY to RF-buffer resulted in greater ($P < 0.03$) initial propionate and total VFA concentrations compared to adding FRE. Total phenolics, CT, degree of polymerization, and specific activity of oak leaves was not affected by oven drying at 60°C or 90°C (Makkar and Singh, 1991). However, drying has reduced CT concentration of other forages (D'Mello and Taplin, 1978; Padmaja, 1989; Palmer et al., 2000). These discrepancies may be due to forage moisture concentration or chemical nature of the tannins (Makkar and Singh, 1991). Therefore, further research is warranted to evaluate effects of drying forages on larvae viability. Adding 4.5% w/v PEG to CNTL, DRY, or FRE RF-buffer did not affect ($P > 0.15$; data not shown) individual or total VFA, pH, or ammonia N.

*Trials 2a and 2b*

Larvae incubated for 16 h in the CNTL RF-buffer had low viability (62.3%) compared to other trials in this study that incubated larvae in CNTL RF-buffer for a maximum of 4 h, but the addition of 4.5% w/v PEG to all of the treatments reduced ($P < 0.009$; data not shown) LV. These results are exactly opposite of what would be expected, considering that PEG is routinely used to eliminate the *in vitro* effects of CT on internal parasites (Molan et al. 2000; Molan et al., 2004). When treatments without PEG were analyzed, no differences in LV were observed ($P > 0.45$; Trial 2a). Due to the high standard error (4.4%) in Trial 2b, LV was similar at all PEG concentrations tested (0 to 2% w/v; 89.4, 78.9, 76.5, 75.5, and 77.5% viable, respectively). The L3 larvae do not feed in the rumen (Gamble and Mansfield, 1996; White et al., 2007), thus larvae motility was probably not related to ingested PEG or CT. Future trials using this assay need to consider the effects of PEG on LMI.

*Trials 3 and 4*

Adding QT at 0%, 0.15%, 0.6%, and 1.2% w/v to the RF-buffer quadratically reduced ($P < 0.001$; Fig. 1) LV. These QT concentrations represented 0%, 0.87%, 3.49%, and 6.98% of daily dry matter intake (dDMI) and approximately 0%, 0.64%, 2.55%, and 5.10% of daily CT intake, respectively. Molan et al. (2004) reported 28% *H. contortus* viability when exsheathed larvae were incubated for 2 h in 1,000 g/mL of CT extracted from sulla (*Hedysarum coronarium* L.) forage; in

relation to the current study (55-kg sheep dDMI), this CT concentration would equate to 0.1% w/v CT. In the current study, approximately 0.1% w/v CT (0.15% w/v QT) resulted in 65.5% LV, which was 2.3 times greater than LV reported when larvae were incubated in 0.58% w/v CT (Molan et al., 2004). Molan et al. (2004) used exsheathed larvae (unknown susceptibility to IVM), but did not incubate the sulla extract in rumen fluid before larvae were incubated. In the current study, ensheathed, IVM-resistant larvae were used and QT was incubated in rumen fluid for 16 h to represent an *in vivo* rumen environment where CT can bind nutrients and alter rumen parameters as previously discussed. Differences between the 2 studies may have been due to: larvae resistance to IVM or being ensheathed vs. exsheathed; different sources of CT or proportions of extractable- and bound-CT; or drying and incubation time of material in RF-buffer. Molan et al. (2000) reported that

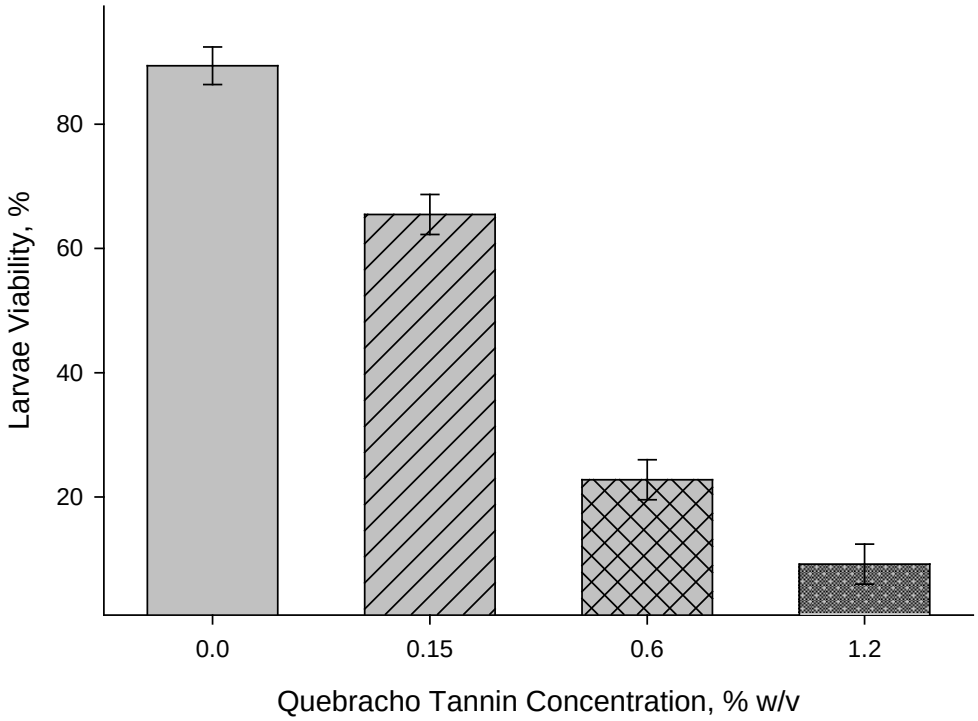


**Fig. 1.** Effects of quebracho tannin concentration in a rumen fluid-buffer solution on *in vitro Haemonchus contortus* larvae viability.

ensheathed deer L3 lungworm larvae were less susceptible to CT than exsheathed larvae. Padmaja (1989) reported a reduction in cassava leaf tannins when leaves were dried at 60 to 70 °C.

When larvae were incubated in 1.2% w/v QT, viability was 9.2%, but this *in vitro* concentration would require an animal to consume QT at 6.98% of dDMI. Performance was reduced when sheep were drenched for 3 days with quebracho extract at 8% w/w of food intake (Athanasiadou et al., 2001), which would relate to approximately 1.38% w/v QT in the current study. In addition, Dawson et al. (1999) reported that QT fed to sheep at 5% dDMI reduced digestibility and performance. In contrast, Paolini et al. (2003) did not report any problems with feed intake when 5% QT (of dDMI) was fed to goats. Even if QT consumption needed to be 3.49% of dDMI (0.6% w/v) to reduce problems associated with intake or growth, it still has potential to significantly reduce *in vivo* LV.

Ivermectin was initially solubilized in 4% DMSO, but this DMSO concentration was toxic to larvae; less than 22% LV (data not shown). *In vitro* assays have incubated larvae for 24-h in 0.3% DMSO (Howell, 2009) and for 2-h in 5% DMSO (Douch and Morum, 1994) without affecting ensheathed *H. contortus* LV. The dramatic decrease currently reported in LV using 4% DMSO cannot be explained. However, the 1% DMSO sufficiently solubilized IVM without negatively affecting LV, thus is recommended for this LMI assay.

Increasing IVM concentrations from 0 to 15 µg/mL quadratically reduced ($P < 0.001$; Fig. 2) LV. Gatongi et al. (2003) reported 50% LV when larvae with previous exposure to IVM were incubated for 3 h in 2.67 µg/mL of IMV, which is fairly similar to results of the current study (Fig. 2) using IVM-resistant larvae. However, Gatongi et al. (2003) reported 10% and 5% LV when 8.2 and

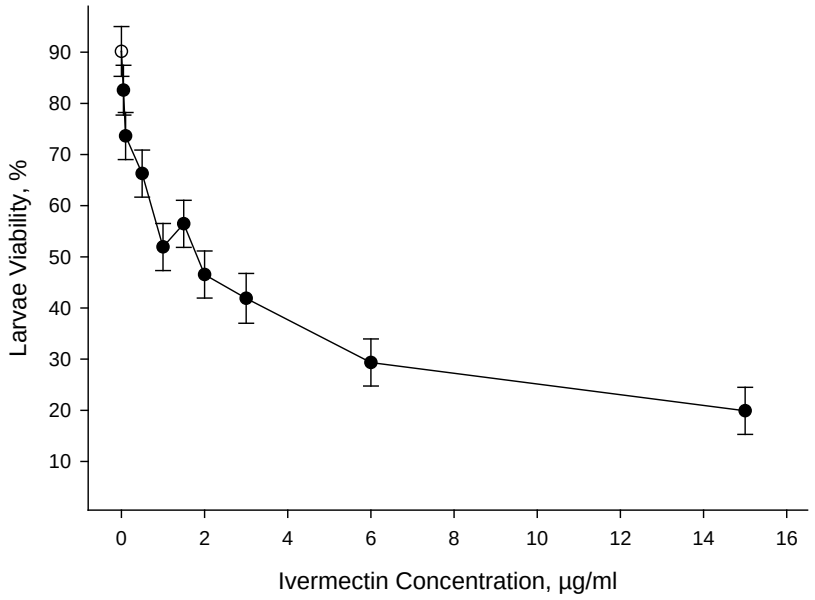


**Fig. 4.** Effects of ivermectin concentration in a rumen fluid-buffer solution on *in vitro Haemonchus contortus* larvae viability.

12.02 µg/mL were used, respectively, values lower than those reported for the current study. Gatongi et al. (2003) do not state if larvae were exsheathed, but differences may be due to the use of exsheathed larvae or different levels of larvae susceptibility to IVM.

## CONCLUSIONS

The *in vitro* LMI assay has been modified and can now be used to evaluate anthelmintic effects of plant secondary compounds that may be occurring in the rumen. The assay was validated by using a known anthelmintic drug (Ivermectin) and a known anthelmintic plant extract (Quebracho tannin). This modified assay will increase effectiveness of future LMI assays and reduce the time associated with preparation and evaluation. Effects of altering *in vitro* rumen fluid pH, ammonia N, and VFA and using PEG and DMSO when evaluating LV need to be further investigated in this assay procedure.

## ACKNOWLEDGEMENTS

This study was funded in part by the Food and Fibers Research Grant Program administered by the Texas Department of Agriculture. Appreciation is expressed to Rick Estell (USDA-ARS Jornada Experimental Range, Las Cruces, NM) for analyzing samples for monoterpenoids. Some of the L3 *H. contortus* larvae were kindly donated by Dr. Anne Zajac, Virginia-Maryland Regional College of Veterinary Medicine at Virginia Tech, Blacksburg.

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## SUPPLEMENTATION WITH ONIONS TO INCREASE BITTERWEED INTAKE BY SHEEP

*Cody J. Bundick, Cody B. Scott, Corey J. Owens*

**ABSTRACT**

Bitterweed (*Hymenoxys odorata*) continues to threaten sheep production in west-central Texas. However, the amino acid cysteine, which is found in onions (*Allium cepa,* L.), can be used to prevent bitterweed poisoning. The objectives of this study were to determine if the supplementation of onions or soybean meal would increase the consumption of bitterweed without increasing incidence of toxicity and to determine if there was any difference in susceptibility to bitterweed poisoning between Rambouillet and Dorper-cross sheep. Two feeding trials were conducted. The first used Dorper-cross (Dorper X Barbado) rams and the second used Rambouillet wethers. Sheep were randomly allocated into three treatments. Treatment 1 was supplemented with onions, Treatment 2 was supplemented with soybean meal, and Treatment 3 only received alfalfa pellets. Bitterweed intake was higher ($P<0.05$) for Dorper-cross sheep fed onions. Bitterweed intake was similar among treatments with Rambouillet wethers. When intake of bitterweed was compared between breeds, Rambouillet wethers consumed more bitterweed. Collectively, these results suggest that Rambouillets can consume more bitterweed while avoiding toxicosis. Supplementation with onions does not seem to improve bitterweed consumption.

**INTRODUCTION**

Bitterweed (*Hymenoxys odorata*) has been recognized as being poisonous to sheep since the early 1920's (Sperry 1949). It is one of the most problematic plant species in western Texas (Hardy et al. 1931), contributing to the decline of sheep production in the Edwards Plateau region. In April 1962, the estimated annual loss from bitterweed poisoning in sheep was over 3.5 million dollars (Dollahite et al. 1973). A sesquiterpene lactone known as hymenoxon 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).

Considerable efforts have attempted to devise ways to continue sheep production on bitterweed-infested rangelands. Some resolutions include the supplementation of activated charcoal, supplementation with the antioxidant Santoquin, and the use of herbicides. These strategies introduce their own problems. 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 (Calhoun et al. 1986, Ueckert and Calhoun 1988). Spraying with the herbicide 2,4-D 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 cysteine increased survival rate up to 12-fold over sheep that were not dosed (Rowe et al. 1980), suggesting that cysteine can be an affective antidote for hymenoxon poisoning. Onions (*Allium cepa,* L.) are high in 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 to onions, soybean meal is high in cysteine. Cysteine levels in soybean meal can reach up to 0.67% on a dry matter basis (Baize 1999).

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 and adaptability. However, little is

known about the Dorpers' susceptibility to hymenoxon, and whether they will be able to survive on bitterweed-infested rangelands. The objectives of this study were to: determine if the supplementation of onions or soybean meal increased the consumption of bitterweed without increasing incidence of toxicity, and determine if there is any difference in susceptibility to bitterweed poisoning between Rambouillet and Dorper-cross sheep.

**MATERIALS AND METHODS**

This study consisted of two trials. The first trial included 27 Dorper-cross rams (9 sheep/treatment) approximately one year of age, and an average weight of 71kg $\pm$ 4.7. 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 1.5m) and allotted 7 days for pen adjustment. Alfalfa pellets were fed at 2% BW to meet the animal's maintenance requirement (NRC 2007). Rams were offered fresh water and a calcium/phosphorus mineral *ad libitum*.

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 (i.e. control), fed only the alfalfa pellets. Supplements were fed from 1400 to 1500 each afternoon for 14 days.

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% with no signs of toxicosis. The amount of onions fed was increased slowly over a 28 day period until sheep were consuming the desired amount. This step-up ration approach began with 25 g $hd^{-1}$ offered daily. Due to low intake levels of onions, an additional 17 days were 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. The average crude protein content of onions is 15.1%.

Assuming an average weight of 73 kg $\pm$ 4.7 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 are iso-nitrogenous.

Immediately after onion or protein supplementation, 50 grams of dried bitterweed, excluding roots and stems, was offered daily for 30 minutes. Intake of bitterweed was monitored daily. If sheep consume all 50 grams, an additional 25 grams was offered the following day. Bitterweed was harvested from the Texas Agrilife Research Center in Barnhart, Texas and then air dried.

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

**Table 1.** Ingredients and nutritional value of Soybean Meal 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)

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 Rambouillet wethers. The second trial consisted of 30 Rambouillet castrated lambs, approximately 3 months old with an average beginning weight of 28$\pm$2.3 kg. Lambs were randomly allocated to the same treatment groups 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 and centrifuged to measure PCV levels.

In experiments, intake (g $kg^{-1}$ BW), weight change and serum levels were compared among breeds and supplement type 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. Means were separated using Tukey's LSD test when $P \leq 0.05$. Data was analyzed using the statistical package JMP (SAS 2007).

**RESULTS**

In Trial 1, Dorper-cross sheep typically consumed all of the alfalfa and supplement that was offered each day in both the preconditioning phase of the trial and when bitterweed was fed during the feeding trial. Sheep were reluctant to consume onions (Fig. 1). The preconditioned phase continued for an additional 31 days (45 days total) before rams would consume enough onions to begin the feeding trial. Once the trial began, sheep generally consumed most of the onions offered (Fig 2). There were a few sheep that consistently refused to consume all of the onions that were presented and intake varied daily for some other individuals as well.

Bitterweed intake differed ($P<0.05$) among treatments. Intake was higher for sheep supplemented with onions. When bitterweed intake was compared among treatments, one major difference was obvious. Sheep 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 (Fig 3). Treatment means for bitterweed intake varied from 0.34$\pm$0.05 g $kg^{-1}$ BW for the treatment group receiving a supplement of SBM, 0.36$\pm$0.05 g $kg^{-1}$ BW for the control group and 0.71$\pm$0.05 g $kg^{-1}$ BW for the treatment group supplemented with onions.

The treatment X day interaction for bitterweed intake was also significant ($P<0.05$) (Fig 4). Initially, Dorper-cross sheep were hesitant to consume bitterweed, (0.03$\pm$0.09 g $kg^{-1}$ BW, SBM and alfalfa; 0.06$\pm$0.09 g $kg^{-1}$ BW, onions); however by day 10, bitterweed intake had

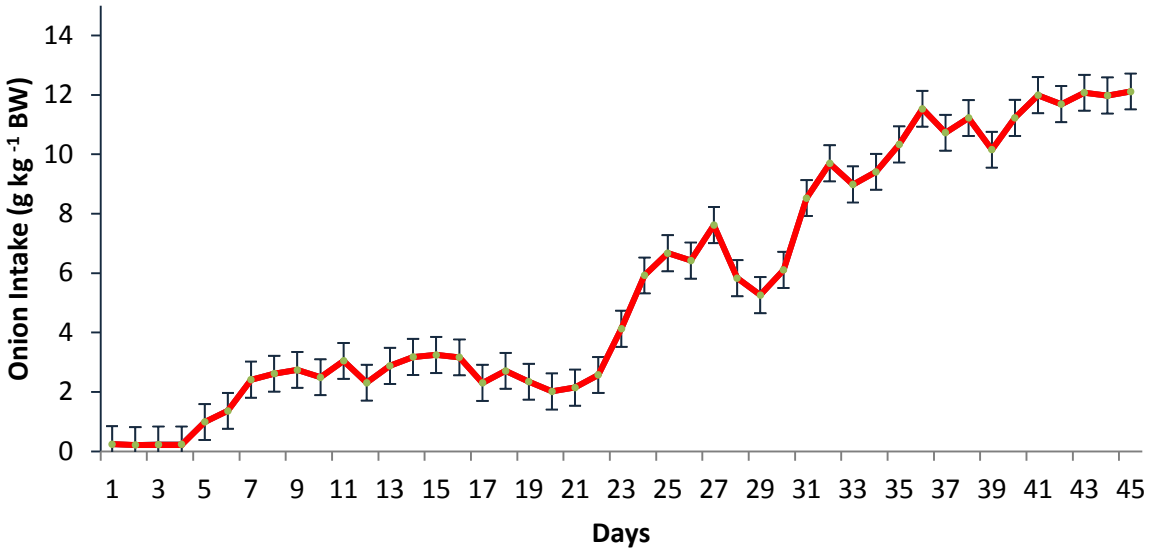


**Figure 1.** Average daily onion intake (g $kg^{-1}$ BW) of Dorper-cross rams throughout a 45-day preconditioning phase.

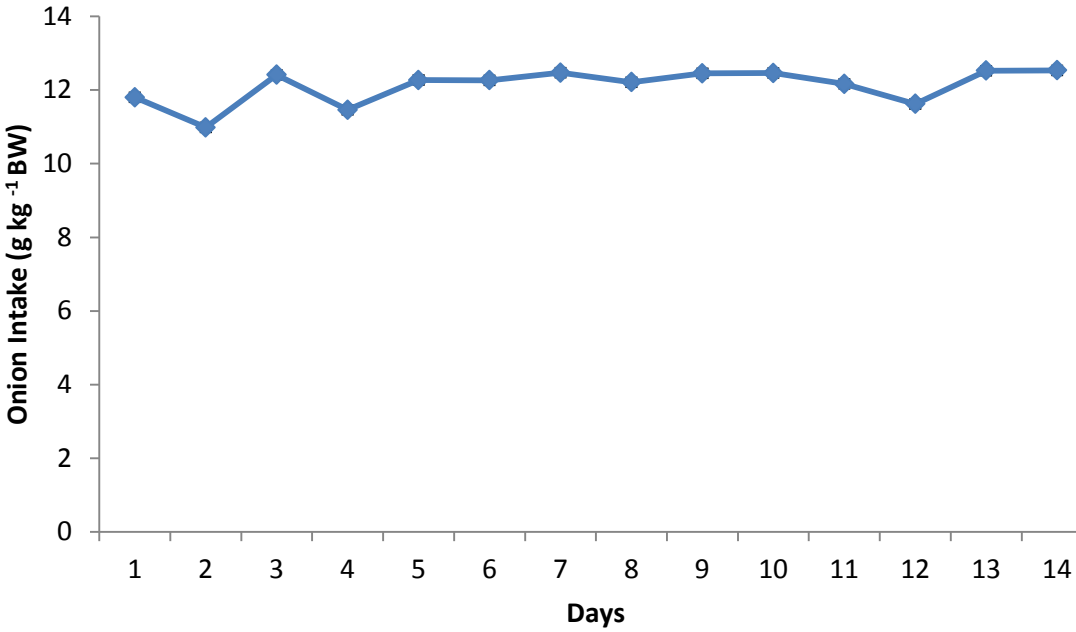


**Figure 2.** Average daily onion intake (g $kg^{-1}$ BW) of Dorper-cross rams that were supplemented with onions immediately prior to being fed bitterweed for 14 days after preconditioning.

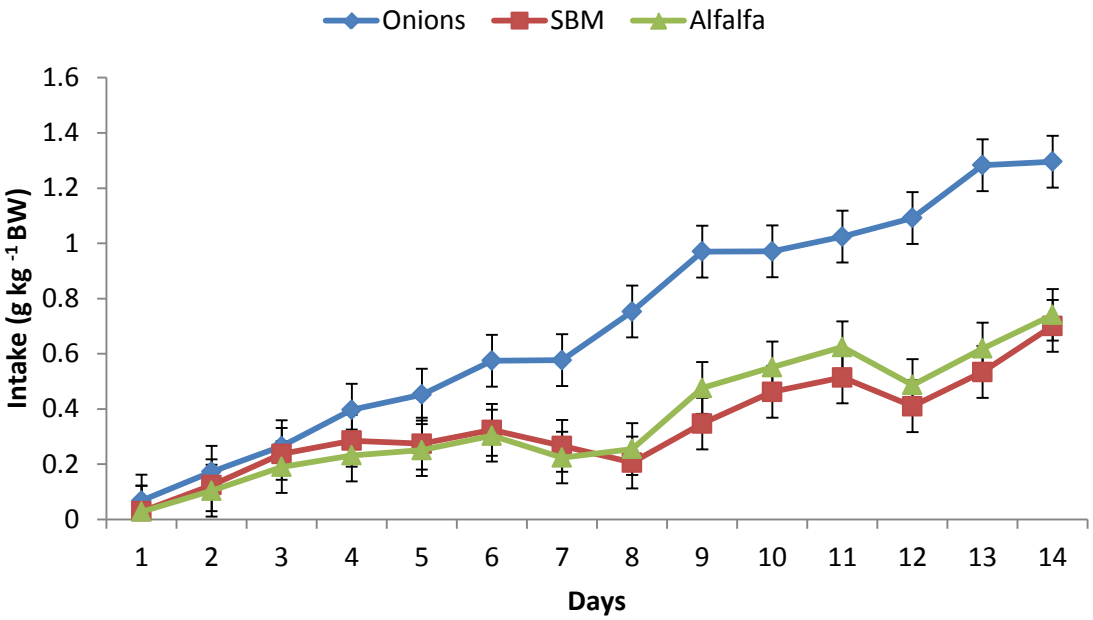


**Figure 3.** Bitterweed intake (g $kg^{-1}$ BW) of Dorper-cross rams when supplemented with onions, soybean meal (SBM), or no supplement (alfalfa only) immediately prior to being fed bitterweed for 14 days.

increased, especially with sheep given onions (0.05±0.09 g $kg^{-1}$ BW, SBM and alfalfa; 0.97±0.09 g $kg^{-1}$ BW, onions).

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 all of the alfalfa and 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 (Fig 4) and during the feeding trial when bitterweed was offered (Fig 5). With the exception of a few individuals, most refused to eat all of the onions throughout preconditioning and the feeding trial even after feeding onions for 27 days. However, sheep given SBM consistently consumed all that was offered.

Bitterweed intake was similar (P>0.05) among all treatments. Much like the Dorper-cross sheep, Rambouillets were hesitant to consume bitterweed (0.06±0.15 g $kg^{-1}$ BW across all treatments). Treatment means for bitterweed intake varied from 0.72±0.1 g $kg^{-1}$ BW for the control treatment group, 0.94±0.1 g $kg^{-1}$ BW for the sheep supplemented with onions and 0.95±0.1 g $kg^{-1}$ BW for the treatment group receiving SBM as a supplement. Bitterweed intake steadily increased throughout the feeding trial (Fig 6) averaging 1.5± g $kg^{-1}$ BW by the end of the trial. There was a significant difference in the treatment X 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 6).

To ensure no anemia was taking place, pack cell volumes were measured and analyzed. There was no significant difference (P>0.05) among treatment groups. Packed cell volume levels were 30.2±1.4 for wethers supplemented onions, 31.9±1.4 for those supplemented with SBM,

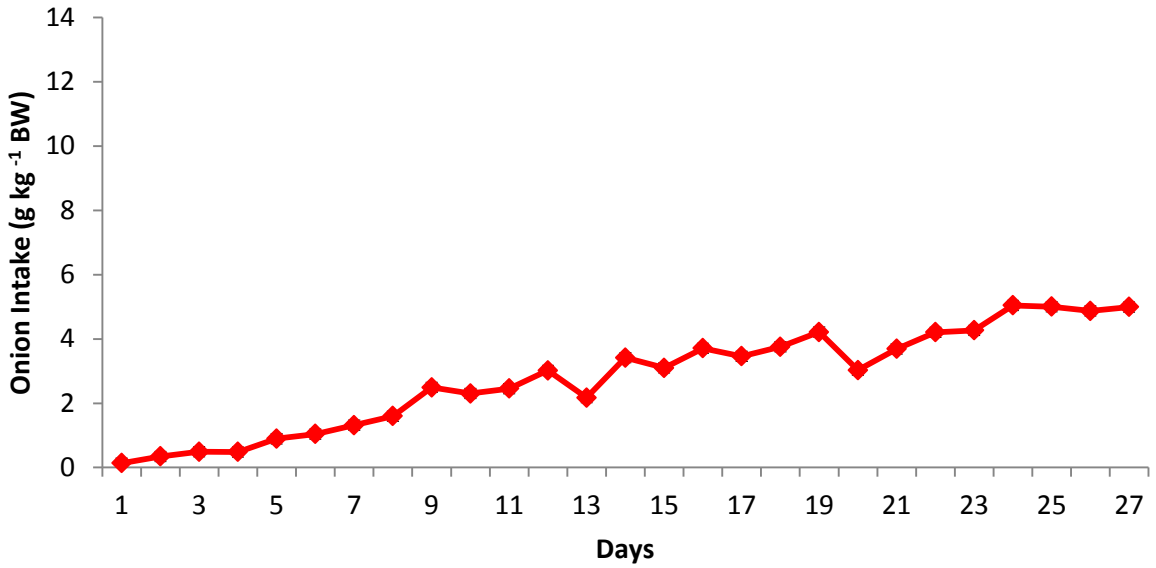


**Figure 4.** Average daily onion intake (g $kg^{-1}$ BW) of Rambouillet wethers throughout a 27-day preconditioning phase.

and 32.1±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.

When weight change was compared, there was a major difference among treatments. Sheep that were supplemented with onions had much lower ending weights when compared to their initial weights. This was true for both Trial 1 and Trial 2 (Table 4). Those individuals that were supplemented with SBM or no supplement had little change in weight.

Data were pooled across breeds and stratified based on average intake of onions. Sheep were assigned to 3 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. 6). Among all breeds, sheep that ate a diet consisting of 25% onions consumed 0.86±0.13 g $kg^{-1}$ BW of bitterweed. Those that ate a diet consisting of 50% onions ate 0.42±0.13 g $kg^{-1}$ BW of bitterweed, while those that ate a diet of 75% onions consumed 0.85±0.13 g $kg^{-1}$ BW of bitterweed.

When data were compared between breeds, Rambouillet wethers ate more bitterweed than Dorper-cross sheep (Table 7). Breed means were 0.86±0.05 g $kg^{-1}$ BW for the Rambouillet and 0.47±0.05 g $kg^{-1}$ BW for the Dorper-cross. Given the significant treatment effect (onion supplementation increase bitterweed intake) and the significant breed effect (Rambouillet wethers ate more bitterweed than Dorper-cross rams), Rambouillet wethers supplemented with onions should have eaten more bitterweed than Dorper-cross sheep supplemented with onions. However, the treatment X breed effect was not significant ($P>0.05$) (Tables 6).

## DISCUSSION

Preliminary study results had suggested that supplementing onions would increase bitterweed intake (E. Campbell et al. unpublished data). Onions that are unfit for human consumption are used as supplemental feeds in other regions of the U.S. (McBride et al. 2004). Sheep supplemented with onions in this study also consumed more bitterweed. The hypothesis

**Table 2.** Serum metabolite levels of Dorper-cross sheep when supplemented with onion, soybean meal (SBM), or no supplement (alfalfa only) immediately prior to being fed bitterweed for 14 days.

| | Treatment | | | |
|---|---|---|---|---|
| | **Onions** | **SBM** | **Control** | **SEM** (standard error) |
| BUN (mg/dl) | 20.1 | 30.2 | 23.9 | 1.38 |
| Creatinine (mg/dl) | 1.33 | 1.05 | 1.12 | 0.03 |
| Bilirubin (mg/dl) | 0.17 | 0.13 | 0.13 | 0.01 |
| AST (U/l) | 76.1 | 80.2 | 79.7 | 4.55 |
| GGT (U/l) | 57.8 | 59.5 | 56.7 | 2.61 |

All were within the normal ranges for sheep.

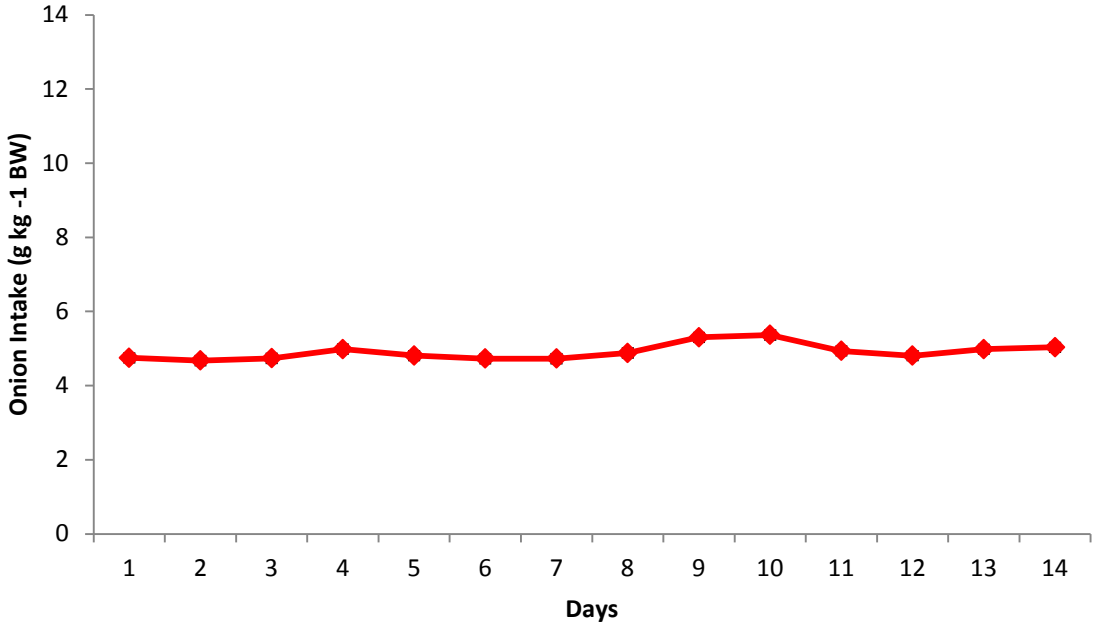


**Figure 5.** Average daily onion intake (g $kg^{-1}$ BW) of Rambouillet wethers that were supplemented with onions immediately prior to being fed bitterweed for 14 days after preconditioning.

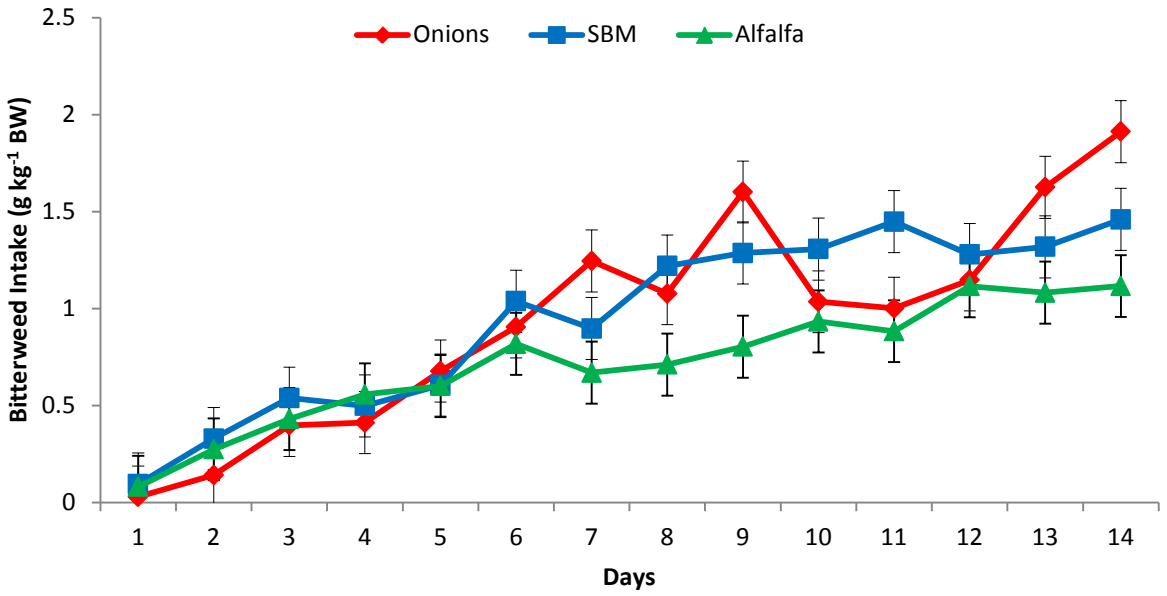


**Figure 6.** Bitterweed intake (g $kg^{-1}$ BW) of Rambouillet wethers when supplemented with onions, soybean meal (SBM), or no supplement (alfalfa only) immediately prior to being fed bitterweed for 14 days.

**Table 3.** Pack cell volume (PCV) for Rambouillet wethers supplemented with either onions, soybean meal (SBM), or no supplement (alfalfa only) immediately prior to being fed bitterweed.

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

[1]All percentages were in the normal range for sheep.

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

| Treatment | Dorper-cross | Rambouillet | SEM (standard error) |
|---|---|---|---|
| Onions | 9.6 | 8 | 1.25 |
| SBM | 0.3 | 2.8 | 1.25 |
| Alfalfa | 2.2 | 3.0 | 1.25 |

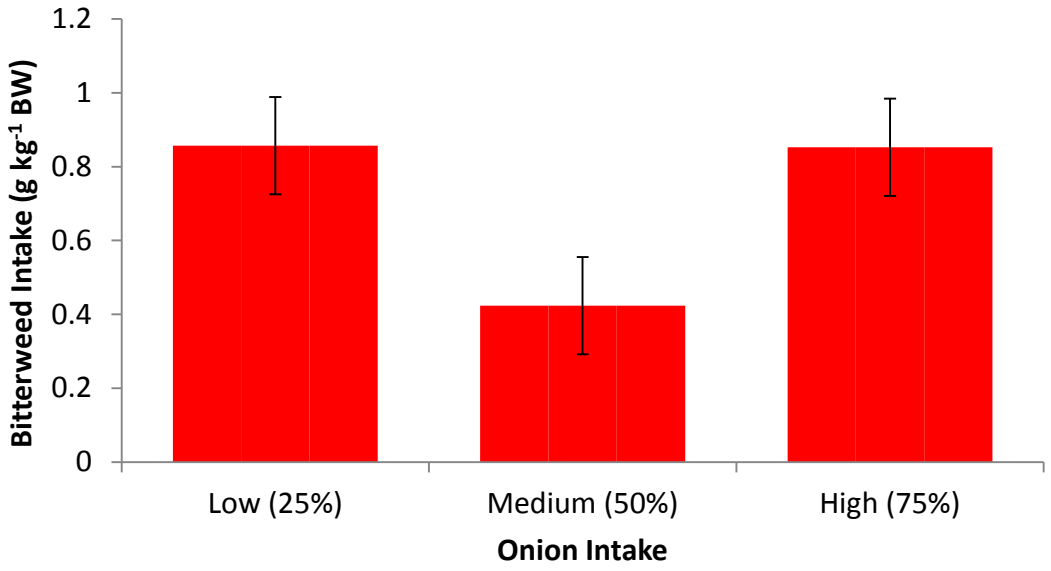


**Figure 7.** Level of onion intake (g $kg^{-1}$ BW) effect on bitterweed intake of both Dorper-cross sheep and Rambouillet wethers.

**Table 5.** Average bitterweed intake (g $kg^{-1}$ BW) of Dorper-cross and Rambouillet breeds.

| Breed | Bitterweed Intake (g $kg^{-1}$ BW) | SEM (standard error) |
|---|---|---|
| Dorper-cross | 0.469 | 0.053 |
| Rambouillet | 0.858 | 0.053 |

**Table 6.** Bitterweed intake (g $kg^{-1}$ BW) of Dorper-cross rams and Rambouillet wethers that were supplemented with onions, soybean meal (SBM), or no supplement (alfalfa only) immediately prior to being fed bitterweed for 14 days.

| Treatment | Dorper-cross intake | Rambouillet intake | SEM (standard error) |
|---|---|---|---|
| Onions | 0.707 | 0.944 | 0.09 |
| SBM | 0.337 | 0.912 | 0.09 |
| Alfalfa | 0.363 | 0.720 | 0.09 |

of this study was that supplements high in L-cysteine would increase bitterweed intake. Both onions and SBM are high in L-cysteine. Other studies have clearly illustrated that supplementation with L-cysteine increased bitterweed intake (Rowe et al. 1980, Calhoun et al. 1986). However, supplementation with SBM apparently had no effect on bitterweed intake in this study.

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 wethers to consume onions, sheep may have increased intake of bitterweed 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). Toxins may encourage animals to eat a variety of foods containing needed nutrients, provided toxins in the foods differ in their physiological effects and are detoxified by different mechanisms (Provenza 1996). Based on the fact that sheep supplemented with onions may have consumed more bitterweed to meet nutritional requirements, the hypothesis that supplementation with onions would improve bitterweed intake was not accepted.

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 (Burke and Miller 2004, Gamble and Zajac 1992, Vanimisetti et al. 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). Results of this study suggest Rambouillet, rather than Dorper-cross sheep may eat a wider variety of plants. The Rambouillet breed may have become more tolerant of this specific plant through generations of selection on bitterweed-infested rangelands.

Rambouillet wethers may have eaten more bitterweed because of higher nutrient requirements. The wethers used in Trial 2 were younger than the Dorper-cross rams used in Trial 1. Younger animals typically have higher nutrient requirements than older individuals, which may have influenced the Rambouillet wethers to consume more bitterweed. However, in a previous study, with Dorper-cross and Rambouillet lambs of similar age, Rambouillet lambs consumed more bitterweed than Dorper-cross lambs as well (Campbell et al. unpublished data). The basal diet in the current study (alfalfa + onions or SBM supplement) was fed to meet nutrient requirements based on average age and body size (NRC 2007). Thus, any differences in age should have been negated by the basal diet.

Future studies should be conducted to fully understand the potential for supplementing onions to prevent bitterweed poisoning. Perhaps the use of a different type of onion, one that is more palatable, would encourage higher intake of the onions. Also, the use of different supplements may be beneficial. Supplementing with cottonseed meal and distiller's grain has shown to increase intake of other toxic plants like juniper in goats (George et al. 2007). Other studies have also illustrated that supplementation will improve intake of poisonous plants (Illius and Jessop 1995, Illius and Jessop 1996, Banner et al. 2000, Burritt et al. 2000).

Many toxins, including hymenoxon, are excreted as conjugates with glucuronic acid or sulfates (Schmidt 1999). Future studies should investigate supplementation with protein sources that are high in glucogenic amino acids. These amino acids escape digestion in the rumen and are absorbed in the small intestine where they are transferred to the liver for further metabolism. Once in the liver, these compounds can be transformed to glucoronic acid for toxin excretion.

## IMPLICATIONS

Based on the results of this study, further investigation is needed before recommending onions as a viable supplement to reduce bitterweed toxicity. This study does suggest that Rambouillet sheep are more bitterweed tolerant than the Dorper-cross breed.

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 and suffering form bitterweed toxicosis.

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**PREFERENCE AND NUTRITIONAL QUALITY OF DIFFERENT VARIETIES OF ONIONS BY SHEEP**

*Kellen A. Cave, Cody B. Scott, and Corey J. Owens*

**ABSTRACT**

The objectives of this study were to determine preferences of sheep for different varieties of onions and differences in nutritional quality between the varieties. There were 3 trials, using 3 types of onions (red, yellow, and white). Each type of onion was fed during each trial with trials occurring at different times during the year. Trials 1 and 2 sheep were fed for 22 days, Trial 3 sheep were fed for 45 days. Treatment 1 received red onions. Treatment 2 received white onions. Treatment 3 received yellow onions. Treatment 4 served as the control group (only alfalfa pellets). Sheep did not have a preference for any one variety of onions. Serum metabolites levels were within normal ranges of healthy animals indicating no toxicity occurred. Packed cell volume of the sheep that were fed onions was higher ($P<0.05$) than the control group, indicating that onions can serve as a viable diet supplement.

**INTRODUCTION**

Bitterweed (*Hymenoxys odorata*) is considered one of the most problematic plant species in western Texas (Hardy et al. 1931), especially in the Edwards Plateau region. The toxin in bitterweed is a sesquiterpene lactone known as hymenoxon (Ueckert and Calhoun 1988). Hymenoxon was first identified by Kim et al. (1975) as the primary toxin in bitterweed. Symptoms of hymenoxon poisoning include bloat, central nervous system recession, termination of rumen activity, and eventually death (Rowe et al. 1973).

There have been several studies conducted in the effort to reduce bitterweed toxicosis. These include supplementing with activated charcoal, with the supplementing antioxidant Santoquin, and applying herbicides. Activated charcoal adsorbs hymenoxon but is hard to supplement because of its fine particle size (Poage et al. 2000). The antioxidant Santoquin reduces bitterweed poisoning; however, it is very unpalatable, which effectively lowers intake (Calhoun et al. 1986; Ueckert and Calhoun 1988). Bunting and Wright (1973) applied 2, 4-D herbicide reducing bitterweed cover, but cost of application is the prohibiting factor. Recent studies have included feeding high protein diets containing cysteine to decrease hymenoxon poisoning (Coffman 2009) and feeding cull onions containing cysteine (Bundick 2008).

Hymenoxon conjugates in the rumen with sulfhydral groups, forming a less toxic compound. The amino acid L-cysteine contains sulfur and may provide sulfhydral groups for conjuagation in the rumen. Rowe et al. (1980) dosed sheep with cysteine increasing the survival rate up to 12-fold over sheep that were not dosed. Onions (*Allium cepa*, L.) contain high levels of cysteine. Sheep will readily consume onions when fed a 100% onion diet and can perform as well on the onions as on a grain diet (Knight et al. 2000). However, onion toxicity can occur with overingestion causing anemia in animals (Cheeke 1998).

Differences have been seen between onion varieties in phenolic content, antioxidant, and antiproliferative activies (Yang et al. 2004). Also, changes in the concentration and total amount of flavor precursors like S-alk(en)yl-L-cysteine sulphoxides during the growth of an onion should affect the flavor and potentially the level of L-cysteine in onions (Lancaster et al. 1983). This may explain why some report ruminants will readily consume onions without any adverse effects (Campbell et al. unpub. data) while others report that ruminants are reluctant to consume onions (Bundick 2008). Campbell et al. also reported that onions reduced the likelihood of bitterweed toxicosis, while Bundick (2008) showed that onions did not offer any protection from bitterweed toxicosis. Accordingly, this proposed study will assess preferences for different onion varieties by sheep,

differences in nutritional quality, and L-cysteine levels between varieties of onions and different seasons of the year.

## MATERIALS AND METHODS

This study consisted of three trials that were conducted at the Angelo State University Management Instruction and Research (MIR) Center. The three trials were conducted seasonally (spring, summer, winter) using three different varieties of onions (red, yellow, white). Trial 1 included 40 recently weaned lambs with 10 lambs per 4 treatments. The lambs were separated into individual pens (1 X 1.5 m) and allowed 5-10 days for pen adjustment. Alfalfa pellets were fed at 2.5% of the total body weight to meet the animal's maintenance requirements as specified by National Research Council (2007). Lambs also received fresh water and a calcium/phosphorus mineral *ad libitum*.

After the adjustment period, the sheep were allocated to one of the four treatments and fed for 22 days. The first treatment was supplemented with red onions, the second with white onions, the third with yellow onions, and the fourth treatment served as the control group receiving alfalfa pellets only. During the 22 day study, the onions were chopped by hand and offered from 1300 to 1400 each afternoon. The amount of onions fed began at 75 g. Once the individual consumed all 75 g twice during the feeding bout, the amount was increased 25 g until the point of refusal. All the sheep received the 2.5% BW alfalfa after the onions had been offered to meet maintenance requirements.

Any changes in weight, blood serum levels, and packed cell volume were recorded during this experiment to monitor for any signs of toxicosis. Serum metabolite levels and packed cell volume were monitored at day 0, 10, and 22 to assess physiological condition. Changes in serum levels are indicative of toxicosis and include levels of serum aspartate transaminase (AST), blood urea nitrogen (BUN), gamma glutamyltrasferase (GGT), creatinine, and bilirubin (Cornelius 1989; Cheeke 1998). Blood was collected via jugular vein puncture, the serum was extracted by centrifugation, frozen, and sent to the Texas Veterinary Medical Diagnostic Lab in College Station, TX for chemical analysis. Packed cell volumes were obtained by transferring fresh blood in capillary tubes, then centrifuging the tubes for 15 minutes and measuring the red blood cells with the scale affixed to the centrifuge.

Subsamples of each variety of onions were sent off for crude protein, digestibility, Acid Detergent Fiber, Neutral Detergent Fiber, Total Digestible Nutrients (TDN) and moisture content to Dairy One in Ithaca, New York. Another subsample of each onion was sent to NP Analytical Labs in St. Louis, MO for L-cysteine levels.

Trial 2 was similar to Trial 1. The second trial consisted of 32 recently weaned lambs. The intake of onions and alfalfa were measured. Lambs were allocated to the same treatment groups and the same variables were measured as in the first trial. Serum levels were not measured in Trial 2, but packed cell volume (PCV) was measured. Blood samples were collected and centrifuged to measure PCV levels. L-cysteine levels of the onions in Trial 2 were not measured because levels were undetectable in Trial 1, but the nutritional analysis was measured.

Trial 3 is similar to Trials 1 & 2 but extended to get sheep to a diet of 100% onions using the 3 varieties of onions. Initially the trial consisted of 40 sheep, but one died of natural causes six days into the trial. Fresh water and a Ca/P mineral supplement were offered *ad libitum* to the sheep. Sheep were randomly allocated to one of four treatments, which were the same as the first two trials.

The first feeding period was over 14 days, alfalfa was offered at 2.5% BW after onions were offered. Onions were offered at 75g, once the amount was consumed entirely the onions were increased 25g. During the second feeding period, onions continued to increase 25g when the amount offered was consumed. Alfalfa was decreased to 2% BW. The third, fourth, and fifth feeding

periods were 7 days long. Alfalfa was decreased to 1.5%, 1%, then to not offering any alfalfa to the onion treatments. The control group received the 2.5% alfalfa throughout the duration of the trial.

Intake of onions and alfalfa were monitored daily along with changes in packed cell volume were recorded through the duration of the trial to monitor for signs of toxicity. Packed cell volumes were taken on days 0, 17, 24, 31, 38, and 45. Blood was collected via jugular vein-puncture, placed in capillary tubes then centrifuged and measured to obtain the packed cell volume. Body weight was measured at the beginning and end of the trial.

This study design was a completely randomized design. Differences between types of onions were assessed using repeated measures analysis of variance. The individual lambs nested within treatments served as replications, and the day of observation was the repeated measure. Means were separated using Tukey's LSD where $P<0.05$ was considered significant. Each trial was analyzed separately using the statistical package JMP (SAS 2007).

**RESULTS**

In Trial 1, intake of onions and alfalfa were similar among the three treatments (Table 1). Sheep were hesitant to consume onions initially, but intake increased for all three treatments across days of feeding (Fig. 1). By the end of the trial, lambs were consuming on average 149 g of onions per day in each treatment.

Some serum levels differed among treatments, but remained with normal ranges for healthy animals (Table 2). For example, creatinine levels differed between the control and the treatment receiving yellow onions. Gamma glutamyltrasferase levels from day 1 collection period differed from the day 11and 22 collections (Table 3).

Regardless of treatment diet, none of the sheep in the spring trial exhibited signs of anemia. Packed cell volume counts were similar among treatments fed onions and remained within normal range for healthy animals (Table 4). The PCV for treatments receiving onions was significantly higher than the control group. Weight change was also similar ($P>0.05$) among treatments.

In Trial 2 (Summer Trial), alfalfa and onion intake among the three treatments were similar (Table 5). Once again, the sheep were initially reluctant to consume onions, but intake for all three treatments increased across days (Fig. 2). The onion treatments were consuming on average 95 g of onions by the end of the trial.

Serum constituents were not measured in Trial 2 because of a lack of differences in Trial 1. Anemia was not detected in the summer trail regardless of treatment diet. Packed cell volume counts were similar ($P>0.05$) and within the normal range among treatments (Table 6). However, onion treatments did not display the higher PCV count than the control as seen in Trial 1. Weight change was also similar ($P>0.05$) among treatments.

Nutritional analysis of the three onion varieties of both trials revealed that there was no difference ($P>0.05$) in nutritional quality of the three onions in or between the two trials (Table 7).

In Trial 3, intake of onions by sheep was similar for the three varieties of onions and for alfalfa intake. As the alfalfa was decreased onion consumption increased to meet nutritional demands that the alfalfa had filled (Fig. 3). By the end of the trial, sheep were consuming on average 621 g of onions per day.

Packed cell volumes for Trial 3 were similar ($P>0.05$) among all treatments (Fig. 4). The sheep in the winter trial were within the normal range for healthy animals, exhibiting no signs of anemia. The packed cell volumes increased from initial collection to the final collection period during the trial (Fig. 4). Weight changes were also similar ($P>0.05$) among the onion treatment with an average loss of 3 to 4 pounds; the control maintained a constant weight throughout the trial.

**Table 1.** Average intake (g·kg $^{-1}$ BW) of onions and alfalfa by sheep receiving different onion supplements for Trial 1.

| | Treatment | | | |
|---|---|---|---|---|
| **Food Item** | **Red** | **White** | **Yellow** | **SEM** |
| Onions | 0.93 | 0.98 | 1.23 | 0.15 |
| Alfalfa | 10.56 | 11.14 | 11.25 | 0.27 |

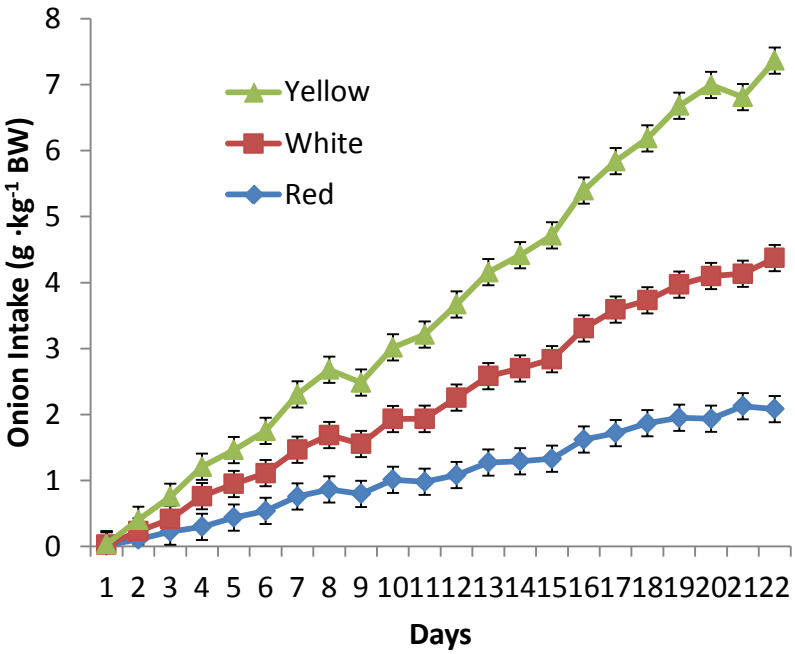


**Figure 1.** Average daily onion intake (g·kg$^{-1}$ BW) by sheep during Trial 1.

**Table 2.** Serum metabolite levels of sheep within treatments in Trial 1.

| | Treatment | | | | |
|---|---|---|---|---|---|
| **Serum Metabolite** | **Red** | **White** | **Yellow** | **Control** | **SEM** |
| GGT (U/l) | 73.70 | 65.08 | 61.21 | 62.14 | 4.77 |
| AST (U/l) | 114.80 | 112.50 | 89.90 | 110.13 | 6.88 |
| Creatinine(mg/dl) | 0.74[ab] | 0.72[ab] | 0.64[b] | 0.74[a] | 0.03 |
| BUN (mg/dl) | 24.03 | 23.53 | 22.27 | 24.79 | 0.81 |
| Glucose(mg/dl) | 58.07 | 60.37 | 59.83 | 61.00 | 2.34 |

[ab] subscripts that differ are significantly different

**Table 3.** Serum metabolite levels of sheep in Trial 1 by collection day.

| | Collection Day | | |
|---|---|---|---|
| **Serum Metabolite** | **Day1** | **Day 11** | **Day 22** |
| GGT (U/l) | 73.9[a] | 60.7[b] | 62.0[b] |
| AST (U/l) | 120.2[a] | 118.0[a] | 82.3[b] |
| Creatinine(mg/dl) | 0.6[c] | 0.8[a] | 0.7[b] |
| BUN (mg/dl) | 23.8[b] | 21.6[c] | 25.6[a] |

[abc] subscripts that differ are significantly different

**Table 4.** Packed cell volume (PCV) for sheep supplemented with either onions or no supplement in Trial 1.

| **Treatment** | **Packed Cell Volume** | **SEM** |
|---|---|---|
| Red | 37.9 | 0.9 |
| Yellow | 38.4 | 0.9 |
| White | 38.3 | 0.9 |
| Control | 35.1 | 0.9 |

**Table 5.** Average intake (g·kg$^{-1}$ BW) of onions and alfalfa for treatments receiving different onion supplements for Trial 2.

| | Treatment | | | |
|---|---|---|---|---|
| | **Red** | **White** | **Yellow** | **SEM** |
| **Food Item** | | | | |
| Onions | 1.4 | 1.2 | 1.2 | 0.3 |
| Alfalfa | 25.0 | 24.7 | 21.9 | 1.8 |

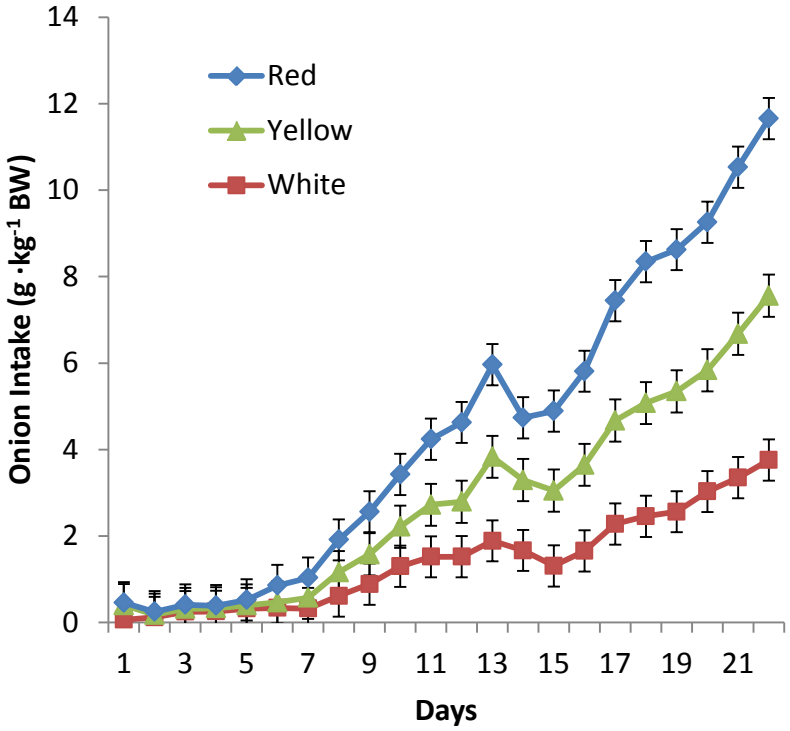


**Figure 2.** Average daily onion intake by sheep (g·kg$^{-1}$ BW) by sheep during Trial 2.

**Table 6.** Packed cell volume (PCV) for sheep supplemented with either onions or no supplement in Trial 2.

| Treatment | Packed Cell Volume | SEM |
|---|---|---|
| Red | 35.25 | 2.28 |
| Yellow | 29.50 | 2.28 |
| White | 29.88 | 2.28 |
| Control | 34.33 | 2.28 |

**Table 7.** Nutritional analysis of the three onion varieties for the spring, summer, and winter trials.

| | Spring Trial | | | Summer Trial | | | Winter Trial | | |
|---|---|---|---|---|---|---|---|---|---|
| Nutrient | Red | White | Yellow | Red | White | Yellow | Red | White | Yellow |
| Crude Protein (%) | 14.0 | 13.4 | 11.5 | 8.6 | 11.3 | 9.3 | 15.2 | 10.8 | 12.7 |
| NDF (%) | 13.3 | 10.4 | 9.5 | 19.6 | 17.5 | 14.8 | 13.3 | 7.7 | 14.1 |
| ADF (%) | 9.7 | 9.9 | 8.4 | 14.9 | 13.8 | 11.0 | 10.0 | 6.7 | 12.0 |
| NEM, (Mcal/Lb) | 0.88 | 0.89 | 0.90 | 0.84 | 0.85 | 0.87 | 0.88 | 0.91 | 0.87 |

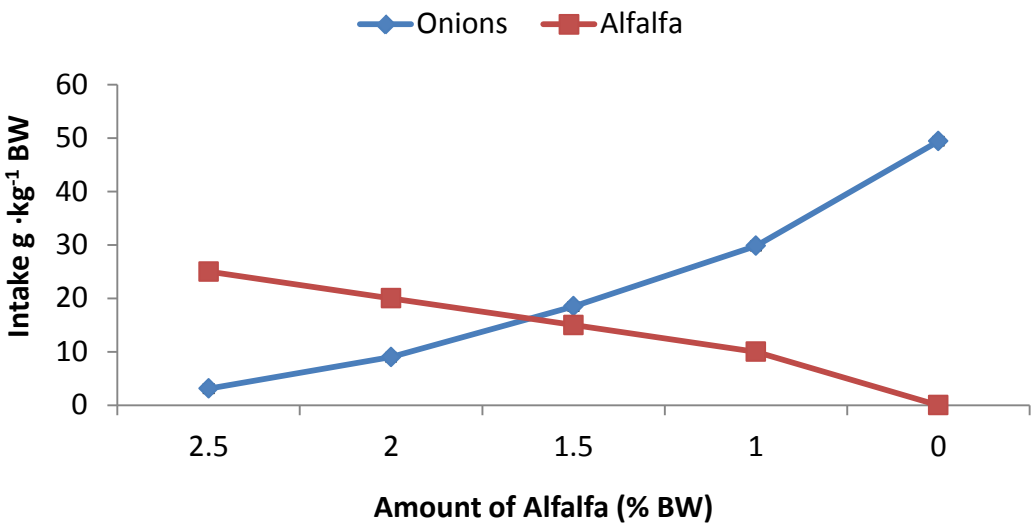


**Figure 3.** Onion and alfalfa intake (g·kg$^{-1}$ BW) of sheep for Trial 3.

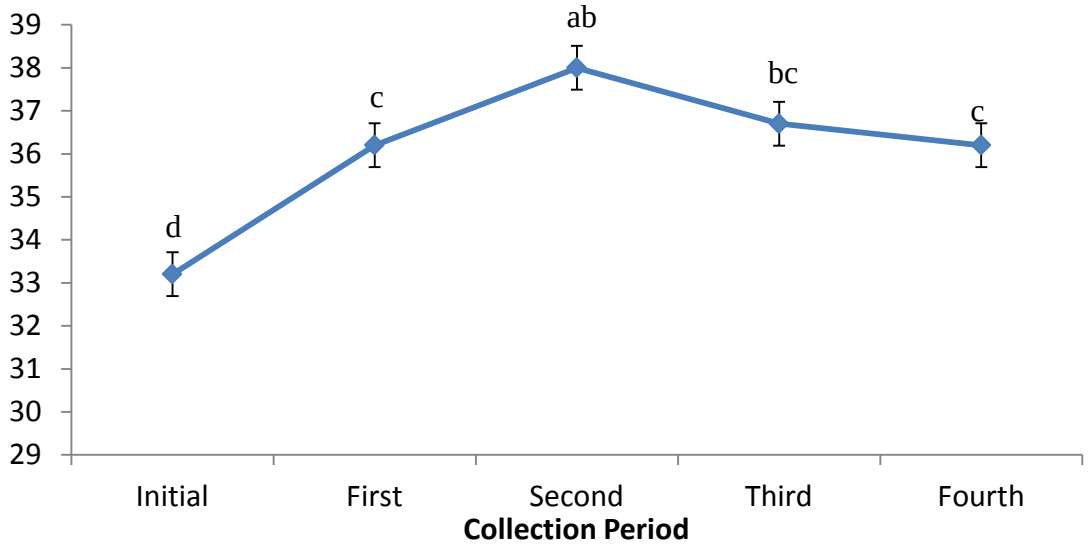


**Figure 4.** Packed cell volume by collection period for Trial 3.
[abcd] subscripts that differ are significantly different

Onions in Trial 3 revealed no nutritional differences between the three varieties that were offered (Table 6). Overall, the onions showed to have no differences nutritionally when the three seasons are compared (P>0.05).

**DISCUSSION**

Results of this study indicate that sheep have no preference for any one of the 3 varieties of onions (red, yellow, white) used in this study. Also, nutritional quality of the three varieties across seasons (spring, summer, winter) was similar. Sheep quickly adapted to eating onions and in many cases prefer eating onions even when other forages are present (McBride 2004). In addition, there were no signs of toxicosis or anemia in any of the trials in this study indicating that onions toxicity may not occur if intake is increased gradually.

In many other regions of the U.S. cull onions are used as a supplemental feed (McBride 2004). Onions reportedly range from 9-13 % crude protein, 83-90 percent TDN, 0.35% Ca, 0.40% P, 0.97 $NE_m$ with a water content of 90% (Lardy and Anderson 2003). The onions in this study had crude protein levels ranging from 8-15%, $NE_m$ from 0.84-0.91%, and 77-81%TDN, with moisture content in the 90$^{th}$ percentile. Calcium and phosphorus were not measured in this study. Forages in the Edwards Plateau region (including grasses, forbs, and browse) range around 5-19% crude protein, 31-70 % digestible organic matter, 0.06-0.22 P and 14-68% water (Clark 1981). A common maintenance ration used at the Angelo State University ranch contains 12% crude protein, 60 % TDN, 0.75% Ca, 0.28% P, and 1.37 $NE_m$. Ruminants need around 6 to 8 percent crude protein to maintain a healthy rumen, which onions easily could supply.

Onions are apparently high in L-cysteine. We attempted to measure L-cysteine levels in the onions used during the spring trial, but levels were undetectable. Dosing with L-cysteine has been shown to increase bitterweed intake while allowing animals to avoid hymenoxon-induced toxicosis (Rowe et al. 1980, Calhoun et al. 1986). Given onions' apparent levels of L-cysteine, others have suggested that onions may be an affective supplement to attenuate bitterweed toxicosis. In a preliminary trial, serum metabolite levels, that are indicative of toxicosis, were lower (P<0.05) in

sheep supplemented with onions (Campbell et al. unpubl. data). Conversely, Bundick (2008) reported that supplementation with onions did not improve intake. Reasons for the differences in results remain unclear. However, Campbell et al. was able to get sheep to consume a diet consisting of 75% onions while Bundick (2008) was not.

Cysteine, a sulfur containing amino acid, reacts with hymenoxon reducing bitterweeds toxicity. This is accomplished by cysteines sulfhydryl group binding with the toxic lactones in the rumen (Kupchan et al. 1970). Unfortunately, the over-consumption of onions can lead to anemia in animals (Cheeke 1998). Onions contain disulfides which are oxidizing agents that cause hemolysis of red blood cells. However, results from this study suggest that sheep may have adapted to the toxic compounds in onions that lead to anemia. Digestive physiology may be altered through exposure to poisonous plants early in life to the point that ruminants can experience toxicosis. This observation was first illustrated by Distel and Provenza (1991). Goats, at 6 weeks of age, were fed blackbrush (*Coleogne ramosissima* Torr.) daily. Blackbrush contains condensed tannins that are toxic to ruminants. Goats introduced to blackbrush early in life consumed 95% ($P<0.01$) more blackbrush than naïve goats, were more efficient at digesting blackbrush, and excreted more uronic acid apparently because of an increased ability to detoxify the tannins in blackbrush.

It is unclear if adaptation to toxic compounds in onions occurred. Furthermore, the mechanism of detoxification of onions is unclear. Toxic compounds are usually altered either through rumen adaptation or altered through liver functions.

Toxins are either detoxified in the rumen or liver. In the liver detoxification occurs by one of two reactions (Williams 1959). Phase I reactions alter existing functional groups or introduce a polar group into the non-polar compound (Bidlack et al. 1986). Phase II reactions conjugate polar groups of foreign compounds with endogenous cofactors (Bidlack et al. 1986). Both Phase I & II reactions alter the compounds making them more hydrophilic and easily excreted from the body (Bidlack et al. 1986; Nebbia 2001). Based on the results of packed cell volumes for each of the trials, anemia from consuming onions never occurred.

Methods to reduce bitterweed toxicosis have been under investigation for several years. The application of herbicide, namely 2, 4-D, to reduce bitterweed cover is very effective; however, the cost of application could prohibit its use (Bunting and Wright 1973). Supplementation with activated charcoal and the antioxidant Santoquin both reduced bitterweed poisoning. However, activated charcoal was difficult to use because it has such a fine particle size (Poage et al. 2000) and Santoquin being very unpalatable lowers intake itself (Calhoun et al. 1986; Ueckert and Calhoun 1988). Bundick (2008) supplemented with onions and soybean meal both high in L-cysteine to increase intake of bitterweed. Intake did not increase with either the soybean meal or the onions. Coffman (2009) used four high protein feeds (soybean meal, soybean meal/dried distillers grain, cottonseed meal, cottonseed meal/dried distillers grain) containing cysteine to increase bitterweed intake. Bitterweed intake increased when supplemented with soybean meal.

## IMPLICATIONS

Based on the results of this study onions could serve as a viable supplement. All three onion varieties are nutritious, and if intake is slowly increased onion toxicity should not occur. Future research should focus on feeding the three onion varieties with bitterweed to see if one variety is a better preventative of hymenoxon poisoning. Also, a pasture study should be conducted using onions as a supplement to determine if it is effective at preventing bitterweed toxicosis in a pasture scenario.

Bitterweed is an annual forb that grows in the late winter and early spring during this time many other forages are dormant. Range animals nutritional requirements are at their highest at this time. Supplementing in the winter would be ideal to prevent bitterweed toxicity in sheep.

Winter supplementation of cull onions is being used in several states in the U.S, especially in areas that grow onions. Use of onions as a supplement in western Texas is possible when cull onions are inexpensive. With the potential that onions have on reducing bitterweed toxicity, supplementation may be advantageous to ranchers in this area.

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## THE EFFECTS OF PROTEIN SUPPLEMENTATION ON BITTERWEED TOXICOSIS IN SHEEP

*Matthew C. Coffman, Cody B. Scott, and Corey J. Owens*

**ABSTRACT**

The objectives of this study were to determine if a supplemented protein diet high in escape protein or high in sulfur-containing amino acids would reduce toxicosis in sheep consuming bitterweed (*Hymenoxys odorata*). Forty Rambouillet lambs (28.3 kg) (n=40) with 8 lambs per treatment were placed in individual pens and allowed 7 days to adjust being fed alfalfa pellets at 2.5% BW, and their treatment diet. Treatments and bitterweed were fed for 15 days. Treatment 1 received a cottonseed meal (CSM) based supplement. Treatment 2 received CSM and DDG. Treatment 3 received a soybean meal (SBM) feed, and Treatment 4 received SBM and DDG. Treatment 5 served as the control group (only alfalfa pellets). Supplements were isonitrogenous (37%). Lambs fed the SBM supplement ate more ($P \leq 0.05$) bitterweed than lambs fed alfalfa alone. CSM, CSM/DDG, or SBM/DDG did not improve bitterweed intake. Based on the results of this study, landowners should consider feeding SBM-based supplements to prevent bitterweed toxicosis in sheep.

**INTRODUCTION**

In 1962, it was estimated that annual losses in livestock production from bitterweed (*Hymenoxys odorata*) toxicity were $3.6 million in the Edwards Plateau Region of Texas alone (Witzel et al. 1974). Calhoun et al. (1980) suggested that bitterweed is the most serious toxic plant problem faced by sheep producers in Texas. With the ever-increasing costs of production in today's livestock industry, there is need to develop methods to prevent these losses and problems associated with bitterweed.

Bitterweed 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. It is a many-branched plant with a slender taproot that can range anywhere from 7 to 50 cm tall when mature. Bitterweed flowers from the early spring to summer, and broken or bruised leaves and stems give off a distinct aromatic odor. Plants retain their toxicity even when dead and dry (Stubbendieck et al. 2003). Historically, bitterweed occupied low-lying areas such as dry lakebeds or areas where silting was common (Ueckert and Calhoun 1988). However, periodic droughts, coupled with the effects of overgrazing rangelands, allow this plant to encroach onto more productive rangelands (Hardy et al. 1931).

Kim et al. (1975) first identified the primary toxin in bitterweed as hymenoxon. Hymenoxon is a sesquiterpene lactone with an exocyclic methylene group conjugated with the lactone carbonyl. 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 (Rowe et al. 1973).

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 cottonseed meal and distiller's dried grain (DDG) based ration (George et al. 2010). Goats also increased juniper consumption when being supplemented with alfalfa, CSM and SBM (Campbell et al. 2007). Supplementation of lambs with barley and activated charcoal increased intake of the shrub big sagebrush (*Artemesia tridentata*) (Banner et al. 2000). In addition, protein supplementation seems to reduce the likelihood of bitterweed toxicity (Calhoun et al. 1989). However, Bundick et al. (2008)

failed to show an increase in bitterweed consumption when sheep were fed SBM or onions as a supplement.

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. Soybean meal is highly soluble in the rumen and contains moderate to high levels of L-cysteine. Feeding supplements containing soybean meal could potentially alleviate some bitterweed toxicity problems.

Protein supplements like cottonseed meal and DDG contain glucogenic amino acids that are not degraded in the rumen. Glucogenic amino acids 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., 1980) 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. Forty recently weaned Rambouillet lambs (28.3 kg, approximately 5 months of age) were used in this experiment with 8 lambs used per treatment. Lambs were separated into individual pens (1 m X 1.5 m), and allowed 7 days for a pen-adjustment period. During this adjustment period they were fed their selected treatment diet and alfalfa pellets. Alfalfa pellets were fed at 2.5% BW daily to meet the animal's 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 and thoroughly mixed before feeding.

Animals in each treatment received the same source of protein each day before bitterweed was fed. Treatment 1 received a supplement with cottonseed meal as the protein source (Table 1). Treatment 2 received a supplement with cottonseed meal and distiller's grain as the protein source (Table 1). Treatment 3 received a supplement with soybean meal as the protein source (Table 1), and Treatment 4 received a supplement with soybean meal and distiller's grain as the protein source (Table 1). 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. Animals were fed one of the 4 treatments daily for 15 days during the bitterweed feeding trial, with intake being recorded daily. Protein supplementation was offered everyday 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

**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% |
| Solulac-Brewers Grain | -- | 16.2% | -- | 26.78% |
| 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% |
| 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

intervals of 25 g on days after that. Sheep were then offered alfalfa pellets at 2.5% BW to meet maintenance requirements from 1500 hours to 1700 hours. The control group was offered dried bitterweed first and then their complete diet of alfalfa pellets afterward. 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. 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 sheep 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 2007).

## RESULTS

### Pretrial

Protein intake was similar ($P>0.05$) across treatments, and across days. Sheep were fed protein supplements for a 7-day pen adjustment period. Animals were reluctant to consume their diets at first but intake was adequate (Fig 1). Sheep readily consumed alfalfa pellets throughout the pre-trial, with only some minor fluctuation in the control group. No variations in intake were significant.

### Bitterweed Feeding Trial

Alfalfa pellet consumption also remained similar ($P>0.05$) for treatments during the feeding trial. Consumption of all protein diets remained relatively constant after day 7, up until

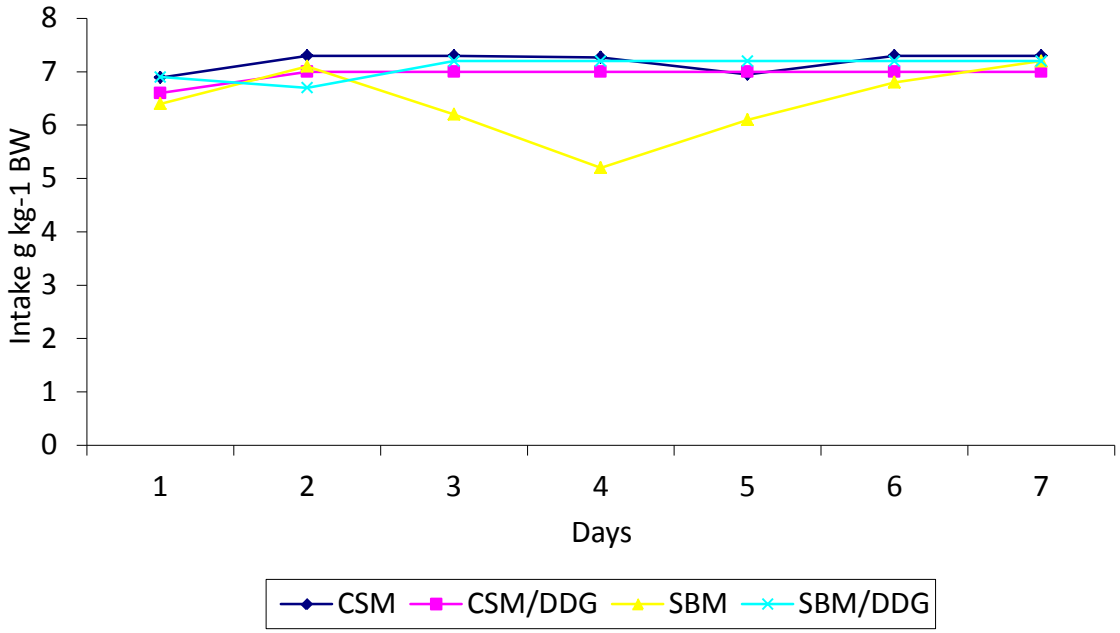


**Figure 1.** Average intake of protein supplements during 7-day pen adjustment period.

day 15 when the CSM treatment group decreased consumption, though not significantly, and slowly increased consumption again up to the end of the trial (Fig 2).

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 3). The treatment X day interaction was not significant when data were analyzed using analysis of variance, however our day effect was significant. Treatment means for bitterweed intake varied from 0.71 $\pm$ 0.09 g/kg BW for the control group, to 1.04 $\pm$ 0.09 g/kg BW for the treatment receiving SBM based diet.

When orthogonal contrasts were used to compare treatment means, differences were evident. Sheep receiving the SBM supplement consumed more bitterweed than those receiving no supplement (Fig 4). Bitterweed consumption of other groups did not differ statistically from the control group (Fig 4).

Treatments were all fed the same amount of protein, which should have been adequate to see an average gain of 150 g/day. However, the CSM treatment group actually showed a trend towards weight loss after the experiment. The control treatment gained an average of 1.4 $\pm$ 0.74 kg. The CSM/DDG treatment group gained an average of 1.7 $\pm$ 0.74 kg. The SBM treatment group gained an average of 1.74 $\pm$ 0.74 kg, and the SBM/DDG treatment group gained an average of 0.21 $\pm$ 0.74 kg. The CSM treatment group lost an average of 0.4 $\pm$ 0.74 kg. No animals displayed clinical symptoms of bitterweed toxicosis during the experiment.

## DISCUSSION

The results of this study indicated that sheep receiving a SBM protein supplement increased bitterweed intake. Sheep typically consume bitterweed to meet nutritional requirements on rangelands when alternative forage is limited. When ruminants are unable to meet their nutritional requirements (i.e., late fall, winter) bitterweed intake increases. 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

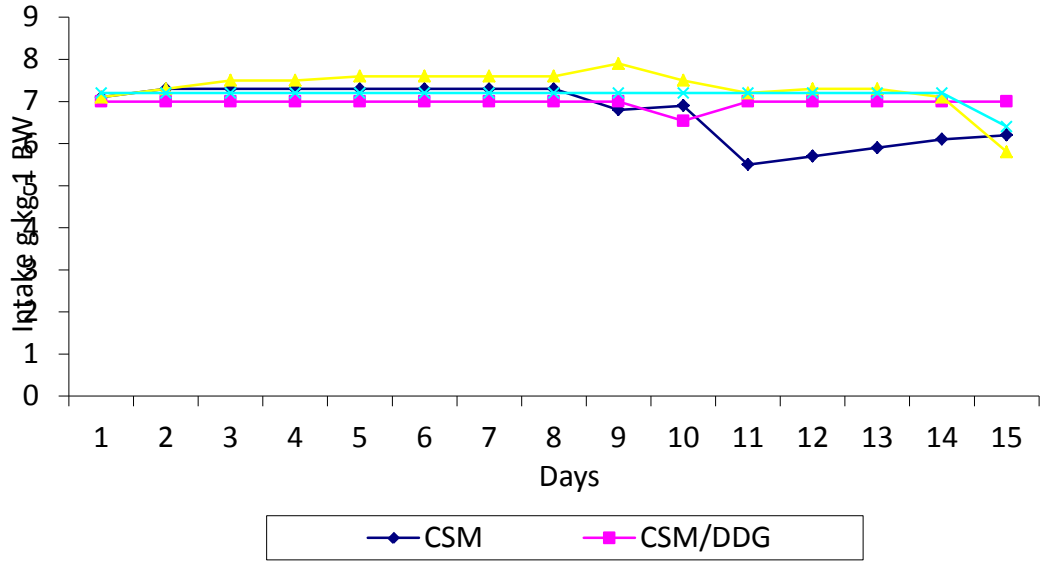


**Figure 2.** Average intake of protein supplements during 15-day feeding trial.

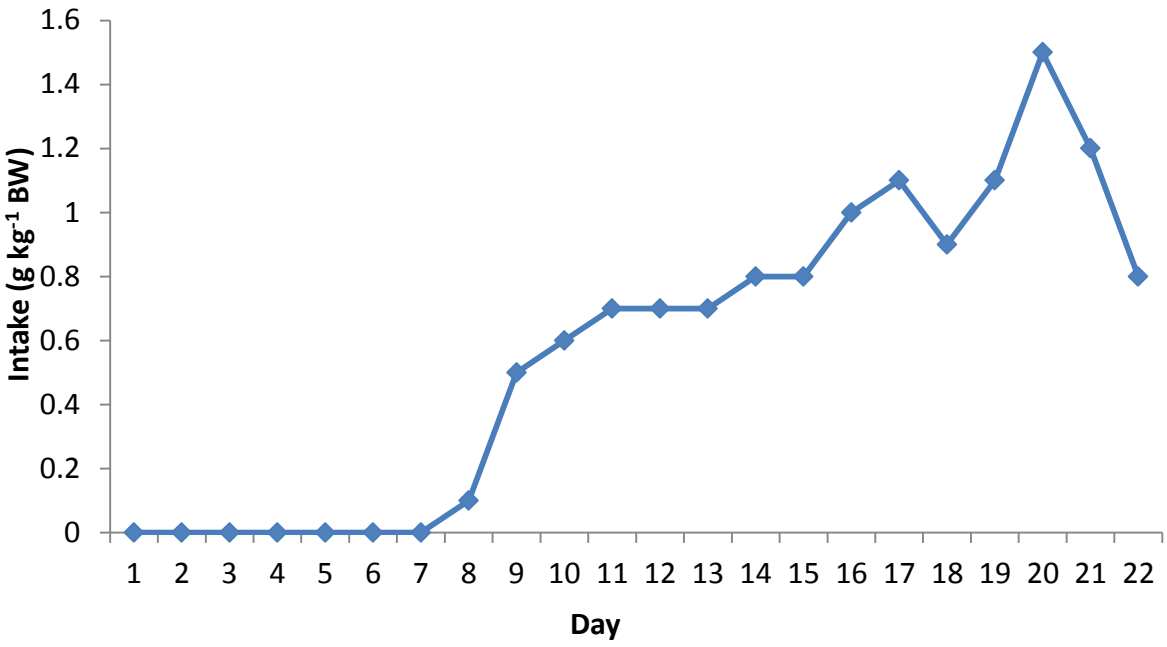


**Figure 3.** Average intake of bitterweed for 22-day feeding trial.

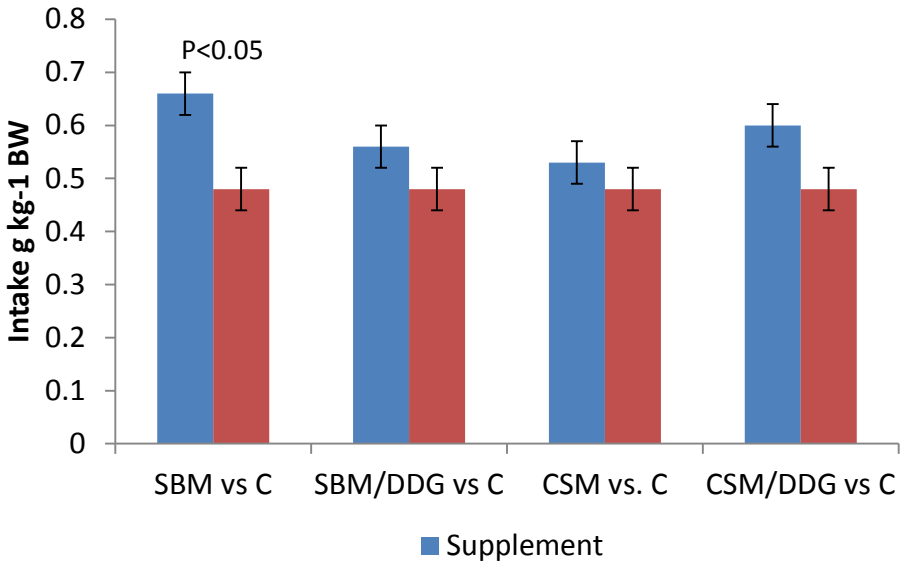


Figure 4. Bitterweed intake from each supplement group compared to bitterweed intake by lambs receiving no supplementation (control).

protein requirements. Bundick (2008) attempted using onions, also high in L-cysteine, or soybean meal as a supplement to increase bitterweed intake. This proved ineffective, however, possibly because of the animal's reluctance to consume a diet of onions. The variety of onions might have contained lower concentrations of L-cysteine as well. It is unclear why protein supplementation with SBM improved intake in this study, but not in the Bundick (2008) study. Future studies should re-evaluate feeding SBM based supplements to reduce bitterweed toxicosis.

Most plant-induced toxicity problems occur in overgrazed situations or when animals are unable to meet their nutritional requirements. For example, cattle avoid locoweed unless alternative forage is unavailable. Recent research has also reported that incidences of “crooked calf disease” from consumption of lupines increases once alternative forages have been removed (Ralphs et al., unpubl. data). Regardless of the supplementation program used, producers should avoid overgrazing rangelands particularly when poisonous plants like bitterweed are present.

The hypothesis that protein sources high in amino acids that escape rumen digestion would improve bitterweed intake was rejected. Previous research illustrated that supplements containing both CSM and DDG improved intake of the toxic shrub redberry juniper. Goats supplemented with a 37% protein supplement that contained both CSM/DDG consumed more juniper that goats receiving alfalfa alone (George et al. 2010).

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 (*Artemisia tridentata*). Supplemented lambs ate an average of 304 g of sagebrush versus control sheep that ate an average of 248 g. Supplemented sheep fed a 20% crude protein diet over a 10% crude protein diet had decreased signs of toxicosis from hymenoxon (Calhoun et al. 1989).

When ruminants ingest toxins, they are typically absorbed, metabolized, buffered and excreted from the body in one or two ways. Soybean meal is high in sulfur containing amino acids, particularly L-cysteine (Baize 1999), that allow for detoxification in the rumen. When sheep were

feed the toxic plant Birds-foot trefoil (*Lotus corniculatus*) that contains condensed tannins, the total and apparent digestibility of S-labeled cysteine was reduced in the small intestines, suggesting interactions of the amino acids and the plant toxins prior to reaching the abomasum (Wang et al. 1996). Similar results were reported by McNabb et al. (1993) when sheep were fed condensed tannins in the plant Big trefoil (*Lotus pedunculatus*). Cysteine reactivity was also lowered in proteolytic digests from sheep fed tannin containing faba beans and lentils. The binding of tannins to the amino acid structures might have protected the disulfide bonds from the reducing agent used in the lab (Carbonaro et al. 1996).

It is important to note that none of the animals in this experiment showed symptoms of bitterweed poisoning. All of these sheep were on an adequate plane of nutrition, and were meeting their requirements for maintenance and growth. It is hypothesized that animals will limit consumption of toxins when nutritionally stressed because their tolerance to secondary plant compounds is lowered (Illius and Jessop, 1995). If a food is toxic to an animal, no amount of exposure is likely to increase intake beyond a point of toxic satiation or a threshold of feedback (Distel and Provenza, 1991). 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).

Another possible reason for the daily increase in bitterweed consumption is that there are physiological and morphological changes that increase the body's ability to metabolize plant toxins. In Distel and Provenza's 1991 study, they showed that goats being fed the toxic plant blackbrush excreted 63% more uronic acids per unit of body weight, and 32% more nitrogen in feces than goats not exposed to blackbrush. The mass of the reticulorumen was also 30% greater than inexperienced goats indicating that goats had adapted mechanisms for conjugating, and excreting the condensed tannins and phenols. Frost et al, (2003) however, failed to detect significant differences in bitterweed consumption from sheep reared in bitterweed infested rangelands, and those reared in bitterweed free rangelands. This could illustrate that the toxic effects of hymenoxon are severe enough that physiological adaptations in sheep are inadequate to protect them. Thus, supplementation may be the only viable option for protecting sheep on bitterweed infested rangelands.

## IMPLICATIONS

Bitterweed is a cool season annual forb that is green and growing in pastures 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 (Rowe et al., 1980, Calhoun et al., 1989, Banner et al., 2000, Campbell et al., 2007, George et al., 2009). Most pasture supplementation in west central Texas begins around mid to late November when forage is becoming less available, and bitterweed plants are growing. Producers that have had bitterweed problems in the past 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 bind with the toxin.

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## THE EFFECTS OF BITTERWEED INGESTION ON REPRODUCTION IN RAMBOUILLET RAMS

*Derrick K. Fuchs, Cody B. Scott, Micheal W. Salisbury, and Corey J. Owens*

**ABSTRACT**

Bitterweed (*Hymenoxys odorata*) is a toxic plant common to central Texas and is known to cause chronic, subacute and acute toxicosis in sheep. However, little is known of bitterweed's effect on reproduction in sheep. This study was conducted to determine if bitterweed ingestion delayed puberty or affected sperm production in yearling Rambouillet rams. Twenty-five rams were used and randomly allocated into either the bitterweed treatment or the control treatment. The bitterweed treatment was dosed with ground bitterweed (0.2% BW) for 3 days via oral gavage. Intake of a novel food was paired with dosing with bitterweed to assess toxicosis. Serum metabolite levels were also monitored to assess soft tissue damage from toxicosis. Bitterweed toxicosis caused reduced intake and elevated serum metabolite levels, but seemed to not impact puberty or sperm production. The control treatment maintained intake and serum metabolite levels remained within the normal levels. Growing ram lambs may consume bitterweed and suffer from toxicosis. As long as they survive toxicosis, reproductive potential should persist.

**INTRODUCTION**

Bitterweed (*Hymenoxys odorata*) is a toxic plant common in central and western Texas (Cheeke 1998). Bitterweed contains hymenoxon, a sesquiterpene lactone, which causes subacute and acute toxicosis (Hill et al. 1979; Terry et al. 1983; Kim et al. 1987). Symptoms range from stiffness of lambs to soft tissue damage in the lungs, kidneys, and liver and even into the central nervous system and eventual death (Witzel et al. 1974; Hill et al. 1980). Animals typically die within a few days after ingesting a lethal dose (Ueckert and Calhoun 1988). Sheep may recover from bitterweed toxicosis if intake does not exceed lethal levels ($LD_{50}$ = 0.264% BW) (Calhoun et al. 1981).

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 nutritious 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. 1998). Because of the lack of alternative forage, sheep are often forced to consume bitterweed, resulting in toxicosis and usually death thereafter.

In the livestock industry, bitterweed is known to cause livestock death, but little is known of its effects on reproductive functions and development of livestock, when sublethal levels are consumed. Dusek (2003) illustrated that bitterweed reduced reproductive development in ewe lambs. Unfortunately, there is still very little known of bitterweed's effects on rams. Broom snakeweed (*Gutierrezia sarothrae*) is known to reduce reproductive performance of cattle when consumed (McDaniel and Ross 2002). Both broom snakeweed and bitterweed contain sesquiterpene lactones (Cheek 1998). Given that both broom snakeweed and bitterweed contain similar toxins, it seems likely that bitterweed could affect reproductive performance/development in sheep as well. It is known that bitterweed toxicity causes central nervous system (CNS) depression and soft tissue damage, which should interfere with hormone levels in both rams and ewes and cause reproductive problems (M.W. Salisbury, pers. comm.). Accordingly, this study was designed to determine if bitterweed reduces reproductive performance of rams. Data from this study will be combined with previous research regarding bitterweed's effect on ewe reproductive performance. The objectives of this study were to determine if bitterweed ingestion interferes with reproductive development in Rambouillet ram lambs.

## MATERIALS AND METHODS

This experiment took place at the Angelo State University Management, Instruction and Research (MIR) Center, San Angelo, Texas, to determine the effect of bitterweed ingestion on reproductive performance of Rambouillet rams. Twenty five recently weaned Rambouillet rams were randomly allocated to 2 treatments. Treatment 1 was the control treatment containing the rams not dosed with bitterweed. Rams in Treatment 2, were dosed with bitterweed at 0.2% BW. Prior to dosing, rams were placed in individual pens (3 m X 6 m) and allowed a 14-day adjustment period. Rams were fed Ram 20 (Table 1), a feed mixed at the MIR (2.5% BW), and provided fresh water to exceed maintenance requirements (NRC 2007).

The bitterweed that was used in this study was collected in the spring 2009 at the Texas Range Station in Barnhart, Texas. Before dosing, bitterweed was ground to pass through a 2 mm screen using a Wylie Mill grinder. During dosing, ground bitterweed was mixed with 200 ml of distilled water and dosed to individual rams using oral gavage. Rams were dosed with bitterweed once per day for 3 days after the allowed adjustment period.

A novel food (rolled barley) was offered to individual rams for 15 minutes each day prior to dosing with bitterweed. The rolled barley also was offered to each ram for 10 days prior to dosing until all 200 g were consumed. Typically, bitterweed results in the formation of a conditioned food aversion (Calhoun et al. 1981; Poage et al. 1998). Intake of a novel food, like rolled barley, should decline because of aversive postingestive feedback from bitterweed dosing (Provenza 1995). Thus, intake of rolled barley was used to quantify the rams' response to toxicosis. In addition, intake of the basal diet of Ram 20 was monitored throughout, prior to and throughout dosing with bitterweed.

Semen quality (concentration, motility, color) was compared among treatments by artificially ejaculating rams throughout the study. In addition, serum was separated from whole blood samples collected throughout the study to identify any soft tissue damage from bitterweed ingestion. Blood samples were taken via jugular venipuncture prior to the animals being exposed to bitterweed on day 2 of dosing and on day 4 of dosing. These samples were placed in a centrifuge to separate the serum and then stored in a -80$^{\circ}$C freezer. Following the completion of the study, the samples were sent to the Texas Veterinary Medical Diagnostic Laboratory in College Station, Texas to be analyzed. These samples were analyzed particularly for serum aspartate transaminase (AST), gamma glutamyltransferase (GGT), blood urea nitrogen (BUN), bilirubin, creatinine, and creatinine kinase (CK). Serum samples were collected initially to establish a baseline for the study and every 24 hours during the 4 days of dosing. Thereafter, semen samples were collected every 14 days over a 56 day period.

This experiment was a completely randomized design, with the individual rams nested with treatments serving as replications. Data was analyzed using repeated measures analysis of variance with day of collection as the repeated measure (Hicks 1993). Means were separated using Tukey's LSD test when $P \leq 0.05$. Data was analyzed using the statistical package JMP (SAS 2007).

## RESULTS

Dosing with bitterweed resulted in rams experiencing aversive postingestive feedback and the formation of an aversion to rolled barley (Fig. 1). 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% body weight 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 (Treatment X Day interaction, $P < 0.05$; Fig. 1). By day 14 of the study (three days of dosing with bitterweed) rams refused to eat barley.

Table 1. Ingredient and nutrient content of the ration (RAM 20) used to meet maintenance requirements of rams. 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 $kg^{-1}$) | 2.8 |
| Crude fiber | 14.1 |
| TDN | 63.0 |

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

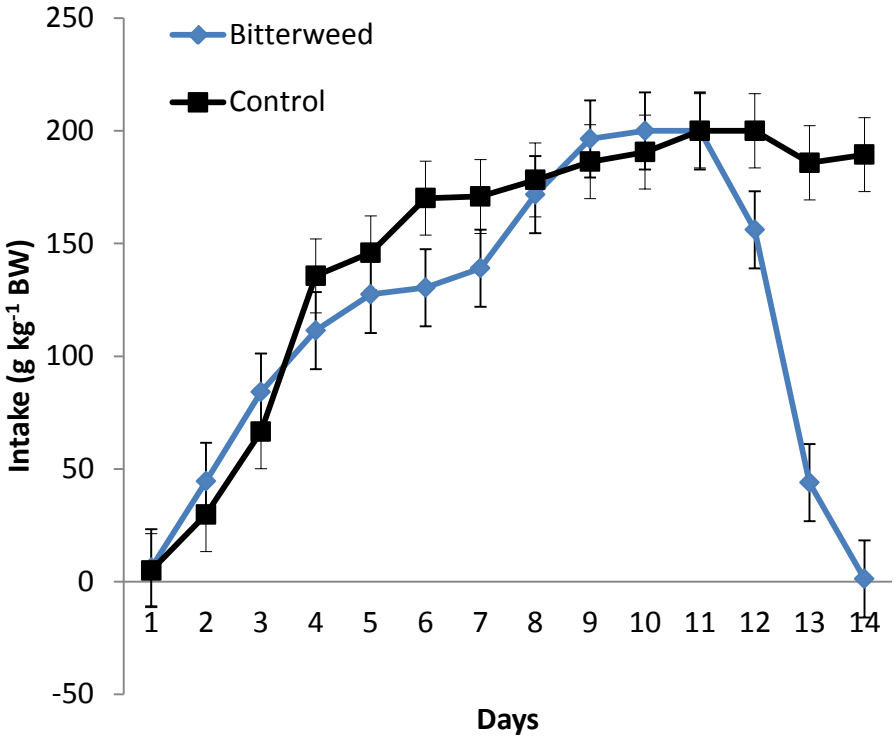


Figure 1. Average daily barley intake (g $kg^{-1}$) for rams dosed with bitterweed (0.2 % BW) or not dosed (control). Dosing began on day 10.

Dosing with bitterweed also reduced ($P < 0.05$) intake of the RAM 20 basal ration at the end of the study (treatment X day interaction differed, $P < 0.05$; Fig. 2). Ram 20 intake for rams dosed with bitterweed declined on days 13 and 14. Dosing with bitterweed stopped after 3 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 lost weight, but weight change was similar ($P > 0.05$) between treatments (Table 2). Serum metabolites differed between treatments and across days of feeding (treatment X day interactions were significant; $P < 0.05$) for all metabolite levels except creatinine kinase (CK) levels. Levels of each increased for rams dosed with bitterweed especially when comparing beginning and ending values (Table 3). By the end of the study, serum metabolite levels were above the normal range of levels for all metabolites except CK. Levels of CK differed for rams in the control but were within the range of healthy animals by the end of the study.

Semen concentration, color and motility were similar ($P > 0.05$) among treatments (Table 4) but differed across days of collection (Table 5). Concentration, color, and motility improved across days of collection for both treatments. The treatment X day interactions for concentration, color and motility did not differ ($P > 0.05$).

## DISCUSSION

Based on the results of this study, it appears that consuming bitterweed does not interfere with sperm production in Rambouillet yearling rams. The semen quality and concentration declined during the last collection period apparently because of homosexual activity that was observed among rams immediately before the collection was taken.

Bitterweed did induce toxicosis as evident from the decline in barley intake, decline in the basal diet intake, and elevated serum metabolite levels. Rams were suffering from soft tissue damage apparently because of bitterweed-induced toxicosis. One ram died on the study. The ram was one of the individuals dosed with bitterweed and died before the end of the study. 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 4 out of 10 sheep while others showed no signs of toxicity even as dosing level increased (Calhoun et al. 1981). The one individual that died in this study may have been more susceptible to bitterweed toxicosis.

Bitterweed causes aversive postingestive feedback and the formation of conditioned food aversions (Calhoun et al. 1989; Poage et al. 1998). Rams apparently associated intake of barley with dosing with bitterweed. This association resulted in a rapid decrease in barley intake. Rams also reduced intake of basal ration in an attempt to avoid aversive postingestive feedback.

The level of toxicosis experienced by rams in the study was sufficient to cause some soft tissue damage given the changes in serum metabolite levels (Calhoun et al. 1981; Frost et al. 2003). When soft tissue damage occurs as a result of plant-induced toxicosis, levels of AST, GGT, bilirubin, blood urea nitrogen, creatinine are typically altered (Cornelius 1989). Levels of each 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 (not measured in this study) decreased while blood urea nitrogen, creatinine, bilirubin increased. In addition, AST levels increased at the highest levels of bitterweed dosing. Results of this study suggest that rams were suffering from toxicosis once dosing with bitterweed began.

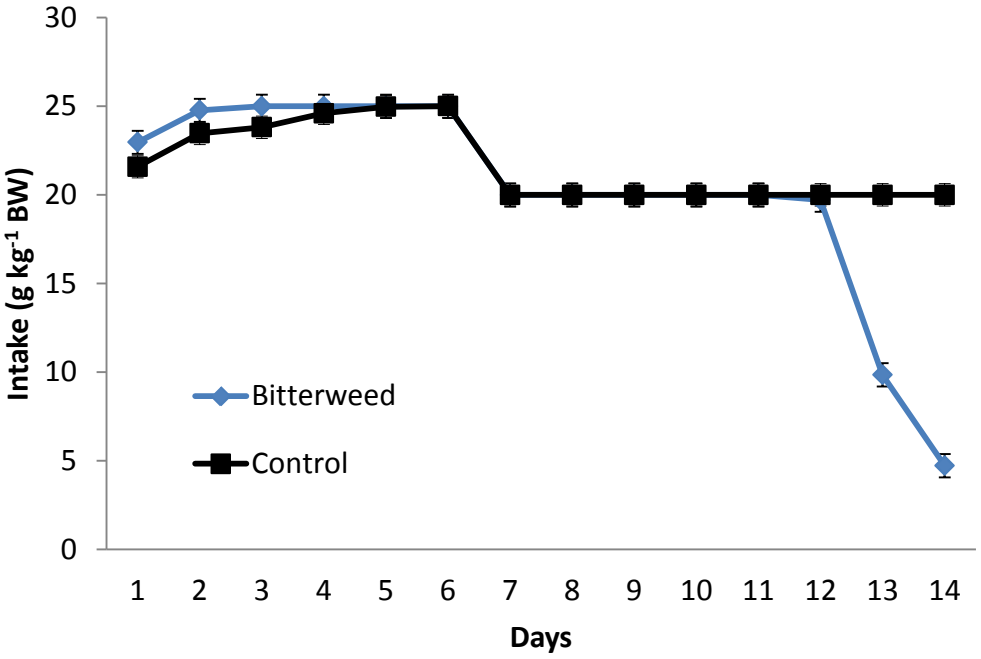


Figure 2. Average daily Ram 20 intake (g $kg^{-1}$) for rams dosed with bitterweed (0.2 % BW) or not dosed (control). Ram 20 was fed as the basal ration to meet maintenance requirements. Dosing began on day 10.

Table 2. Average weight (kg) of rams at the beginning and end of the feeding trial. Rams were either dosed with bitterweed or not dosed (control).

| Treatment | Initial | Final |
|---|---|---|
| Bitterweed | 54.8 | 52.2 |
| Control | 55.8 | 58.4 |

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

| Treatment/Serum Metabolite | Serum Collection | | | |
|---|---|---|---|---|
| | Initial | Middle | End | Normal Range |
| **Dosed with bitterweed** | | | | |
| BUN (mg/dl) | 17.2[b] | 33.1[a] | 37.1[a] | 12-32 |
| Creatinine (mg/dl) | 0.6[c] | 1.2[b] | 1.6[a] | 0.3-1.3 |
| Bilirubin (mg/dl) | 0.1[b] | 0.1[b] | 0.4[a] | <0.3 |
| CK (U/l) | 172.7 | 174.0 | 185.7 | 15-213 |
| AST(U/l) | 105.6[b] | 236.1[b] | 435.4[a] | 51-130 |
| GGT (U/l) | 72.9[b] | 136.8[b] | 241.2[a] | 34-82 |
| **Control** | | | | |
| BUN (mg/dl) | 19.9 | 17.2 | 16.7 | 12-32 |
| Creatinine (mg/dl) | 0.6 | 0.7 | 0.8 | 0.3-1.3 |
| Bilirubin (mg/dl) | 0.1 | 0.1 | 0.1 | <0.3 |
| CK (U/l) | 222.0[a] | 235.8[a] | 173.8[b] | 15-213 |
| AST (U/l) | 104.8 | 80.2 | 82.6 | 51-130 |
| GGT (U/l) | 69.7 | 72.4 | 74.5 | 34-82 |

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

Table 4. Semen color, motility, and concentration (million cells $ml^{-1}$) of rams dosed with bitterweed (0.2% BW) or not dosed. Samples were collected 14, 29, 44, and 56 days after dosing with bitterweed. Semen color and motility were visually scored on a scale of 0 to 5, where 0=clear or no movement and 5=cloudy or excellent motility.

| Treatment | Color | Motility | Concentration |
|---|---|---|---|
| Bitterweed | 2.4 | 2.4 | 98.2 |
| Control | 2.4 | 2.3 | 84.3 |

Table 5. Pooled average semen color, motility, and concentration (million cells $ml^{-1}$) of rams dosed with bitterweed (0.2% BW) or not dosed. Samples were collected 14, 29, 44, and 56 days after dosing with bitterweed. Semen color and motility were visually scored on a scale of 0 to 5, where 0=clear or no movement and 5=cloudy or excellent motility. Treatment means were similar (P>0.05).

| Day of Collection | Color | Motility | Concentration |
|---|---|---|---|
| 1 | 1.4 | 1.2 | 38.6 |
| 2 | 2.1 | 2.1 | 90.1 |
| 3 | 3.2 | 3.4 | 162.5 |
| 4 | 3.0 | 2.8 | 73.7 |

Given that both bitterweed and broom snakeweed contain toxins classified as sesqueterpine lactones, it seemed likely that bitterweed would impair reproductive performance. Dusek (2003) suggested that bitterweed intake reduced reproductive development in female Rambouillet ewe lambs. Conversely, the current study failed to agree with those of Dusek (2003). The results of the current study are consistent with Calhoun et al. (1981) who reported that intake and serum metabolite levels are altered because of bitterweed ingestion and subsequent toxicosis. 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 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. Similar observations have been noted with other toxins resulting in reproductive issues (Cheeke 1998; Panter et al. 2002). At the very least, libido should decline as males experience internal malaise.

## IMPLICATIONS

Although the study did not show any affect on reproduction in males, bitterweed ingestion could cause production and growth problems when it is consumed at a toxic amount. Animals typically avoid plants that cause aversive postingestive feedback (Provenza 1995). Given that bitterweed ingestion causes aversive postingestive feedback and the formation of conditioned food aversions (Calhoun et al. 1981; Poage et al. 1998), 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 (Taylor and Ralphs 1992).

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# MESQUITE SEEDLING DISAPPEARANCE UNDER GOAT BROWSING

*Landon W.T. Pyle, Cody B. Scott, and Corey J. Owens*

## ABSTRACT

Mechanical and chemical treatments of mesquite (*Prosopis glandulosa)* reduce cover but are expensive with limited longevity of control. Biological control of mesquite is difficult because toxins within the plant limit intake. Objectives of this study were to determine if goats could control mesquite from the seedling stage and determine if repeated defoliation would lessen seedling survivability. Trial 1 was a pasture study utilizing four pastures. Seedlings (n=40) were transplanted to two 10 x 10 m plots per pasture. Each plot was monitored twice weekly for herbivory and mortality. Trial 2 consisted of seedlings that were subjected to different frequencies of defoliation in a greenhouse. There were no differences ($P>0.05$) in seedling disappearance between treatments. Root mass was greater ($P<0.05$) for seedlings that were not clipped and foliage mass did not differ. We contend that biological control of mesquite at the seedling stage by goats is not an effective control method. Mesquite seedlings will recover from browsing under optimal growing conditions as long as browsing occurs above the cotylodons.

## INTRODUCTION

Honey mesquite (*Prosopis glandulosa*) is the largest brush problem in Texas, infesting around 22 million ha of rangeland (Jacoby et al. 1990). Mesquite invades range sites by means of seeds, which are transported by herbivores, wind, and water (Scifres and Brock 1970). Chemical and mechanical control efforts can reduce mesquite cover but are expensive treatments with limited longevity of control (Jacoby et al. 1990; Ansley et al. 2001, 2004). Treated areas may have to be retreated in 3-5 years due to rapid reinvasion. Biological control is effective in reducing cover of other invasive shrubs like juniper (*Juniperus* sp.), but biological control of mesquite is difficult because the toxic agents within the plant limit intake (Alder 1949; Dollahite and Anthony 1957; Tabosa et al. 2000). Although these toxins have not been completely identified, it is now known that they are very potent in nature (Alder 1949; Dollahite and Anthony1957; Tabosa et al. 2000).

Mesquite seeds are encapsulated in a hard coat that must be scarified before rapid germination will take place; passage of seeds through ruminant digestive tract (i.e scarification) enhances germination (Scifres and Brock 1972). Consumption of mesquite seeds by livestock and wildlife appears to be the primary vector of seed dispersal (Kramp et al. 1998). Although this holds true for most species of livestock, goats appear to disperse few viable seeds in feces (Kneuper et al. 2003).

Cook et al. (2008) showed that goats can consume large amounts of mesquite pods, up to 60% of their diet for short periods of time without experiencing a decrease in intake, a decrease in fecal output, or soft tissue damage.

When toxicosis occurs from mesquite pod ingestion, it is typically after 2-10 months of pod consumption (Burrows et al. 2001). According to Cook et al. (2008), long-term control of mesquite will only be successful when seed dispersal and seedling establishment are suppressed.

Weltzin et al. (1998) showed that mesquite seedlings clipped several times in field trials were much less likely to survive than seedlings clipped in the laboratory. This is to be expected simply because of abiotic stress or resource limitations that mesquite seedlings would naturally experience in a rangeland setting. With this knowledge, future management techniques should focus on slowing the reproduction and establishment of mesquite. This experiment focused on controlling mesquite from the seedling stage and determined whether or not repeated defoliation by browsing goats lessened seedling survivability. If repeated herbivory by goats lowers survivability of seedlings, this coupled with mesquite pod consumption by goats, may offer an effective suppression technique that reduces mesquite establishment and invasion after brush control

techniques have been applied. The objectives of this study were to: determine if goats browsed mesquite seedlings in a pasture setting, and determine if herbivory increased the mortality rate of mesquite seedlings.

## MATERIALS AND METHODS

This study consisted of two trials. Trial 1 was a pasture study, where mesquite seedling disappearance/survivability was monitored both with and without goat browsing. For Trial 2, mesquite seedlings were subjected to different frequencies of defoliation in a greenhouse with biomass accumulation and mortality rates recorded.

Four pastures (22a, b, c, & d) ranging from 24-28 ha were utilized on the Angelo State University Management, Instruction, and Research (MIR) Center. Two of the pastures were stocked at a moderate stocking rate (1 AU/ 8.09 ha) with Boer nannies, while the other two served as controls (i.e., no goat browsing). Mesquite seedlings were germinated in a greenhouse located at the MIR Center. Mesquite pods were collected from randomly selected trees on the MIR Center in the summer when pod senescence occurred. Seeds were separated from pods by removing the exocarp. Seeds where planted at a depth of 0.6 cm in a 10.2 cm by 10.2 cm plastic container containing a commercial potting mix and watered daily. Once germinated, seedlings were transplanted into two 10 x10 m plots in each pasture. Watering took place as necessary to encourage seedling establishment. One plot was constructed close to water to encourage herbivory activity, while the other was constructed at a random location in the pasture. All plots were placed in locations that have comparable herbaceous and woody cover. There were five randomly placed mesquite seedlings within each plot. Each seedling was flagged and numbered for easy identification. To determine available forage and herbaceous species composition, five 1/3m$^2$ clip samples were collected per plot. All herbaceous forage was hand-clipped to ground level by species. Samples were dried at 60° C for 48 hrs for dry matter determination. Shrub density and composition where recorded as well.

Each plot was monitored twice weekly for herbivory activity and seedling mortality until frost. Seedling mortality was determined with the observance of a dead seedling after being browsed upon or a seedling that had been uprooted. Seedlings that died or were uprooted were replaced weekly. Overall weather conditions were monitored by utilizing the weather station located at the MIR Center.

Mesquite seedlings were germinated using the same method as in Trial 1. Seedlings remained in the greenhouse where the climate was controlled and watered daily. There were four separate treatments containing 10 seedlings each and two replicates. Seedlings that germinated in the 10.2 cm by 10.2 cm pots were transplanted into 17.8 cm by 17.8 cm containers after germination to insure sufficient room for root development. Treatment 1 served as a control (no clipping), and Treatment 2 was clipped above the cotyledonary node three times. Treatment 3 was clipped twice, and Treatment 4 once. Seedling mortality and regeneration was observed. The trial lasted for 127 days with total above ground and below ground biomass recorded on a dry matter basis. Roots and above ground biomass was separated and placed in a drying oven at 60° C for 48 hrs for dry matter determination.

Data from Trial 1 and 2 was analyzed using repeated measures analysis of variance. Treatments served as a fixed effect, seedlings within treatments as a random effect, and day of observation as the repeated measures. Means were separated using Tukey's LSD when $P \leq 0.05$. Data was analyzed using the statistical package JMP (SAS 2007).

## RESULTS

This trial was conducted during the months of July through November. The average temperature was 23° C and the overall total precipitation reached 36.4 cm. Available herbaceous forage and shrub density and composition were similar between all plots utilized for this study (Table 1). Of the seedlings that where browsed upon, all regenerated new top growth. All mortality that was observed was from uprooted seedlings. With an average mortality percentage of 5.5% for control treatments (unstocked) and a 6.3% for goat treatments (stocked), there were no differences ($P>0.05$) in seedling disappearance between the two treatments (Fig. 1). There were no statistical differences ($P>0.05$) between days of observation for control and treatment plots (Fig. 2). The rate of seedling disappearance varied throughout the grazing season with no clear pattern of seedling disappearance in either the grazed or ungrazed treatments.

Sixty seedlings were used in clipping treatments. Of the 60 seedlings clipped, mortality was observed from only one seedling. Average root mass in grams was greater ($P<0.05$) for seedlings in control treatments when compared to seedlings that were subjected to different frequencies of defoliation (Fig. 3). There were no differences ($P>0.05$) between root mass of seedlings from the three separate clipping treatments. Average foliage mass from all clipping treatments was similar to the control treatments (Fig. 4).

## DISCUSSION

### Trial 1: Pasture Study

Results of this study indicate that biological control of mesquite at the seedling stage by goats is not an effective control method. Goats avoid consumption of mesquite apparently because of the toxic agents found within the plant (Alder 1949; Dollahite and Anthony 1957; Tabosa et al. 2000). Ingestion of both seeds and foliage can produce toxic effects. Little data exists on the toxic effects of consuming mesquite foliage. However, one preliminary study illustrated that goats would consume small amounts of mesquite foliage for 5 days and then had to be removed from the study because of adverse physiological effects, including severe scours, apparently from mesquite-induced toxicity.

Mesquite pod ingestion by goats has shown promise because goats consume more seeds per kilogram of body weight and disperse fewer viable seeds than any other species of livestock (Kneuper et al. 2003). However, some evidence shows that continuous consumption of mesquite pods as the primary dietary component may lead to rumen compaction and toxicological effects (Tabosa et al. 2000, 2004). High sucrose production from the digestion of pods decreases cellulose digestion, leading to rumen compaction, rumen stasis, and decreased blood glucose levels (Alder 1949). In recent research, goats have shown to consume large amounts of mesquite pods, up to 60% of their diet for short periods of time without experiencing toxicological effect (Cook et al. 2008). This is promising because mesquite beans in west central Texas usually disappear within two to four weeks after fruit senescence (Cook et al. 2008). Therefore, goats could be exposed to areas of high pod production for short amounts of time to ingest mesquite beans that would otherwise be available for other species of livestock as well as wildlife.

When goats do browse on a mesquite plant, overall foliage reduction is low and regeneration is observed with no signs of mortality. The only mortality observed in this study was when goats would uproot the plant; however, there was no difference in mortality between seedlings uprooted by goats and seedlings uprooted by wildlife species in control plots. The inclusion of goat browsing does not appear to further reduce mesquite seedling survival.

**Table 1.** Average herbaceous forage production (kg $ha^{-1}$) and shrub density (total trees per treatment) of control treatments (unstocked pastures) and goat treatments (stocked pastures).

| Treatment | Pasture | Average Herbaceous Forage (kg $ha^{-1}$) | Shrub Density (total trees per treatment) |
|---|---|---|---|
| Goat | 22A | 780.2 kg | 32 |
| Goat | 22D | 1015.08 kg | 13 |
| Control | 22B | 929.28 kg | 11 |
| Control | 22C | 1088.79 kg | 10 |

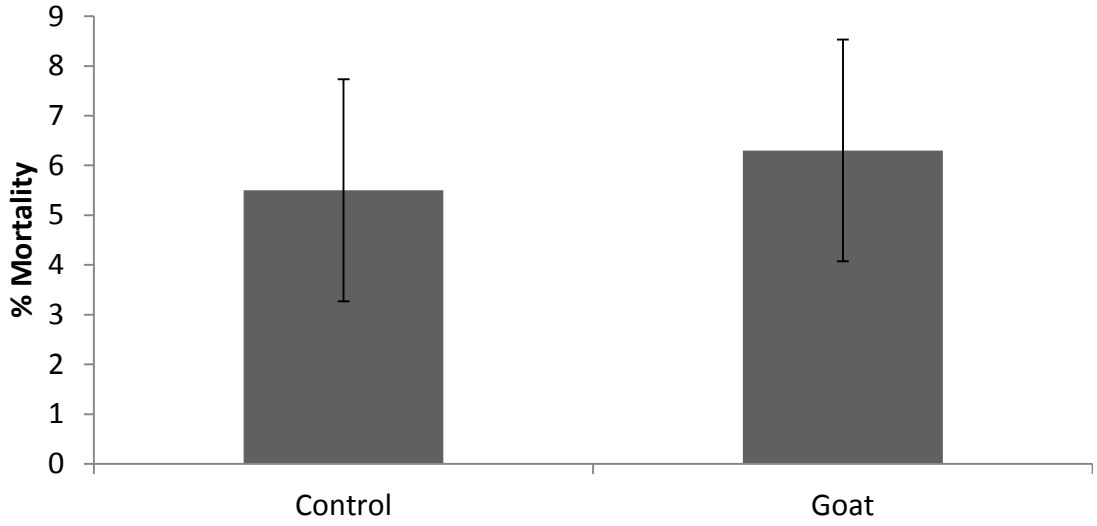


**Fig 1.** Average seedling mortality percentage observed between control treatments (unstocked pastures) and goat treatments (stocked pastures).

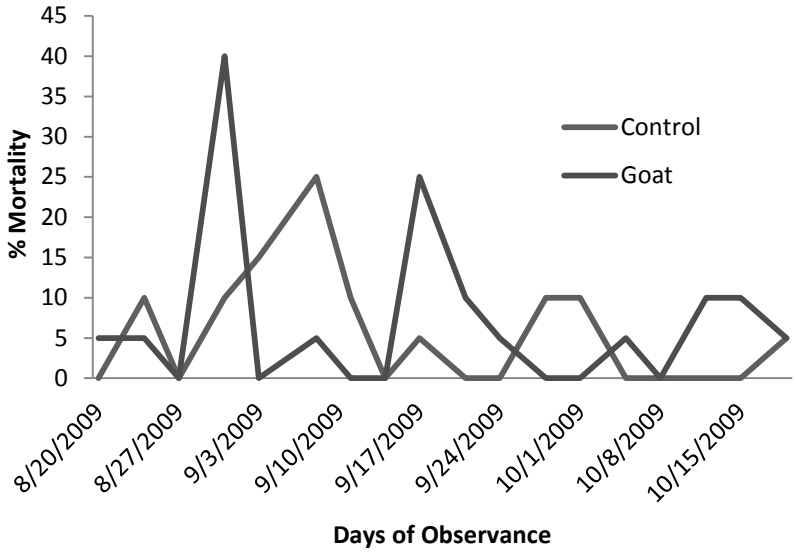


**Fig 2.** Average seedling mortality percentage based on day of observance between control treatments (unstocked pastures) and goat treatments (stocked pastures).

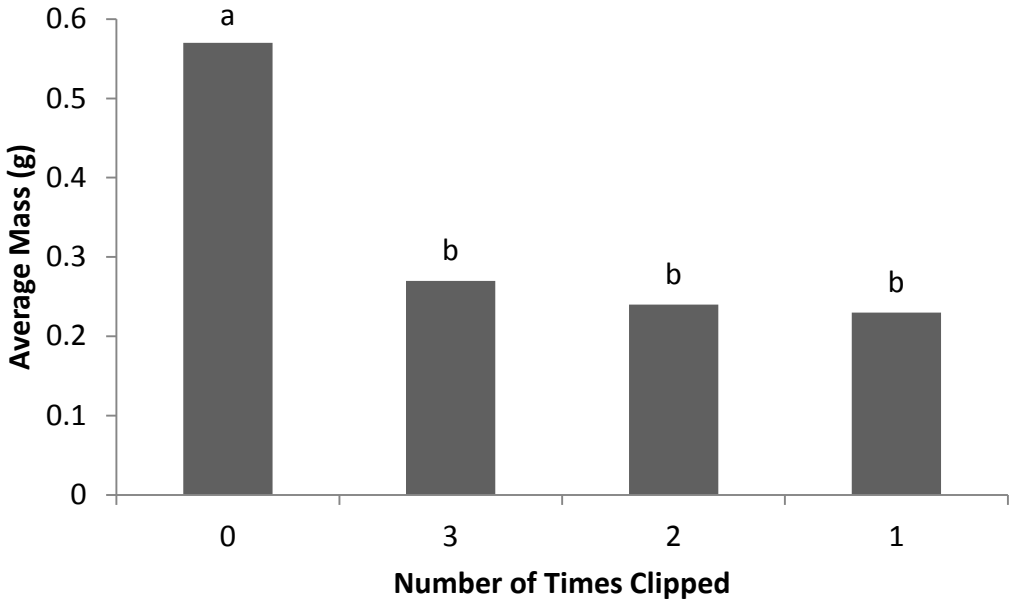


**Fig 3.** Average root mass in grams for Treatment 1 (control), Treatment 2 (clipped 3 times), Treatment 3 (clipped twice), and Treatment 4 (clipped once) after being dried at 60°C for 48 hours.

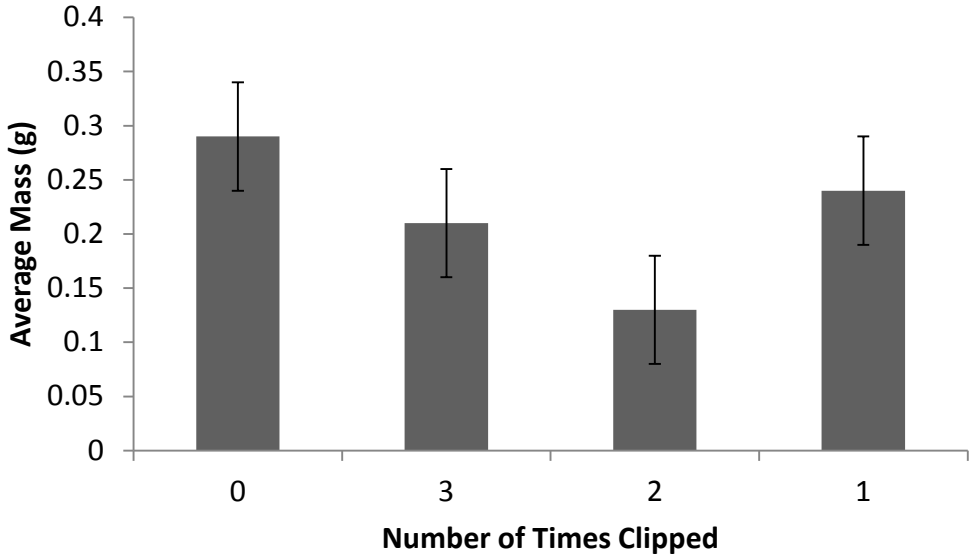


**Fig 4.** Average above ground foliage mass in grams for Treatment 1 (control), Treatment 2 (clipped 3 times), Treatment 3 (clipped twice), and Treatment 4 (clipped once) after being dried at 60°C for 48 hours.

Mortality results of seedlings after herbivory may have varied if water supplementation was not provided during periods of dry weather. Weltzin et al. (1998) showed that mesquite seedlings clipped several times in field trials were much less likely to survive than seedlings clipped in the laboratory. However, repeated evidence of herbivory on the same seedling was not frequently observed. Apparently, it is rare that mesquite seedlings are browsed by wildlife or livestock, and the likelihood of regeneration is high unless seedlings are repeatedly defoliated under dry conditions. Arguably, repeated defoliation of mesquite seedlings would probably require excessive stocking rates of goats that would adversely affect herbaceous species composition as well as reduce overall range condition.

Results of this study indicate that little or no mortality of mesquite seedlings will be observed from clipping in a greenhouse or laboratory setting. Root mass was greater for seedlings that are not clipped when compared to seedlings that are repeatedly clipped. This is to be expected since root growth is secondary to vegetative growth. Seedlings that were in clipping treatments were constantly reallocating resources and growth efforts towards top growth regeneration at the expense of overall root mass loss. Even with a loss of overall root mass, mesquite seedlings still possessed 2-3 times the amount of root mass to efficiently uptake nutrients and survive.

Foliage mass may have differed if this trial was conducted in the spring and summer months of the year. This trial was conducted during the fall and winter months. In its natural habitat, at these times mesquite is entering into a state of dormancy and remains that way until the following spring. During dormancy, mesquite is not undergoing any new top growth. Even though this study was conducted in a greenhouse environment, the seedlings were still subjected to cooler day and

night temperatures, as well as shorter day lengths which suppressed and stunted foliage regeneration.

Reducing mesquite seedling success using goats is not a viable management tool. On healthy rangelands, goats would have an ample supply of more palatable and nutritious browse species to choose from. Large grass stands and dense browse plants offer mesquite seedlings protection from browsing goats. Using increased stocking rates of goats to try and suppress mesquite seedling survivability would ultimately lead to an overall degradation of rangelands because more desirable browse species would have to be heavily browsed upon and lowered in overall abundance before goats would shift their foraging preferences. Preconditioning goats to consume mesquite in a pen setting and forming a preference for mesquite is not possible due to the violent nature of the toxins within the foliage. Mesquite is a very adaptive species and has found an ideal home on rangelands in Texas. This is more of a natural succession of our rangelands into a mesquite dominated shrub land than management practices including goat dominated stocking rates or suppression of fire.

## IMPLICATIONS

Given the lack of evidence of herbivory induced mortality by goats and seedling survivability after clippings, goats will not be sufficient in control and suppression of mesquite seedlings. However, goats have shown to be able to consume mesquite beans up to a level of 60% of their overall diet without experiencing toxicosis for short periods of time (Cook et al. 2008), and digestion of mesquite seeds by goats greatly lowers the seed's viability (Kneuper et al. 2003). Stocking rangelands with multiple species of livestock is an important management technique to utilize. Even though this would not result in lower mesquite seedling survivability, it would lead to better forage utilization and in some cases higher stocking rates. Mesquite control should continue to implement chemical and mechanical methods and in available situations use the addition of goats to lower the amount of viable seeds that could germinate. If this approach is taken, there is a possibility of broadening the gap between chemical and mechanical applications due to reduced viable seed availability.

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## EFFECTS OF ISOFLAVONES ON PUBERTY AND PREGNANCY RATES IN EWE LAMBS

*Kris C. Ede, Micheal W. Salisbury, Gil R. Engdahl, and Brian J. May*

**ABSTRACT**

Soy isoflavones in humans have shown to increase circulating estrogen levels. Female infants consuming formula high in soy proteins tends to cause early onset of puberty and the development of secondary sex characteristics from high levels of estrogen in their blood. This study was designed to determine if ewe lambs consuming diets high in soy proteins would have increased levels of circulating estrogen and have an earlier onset of puberty and ultimately higher conception rates. Sixty-six Rambouillet and 15 Suffolk ewe lambs (98 d of age) were blocked by breed and randomly assigned to one of three treatments. Treatment differences were only the source of protein ingredient in the diet, either cottonseed meal (CSM) or soybean meal (SBM). Treatment 1 was the control diet without any SBM, only CSM as the protein, treatment 2 diet had half SMB and half CSM as the protein and treatment 3 had only SMB as the protein. Diets were formulated to be isonitrogenous and isocaloric. At weaning ewe lambs were weighed and a serum sample was collected to measure estrogen. Ewe lambs had ad libitum access to their diets for 90 d. At which time they were weighed and another serum sample was collected to measure change in circulating estrogen levels. Additionally, twice per week serum samples were collected to measure serum progesterone levels as an indicator of attainment of an estrous cycle. On d90 fertile males were placed with the ewe lambs for 45 d while remaining on their treatment diets. Following ram removal ewes were joined into one group until lambing. On d175 pregnancy rates and number of multiple fetuses were determined using ultrasound. No differences *($P > 0.05$)* in weight gain or conception rates were observed among treatments. However, ewe lambs receiving soy protein in their diets, regardless of level, had 7 times higher *($P < 0.05$)* levels of serum estrogen levels than those not receiving soy proteins in their diet. Therefore, results indicate that consumption of diets containing soy proteins, post-weaning, will result in elevated serum estrogen levels.

**INTRODUCTION**

In recent years the advances in technology have been a major factor for the current progressive turn in the livestock industry, and more importantly, the field of reproductive physiology. Not only have advances in technology increased the quality of livestock, but also have enabled producers to consequently increase the amount of revenue changing hand, and in turn, aid an already recessive economy. The particular area in which major improvement could be made, particularly in the sheep and goat industry, is allowing the producer to have the ability to maximize the potential of ewe lambs being able to reach puberty, and ultimately conceive and give birth to offspring at an earlier age. This would not only allow the industry to produce more fall born lambs, but also allow the numbers to increase, which to an industry whose numbers are declining, higher volume, higher quality livestock is a must.

Unfortunately, there have been few studies on the effect soy protein isoflavones have on animals. There have been limited studies on the effects they have in humans, but general observations have been made and an attempt to correlate them to female puberty has been made. In a study by Fallon and Enig (2009), only 14.7 percent of Caucasian girls and almost 50 percent of African-American girls showed signs of early puberty. This has been linked to soy based infant formula in which the African-American girls had consumed at a higher rate. The formula in question has boosted plasma estradiol concentrations anywhere from 13,000 to 22,000 times higher than estradiol concentrations in infants on cow's milk formula. It is estimated that an infant exclusively fed soy formula receives the estrogenic equivalent (based on body weight) of at least five birth

control pills per day (Fallon and Enig, 2009). Therefore the objectives of this study were to determine the effects isoflavones in the diets of developing pre-pubertal Rambouillet and Suffold ewe lambs have on the attainment of puberty and early reproductive activity.

## MATERIALS AND METHODS

Sixty-six freshly weaned Rambouillet ewe lambs and 15 Suffolk ewe lambs were randomly assigned to one of three treatments. Treatments consisted of all ewes receiving a mixed diet that meets the NRC (NRC, 2007) requirements for weaned, young, growing ewes with varying levels of isoflavones; 1) containing no isoflavones, 2) containing ½ the level as reported to impact developing young girls (Table 1), 3) level reported to impact growing young girls (Table 1). Actual level was reviewed by the human nutrition lab at Iowa State University. Soybean meal replaced cottonseed meal in the control diet to meet the required isoflavone levels needed while maintaining similar crude protein levels. All diets were isonitrogenous and isocaloric to eliminate nitrogen and energy as the factor influencing development (Table 1). Isoflavone analysis was conducted by the human nutrition laboratory at Iowa State University. The three main isoflavones relevant to this study were daidzein, genistein, and glycitein, and to be activated must have met levels of 477, 666, and 150 mg/g respectively. Animals were housed in small groups with ad libitum access to shade, clean fresh water, and their respective diets. Following 120 days of feeding they were placed, in treatment groups, with intact rams for mating. Rams were fitted with marking harnesses designed to place a colored mark on the female's back when they are mated. Mating activity was monitored daily. Body weights were taken at d0 and d90 to monitor growth and development.

Data was analyzed using the GLM procedure of SAS (SAS Institute, Cary, NC). Individual animal served as the experimental unit, and differences were considered significant when $P \leq 0.05$. Variables measured were initial weights, ending weights, total and periodic gain. Reproductive activity was analyzed using the Categorical models of SAS since data was collected as yes or no data.

## RESULTS AND DISCUSSION

The goal of this research and the following results of this research are to have the ability to relay benefits or non-beneficial messages correlating to the feeding of diets high in soy isoflavones. There are both positive and negative connotations dealing with high soy protein related diets. With females being our primary concern, growth and reproductive performance was of importance

During the experiment, several ewe lambs experienced rectal prolapses and were removed from the data. Fortunately, the numbers of affected by prolapses were equal across all treatments (two/treatment). Additionally, approximately mid-way through the trial, two dogs attacked the ewes in treatment three, resulting in three being removed from the trial and the remaining ewe lambs in this treatment temporarily reduced feed intake. Therefore, results involving treatment three should be interpreted with caution.

### *Performance*

No differences ($P > 0.05$) were identified in growth performance (Table 2). All treatments had similar initial weights, final weights, gain and average daily gain. Therefore, diet composition had little or nothing to do with animal growth and any differences in reproductive performance are related to ingredients rather than nutrient levels.

### *Pregnancy Rate*

No statistical differences ($P > 0.05$) in pregnancy rate were detected; however, a trend was evident (Table 2). Unfortunately, in this project, total number of ewes was relatively small when using a Qui-square analysis ( Categorical Model in SAS) and becomes a relatively weak statistical test. There was a 17% difference in conception rates between treatments 1 and 3 for the Rambouillets while the Suffolk lambs were identical across all treatments. Realizing that within our study, this was not statistically significant, it could be economically important to a larger producer.

Table 1. Ingredient composition and nutrient composition of experimental diets on an as fed basis

| Ingredient | Treatment 1 | Treatment 2 | Treatment 3 |
|---|---|---|---|
| Corn% | 45.00 | 45.00 | 45.00 |
| Cottonseed Hulls% | 22.00 | 29.00 | 30.00 |
| Soy Bean Meal % | 0.00 | 9.00 | 17.00 |
| Cottonseed Meal % | 15.00 | 9.00 | 0.00 |
| Alfalfa pellets % | 12.50 | 2.50 | 2.50 |
| Molasses % | 3.00 | 3.00 | 3.00 |
| Sheep Mineral Premix % | 2.50 | 2.50 | 2.50 |
| TDN | 62.31 | 63.46 | 64.43 |
| CP | 14.04 | 14.07 | 14.00 |
| Neg | 0.91 | 0.94 | 0.97 |

Table 2. Growth, performance and pregnancy differences in Rambouillet and Suffolk ewe lambs consuming diets with differing levels of soy isoflavones, no statistical differences among treatments ($P \leq 0.05$) .

| | TREATMENT 1 | | TREATMENT 2 | | TREATMENT 3 | |
|---|---|---|---|---|---|---|
| | Rambouillet | Suffolk | Rambouillet | Suffolk | Ramboullet | Suffolk |
| Initial Weight, kg | 36.95 | 35.00 | 38.95 | 36.14 | 38.23 | 35.45 |
| Final Weight, kg | 50.77 | 51.59 | 54.64 | 51.05 | 52.23 | 47.95 |
| Total Gain, kg | 13.82 | 16.59 | 15.68 | 14.89 | 14.00 | 12.50 |
| Daily Gain, kg/d | 0.15 | 0.18 | 0.17 | 0.17 | 0.16 | 0.14 |
| Conception Rate, % | 60 | 75 | 70 | 75 | 77 | 75 |

For example, an operation with 1000 head of ewes had a 17% increase in conception rate, this could easily mean that there would be 170 more lambs to be born. Inevitably, a larger sample size would dictate that a 17% increase in conception rate would suggest a significant difference and therefore should be investigated a larger scale than the current experiment.

***Reproductive Activity***

Reproductive activity as measured by circulating progesterone showed no difference ($P > 0.05$) among treatments. In fact, only 8% of all ewe lambs, regardless of treatment, actually showed progesterone levels adequate for a reproductive cycle (Salisbury et. al., 2000). These results are

quite interesting considering the fact that over 70% of all ewes bred and conceived within 30 days of taking the final blood sample. However, this discrepancy is similar to that observed by Salisbury et. al (2000) where they had similar results in a study comparing
reproductive rates in ewe lambs on different protein levels when measuring progesterone levels in the serum. As in ours, their ewe lambs actually conceived and gave birth to lambs. It can be speculated that the lack of exposure to a ram may have played a role in the lack of hormonal evidence of cyclicity when in fact they bred once they had a male placed with them. This phenomenon is described in detail by Bearden et. al. (2004) where he described the impact of the male on reproductive activity in all species. They explain that the impact is even greater on prepubertal females in their first breeding season.

Additionally, blood sampling ceased at d 47 which was the day prior to the attack by dogs in order to reduce stress on the ewes. Therefore, if blood sampling was to have continued there may have been a difference in progesterone levels because the ewes would have been closer to their normal breeding season. It is obvious by the conception rates that ewes experienced reproductive activity, but it was just not detected in the serum progesterone levels.

***Isoflavone Concentrations***

Isoflavones are found in any plant product and are defined as a substance that has a hormonal effect on mammalian beings (Ursin et. al., 2006; Higdon, 2009). It is also stated upon ration analysis at the Iowa State University Food Science and Human Nutrition Laboratory that the three main isoflavones that concerns our study were daidzin, genistin, and glycitin. Daidzin, genistin, and glycitin had an activation rate of 477, 666, and 150 mg/g, respectively. Treatment 1 was the control and contained no isoflavones. Treatment 2 was the low soy diet and the sheep in treatment 2 ingested 48,785 mg/d of daidzein, 67,932 mg/d of genistein, and 15,300 mg/d of glycitein (Table 3). While the ewe lambs in treatment 3 ingested 92,061 mg/d of daidzein, 128,538 mg/d of genistein, and 28,950 mg/d of glycitein (Table 3).

***Circulating Estrogen***

Estrogen level is a major factor in female reproductive tract growth Bearden et. al. (2004). Therefore, serum estrogen levels were measured in the ewes prior to the feeding treatments and again at the conclusion of the feeding treatments as an indicator of potential reproductive tract growth and the ability to conceive. Differences ($P < 0.05$; Table 4) were indentified in the initial samples (d0) between the control ewe lambs and the low soy treatment. There were also differences ($P < 0.05$) on d47 between the control and both soy treatments. Since there were differences at the initiation of the experiment, the difference between the initial and final concentrations was calculated to determine the
actual change in concentration level following the feeding of diets with differing level of soy isoflavones. Change in concentration followed the same pattern as final concentration with the control ewes being lower than both soy treatments ($P < 0.05$; Table 4). These findings are consistent with that reported by Fallon and Enig (2009) when they found that young females consuming even moderate amounts of the soy isoflavones had a considerable increase in circulating estrogen levels. Therefore, females should see an increase in reproductive tract development consistent with early puberty (Bearden et. al. 2004).

**IMPLICATIONS**

The results of this study suggest that ingestion of an increased level of soy isoflavones will have an effect on estrogen levels. In addition, it seems that conception rate was dictated at a lower degree, however, the use of soy isoflavones could still be used in a practical sense. Producers that run large operations would obtain a greater benefit than those raising farm flocks, primarily due to volume of sheep. Instead of feeding out ewe lambs on a traditional West Texas diet with a cottonseed meal base, producers could feed them out using a soy bean meal based diet. This

Table 3. Soy isoflavone intake by Rambouillet and Suffolk ewe lamb consuming diets containing three different levels of soy proteins.

| | Daidzein | Genistein | Glycitein | Totals |
|---|---|---|---|---|
| Isoflavone Levels, mg/g of SBM | 477.0 | 666.0 | 150.0 | N/A |
| Ration 1 (control), mg/d | 0.0 | 0.0 | 0.0 | 0.0 |
| Ration 2 (low soy- 102 g/d), mg/d | 48.9 | 67.9 | 15.3 | 132.0 |
| Ration 3 (high soy-193 g/d), mg/d | 92.1 | 128.5 | 28.9 | 249.6 |

Table 4. Serum estrogen concentration in Suffolk and Rambouillet ewe lambs consuming diets with different levels of soy isoflavones.

| | Treatments | | | |
|---|---|---|---|---|
| | Control | Low Soy | High Soy | SE[a] |
| Day 0, ng/ml | 2.0[b] | 8.1[c] | 4.6[bc] | 2.15 |
| Day 47, ng/ml | 2.6[b] | 19.9[c] | 17.4[c] | 6.02 |
| Change, ng/ml[d] | 0.6[b] | 11.7[c] | 12.9[c] | 5.11 |

[a]Most conservative standard error of the least squares mean.
[b,c]Mean in the same row with differing super scripts are different ($P < 0.05$).
[d]Change in estrogen concentrations from d0 to d47.

should allow the ewe lambs to ingest a relative amount of soy isoflavones, consequently increasing their estrogen levels. Ultimately, this should allow the ewe lambs to reach puberty in an earlier time frame, thus allowing the producer to likely have a higher conception rate in his ewe lambs. Consequently, if this happened that likelihood of more lambs being on the ground would in turn suggest that more revenue would be generated for the producer. One more lamb per ewe per season of service would allow the female to produce more lamb throughout her productive life, ultimately maximizing her profit potential.

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# MONITORING THE PLASMA PROGESTERONE LEVELS IN BOER CROSS NANNIES POST WEANING AND THE EFFECTS OF SUMMER CONDITIONS ON GOAT CYCLICITY

*Dustin W. Vick and Micheal W. Salisbury*

## ABSTRACT

When dealing with meat goats, reproductive rate and desired markets are two of the most important factors that must be considered. Producers must also determine their markets prior to breeding. Goat meat is widely popular during certain ethnic and religious holidays such as Easter. In order to produce a market goat that is ready to sell prior to some of these holidays, females often need to be bred outside of their typical breeding season. Although goats, Boer goats in particular, are considered to be far less seasonal than sheep in this part of the world, the extreme heat and increased day length can have a negative effect on cyclicity. Little research has been done testing the potential fertility of female goats during the summer months. The purpose of this study was to determine the likelihood or the ability of Boer cross nannies to ovulate during the summer months based on plasma progesterone levels. Twelve Boer cross nannies, post weaning, from the Angelo State University ranch, were utilized in this study. These twelve nannies were pastured together with the rest of the ASU goat herd to ensure the same environmental conditions and diet. Blood samples were obtained in serum separator tubes via jugular venipuncture twice a week for approximately ten weeks. The serum was then harvested following centrifugation. The serum was then sent to the New Mexico State Endocrinology Lab to be analyzed for progesterone. According to Schneider and Hallford (1996), goats experiencing plasma progesterone concentrations of 1 ng/ml or greater over two consecutive sample days have experienced a fertile ovulation. Variables in this study that could potentially effect heat cycle include day length, temperature, humidity, body condition (diet and parasites), and lack of exposure to males. Serum progesterone levels indicate that no female experienced a fertile cycle during the experiment.

## INTRODUCTION

In all species of livestock, reproduction efficiency is the first, and one of the most important factors of production that must be considered. Knowing the ideal times to breed livestock and the proper markets for maximizing income varies between and among species. Most livestock are seasonal breeders, typically breeding during the short days of the year, from August to April. Although sheep and goats are both, for the most part, seasonal short-day breeders, Boer goats are seen as less seasonal than other breeds. According to Hutchens et al. (2003), Boer goats are considered partially seasonal. This means that there is typically no period of anestrus if conditions are favorable (Lu, 2002). Most research suggests that heat cycles are regulated by day length alone when dealing with the summertime. There is very little information to support temperature having an impact on cyclicity. In this study, we investigate the likelihood that Boer cross nannies will breed during the summertime months based on a fertile ovulation which is tested via serum progesterone ($P_4$) levels. Drawbacks to breeding during this time of year include extreme heat and very long daylight. Extreme heat can have an abnormally negative effect on reproduction. It can lead to lower conception rates, decreased embryo survival, and poor fetal development (Gimenez and Rodning, 2007). Variables that can alter the heat cycle in goats and other livestock species include day length, temperature, body condition scores, exposure to males, and over climactic conditions.

## MATERIALS AND METHODS

12 Boer cross nannies, ranging from 2 to 4 years of age, were utilized. These nannies were randomly selected from a group that had weaned at least one kid the previous kidding season. All nannies used were from the Angelo State University goat herd. Each nanny utilized had recently weaned at least one kid at approximately 80 days of age. This study was conducted at the Angelo State University Management and Instructional Research Center in San Angelo, Texas. The study began 14 days post weaning. Blood samples were taken twice a week for approximately 10 weeks beginning the final week of May. Temperature, day length, and other notable conditions were recorded and considered for deliberation of the final outcome of this study. Blood was collected every 3 to 4 days into serum separator tubes via jugular venipuncture. Once the blood was collected, it was allowed to clot for approximately 30 minutes at room temperature. The serum was then harvested from the blood after centrifugation at 1500 x g for 15 minutes at 4°C. The serum was then stored at -20°C and sent to the New Mexico State Endocrinology Lab to be analyzed for progesterone using a radioimmunoassay, as described by Schneider and Hallford (1996). The serum progesterone was quantified using a commercial RIA kit (Schneider and Hallford, 1996). This procedure is a solid phase, anti-body-coated tube assay using $^{125}$I-$P_4$ as the tracer (Schneider and Hallford, 1996). This process requires no extraction of the serum (Schneider and Hallford, 1996). The tracer solution also liberates $P_4$ from its binding protein, giving us our plasma-progesterone concentration (Schneider and Hallford, 1996). According to Schneider and Hallford (1996), goats experiencing plasma progesterone concentrations of 1 ng/ml or greater over two consecutive sample days have experienced a fertile ovulation. As a result, these goats are considered to be cycling. If a goat experiences a fertile ovulation, they do so because they have a functional corpus luteum.

## RESULTS

Cyclicity data was analyzed using the CATMOD procedure of SAS (SAS Institute, Cary, NC) with individual animals serving as the experimental unit. Only blood serum samples through mid-July were sent off for analysis, as goats were expected to resume normal cyclicity near the end of July and the beginning of August; which they did, as all were bred and kidded with a 100 % conception rate in January. No goats experienced even one plasma progesterone concentration greater than 1 ng/ml. Two consecutive samples reaching 1 ng/ml were needed to confirm a fertile ovulation. Differences within the experiment were not significant. All goats tested remained in a period of anestrous throughout the testing period. Although some animal units exhibited some plasma progesterone concentration fluctuation, There was enough to show any exhibition of a fertile ovulation. Figure 1 shows the two extreme animal units in regards to plasma progesterone concentration variation in ng/ml. Animal unit 272 exhibited the highest degree of variance in plasma progesterone concentration, while animal unit 263 remained flat lined at 0.1 ng/ml of plasma progesterone throughout the experiment. Several animal units showed similar trends in their plasma progesterone concentration as animal unit 272, proving that there was some cyclic activity occurring, but nothing fertile.

Figure 2 shows the average day length for all twelve months of the year, and highlighted in red, are the four months considered in this experiment. This figure illustrates the vast increase in day length during the summer months for San Angelo, Texas. Day length is important to cyclicity in that sunlight inhibits melatonin production. Therefore, melatonin production and secretion is increased with increases in periods of darkness. Melatonin is a chemical compound that is synthesized and secreted by the pineal gland. Melatonin levels aid in regulating a number of biological functions in the body including reproduction. Melatonin

Figure 1: Plasma progesterone concentrations in ng/ml for the two extreme animal units measured, by date.

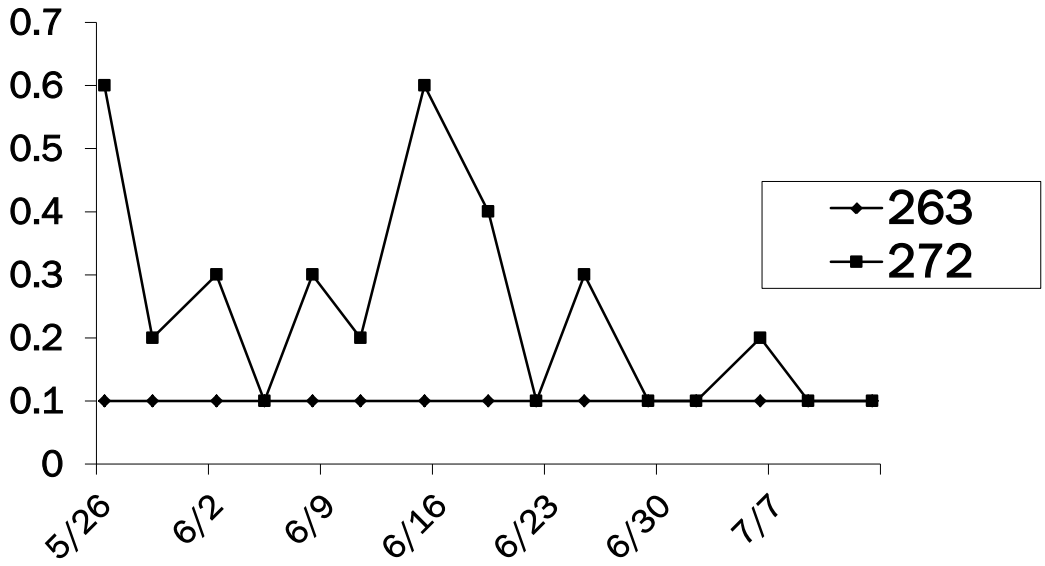
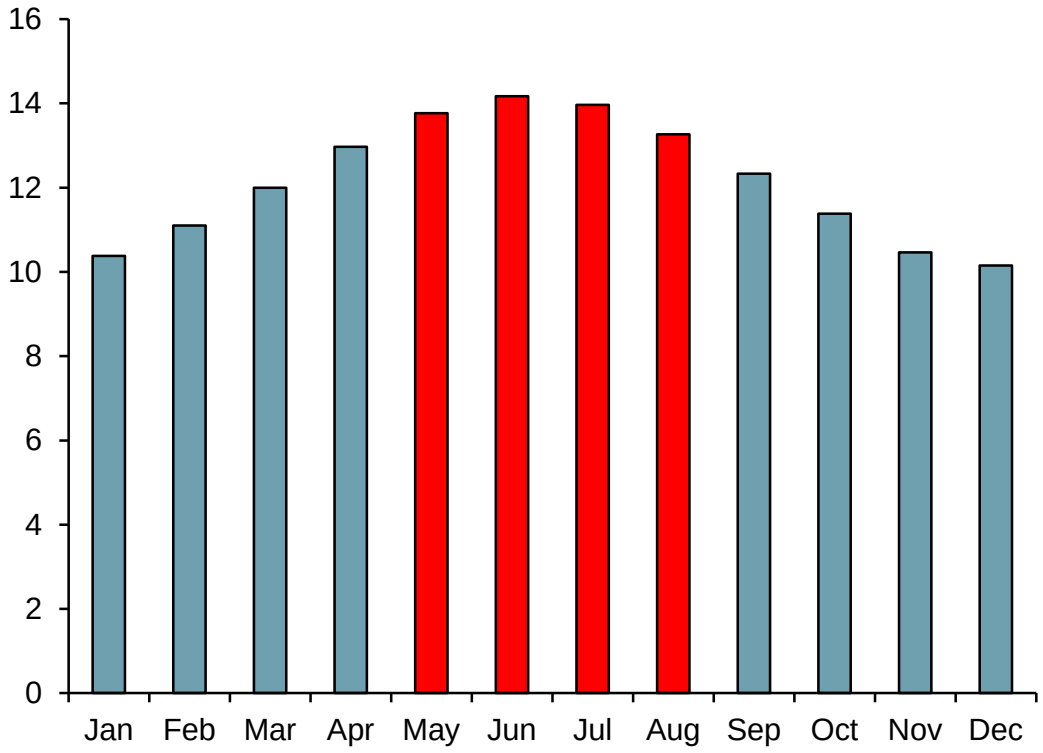



Figure 2: Average day length in hours for each month in 2009.



targets the hypothalamus, triggering the release of gonadotropin releasing hormone (GnRH). GnRH signals the anterior pituitary which produces and secretes lutinizing hormone (LH) and follicle stimulating hormone (FSH). LH and FSH stimulate a sharp drop in $P_4$ in the body via the gonads. At this point, the ovarian follicle(s), that was released bursts, forming the functional corpus luteum. A sharp increase in LH and FSH occurs simultaneously. Estrogen ($E_2$) levels spike due to the formation of the CL. It is at this point that the female is in standing heat. This progression of hormonal release is what sets up the standing heat experienced by the female animal. This hormonal cycle is what enables us to distinguish periods of estrus using plasma progesterone concentrations.

In short-day breeders increased melatonin production and secretion stimulates cyclicicty; and in long-day breeders, increased levels of melatonin suppress cyclicity.

Figure 3 shows the average temperature in °F for each particular day in 2009 as well as the actual high temperature in °F for 2009. The most elevated temperatures in this particular graph correspond with the time period in which this experiment took place. Elevated temperatures can reduce fertility in both males and females. The highest temperatures for the year correspond with the longest days of the year. These two factors quite possibly work in conjunction with each other to inhibit fertility in Boer cross nannies during the summer months as was seen in the final outcome of this experiment.

**Conclusion**

Under the environmental conditions present during this study, plasma progesterone levels indicated no fertile ovulations or functional C.L.'s in the female Boer cross goats. These results could be due to several factors associated with the summer season including extreme heat and increased day lengths. Further research is necessary to confirm the findings within this experiment. Year long testing would increase the validity of a study such as this. Studies over multiple, consecutive years would likely yield more convincing results of cyclicity in Boer cross nannies. The effect of the presence of a male is also a factor in bringing upon the onset of estrus. The addition of a male (teaser or intact) could also be incorporated into a study such as this. Although none of the females utilized within this study experienced no fertile estrus cycles, the presence of a male within the group could yield different results in future studies. A study such as this may also be region specific in its results. Extreme heat conditions, however, do not guarantee successful conception. If the same study was performed in a cooler environment with similar day lengths, the findings may change. On a side note related to this study, every Boer cross nanny utilized in this study was successfully bred in August, 2009 and gave birth in January, 2010.

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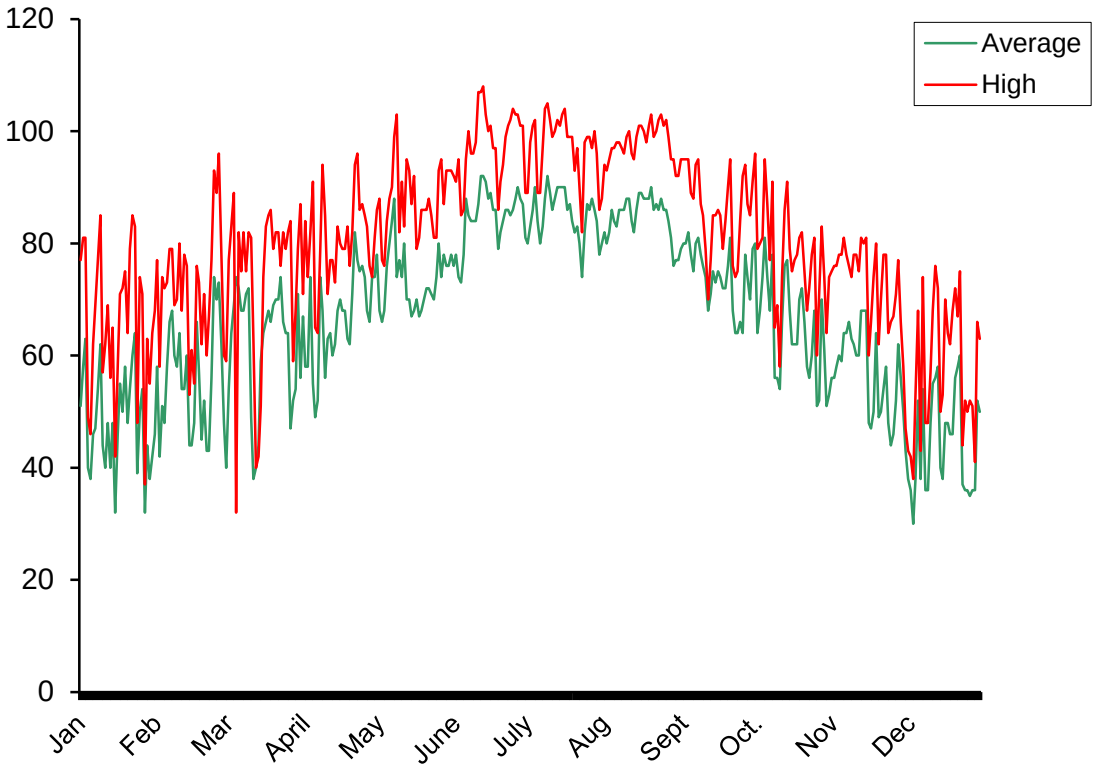
Figure 3: Average and daily high temperatures in °F for each day in 2009.



# EFFECTS OF SUPPLEMENTATION OF TASCO (KELP SEAWEED) ON INFERTILITY RELATED TO HEAT STRESS IN MALE SHEEP

*Chase E. Settle, Micheal W. Salisbury, Gil R. Engdahl*

## ABSTRACT

*Ascophyllum nodosum* (Kelp Seaweed; Tasco) has gained popularity as an agent to reduce heat stress and improve reproductive performance in livestock. This study was conducted to determine if supplementation of Tasco would improve the breeding ability of mature Suffolk and Rambouillet rams. Rams were penned by age and breed in groups of 2-3 and were fed a diet at 2.5% of their body weight and received just the diet alone or the diet plus Tasco at 5 $g \bullet hd^{-1} \bullet d^{-1}$ for 56 days. Following the 56 day feeding period they were placed on pasture to simulate a normal breeding period for 56 days. Weights were taken on days 0, 28, 56, 84 and 112 and semen samples and scrotal circumference were collected on days 0, 56, 84 and 112. Results from the current study showed no differences in weight, semen quality parameters and scrotal circumference ($P > 0.05$). However, during the testing period, the ambient temperatures were exceptionally low and may have had an impact in the results since a heat stress environment was never experienced by the rams.

## INTRODUCTION

Heat stress is caused primarily by high ambient temperatures but can be intensified by humidity, thermal radiation, and air movement (Morrison, 1983). Heat stress is caused from the inability to transfer the heat absorbed by the body from the air temperature back out of the body. High ambient temperatures negatively affect the reproductive ability of several mammalian species (Wettemann and Desjardins, 1979).

High ambient temperatures are the cause of heat stress, but they don't affect the testicular germ cells directly. Caspase enzymes 3 and 9 are responsible for the direct disassembly and apoptosis of the testicular germ cells (Vera et al., 2005). With increased heat from ambient temperature, the body opens the intrinsic pathway and extrinsic pathway which allows for caspase 9 to start initiating germ cells for disassembly, and then caspase 3 comes in and does the actual destruction of the cells. Vera et al. (2005) concluded that many triggers can cause apoptosis to begin in the testicular tissue of mice, with heat-induced apoptosis causing the release of caspase 9 and then the release of caspase 3. In the study they (Vera et al., 2005) showed a caspase inhibitor caused a reduction in the number of cells experiencing apoptosis and a reduction in the number of cells expressing caspase 3 and 9 in mice.

Vera et al. (2004) showed that the main cause for heat stress and apoptosis was the heating of the internal body temperature and chemical pathways being opened. Enzymes were shown to block these pathways and prevent apoptosis from occurring, therefore preventing infertility in mice. Kelp seaweed has been shown to decrease respiratory rates and increase immunity when supplemented during periods of high ambient temperature (Allen et al., 2001a). Kelp seaweed is high in antioxidants and therefore affects the free radical production that is at a high level during increased temperatures (Allen et al., 2001b). Allen et al. (2001b) also found that the antioxidants prevented the formation of the free radicals, which eliminated them from the heat stress equation. With the levels of free radicals increasing as temperature increases (Vera et al., 2004), there is an increased need for high levels of antioxidants that can counteract these free radicals and improve immunity and lower respiratory rates (Allen et al., 2001b). Increases in α-tocopherol, β-carotene, ascorbic acid and superoxide dismutase have been seen in studies to increase with the treatment of *Ascophyllum nodosum* on several different species of grasses besides just tall fescue (Montgomery et al., 2001; Zhang and Schmidt, 1999). With these results from plants also come results with

livestock. *A. nodosum* has been shown to lower core body temperatures of cattle in hot weather and also heat the core temperature of cattle in cold weather (Archer et al., 2007; Allen et al., 2001a,b). It has also been shown to increase immunity by increasing cell mediated function (Archer et al., 2007; Allen et al., 2001b), as well as increase weight gain (Turner et al., 2002) and marbling. In a study using crossbred Rambouillet/Suffolk lambs, Archer et al. (2007), found that a bolus supplementation of *A. nodosum* at a 1 and 2% supplementation rate, decreased the cortisol concentrations of lambs prior to and through the completion of the walking and transport study compared to the control group that received no bolus. Normally a decreased cortisol concentration is indicative of a low stress situation. This suggests that *A. nodosum* has a positive effect on the limiting of cortisol release from the adrenal gland. *A. nodosum* has also been shown to have a positive effect on semen concentration, motility, and scrotal circumference of young male goats during a period of high ambient temperature (Yates et al., 2010). Yates et al. (2010) study on the affects that Tasco-EX had on male goats during high ambient temperatures contradicted the study done by Turner et al. (2002) showing that there was no difference in weight gain between goats supplemented with Tasco-EX and the control group. The study by Yates et al. (2010) is the basis for this study to determine if the same positive effects of increased semen concentration, motility, and scrotal circumference will be duplicated on a similar ruminant species, male sheep.

**MATERIALS AND METHODS**

The study was conducted at the Angelo State University Management, Instruction and Research Center in San Angelo, Texas of Tom Green County.

Forty-four intact rams were gathered from two producers (Angelo State University and Cal Hengst) two weeks before the study began. Thirty-five Rambouillet rams and nine Commercial Suffolk rams, ranging in age from 1 to 6 years of age, were used for the study. During these two weeks the rams were adjusted to the complete ration (Table 1) that was fed for the duration of the study. They were slowly adjusted up from a minimal level of feed to the level that was used during the study (2.5% of BW). Also this time was used to make sure all rams were healthy and physically sound.

After two weeks of acclimation, on day 0, the rams were randomly assigned within breed to one of 22 pens containing 2-3 rams per pen. There was one pen of 3 Suffolk rams and one pen consisting of 1 Rambouillet ram. The pens were then randomly assigned to one of two treatments. The two treatments consisted of: Treatment 1) grain diet to serve as control; and Treatment 2) grain diet plus Kelp (Brown) Seaweed at 5 $g \bullet hd^{-1} \bullet d^{-1}$ from day 0 to day 56. There were 11 pens per treatment and the pen served as the experimental unit. All rams consumed a feed mixed at the Angelo State University Management, Instruction, and Research Center. All rams were fed from day 0 to day 56 at 2.5% of the average body weight of the pen of rams. Rams were weighed on days 0 and 28, when feed amount was calculated and adjusted. All rams were consistently fed at the same time (1600 hours) each day and the Kelp Seaweed was hand mixed into the feed for the pens receiving Treatment 2. All rams were allowed *ad libitum* access to water and shade.

On day 57, all rams were placed on a pasture in one large group and taken off of feed to simulate the natural breeding environment. The rams remained on pasture through the duration of the study (remaining 56 days). The rams were monitored daily for health issues.
Weights of all rams were taken on day 0, 28, 56, 84, and 112 during which time semen samples were also taken (except for day 28). Semen was collected from the rams using a Ram-O-Jack

Table 1. Ingredient and nutrient composition of diets used in the experiment.

| Ingredient | % of Diet |
|---|---|
| Alfalfa Pellets | 17 |
| Corn, Yellow Dent | 45 |
| Cotton Seed Meal | 10 |
| Soybean Hulls | 22.5 |
| Mineral Premix | 2.5 |
| Molasses | 3 |
| Nutrients, % | |
| Crude Protein | 13.4 |
| Total Digestible Nutrients | 59.8 |

electro ejaculator (Lane Manufacturing, Denver, CO). Semen was then assigned a motility score from 0-5 and a visual concentration score of 0-5 (Herman et al., 1999a), with 0 being the lowest quality and 5 being the best quality for both measurements. Semen concentrations were then calculated for each ram using the sperm count with hemocytometer method (Herman et al., 1999b) to determine the number of cells per ml. Cells per milliliter were determined by: ((sperm cell count x 2.5) x 10,000) x 200). Scrotal circumference was also taken on all rams on day 0, 56, 84 and 112.

Rams were randomly assigned to a pen within breeder and breed, with 11 pens control and 11 pens receiving Tasco supplementation (n = 11/treatment). There were 2-3 rams per pen (except one pen with 1 ram) with the pen being the experimental unit. Scrotal circumference, semen color, semen motility, and semen concentration were analyzed using analysis of variance (ANOVA) of SAS (SAS Institute, Cary, North Carolina). The initial semen collection on day 0 acted as the covariant that the data was compared with to determine if the treatments had an impact on semen quality.

## RESULTS AND DISCUSSION

*Weight gain*

Tasco Kelp Seaweed supplementation did not have a significant difference ($P < 0.05$; Figure 1) on the weight of the rams being supplemented vs. the rams that received the control diet. This was the expected outcome since all rams were fed at a constant 2.5% body weight for the duration of the trial. Therefore, we expected the rams to gain, lose, and maintain weights similar to each other regardless of treatment. However, questions we wished to answer were whether the Tasco Supplementation would play a role on the residual pasture effect of the study (maintain higher semen quality after supplementation was stopped) and if the supplemented rams would maintain a heavier weight. The data from this study had similar results as those reported by Yates et al. (2010) and Saker et al. (2001) in that Tasco supplementation did not have an effect on weight gain.

*Scrotal circumference*

As with weight gain, Tasco supplementation had no significant difference ($P < 0.05$; Table 2) on scrotal circumference between the supplemented rams and the control rams. These results again confirm the same result that Yates et al.(2010) encountered. In their study there were some variations among individual animals as in this study. The lowest probability value ($P = 0.12$) occurred on day 56 with control rams averaging 35.1 cm and supplemented rams averaging 36.6 cm. Day 84 and 112 were both at a $P > 0.6$ with a control average of 36.4 and 6.8 cm vs. a supplemented average of 36.8 and 37.2 cm.

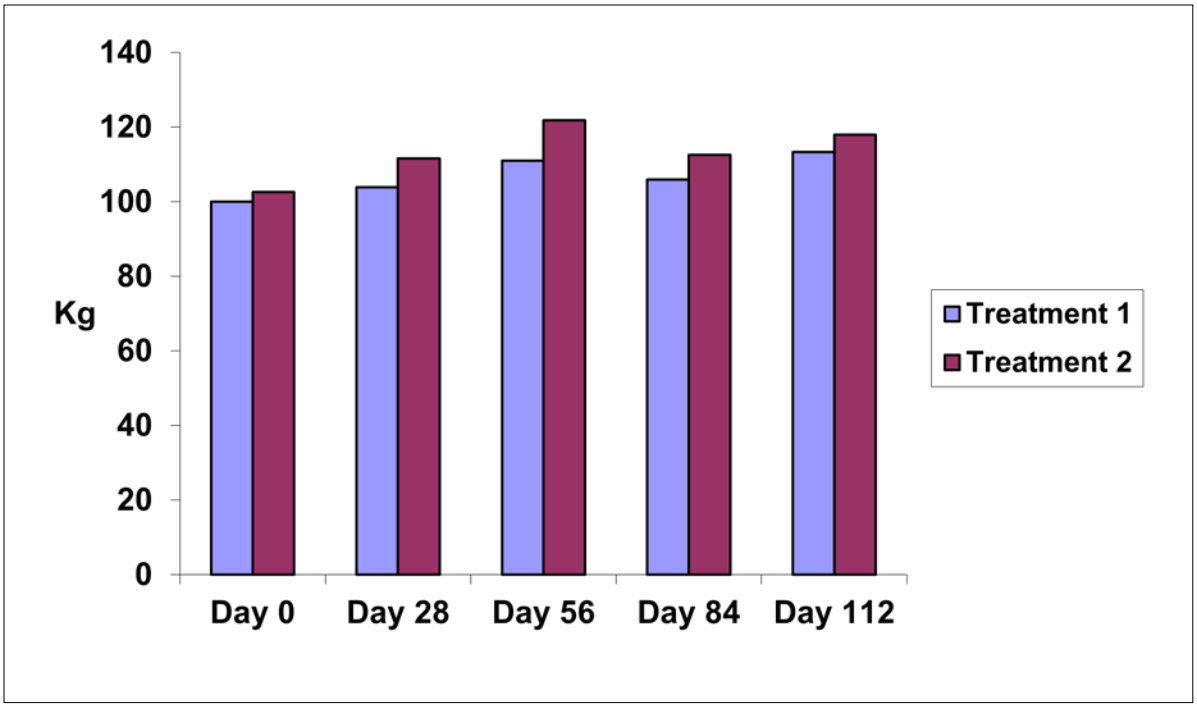



Figure 1. Mean body weights of Rambouillet and Suffolk rams receiving a complete diet at 2.5% of body weight (Treatment 1) or receiving diet plus Tasco (Treatment 2)

Table 2. Mean Scrotal Circumference, sperm cell motility, and visual concentration of rams receiving a complete diet at 2.5% of body weight (treatment 1) or receiving the diet plus Tasco (treatment 2)

| | Treatments[1] | | | |
|---|---|---|---|---|
| | 1 | 2 | P-value | SEM[2] |
| Scrotal Circumference, cm | 35.84 | 36.40 | 0.50 | 0.61 |
| Motility[3] | 4.05 | 4.32 | 0.60 | 0.26 |
| Visual Concentration[4] | 4.58 | 4.75 | 0.32 | 0.17 |

[1]Treatment 1 received a complete diet at 2.5% of body weight and treatment 2 received the diet at 2.5% of body weight plus 5 grams per day of Tasco, Brown Seaweed.
[2]Standard error of the least squares mean.
[3]Motility score is a visual score of 1-5 of the movement of sperm cells viewed under a microscope at 100 magnification. 1=dead, no movement and 5=rapid swirling of the cells
[4]Visual concentration is a visual score of the color of the semen sample 1-5. 1=clear with no color and 5=thick milky white appearance.

*Semen quality*

Semen quality was also not significantly increased by supplementation or inhibited by the lack of supplementation. Motility scores varied among individual animals, but remained in the normal 3-5 score range (Table 2). However, motility was where the greatest significance in the study occurred with a P = 0.059 on the day 112 collection. The average score for the control group was 3.7 and the average for the supplemented rams was 4.5 with a standard error of 0.3. On day 0 and 84 the average for both treatments was above a 4.0, with the only other average below 4.0 being on day 56 with the control group having a 3.8 average where the supplemented group had an average of 4.0 while the standard error was 0.3. Besides day 0, the supplemented rams did

consistently average a higher motility score than the control group. Yates et al.(2010) reported a similar trend among the goats that had received Tasco-EX when compared to the control group of goats. They also saw no significant difference in motility among treatments. Visual concentration followed the same trend as motility with the supplemented rams having a slightly higher average on all collection days except day 0. The average was always above 4.0 (Table 2) for both treatment groups with the lowest P value occurring on day 56 with a P = 0.17. Again this coincided with Yates et al. (2010) who saw no significant difference in visual concentrations among treatments.

As with the other semen qualities that were analyzed, there was no significant difference in semen concentrations between the two treatments. The lowest probability value (P = 0.35) occurred on the onset of the study on day 0, with the control rams having an average of 2.8 billion cells/mL (Table 3) and supplemented rams having an average of 2.4 billion cells/mL where the standard error was 0.3. Once again, the same trend was seen for the remaining collections, with the supplemented rams being slightly greater but not to a significant degree. All the average concentrations for both treatments fell in the normal concentration range of 2-3 billion cells/mL (Herman et al. 1999b; Table 3). This contradicts Yates et al.(2010) study which saw significant increases in cell concentration levels among the supplemented and control goats. However, this difference may be due to the fact Yates' study utilized young, immature and growing male goats, while the current study used mostly mature male sheep that were no longer growing. Additionally, as covered in the next section, ambient temperature may have played a role in the differences of results.

*Lack of high ambient temperature*

By far the largest question during and after the completion of the study was "Did it ever get hot enough?" It was an abnormally cool spring/summer breeding season in San Angelo, TX during the duration of this study. The previous year when Yates et al.(2010) performed their study in the same pens, there were 33 days above 37.78˚C (Table 4), whereas this study had 0 days above 37.78˚C (National Weather Service Climatological Report, Norman, Oklahoma).

Also the previous year, the average temperature highs and lows were all above the average temperature highs and lows for this study. For there to be a definite answer to whether Tasco Kelp Seaweed will positively affect semen quality due to heat stress, there must first be enough heat to cause stress. With the abnormally cool weather that was experienced during this study, there is room to speculate whether or not the ambient temperature ever elevated enough to cause the rams to experience heat stress. It also raises the question if the temperatures were average or above average if the results would have been the same or if they would have been similar to those reported by Yates et al. (2010).

*Species effects in relation to Tasco Kelp Seaweed*

Often we take for granted the similarities between sheep and goats. This may be a case in which they are more different than we think, as it is with the relation of sheep and goats to copper supplementation. We know that goats can consume a significantly larger quantity of copper (100-300mg Cu/kg DMI) without having the adverse effects that sheep experience. Not only can the goats tolerate the copper better, but they have been known to consume amounts that would kill sheep (20 mg Cu/kg DMI; NRC, 2007). So this could be one of the cases where sheep and goats are physiologically more different than we realize. We know that both male sheep and goats experience heat stress, but maybe the effects of the Kelp Seaweed

Table 3. Mean actual sperm cell concentrations of rams receiving a complete diet at 2.5% of body weight (treatment 1) or receiving the diet plus Tasco (treatment 2)

| | Treatments[1] | | | |
|---|---|---|---|---|
| Concentration, bill/ml[3] | 1 | 2 | P-value | SEM[2] |
| Day 0 | 2.78 | 2.38 | 0.35 | 0.29 |
| Day 56 | 1.84 | 1.87 | 0.95 | 0.30 |
| Day 84 | 2.34 | 2.71 | 0.41 | 0.32 |
| Day 112 | 2.71 | 2.76 | 0.90 | 0.27 |

Normal Sperm Cell Concentrations for male sheep, cells per mL
2.0 to 3.0 billion cells per mL

[1]Treatment 1 received a complete diet at 2.5% of body weight and treatment 2 received the diet at 2.5% of body weight plus 5 grams per day of Tasco, Brown Seaweed.
[2]Standard error of the least squares mean.
[3]Actual sperm cell concentration is sperm cells per mL of semen in billions per mL.

Table 4. Average monthly high/low ambient temperatures for the current study and study conducted by Yates et al.(2010)

| | Months | | | | | |
|---|---|---|---|---|---|---|
| | March | April | May | June | July | August |
| Current Study | 23.2/9.3 | 24.6/9.6 | 27.1/16.0 | 31.1/19.9 | Study Complete | Study Complete |
| Days over 37.8 C | 0.0 | 0.0 | 0.0 | 0.0 | | |
| Yates' Study | Prior to Study | Prior to Study | 35.3/16.9 | 35.9/19.9 | 36.1/21.9 | 36.2/22.8 |
| Days over 37.8 C | | | 0.0 | 9 | 12 | 12 |

supplementation are more greatly increased in the physiological functions of goats rather than sheep.

*Age and maturity in relation to Tasco Kelp Seaweed effects*

This study consisted of rams from 1 to 6 years of age, with the majority of them around the 2-4 year old range. These rams have had previous experience with the summer heat and may have adapted to the heat better than a male who has not yet experienced heat or heat stress. So with this knowledge in mind, it stands to reason that the majority of the rams on this study had previous experience with heat and heat stress, and that may have been one reason the Kelp Seaweed showed less of an effect than in other studies. In the Yates et al.(2010) experiment, the study subjects were young male goats who were experiencing their first summer heat and had no previous experience with heat stress. This may explain why the Tasco-Ex showed such good results in that particular study. Previous heat experience and the fact they are mature (not still growing) may have allowed them to cope with the spring and summer heats more effectively. However, the below average ambient temperature coupled with the mature growth may have been the reason for the lack of differences.

## IMPLICATIONS

This study demonstrated that Tasco Kelp Seaweed supplementation did not impact weight gain, scrotal circumference, or improved semen quality. But it also did not show any adverse effects to the rams, instead it displayed a trend for the supplemented rams to be just a little better in all the semen quality categories. With this study taking place during such abnormally cool ambient temperatures, it is difficult to base all management decisions on this one study alone. With higher ambient temperatures there may be a greater extreme for the Tasco Kelp Seaweed to perform more productively. But with this study alone, there is no reason to supplement with Tasco Kelp Seaweed when rams performed normally without it. Additional research is needed to verify that the cool ambient temperatures and the age of the rams had no effect on the performance of the Tasco Kelp Seaweed as a combatant to heat stress.

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# EFFECTS OF PRENATAL SUPPLEMENTATION WITH AVAILA 4® ON FETAL GUT DEVELOPMENT, MINERAL STATUS AND CELL MEDIATED IMMUNE RESPONSE IN BOER CROSS MEAT GOATS

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***Project was funded by Zinpro Corporation, Eden Prairie, Minnesota***

## ABSTRACT

Forty 4 to 5 year old Boer Cross does pregnant with twins were randomly assigned to receive a mineral of Availa 4® (AV4) or inorganic (IN) forms of mineral. Does had free choice Sorghum Sudan hay and received the mineral supplement in a pelletted form for 45d prebreeding until weaning of their kids daily at 1300h. During the last trimester whole corn was added to the supplementation to meet nutrient requirements. Blood samples were collected, from each doe, at the beginning of the study (baseline), d45 of gestation and at parturition to analyze for Vitamin $B_{12}$ and circulating minerals. At parturition, birth weights were collected from each kid, colostrums samples collected and blood collected from each kid prior to nursing for the first time. Each kid was then dosed, by stomach tube, with 25 ml of colostrum collected from their dam. At 24h of age, another blood sample was collected from each kid and the lightest kid was euthanized using Uthasol®. The sacrificed kid was then dissected to remove a 10cm section of the duodenum and jejunum for histological analyses. Additionally, the right femur was removed and dried for mineral analysis and the liver was collected, divided into two separate sections and frozen until mineral analysis. On d75 postpartum, the remaining kids were weaned and body weights taken to determine growth rate. Twenty-one days after weaning, kids were injected intradermally in the tale web with kidney bean extract to measure cell mediated immune response every 3 h for 24 h. In all weight measurements, there were no differences between supplementation types. Supplementing with AV4, however, increased levels of Fe, Ca, Ph and Mg ($P<0.05$) in the femur while IN supplementation had higher ($P<0.05$) total ash content. In both colostrum and milk AV4 had higher ($P<0.05$) Co and Mn levels while IN had higher ($P<0.05$) Se contents. In all plasma sample for both the doe and kids, Co was higher ($P<0.05$) in the AV4 supplemented does. Intestinal morphology was similar between supplements. Additionally, cell mediated response to a foreign protein tended to be higher at all collection times with total inflammation and 24 h inflammation being higher ($P<0.05$) in AV4 supplemented kids. Mineral supplementation with AV4 or IN didn't result in higher levels in all measurements, but AV4 had an increase in, especially Co, several minerals measured with greater immune response to a foreign protein.

## INTRODUCTION

Microminerals are essential to numerous biological processes. Trace mineral deficiencies can lead to weight loss, lowered immunity responses and increased disease vulnerability (Suttle and Jones, 1989). While most goats receive adequate amounts of microminerals from forages or feed supplements, the vast majorities are not receiving the required amount of minerals for high levels of production. The development of the fetus in the last few weeks of pregnancy is influenced by the health and nutritional status of the animal (Sheep Production Handbook, 2002).

Organic mineral source has been shown to be more effective in decreasing or preventing mineral deficiencies (Greene, 1995). Transition metals (copper, cobalt, iron, manganese and zinc) are the only minerals that can be chelated to an organic complex.

Limited studies have evaluated the effects of organic mineral supplementation in goats. Therefore, this study compared an organic amino-acid complex mineral supplement to a non-organic mineral supplement. The organic mineral supplement that was used in the study is Availa-4, which consists of four trace minerals: zinc, copper, manganese and cobalt attached to an amino-acid

complex. Availa-4 is an industry mineral supplement produced by Zinpro© and widely used in the dairy cattle industry.

*Zinc, Copper, Manganese and Cobalt*

Zinc is the main trace mineral for DNA synthesis and protein metabolism in animals (Underwood, 1981). Immune function and hormone levels are influenced by zinc levels (Cousins and Hempe, 1990). The National Research Council (2007) recommends 10 mg Zinc/kg diet dry matter for adequate growth performance and reproduction. Zinc deficiency can greatly affect the growth and synthesis of cells in the body (Underwood, 1981). Deficiencies in copper and zinc, will lead to decreased immune response and increased disease susceptibility (Suttle and Jones, 1989). For this reason adequate volumes of zinc are extremely important during and after parturition.

Copper plays a vital role in proper nervous and immune system functionality (Machen, 2008). The liver is the main storage organ for copper. Metallothionein, which is found in the liver, prevents toxicity by binding and storing excess minerals such as copper and zinc. (Underwood, 1981). Copper is then released from the liver and used to build up an immune response (NRC, 2007). Copper may be particularly important for goats, because goats do not tolerate copper deficiencies very well, but are able to tolerate copper toxicity (Merck, 1991). The National Research Council recommends 7-11 mg/kg diet DM of copper in the diet.

Manganese is essential to several enzyme systems and is widely distributed in low concentrations in animal cells and tissues. This trace mineral is essential for bone development and reproduction (Underwood, 1981) and is found in high concentrations in bones, liver and kidneys. (Hidiroglou, 1979).

Animal tissues have no requirement for cobalt, but require Vitamin $B_{12}$, which is synthesized by rumen microbes. Rumen microorganisms require 0.5 to 1.0 mg cobalt/kg diet dry matter for growth (NRC, 2007). Vitamin $B_{12}$ production is restricted not only by the availability of copper. Vitamin $B_{12}$ deficiencies may occur when copper levels are below recommended levels (NRC, 2007). Cobalt deficiencies will lead to low growth performance because of lack of energy and protein metabolism (Kadim et al., 2006).

*Passive Immunity Transfer*

Does must receive proper nutrition to properly transfer immunoglobulins to their kids. Antibodies (immunoglobulin) are gamma globulin proteins found in blood and other bodily fluids. Immunoglobulins are used by the immune system to identify and neutralize foreign objects such as bacteria and viruses. Passive immunity is the transfer of serum antibodies from donor to recipient and can be natural or artificial. Passive immunity transfer of antibodies occurs through the placenta, colostrum and milk. The two immunoglobulins that were studied during this project were Immunoglobulin Gamma (IgG) and Immunoglobulin Mu (IgM). The most common antibody found in the body is IgG and it is the only immunoglobulin that can be passively transferred across the placenta. IgG transfer to neonatal lambs was positively correlated to greater survival of newborn lambs at birth and 6 months of age (Halliday, 1974). Research has indicated that total IgG levels in the milk quickly dropped at 36 hr after birth (Arguello et al., 2006). The most complex immunoglobulin is IgM, which does not cross the placenta and must be transferred through milk. The kid must receive adequate amounts of IgM via colostrum to build up enough antibody to fight bacteria and viruses.

*Cell Mediated and Humoral Immune Responses*

Immunity is split into cellular and humoral components, which are activated after infections and injuries (Fiolka, 208). Immunity transfer was tested through cell-mediated and humoral immune responses.

*Colostrum and Milk Quality*

Early intake of adequate volumes of good quality colostrum is the most effective way to obtain sufficient immunoglobulins in neonates. Kids that do not receive adequate volumes of colostrum have a greater chance of developing infectious diseases. Survival rates of newborn kids is directly correlated with the amount of colostrum fed in the first two days of life (Morand-Fehr, 1989). A 20% mortality rate was observed in colostrum-deprived lambs (Vihan, 1988). Milk with high levels of protein and butter fat content are good indicators of high quality milk.

*Somatic Cell Count*

Clinical studies have indicated that the infiltration of bacteria within the mammary gland induces an inflammatory reaction leading to increased somatic cell count in the milk (Ferrer et al., 1997). In the United States, the legal milk somatic cell count (MSCC) limit determined by the Food and Drug Administration for goats and sheep is 1,000 x $10^3$ $ml^{-1}$. Intramammary infections have been found to be the main indicator of poor milk quality and quantity (Gonzalez-Rodriguez et al., 1995; White and Hinckley, 1999). Because, sheep and goats have only two mammary glands, MSCC counts will not be lowered by the mixing of the milk of one infected gland with the other gland that is not infected (Leitner et al., 2008). The elevation of MSCC can also be attributed to inefficient management of the flock, age of animal, or lactation stage (Paape, et al., 2007).

This study evaluated the ability of Boer-Spanish cross does to pass on immunity to their neonates based on pre-natal supplementation of the does. Immunity levels were evaluated by IgG and IgM levels and cell mediated & humoral immune responses in the kid. Growth performance of the kids was also measured. The study also measured mineral levels, milk quality and Vitamin $B_{12}$ levels produced in the does.

## MATERIALS AND METHODS

Animal Management: Eighty Boer cross females 4-5 years of age were randomly assigned to one of two treatments. Treatments consisted of does having ad libitum access to Sudan hay and receiving a mineral supplement in a pellet (Table 1) at 150 g/d at 1300h. They received treatment for 45 days prior to bucks being placed with the does (1 buck/15 does) for a 35 day breeding period. Daily breeding activity was recorded. Does stayed in supplementation groups until 30 post breeding. At which time 20 does from each group that were carrying twins and relatively close in breeding dates (+/- 10days) were then place in pens (3 does/pen). Pens measured 3.3 X 12.9 m with one half covered for shade and protection. Pens contained automatic waterers that were cleaned twice per week. Does were then individually fed their supplement daily at 1300h and during the last trimester they were also fed an additional 200 g of whole corn to meet their energy demands. Does remained in smaller pens until 5 days after parturition, at which time they were placed in larger pens with their single kid. At parturition, birth weights were recorded for each kid and kids were indentified with ear tags. The lightest weight kid was then chosen to be euthanized at 24h for further samplings. At birth, colostrum was collected from the doe and each kid was dosed with 25 ml. The remaining kid was weaned at 75 days of age and weighed.

Table 1. Mineral analysis (As-Fed, amount per day)

| | Organic | Non-Organic |
|---|---|---|
| Dry Matter, % | 90.60 | 90.27 |
| Crude Protein, g/d | 21.30 | 20.05 |
| ADF, g/d | 25.84 | 26.90 |
| NDF, g/d | 35.51 | 38.95 |
| TDN, g/d | 71.75 | 70.50 |
| Calcium, g/d | 0.77 | 0.83 |
| Phosphorous, g/d | 0.43 | 0.42 |
| Cobalt, ppm | 1.60 | 0.70 |
| Copper, ppm | 33.70 | 38.50 |
| Iodine, ppm | 1.19 | 1.20 |
| Iron, ppm | 45.90 | 43.60 |
| Manganese, ppm | 55.00 | 65.70 |
| Selenium, ppm | 0.32 | 0.29 |
| Zinc, ppm | 75.60 | 89.40 |

*Sampling*

Plasma and serum samples were collected from each doe at the initiation of the experiment, when placed in smaller pens, at parturition and at weaning for mineral and vitamin analysis. At parturition colostrum sample was collected for analysis of minerals, vitamins and IgG. Plasma and serum samples were also collected from both kids at parturition and 24h of age for mineral, vitamin and IgG analysis. The remaining kid was also sampled at 96h. The lightest weight kid was euthanized with euthasol at 24h. At this time the right femur was removed, dried and divided into two equal parts for mineral analysis. The liver was removed divided and frozen for later mineral analysis. Small intestinal samples were collected 1 meter from the pylorus and one meter from the cecum, fixed in 5% formalin for microhistological analysis of villous length and crypt depth. At weaning kids were immunologically challenged with a commercial clostridium vaccine. Serum samples were collected at d0 and every 7 d for 28 d. Additionally, at 21 d a cell mediated response to a foreign protein injected under the tail web intradermally. Swelling response was measured a 0h and at 3h intervals for 12h and again at 24h.

## RESULTS AND DISCUSSION

*Weight gain and growth performance*

Doe weights were similar throughout the study (Table 2). Does from both treatments had a slight decrease in weight during the first trimester, but doe weights increased back to baseline weights during the third trimester. The mean weight at weaning showed a large decrease. The organic group showed a decrease of 10.5 kg from the baseline weight to the weaning weight. The non-organic group showed a decrease of 7.5 kg during the same time. These results show that the does were evenly distributed in regard to weight from the beginning of the study.

*Kid Growth Performance*

Kid weights at birth and weaning were not significantly different (Table 3). Mean birth weights were exactly the same in both treatments. Mean weight gains were 13.6 ± 0.8 for the organic treatment and 12.8 ± 0.6 for the non-organic treatment. These results indicate that

Table 2. Doe Weights (kg)

| Time Period | Mean | SE | *P-Value* |
|---|---|---|---|
| **Baseline** | | | |
| Organic | 63.3 | 1.6 | 0.48 |
| Non-organic | 61.7 | 1.5 | |
| **1st Trimester** | | | |
| Organic | 59.3 | 1.9 | 0.58 |
| Non-organic | 60.7 | 1.8 | |
| **3rd Trimester** | | | |
| Organic | 62.9 | 2.1 | 0.86 |
| Non-organic | 62.4 | 2.0 | |
| **Weaning** | | | |
| Organic | 52.8 | 2.4 | 0.65 |
| Non-organic | 54.2 | 2.0 | |

Table 3. Kid birth weights, weaning weights and weight gain (kg)

| | **Mean** | **SE** | ***P-Value*** |
|---|---|---|---|
| **Birth** | | | |
| Organic | 3.0 | 0.1 | 0.98 |
| Non-organic | 3.0 | 0.1 | |
| **Weaning** | | | |
| Organic | 17.0 | 0.8 | 0.39 |
| Non-organic | 16.1 | 0.6 | |
| **Weight Gain** | | | |
| Organic | 13.6 | 0.8 | 0.44 |
| Non-organic | 12.8 | 0.6 | |

prenatal mineral supplementation of an organic mineral supplement does not affect growth performance.

*Serum Mineral Profile*

Mineral analysis of the femur showed differences in various minerals (Table 4). Copper, Manganese, Cobalt, Zinc, Selenium, and Molybdenum were similar (P>0.05). Iron tended to be higher (P=0.09) while differences were noted for Ash (P=0.001), Calcium (P=0.01), Phosphorus (P=0.002), and Magnesium (P=0.03) where Availa tended to have higher mineral concentrations than the inorganic treatment.

Plasma samples taken from the does showed trace mineral concentrations for the baseline sample to be higher in the inorganic treatment (Table 5). Zinc had elevated levels (P=0.08). Cobalt (P<.0001) differed while Manganese (P=0.06) was higher at the 0 hour sample (Table 5). Iron, Copper, Zinc, Selenium, and Molybdenum held at P>0.05. Mineral concentrations during the third trimester were similar (P=0.05) except for Cobalt (P=0.009). Plasma at weaning

Table 4. Right femur removal, weighed, dried, and analyzed for mineral profile

| | Treatment[a] | | | |
|---|---|---|---|---|
| | Availa | Inorganic | SE[b] | P-value |
| Mn[c] | 4.80 | 4.83 | 0.89 | 0.98 |
| Fe[c] | 236.04 | 180.01 | 23.61 | 0.09 |
| Co[c] | 0.95 | 1.92 | 0.66 | 0.30 |
| Cu[c] | 9.68 | 12.36 | 1.32 | 0.15 |
| Zn[c] | 96.36 | 109.06 | 6.76 | 0.18 |
| Se[c] | 0.08 | 0.06 | 0.01 | 0.26 |
| Mo[c] | 0.41 | 0.40 | 0.05 | 0.82 |
| Ash[e] | 51.74 | 55.81 | 0.79 | 0.001 |
| Ca [f] | 33.24 | 30.95 | 0.58 | 0.01 |
| Ph[f] | 16.60 | 15.32 | 0.28 | 0.002 |
| Mg[d] | 6.57 | 6.12 | 0.14 | 0.03 |

[a]Availa-4 is the organic treatment under analysis to the traditional inorganic mineral supplementation

[b]Most conservative standard error of the least square means.

[c]ug/gram of ash

[d]mg/grams of ash

[e]g/100 gram of fat free dry bone

[f]g/100 gram of ash

Table 5. Mineral analysis taken from plasma samples of does at beginning of study, parturition, third trimester and weaning.

| | Treatment[a] | | | |
|---|---|---|---|---|
| | Availa | Inorganic | SE[b] | P-value |
| | | Baseline | | |
| Mn, ng/ml | 2.37 | 2.70 | 0.14 | 0.10 |
| Fe, ug/ml | 1.50 | 1.59 | 0.11 | 0.59 |
| Co, ng/ml | 0.76 | 0.82 | 0.05 | 0.49 |
| Cu, ug/ml | 0.97 | 0.98 | 0.03 | 0.78 |
| Zn, ug/ml | 0.57 | 0.62 | 0.02 | 0.08 |
| Se, ng/ml | 148.05 | 141.04 | 2.94 | 0.10 |
| Mo, ng/ml | 55.79 | 22.73 | 8.81 | 0.01 |
| | | O Hr[c] | | |
| Mn, ng/ml | 2.26 | 2.56 | 0.11 | 0.06 |
| Fe, ug/ml | 1.48 | 1.88 | 0.36 | 0.42 |
| Co, ng/ml | 31.90 | 10.97 | 2.88 | <.0001 |
| Cu, ug/ml | 1.08 | 1.08 | 0.05 | 0.99 |
| Zn, ug/ml | 0.46 | 0.43 | 0.04 | 0.70 |
| Se, ng/ml | 146.59 | 145.79 | 3.56 | 0.87 |
| Mo, ng/ml | 37.85 | 39.37 | 3.04 | 0.72 |
| | | Third Trimester | | |
| Mn, ng/ml | 2.56 | 3.21 | 0.36 | 0.20 |
| Fe, ug/ml | 1.62 | 1.50 | 0.10 | 0.39 |
| Co, ng/ml | 16.63 | 8.61 | 1.62 | 0.009 |
| Cu, ug/ml | 1.19 | 1.20 | 0.05 | 0.85 |
| Zn, ug/ml | 0.47 | 0.63 | 0.07 | 0.14 |
| Se, ng/ml | 158.20 | 160.10 | 2.85 | 0.64 |
| Mo, ng/ml | 30.58 | 29.87 | 2.08 | 0.81 |
| | | Weaning | | |
| Mn, ng/ml | 3.83 | 4.24 | 0.41 | 0.44 |
| Fe, ug/ml | 2.30 | 1.91 | 0.15 | 0.05 |
| Co, ng/ml | 3.88 | 2.92 | 0.74 | 0.32 |
| Cu, ug/ml | 1.58 | 1.40 | 0.07 | 0.07 |
| Zn, ug/ml | 0.60 | 0.62 | 0.09 | 0.88 |
| Se, ng/ml | 177.05 | 172.18 | 8.27 | 0.64 |
| Mo, ng/ml | 85.27 | 70.91 | 10.35 | 0.28 |

[a]Availa-4 is the organic treatment under analysis to the traditional inorganic mineral supplementation

[b]Most conservative standard error of the least square means.

[c]0 Hr is taken the moment of birth

mineral analysis shows Iron (P=0.05) as a notable difference and higher levels of Copper (P=0.07). Manganese, Cobalt, Zinc, Selenium, and Molybdenum were similar (P>0.05) and showed higher levels in the Availa treatment (Table 5.)

Mineral analysis for doe colostrums samples show mineral concentrations for Iron, Copper, Zinc, Selenium, and Molybdenum to be consistent (P>0.05). Manganese (P=0.01) and Cobalt (P=0.0008) are different (Table 6). Milk sample mineral concentrations are also all consistent (P>0.05) except for Cobalt (P<.0001) and Selenium (P=0.05) (Table 6).

Plasma samples of kids at birth, 24 hours, and 96 hours of age were analyzed (Table 7). 0 hour shows a significance for Manganese (P=0.0001) and Cobalt (P=0.0001). Iron, Copper, Zinc, Selenium, and Molybdenum were consistent (P>0.05). At 24 hours of age Cobalt (P=.0001) was the only difference between the minerals. Mineral concentrations at 96 hours of age show a difference in Cobalt (P=0.0006) and Copper (P=0.0002). A higher rate was noted in Selenium (P=0.08). Mineral concentration levels were higher at 24 hours in the inorganic treatment and vice versa at 0 hour and 96 hour.

*Vitamin $B_{12}$*

Vitamin $B_{12}$ concentrations in the blood were analyzed to determine if prenatal supplementation of an organic trace mineral would affect cobalt and Vitamin B12 production transfer from the doe to the kid (Figure 1). Doe vitamin $B_{12}$ serum concentrations were not significantly different at birth (*P=0.48*). The serum Vitamin $B_{12}$ levels were 756.4 pg/ml ± 46.1 for the organic group and 711.7 pg/ml ± 43.1 for the inorganic group. The heaviest weight kids in the organic group had significantly higher Vitamin $B_{12}$ serum concentrations (*P = 0.008*) than the non-organic group. The serum Vitamin $B_{12}$ levels were 980.7 pg/ml ± 72.0 for the organic group kids and 702.1 pg/ml ± 67.6 for the inorganic group kids. The Vitamin $B_{12}$ serum concentrations for the lightest weight kid at zero hour was not significantly different (*P = 0.44*). The serum Vitamin $B_{12}$ levels were 783.7 pg/ml ± 89.1 for the organic group kids and 881.9 pg/ml ± 87.0 for the inorganic group kids. Vitamin $B_{12}$ concentration levels at 24 hours of birth were significantly different for the lightest weight kid in the non-organic group (P = 0.02). The serum concentration levels for the non-organic group were 1035.7 *pg*/ml ± 100.4 and 712.1 ± 96.1. The heaviest weight kids at 24 hours of birth for both treatments had similar Vitamin $B_{12}$ serum concentrations. The serum concentrations for the heaviest weight kids were 751.0 pg/ml ± 78.6 for the organic group and 748.5 ± 71.7 for the non-organic group.

Vitamin B12 serum concentrations in the doe were significantly different at the beginning of the study (*P < 0.001*). The baseline serum concentrations were 1776.0 pg/ml ± 85.3 for the organic group and 1116.7 ± 83.6 for the non-organic group (Fig. 3). The Vitamin B12 concentrations decreased during the third trimester. The organic group had a concentration level of 676.4 ± 40.3 at the third trimester, which was a decrease of 1100 pg/ml from the baseline concentration. The non-organic group had a concentration level of 637.7 ± 39.5 at the third trimester, which was a decrease of 479 *p*g/ml from the baseline concentration. The serum concentrations at weaning showed an increase in Vitamin $B_{12}$ levels during the third trimester, but never reached the baseline serum concentration levels. The Vitamin $B_{12}$ concentrations at weaning were not significantly different for the does (*P = 0.21*). The Vitamin $B_{12}$ concentration levels were 1050.3 ± 71.5 for the organic group and 932.8 ± 57.1 for the non-organic does.

Table 6. Mineral analysis for doe colostrum and milk samples

| | Treatment[a] | | | |
|---|---|---|---|---|
| | Availa | Inorganic | SE[b] | P-value |
| | | Colostrum | | |
| Mn, ng/g | 180.92 | 95.01 | 24.45 | 0.01 |
| Fe, ug/g | 0.89 | 0.93 | 0.14 | 0.83 |
| Co, ng/g | 14.34 | 6.90 | 1.52 | 0.0008 |
| Cu, ug/g | 1.07 | 1.08 | 0.14 | 0.93 |
| Zn, ug/g | 38.09 | 34.34 | 4.91 | 0.57 |
| Se, ng/g | 217.05 | 198.02 | 18.65 | 0.45 |
| Mo, ng/g | 20.53 | 23.50 | 2.73 | 0.42 |
| | | Milk | | |
| Mn, ng/g | 52.65 | 58.05 | 4.56 | 0.36 |
| Fe, ug/g | 0.51 | 0.53 | 0.06 | 0.84 |
| Co, ng/g | 1.39 | 0.35 | 0.11 | <.0001 |
| Cu, ug/g | 0.10 | 0.12 | 0.03 | 0.61 |
| Zn, ug/g | 3.52 | 3.66 | 0.22 | 0.61 |
| Se, ng/g | 32.03 | 38.48 | 2.53 | 0.05 |
| Mo, ng/g | 30.50 | 25.45 | 4.03 | 0.33 |

[a]Availa-4 is the organic treatment under analysis to the traditional inorganic mineral supplementation

[b]Most conservative standard error of the least square means.

Table 7. Mineral analysis taken from plasma samples of kids at birth, 24 hours, and 96 hours of age.

| | Treatment[a] | | | |
|---|---|---|---|---|
| | Availa | Inorganic | SE[b] | P-value |
| | | O Hr | | |
| Mn, ng/ml | 2.95 | 4.14 | 0.20 | <.0001 |
| Fe, ug/ml | 3.14 | 3.73 | 0.27 | 0.10 |
| Co, ng/ml | 1.71 | 0.88 | 0.14 | <.0001 |
| Cu, ug/ml | 0.29 | 0.28 | 0.01 | 0.73 |
| Zn, ug/ml | 0.43 | 0.35 | 0.07 | 0.43 |
| Se, ng/ml | 75.06 | 74.31 | 2.04 | 0.79 |
| Mo, ng/ml | 34.16 | 35.17 | 3.86 | 0.85 |
| | | 24 Hr | | |
| Mn, ng/ml | 3.10 | 3.32 | 0.18 | 0.39 |
| Fe, ug/ml | 1.87 | 3.20 | 0.82 | 0.25 |
| Co, ng/ml | 1.90 | 0.94 | 0.14 | <.0001 |
| Cu, ug/ml | 0.36 | 0.38 | 0.01 | 0.33 |
| Zn, ug/ml | 0.68 | 0.69 | 0.07 | 0.93 |
| Se, ng/ml | 89.35 | 93.15 | 2.33 | 0.24 |
| Mo, ng/ml | 28.16 | 29.03 | 3.98 | 0.88 |
| | | 96 Hr | | |
| Mn, ng/ml | 3.15 | 2.98 | 0.23 | 0.59 |
| Fe, ug/ml | 1.45 | 1.44 | 0.13 | 0.97 |
| Co, ng/ml | 1.58 | 0.73 | 0.17 | 0.0006 |
| Cu, ug/ml | 0.57 | 0.78 | 0.04 | 0.0002 |
| Zn, ug/ml | 0.81 | 0.93 | 0.05 | 0.12 |
| Se, ng/ml | 63.87 | 71.14 | 2.98 | 0.08 |
| Mo, ng/ml | 14.11 | 11.19 | 4.28 | 0.61 |

[a]Availa-4 is the organic treatment under analysis to the traditional inorganic mineral supplementation

[b]Most conservative standard error of the least square means.

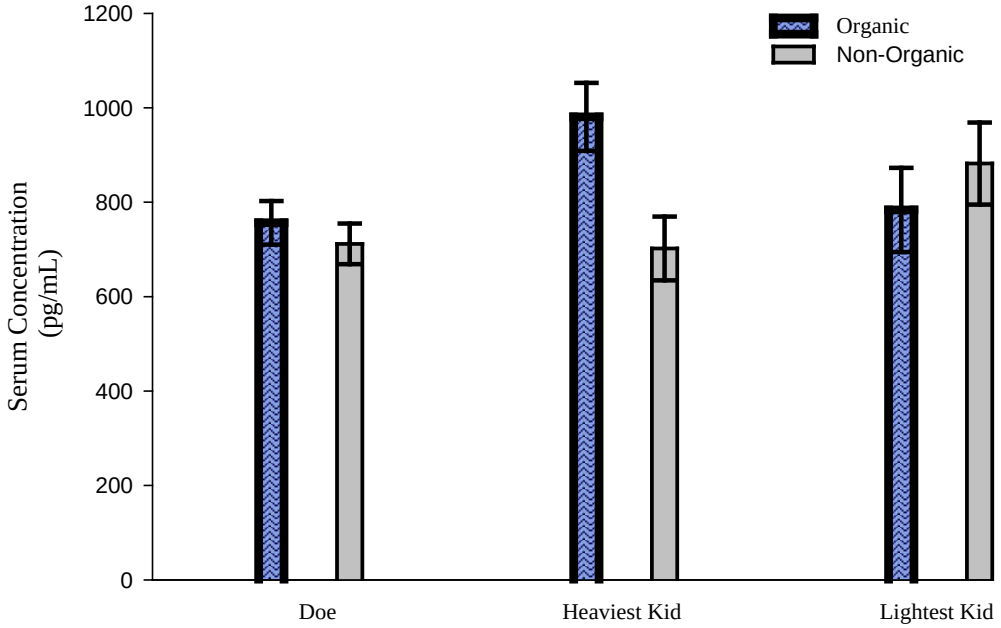


Fig. 1 Vitamin $B_{12}$ Serum Concentrations at birth.
Doe (*P = 0.48*)
Heaviest Kid (*P = 0.008*)
Lightest Kid (*P = 0.44*)

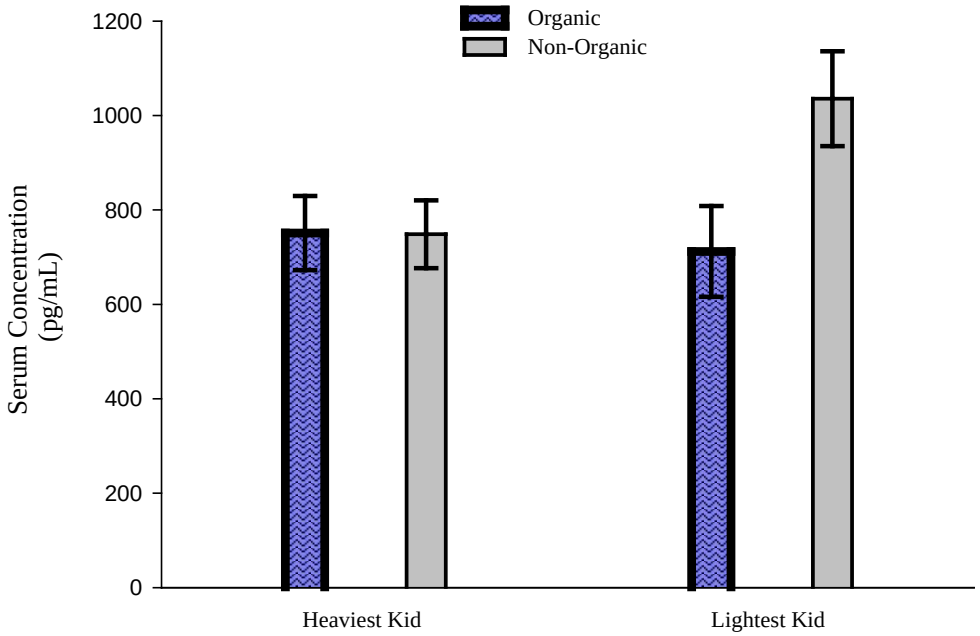


Fig. 2 Vitamin $B_{12}$ Serum Concentrations of kids at 24hrs old

Heaviest Kid (*P = 0.98*)
Lightest Kid (*P = 0.02*)

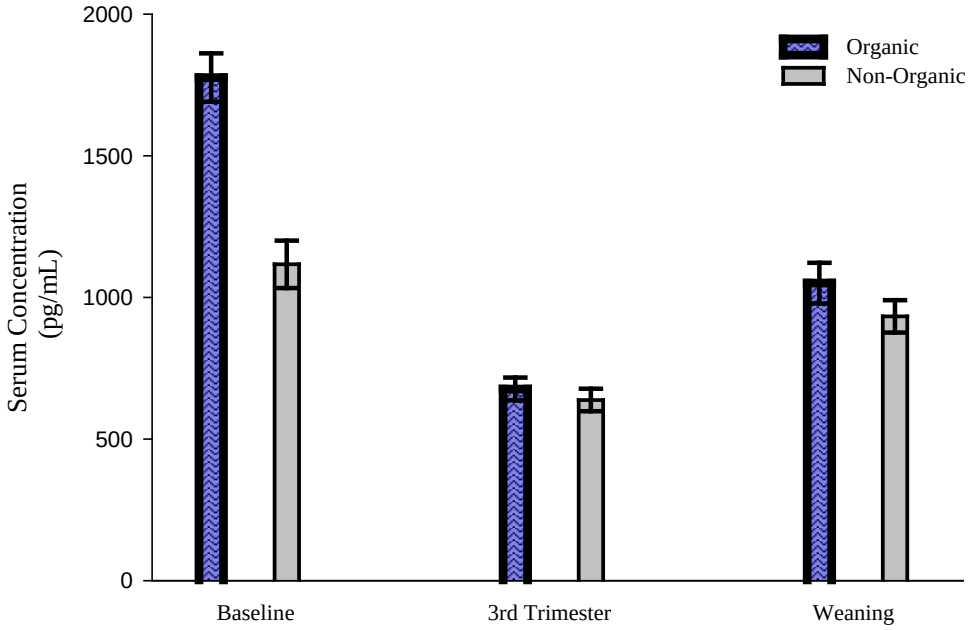


Fig. 3 Vitamin B12 Serum Concentrations of Does
Baseline (*P < 0.001*)
3rd Trimester (*P = 0.50*)
Weaning (*P = 0.21*)

*Cell Mediated Immune Response*

The cell mediated immune response of the heaviest kids at weaning showed a significant difference 24 hours after the injection of PHA-P into the tail-web of the kid. There was no difference in skin swelling at 3, 6, 9 or 12 hr post injection (*P > 0.14*). The base measurement before the injection was 2.25 mm ± 0.09 for the organic group and 2.39 mm ± 0.07 for the non-organic group. The 3 hr measurement after the injection was 10.28 mm ± 0.77 for the organic group and 9.65 mm ± 0.62 for the non-organic group (Fig. 4). The 3 hr measurement after the injection showed an increase in swelling with increases of 8.04 mm ± 0.75 for the organic group and 7.26 mm ± 0.56 for the non-organic group (Fig. 5). The 6 hr measurement after the injection was 9.32 mm ± 0.67 for the organic group and 9.15 mm ± 0.54 for the non-organic group (Fig. 4). The 6 hr measurement after the injection showed an increase in swelling from the base measurement but a decrease from the 3 hr measurement (Fig. 5). The difference from hour 6 and the base measurement was 7.08 mm ± 0.65 for the organic group and 6.71 mm ± 0.52 for the non-organic group (Fig. 5). The 9 hr measurement after the injection were not significantly different between treatments (*P = 0.29*). The 9 hr measurement stayed the same as the 6 hr measurement for the organic group, but decreased slightly for the non-organic group (Fig. 4).

The 9 hr measurement after the injection was 9.38 mm ± 0.58 for the organic group and 8.60 mm ± 0.46 for the non-organic group (Fig. 4). The 9 hr measurement after the injection showed a slight increase over the 6 hour measurement for the organic group. The 9 hour

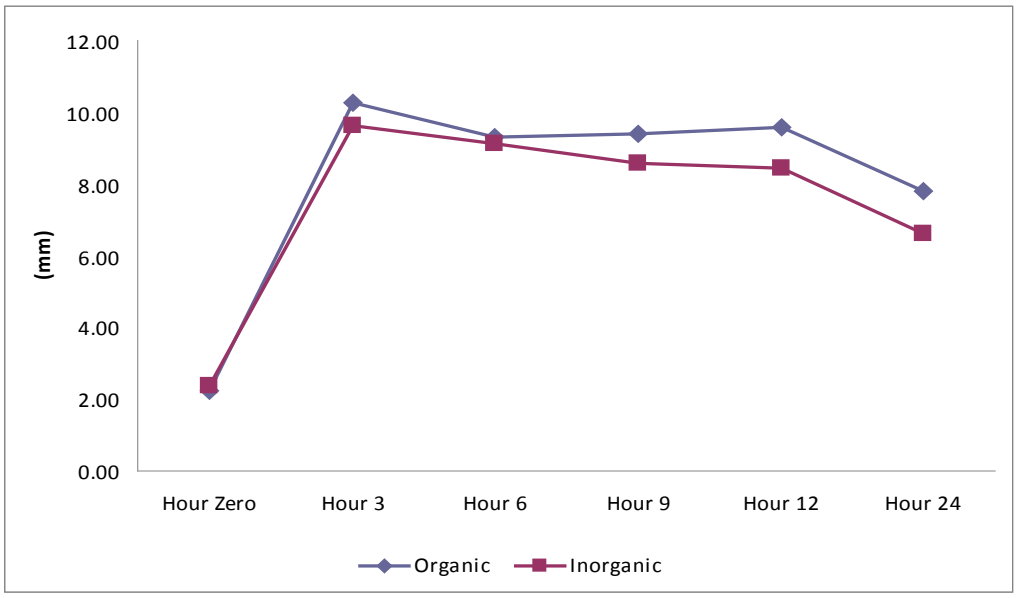


Figure 4 Cell Mediated Response of heaviest kid at 45 days of age

Hour Zero (*P = 0.21*)
Hour 3 (*P = 0.53*)
Hour 6 (*P = 0.80*)
Hour 9 (*P = 0.30*)
Hour 12 (*P = 0.14)*
Hour 24 (*P = 0.03*)

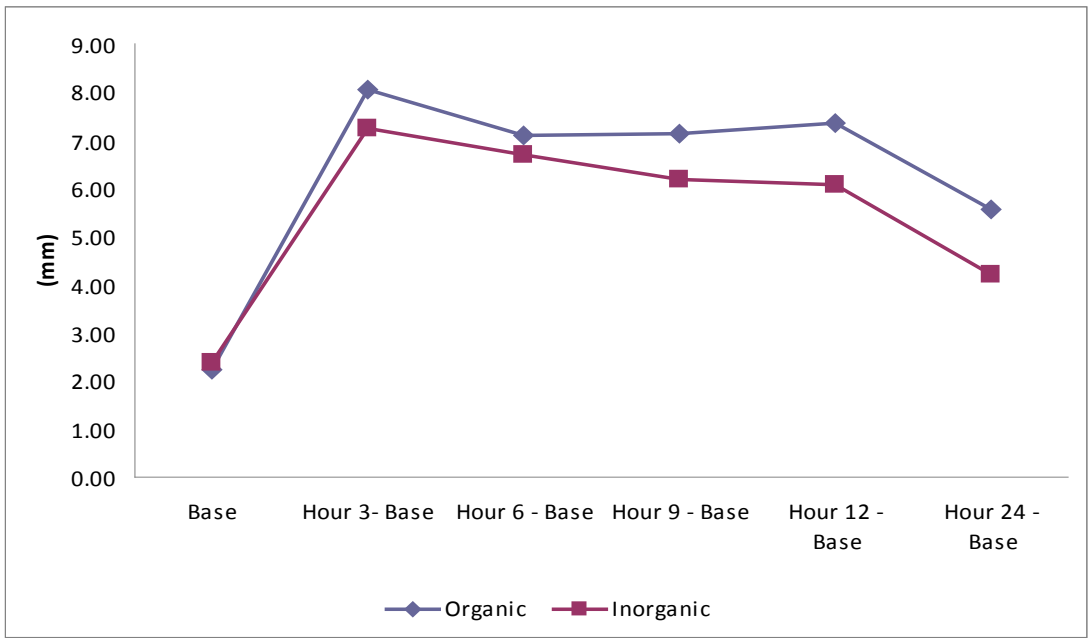


Figure 5. Cell Mediated Response at 45 days of age: Increase in Swelling (Hour– Base Measurement)

Hour 3 - Base (*P = 0.42*)
Hour 6 - Base (*P = 0.66*)
Hour 9 - Base (*P = 0.22*)
Hour 12 - Base (*P = 0.14*)
Hour 24 - Base (*P = 0.03*)

measurement was noticeably lower than the 6 hour measurement for the non-organic group (Fig. 4). The difference from hour 9 and the base measurement was 7.14 mm ± 0.58 for the organic group and 6.20 mm ± 0.46 for the non-organic group.

The 12 hr measurement after the injection was not significantly different between treatments (*P = 0.14*). The 12 hr measurement stayed about the same as the 9 hr measurement for the organic group and non-organic group (Fig. 4). The 12 hr measurement was 9.59 mm ± 0.59 for the organic group and 8.45 mm ± 0.47 for the non-organic group (Fig. 4). The difference from 12 hr and the base measurement was 7.34 mm ± 0.59 for the organic group and 6.06 mm ± 0.47 for the non-organic group (Fig. 5).

The 24 hr measurement showed a significant difference between treatments for the 24 hr measurement (*P = 0.03*). The 24 hr measurement was 7.81 ± 0.40 for the organic group and 6.61 ± 0.32 for the non-organic group. A decrease in skin swelling at 24 hr compared to previous measurements was noticeable. The overall measurement, 24 hr measurement minus base measurement, was significantly higher in the organic group (*P = 0.01*). The difference from 24 hr and the base measurement was 5.57 mm ± 0..40 for the organic group and 4.22 mm ± 0.32 for the non-organic group (Fig. 5). The organic group skin swelling was higher throughout the 24 hr time period. This higher skin swelling indicates that the organic kids had built up a stronger cell-mediated response than the non-organic kids. In contrast, trace mineral supplementation did not elicit a cell-mediated immune response in cows (Stanton et al., 2000). Results suggest that prenatal organic mineral supplementation does improve cell-mediated immune response. Higher vitamin $B_{12}$ concentrations at birth may be positively correlated to a stronger cell mediated response.

*Humoral Immunity Responses*

Humoral immunity of weaned kids 0, 7, 14, 21 days after a vaccination of enterotoxaemia showed no differences in titer levels (Table 8). Day zero was used as a baseline measurement of titer levels in the kids. The zero hour data indicates the antibody level that the kid had before the vaccine was administered. The organic kids at day zero had a mean titer level of 234.3 ± 66.9 and showed a decrease in titer levels at day 7 and then increased on day 14 and day 21. These data indicate that the organic kids had more naturally acquired passive immunity than the non-organic kids. The non-organic kids had a lower titer level at day zero and day 7 and were able to build up immunity responses to the antigen at day 21.

*Passive Immune Transfer*

Plasma samples collected from the kids show that does consuming the Availa-4 supplement had similar (*P*>0.05; Table 9) levels of circulating IgG at birth and 24h of age. However, IgM levels were significantly greater (*P*<0.004) at both birth and 24h of age, indicating an immune system that should be healthier. These results were without a subsequent increase (Table 10) in IgM levels in the colostrum, which appears to indicate that the Availa kids were more capable of absorbing IgM than the kids consuming the traditional inorganic mineral supplement.

*Liver Mineral Analysis*

Copper mineral levels in the lightest (sacrificed) kid tended to be lower in the organic kids (*P = 0.06*). Lower liver copper levels in the lightest kid at 24 hr indicates that the lightest weight kid in the organic group did not receive adequate amounts of copper and was unable to store enough copper in the liver. Manganese, cobalt and zinc levels were not significantly different in the liver (Table 11).

*Milk Analysis*

Milk butter fat, protein and somatic cell count analysis showed that there were no differences between treatment (Table 11). The non-organic does tended to have higher MSCC at 12 hr,

Table 8. Humoral immunity of weaned kids at 75 d, titer level.

| | **Mean** | **SE** | ***P-Value*** |
|---|---|---|---|
| **O day post vaccination** | | | |
| Organic | 234.3 | 66.9 | 0.30 |
| Non-organic | 143.6 | 53.3 | |
| **7 day post vaccination** | | | |
| Organic | 134.3 | 29.8 | 0.92 |
| Non-organic | 130.9 | 23.7 | |
| **14 day post vaccination** | | | |
| Organic | 148.6 | 23.0 | 0.35 |
| Non-organic | 176.4 | 18.3 | |
| **21 day post vaccination** | | | |
| Organic | 188.6 | 36.5 | 0.28 |
| Non-organic | 240.0 | 29.1 | |

Table 9. Immunoglobulin Gamma (IgG) and Mu (IgM) levels (g/dl of serum) in serum collected from kids at birth and 24h of age.

| | Availa | Inorganic | SE | P-value |
|---|---|---|---|---|
| | | 0 hour, Birth | | |
| IgG | 0.458 | 0.364 | 0.059 | 0.26 |
| IgM | 0.052 | 0.012 | 0.007 | 0.001 |
| | | 24 hours of age | | |
| IgG | 1.74 | 1.88 | 0.169 | 0.56 |
| IgM | 0.323 | 0.170 | 0.036 | 0.004 |

Table 10. Immunoglobulin Gamma (IgG) and Mu (IgM) levels (g/dl of colostrum) in colostrum collected from does at parturition.

| | Availa | Inorganic | SE | P-value |
|---|---|---|---|---|
| IgG | 10.82 | 8.98 | 0.988 | 0.185 |
| IgM | 0.487 | 0.478 | 0.071 | 0.927 |

Table 11. Sacrificed Kid – Liver Mineral Analysis, ug/g of dry liver.

| | Mean | SE | *P-Value* |
|---|---|---|---|
| **Manganese** | | | |
| Organic | 18.4 | 1.4 | 0.51 |
| Non-organic | 19.8 | 1.5 | |
| **Cobalt** | | | |
| Organic | 0.24 | 0.02 | 0.39 |
| Non-organic | 0.21 | 0.02 | |
| **Copper** | | | |
| Organic | 118.1 | 18.6 | 0.06 |
| Non-organic | 170.9 | 19.6 | |
| **Zinc** | | | |
| Organic | 116.6 | 11.6 | 0.39 |
| Non-organic | 101.9 | 12.2 | |

5 and 75 d after parturition. The non-organic does milk somatic cell count (MSCC) was above the limit determined by the Food and Drug Administration. The results indicate that the organic mineral supplement tended to decrease MSCC. The milk butter fat and protein levels of both treatments were the same throughout the lactation period (Table 12).

*Doe and kid losses during the study*

Doe losses and kid losses during the study are worth commenting about. The organic group did have 3 set of triplets that were alive at birth, while the non-organic had 5 sets of triplets that were alive at birth. The organic group also had 3 sets of triplets and the non-organic had 2 sets that had a dead fetus at birth. The organic group also had 2 sets of twins that had one of the fetuses dead at birth. The non-organic group had 3 sets of twins that had one fetus dead at birth. Three does in the organic group had one kid die shortly after birth, while the non-organic group had one kid die after birth. An organic doe did die before giving birth because of the possibility of her having ketosis. Two more does in the organic group were suspected to have ketosis and had kids that died because of lack of milk.

*Intestinal Morphology*

Fetal gut analysis does not show a difference for illeum villus and crypt depth or jejunum villus depth (P>0.05). Jejunum crypt was higher (P=0.07) (Table 13).

**IMPLICATIONS**

Availa-4 supplementation for goats appears to be a viable alternative to traditional mineral supplementation for improving mineral status in several of the essential minerals in both the doe and offspring. Additionally, vitamin B12 is increased in goats which should improve immune status. Cell mediated immune response to a foreign protein seems to be improved and the response to vaccines occurred faster in kids supplemented with Availa-4 which should result in stronger vaccine efficacy. Passive immune (IgM) is improved in the Availa treatment, and this coupled with the increase other responses to the immune system suggest Availa supplementation would result in healthier kids and ultimately stronger faster growing kids. Therefore, Availa-4 supplementation seems to improve the health and immune status in Boer Cross does and their offspring.

Table 12. Milk Values

| | Butter Fat % [A] | | Protein % [B] | | MSCC % [CD] | |
|---|---|---|---|---|---|---|
| | **Mean** | **S.E.** | **Mean** | **S.E.** | **Mean** | **S.E.** |
| **12 Hour** | | | | | | |
| Organic | 6.08 | 0.68 | 6.90 | 0.51 | 668.0 | 324.7 |
| Non-organic | 6.67 | 0.65 | 7.63 | 0.49 | 1065.6 | 309.6 |
| **Day 5** | | | | | | |
| Organic | 6.64 | 0.70 | 6.20 | 0.45 | 791.8 | 316.2 |
| Non-organic | 7.21 | 0.68 | 5.80 | 0.44 | 1042.8 | 307.8 |
| **Day 45** | | | | | | |
| Organic | 5.71 | 0.35 | 3.60 | 0.11 | 757.1 | 176.9 |
| Non-organic | 5.55 | 0.29 | 3.61 | 0.09 | 441.8 | 144.4 |
| **Day 75** | | | | | | |
| Organic | 4.76 | 0.60 | 3.81 | 0.23 | 1331.2 | 958.0 |
| Non-organic | 5.09 | 0.41 | 3.92 | 0.16 | 2594.5 | 659.3 |

[A] Butter Fat - 12 hr, Day 5, 45 and 75 ( P > 0.52)

[B] Protein - 12 hr, Day 5, 45 and 75 ( P > 0.31)

[C] Milk Somatic Cell Count (MSCC) - 12 hr, Day 5, 45 and 75 ( P > 0.17)

[D] SCC (x1000)

Table 13. Fetal gut analysis, µm[c]

| | Treatment[a] | | | |
|---|---|---|---|---|
| | Availa | Inorganic | SE[b] | P-value |
| Ileum Villus | 574.24 | 622.91 | 36.98 | 0.35 |
| Ileum Cyrpt | 151.38 | 109.52 | 33.77 | 0.38 |
| Jejunum Villus | 536.28 | 575.30 | 32.68 | 0.40 |
| Jejunum Crypt | 100.42 | 109.54 | 3.54 | 0.07 |

[a]Availa-4 is the organic treatment under analysis to the traditional inorganic mineral supplementation

[b]Most conservative standard error of the least square means.

[c]Intestinal tissue samples taken two meters from the pylorus, from the measured middle of the small intestine, and 75% towards the terminal ileum.

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## THE EFFECTS OF SEASONALITY ON RAM FERTILITY

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**ABSTRACT**

The objective was to determine how seasonality affects reproductive activity in rams of different breeds, comparing wool, black face, and hair sheep. Fourteen rams were included: 5 Rambouillet, 5 Suffolk, 2 Black-headed Dorper, and 2 St. Croix. Once a month, semen was collected, and the rams were evaluated for a body condition score, scrotal circumference, and rectal body temperature. Samples were evaluated for color, motility, and sperm concentration. A libido test was performed two weeks after every collection. It involved counting ewes a ram heat checked within a two-minute period. The experiment covered 12 months to cover all variations in season, temperature, and day length. Because of small sample size, differences were considered significant at $P \leq 0.10$. It was concluded that there is more variation among individuals within a breed than among the three breeds as shown by high standard errors.

**INTRODUCTION**

Seasonality has effects on the breeding cycles of wool breeds of ovine. The change in the photoperiod causes a change in the hormones that leads to the ovine cycling only during short days. During these short photoperiods, there is a decrease in activity in the excitatory pathways. This causes less inhibition on the pineal gland and the pinealocyotes, which allows for melatonin to be released. The melatonin then stimulates the release of GnRH in the hypothalamus, thus initiating cyclicity in the ewe. Wool breeds are considered to be short day breeders; therefore, breeding season is normally scheduled for the fall and winter. The breeding season of wool breeds results in lambing in the late winter and spring. However, it is unknown if the seasonality and length of the photoperiod have as much of an impact on the ram as they do on the ewe.

With the introduction of hair sheep and the increased popularity of the breed, it has been shown that ovine can breed during other seasons and lengths of photoperiods. Because hair sheep breeds will mate throughout the year, they have been known in some cases to have multiple lamb crops in a single year; therefore, the ewe becomes more productive for the producer. In these cases, hair sheep lambs have been born not only in the spring, but also in the summer, fall, and winter.

*Spermatocytogenesis*

Spermatogenesis is defined as the process whereby spermatozoa are formed. This process consists of proliferation (mitosis), meiosis, and differentiation. This process begins in the testes where there is a large potential output of spermatozoa that can range in mammals from less than 1 to 25 billion spermatozoa per day for both testes in normal males. Once these spermatozoa are produced, they are then passed through the rete tubules and then efferent ducts and enter the head caput (head) and corpus (body) of the epididymis. Here the spermatozoa undergo changes that allow them to become fertile. The descent through the caput and corpus is gradual and can take several days. The spermatozoa then enter the cauda (tail) of the epididymis where the spermatozoa are stored until needed. The spermatozoa in the cauda of the epididymis are capable of fertilization and are stored here until ejaculation. Upon sexual excitation, the spermatozoa in the cauda are transferred to the pelvic urethra where they are mixed with the seminal plasma and become available for delivery by ejaculation (Senger, 2003).

*Out of Season Breeding of Sheep*

There have been some studies on breeding sheep out of their normal breeding season of the short days of the fall. One study found that fertility rates of sire breeds of Romanov sheep that were bred in March were 92% and were 89% in May. This study also looked at Finnsheep with fertility rates of 91% in March and 72% in May, Texel sheep with 90% in March and 52% in May, Montadale sheep with 88% in March and 52% in May, and, lastly, Dorset sheep with 83% in March and 62% in May. This study shows that fertility rates were much higher for all breeds examined in March than in May (Casas et al., 2005). Another study showed that the intervals that lead to the first estrus cycle of the ewe and conception lengths were longer during an August breeding season compared to an October, November, and December breeding season. This study also showed that lambing rates were highest during the October breeding season and that Rambouillet and Navajo breed ewes tended to show estrus and conceive earlier than Suffolk, Hampshire, Targhee, and Corriedale breeds (Glimp, 1971).

*Impact of Day Length on the Ram*

Many studies have been done on seasonality effects of the ewe but not on the ram. One study examined mating Romney and East Friesian Composite ewes during all seasons. In this study, deNicolo et al. (2006) stated that even through there were poor results in pregnancy rates, the ability of out-of-season breeding regimens to induce the behavioral oestrus (the number of ewes marked by the ram per ewe exposed to the ram) were typically good. This procedure indicates that though the conception rates were low, the libido of the ram and ewe were good even when they were attempting to be bred during a non short-day time period.

Other studies have also examined the hormone levels in rams when exposed to ewes and during the breeding season. The hormones LH and testosterone are in direct correlation with libido and sperm cell production in the male. One particular study concluded that rams that were used to stimulate ewes out of anestrous had an increase in the hormone levels of LH and testosterone starting at hour 12 after being placed with the
ewes. The concentrations of these two hormones increased when ewes came into estrous and mating was allowed. Therefore, this study concluded that the most influential factors on ram hormone concentrations were that of ewes in estrous and times of mating (Ungerfeld and Silva, 2003).

Not many studies have focused on hair sheep to determine the differences between their reproductive habits and those of more traditional ovine such as wool breeds. However, one study marked an entire life-cycle of a Mouflon ram's reproductive seasonality and maturation. Lincoln, et.al showed that there was a consistent cycle in the blood plasma concentration of FSH and testosterone, and the growth of the testes. There was a peak in the reproductive function during September to October, which is associated with the rut and short photoperiod. This study also indicated that there was a slow increase in the hormone levels of FSH and testosterone correlated with the increase in the testes from the age of 1-6 years. From here, the levels hit a plateau then slowly started to decrease as the animal aged (Lincoln, 1998).

More studies need to be conducted to determine the differences in the rams and to determine how the seasonality truly affects the rams (not just the ewes) in all breed types. This could lead to a change in herd composition and production goals of producers.

## MATERIALS AND METHODS

This experiment compared Rambouillet, Suffolk, and Dorper/St. Croix rams, which are an example of wool, black face, and hair sheep, respectively. A total of 14 intact rams were included in the experiment, which included 5 Rambouillet rams, 5 Suffolk rams, 2 Black-headed Dorper rams, and 2 St. Croix rams. These rams were from the Angelo State University sheep flock. Rams ranged in age from one to five years, and all rams had reached puberty prior to the start of the study. One

month prior to the first collection, all rams were ejaculated for semen samples to ensure accurate data. Throughout the study, rams were placed in the same pasture at the Angelo State University Management, Instruction, and Research Center, located in Tom Green County, north of San Angelo. All rams were fed the same diet throughout the study and were supplemented when range conditions warranted at the rate of one pound per head per day of twenty percent range cubes. All wool breed rams were shorn on August 1, 2008.

Each month, utilizing a Ram-O-Jack electro ejaculator (Lane Manufacturing, Denver, CO), rams were semen collected. The semen collected was assigned a color score (Col) from 0-5, and a motility (Mot) score from 0-5, with 0 being the lowest score and 5 being the highest score (Herman et al., 1993). Sperm cell concentrations (Con) were calculated utilizing the hemocytometer method (Herman et al., 1993). At the time of collection, rams were also evaluated for a body condition score (BCS), scrotal circumference (SC) in cm, and rectal body temperature (Temp), to ensure ram health. The dates that the semen collection was performed included January 28, March 4 and 28, April 28, June 5, July 29, and December 12 of 2008. The samples will be referred to as Temp, BCS, SC, Col, Mot, and Con 1-7, respectively.

Approximately fourteen days following every semen collection, rams participated in a monthly libido check (LC). The libido checks will be referred to as LC 1-7, respectively. The libido check was conducted by allowing a single ram into a pen with multiple ewes while the number of ewes the ram heat checked within a two- minute period starting when the ram checked the first ewe for heat. Ewes used for all libido checks included wool and hair breeds and a variety of locations through their reproductive cycle. Prior to the libido test, all rams were secluded in a solid wall trailer so that they were not able to see or smell the ewes.

The duration of the experiment was 12 months to cover all variations in season, temperature, and day length.

Death occurred with two of the original Rambouillet rams. The death loss of one ram was due to urinary calculi and fighting. One of the rams was replaced with another Rambouillet ram that was already included in the herd. This ram was within the same experimental qualifications as the other rams within the study in terms of breed, age, diet, and prior handling. The second ram was not replaced in the study because of lack of rams that met the study qualifications. Another Rambouillet ram was treated for 30 days for fighting and was held and fed in a separate pen for the duration of his antibiotic and ointment treatment. He remained in the study and was returned to the herd following treatment.

Individual rams served as the experimental unit. Data was analyzed using the GLM procedure in SAS (SAS Institute, Cary, NC). Treatments were considered different at $P \leq 0.10$. An alpha level of 0.10 was used, instead of 0.05, because the sample size was small and differences at the 0.10 level would be important to commercial sheep production.

**RESULTS AND DISCUSSION**

Mean libido checks (LC; Table 1) generally were not different except for LC 6. During this period, Suffolk rams were greater ($P = 0.08$) than both Rambouillet and Hair, which were similar ($P > 0.10$). This difference could be because of the change in the temperature or that the Suffolk rams were more active because of the season change and the beginning of the change in day length to shorter periods. This change in day length is known to have an effect on ewe reproduction and the ewes were coming into natural cycling period (Rasby and Vinton 2002), therefore causing the Suffolk rams to be more active.

Mean rectal body temperatures (Temp; Table 2) were not significantly different for the Temp 1, Temp 2, or Temp 3. However, there was a significant difference ($P<0.10$) for the Temp

Table 1: Least Squares Means of libido (ewe checks per 2 minutes) in Suffolk, Rambouillet, and Hair rams throughout a 12 month period in the year 2008.

| | Treatment | | | | |
|---|---|---|---|---|---|
| | **Suffolk** | **Rambouillet** | **Hair** | **SE[a]** | **P-value** |
| **LC1[b]- Feb 11** | 9.0 | 9.6 | 9.8 | 2.76 | 0.98 |
| **LC2- March 24** | 3.8 | 7.0 | 6.3 | 1.58 | 0.29 |
| **LC3- April 8** | 7.4 | 8.8 | 5.3 | 1.51 | 0.26 |
| **LC4- May 18** | 10.3 | 5.6 | 4.3 | 2.22 | 0.18 |
| **LC5- June 20** | 6.0 | 3.0 | 3.3 | 1.33 | 0.24 |
| **LC6- Aug 26** | 11.0[c] | 4.8[d] | 5.3[d] | 2.00 | 0.08 |
| **LC7- Dec 1** | 9.5 | 8.2 | 4.5 | 3.52 | 0.53 |

[a]: Standard Error of the least square means
[b]: Libido Check assessed by number of ewes checked within a 2 minute period
[c, d]: Mean is the same with differing superscripts are different by indicated P-value

Table 2: Least Squares Means of Rectal Temperature ($^{o}$C) in Suffolk, Rambouillet, and Hair rams throughout a 12 month period in the year of 2008.

| | Treatment | | | | |
|---|---|---|---|---|---|
| | **Suffolk** | **Rambouillet** | **Hair** | **SE[a]** | **P-value** |
| **Temp 1[b]- Jan 28** | 39.4 | 39.4 | 39.4 | 0.19 | 0.98 |
| **Temp 2- March 4** | 39.2 | 39.6 | 39.2 | 0.18 | 0.25 |
| **Temp 3- March 28** | 39.6 | 39.6 | 38.9 | 0.30 | 0.24 |
| **Temp 4- April 28** | 39.1[c] | 39.6[d] | 39.6[d] | 0.39 | 0.03 |
| **Temp 5- June 5** | 39.2[c] | 39.8[d] | 39.9[d] | 0.18 | 0.02 |
| **Temp 6- July 29** | 39.3[c] | 39.7[d] | 39.9[d] | 0.20 | 0.10 |
| **Temp 7- Dec 12** | 39.4[c] | 39.3[c] | 39.9[d] | 0.15 | 0.02 |

[a]: Standard Error of the least square means
[b]:Rectal Temperature in degrees Celsius
[c, d]: Mean is the same with differing superscripts are different by indicated P-value

4, Temp 5, Temp 6, and Temp 7. During the Temp 4, there was a significant difference (P = 0.03) where the Suffolk has a lower temperature than both the Rambouillet and Hair rams. This difference could be due to the Suffolk rams being more susceptible to colder atmospheric temperatures or Suffolk rams naturally having a lower rectal body temperature. The significant difference (P=0.02) at Temp 5 showed that again the Suffolk had a lower temperature than both the Rambouillet and Hair rams. This result could be caused for the same reason as in Temp 4. At Temp 6, all breeds were different (P=0.10) from one another with Suffolks being lowest, Hair as highest, and Rambouillet being intermediate. This difference could be because of the rise in the atmospheric temperature and its effects on the different breeds. However, there is not any data available in the literature to support these findings or the speculation. There was also a significant difference (p=0.02) at Temp 7 that showed the Hair rams averaged higher than the Suffolk and Rambouillet rams. This difference could be attributed to Hair
rams having a naturally higher rectal body temperature than the other breeds because of the lack of wool covering to act as insulation against the environment. According to the data, the Hair rams were shown to have a higher rectal temperature average throughout the year, and generally the Suffolks were the lowest.

Body condition scores (BCS; Table 3) were based on a scale of 1 to 5, with one being extremely thin and five being extremely obese. There was a significant difference (P<0.10) in six of the seven observations. Body Condition Score (BCS) 1 showed a significant difference (P=0.002) with the Suffolk rams having the lowest of 3.0, followed by the Rambouillet rams with 3.2, and the highest was the Hair rams with 4.1. This trend was also shown at the BCS 2 (P=0.02), BCS 3 (P<0.001), BCS 4 (P=0.10), and at BCS 7 (P=0.07). There was also a significant difference (P=0.01) shown at the BCS 5, with the Rambouillet rams with the lowest of 2.7, followed by the Suffolk rams with an 3.4, and the Hair rams with the highest of 4.0. Throughout the study, the Hair rams had the highest body condition score. This could be because of the breed characteristics of the Hair rams and their lack of wool growth. Hair breeds are characteristically smaller framed, faster growing animals that fill out quickly and are bulkier, more muscled, and more obese. The Suffolk breed is characteristically a thinner breed as an adult animal. Suffolks have a large frame and as a young animal are heavy muscled, but they are affected greatly by heat and humidity. Suffolk sheep grow a light-weight, coarse wool that covers only their body. Rambouillet breed sheep are a characteristically medium frame animal with medium muscling. They grow a medium weight wool fleece that consists of fine fibers (Bradford, 2002).

There was also a significant difference among six of the seven observations for scrotal circumference (SC; Table 4). There was a significant difference (P=0.03) at SC 1 with the trend being the Rambouillet rams the lowest with 33.0cm, followed by the Hair rams with 35.5cm, and Suffolk rams being highest with 40.6cm. This trend was also shown for SC 2 (P=0.004), SC 3 (P=0.003), SC 4 (P=0.002), SC 5 (P=0.006), and SC 7 (P=0.07). From these observations, it can be concluded that compared to Rambouillet and Hair breed rams, Suffolk rams have a higher scrotal circumference. It can also be concluded that compared to Suffolk and Hair breed rams, Rambouillet rams have a lower scrotal circumference. However, all scrotal circumference measurements are well within the normal expected range for reproductively mature rams (Bearden, et al. 2004).

There were no significant differences shown among observed semen colors (Col; Table 5). Each collection of all rams was consistent in color within all breeds. There was also no significant difference shown among observed visual motility (Mol; Table 6). Each collection of all rams was consistent in motility within all breeds. Both outcomes are expected, which is most likely because all the rams were fertile and reproductively mature.

There was only one sperm cell concentration (Con; Table 7) that showed a significant difference. The Con 4 had a significant difference (P=0.06) among all breeds. The lowest concentration was the Suffolk rams with 1088.0 x $10^6$, followed by the Rambouillet rams with 2158.0 x $10^6$, and the highest concentration was the Hair rams with 2920.0 x $10^6$. This difference could be because of the environmental conditions and photoperiod having an effect on the Suffolk and Rambouillet rams (Rasby and Vinton, 2002).

**IMPLICATIONS**

Based on data collected from the experiment, it was concluded that there is more variation among individuals within a breed than among breeds as evidenced by high standard errors. The data showed that within breeds, there were certain individuals that were outside the normal range, which created the high standard errors. Therefore, it was shown that certain individuals varied more than breed types. From the data, it can be concluded that the photoperiod most likely has more effect on the ewe than the ram and that the ewe dictates the breeding season, not the ram. However, more research needs to be completed with increased numbers of rams of all breeds, and they will need to be tested and analyzed at more constant intervals and over a longer term to ensure conclusions.

Table 3: Least Squares Means of Body Condition Scores in Suffolk, Rambouillet, and Hair rams throughout a 12 month period in the year 2008.

| | Treatment | | | | |
|---|---|---|---|---|---|
| | **Suffolk** | **Rambouillet** | **Hair** | **SE[a]** | **P-value** |
| **BCS 1[b]- Jan 28** | 3.0[c] | 3.2[c] | 4.1[d] | 0.18 | 0.002 |
| **BCS 2- March 4** | 2.8[c] | 2.7[c] | 3.9[d] | 0.27 | 0.02 |
| **BCS 3- March 28** | 2.4[c] | 3.0[d] | 4.1[e] | 0.10 | <0.0001 |
| **BCS 4- April 28** | 2.7[c] | 3.9[d] | 4.4[e] | 0.55 | 0.10 |
| **BCS 5- June 5** | 3.4[c] | 2.7[d] | 4.0[e] | 0.25 | 0.01 |
| **BCS 6- July 29** | 3.4 | 3.2 | 4.1 | 0.32 | 0.15 |
| **BCS 7- Dec 12** | 2.6[c] | 2.4[c] | 3.5[d] | 0.34 | 0.07 |

[a]: Standard Error of the least square means
[b]: Body Condition Score is measured on a scale of 1= extremely emaciated, 5= extremely obese
[c, d, e]: Mean is the same with differing superscripts are different by indicated P-value

Table 4: Least Squares Means of Scrotal Circumference (cm) in Suffolk, Rambouillet, and Hair rams throughout a 12 month period in the year 2008.

| | Treatment | | | | |
|---|---|---|---|---|---|
| | **Suffolk** | **Rambouillet** | **Hair** | **SE[a]** | **P-value** |
| **SC 1[b]- Jan 28** | 40.6[c] | 33.0[d] | 35.5[d] | 1.97 | 0.03 |
| **SC 2- March 4** | 39.2[c] | 31.3[d] | 34.6[e] | 1.42 | 0.004 |
| **SC 3- March 28** | 41.1[c] | 32.4[d] | 34.8[d] | 1.36 | 0.003 |
| **SC 4- April 28** | 41.1[c] | 32.8[d] | 35.6[d] | 1.35 | 0.002 |
| **SC 5- June 5** | 40.6[c] | 33.3[d] | 34.5[d] | 1.34 | 0.006 |
| **SC 6- July 29** | 37.6 | 33.1 | 34.7 | 1.61 | 0.19 |
| **SC 7- Dec 12** | 34.7[c] | 28.8[d] | 30.6[d] | 2.31 | 0.07 |

[a]: Standard Error of the least square means
[b]: Scrotal Circumference measured in cm
[c, d, e]: Mean is the same with differing superscripts are different by indicated P-value

Table 5: Least Squares Means of Observed Semen Color in Suffolk, Rambouillet, and Hair rams throughout a 12 month period in the year 2008.

| | Treatment | | | | |
|---|---|---|---|---|---|
| | **Suffolk** | **Rambouillet** | **Hair** | **SE[a]** | **P-value** |
| **Col 1[b]-Jan 28** | 4.8 | 5.0 | 4.3 | 0.28 | 0.18 |
| **Col 2- March 4** | 5.0 | 4.3 | 4.8 | 0.49 | 0.54 |
| **Col 3- March 28** | 4.8 | 4.6 | 4.5 | 0.35 | 0.88 |
| **Col 4- April 28** | 3.7 | 4.6 | 4.9 | 0.64 | 0.38 |
| **Col 5- June 5** | 3.8 | 3.9 | 3.8 | 1.19 | 0.99 |
| **Col 6- July 29** | 3.8 | 4.0 | 4.5 | 0.46 | 0.53 |
| **Col 7- Dec 12** | 4.5 | 4.5 | 5.0 | 0.53 | 0.72 |

[a]: Standard Error of the least square means
[b]: Observed semen color is measured on a scale of 0=clear, 5= cloudy white

Table 6: Least Squares Means of Visual Motility in Suffolk, Rambouillet, and Hair rams throughout a 12 month period in the year 2008.

| | Treatment | | | | |
|---|---|---|---|---|---|
| | **Suffolk** | **Rambouillet** | **Hair** | **SE[a]** | **P-value** |
| **Mot 1[b]- Jan 28** | 4.3 | 3.3 | 3.9 | 0.66 | 0.51 |
| **Mot 2- March 4** | 4.7 | 3.6 | 4.0 | 0.64 | 0.42 |
| **Mot 3- March 28** | 4.8 | 4.6 | 4.5 | 0.35 | 0.88 |
| **Mot 4- April 28** | 4.0 | 4.6 | 5.0 | 0.54 | 0.40 |
| **Mot 5- June 5** | 3.3 | 3.9 | 3.8 | 1.14 | 0.90 |
| **Mot 6- July 29** | 3.0 | 3.0 | 2.4 | 1.10 | 0.90 |
| **Mot 7- Dec 12** | 4.3 | 3.2 | 5.0 | 1.11 | 0.38 |

[a]: Standard Error of the least square means

[b]: Observed semen motility measured on a scale of 0= no movement, 5= extreme circular movement

Table 7: Least Squares Means of Counted Sperm Concentration ($x10^6$) in Suffolk, Rambouillet, and Hair rams throughout a 12 month period in the year 2008.

| | Treatment | | | | |
|---|---|---|---|---|---|
| | **Suffolk** | **Rambouillet** | **Hair** | **SE[a]** | **P-value** |
| **Con 1[b]- Jan 28** | 3298.0 | 1936.0 | 2213.8 | 772.20 | 0.38 |
| **Con 2- March 4** | 1724.0 | 1332.0 | 2635.0 | 603.80 | 0.30 |
| **Con 3- March 28** | 910.0 | 2012.0 | 2182.5 | 628.17 | 0.33 |
| **Con 4- April 28** | 1088.0[c] | 2158.0[d] | 2920.0[e] | 511.01 | 0.06 |
| **Con 5- June 5** | 2380.0 | 2016.0 | 785.0 | 807.58 | 0.37 |
| **Con 6- July 29** | 480.0 | 1172.5 | 1880.0 | 813.90 | 0.50 |
| **Con 7- Dec 12** | 702.5 | 2326.0 | 1790.0 | 764.55 | 0.14 |

[a]: Standard Error of the least square means

[b]: Counted sperm concentration per mL

[c, d, e]: Mean is the same with differing superscripts are different by indicated P-value

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**FINAL REPORT: EFFECTS OF TEMPERATURE ON MICROBIAL SHELF-LIFE CHARACTERISTICS OF BEEF SNACK STICK PRODUCT**

*Loree A. Branham and Kirk W. Braden*

## INTRODUCTION

With the demand for 'convenience foods' on the rise, the meat industry has attempted to meet this demand with shelf stable processed meat products which do not have to be refrigerated. *The Meat Market*, the retail outlet of the Angelo State University Food Safety and Product Development Laboratory, currently offers both a fully cooked beef snack stick and a beef jalapeno cheese snack stick. These products are thermally processed according to guidelines set by the United States Department of Agriculture to control potential pathogens which may be present in the raw ingredients. The objective of this study was to measure shelf stability of a beef snack stick product in a retail environment at room temperature compared to product stored at refrigerated temperatures. Classification of a processed meat product as shelf stable at room temperature allows for marketing of that product for a longer period of time and in a wider variety of retail venues; this increases the potential for sale of that product, as well as, convenience of the product to the consumer. Currently the beef snack stick produced at the Angelo State University Food Safety and Product Development Laboratory (FSPD) is federally approved for sale under the stipulation that it remain refrigerated. While certain product characteristics, including water activity and pH, indicate that this product is shelf stable at room temperature, the Food Safety and Inspection Service requires further microbial validation in order for it to be marketed as such. By measuring microbial characteristics including total aerobic bacteria and *Pseudomonas* spp., graduate student researchers were able to not only learn and perfect laboratory techniques utilized in the food industry but also provide scientific documentation which will allow the beef stick product to be marketed as a room temperature, shelf stable product.

## MATERIALS AND METHODS

Microbial characteristics of beef snack sticks stored at refrigerated (4±1 °C) and room temperatures (21±2 °C) were evaluated. These microbial indicators include aerobic bacteria counts and *Pseudomonas* spp. counts. Total aerobic bacteria were enumerated using 3M™ Petrifilm™ Aerobic Count Plates. *Pseudomonas* spp. were enumerated using Pseudomonas F agar. This research project consisted of two trial replications (n=64). Trial 1 began mid-July, 2009. Trial 2 began approximately one week later. Preliminary data from both trials was collected by the last week in September.

In both trials, a 20lb batch of beef snack stick (Figure 1) was prepared using federally inspected and approved processing methods. Figures 2 and 3 show the thermal processing parameters of the batch of beef snack sticks sampled in the first and second trials of the shelf life project. Beef snack sticks were cooked in a commercial smokehouse using approved time and temperature parameters provided in the USDA Appendix A[4]. After the product was fully cooked and initially chilled, a portion of the snack sticks were randomly placed in 32 vacuum packages, approximately 0.25lb each, utilizing industry standard sanitary techniques. The packages were randomly allocated to one of the two temperature treatments and one of the four sampling periods. Treatments include: 1) storage temperature of 21±2 °C – Room Temperature and 2) 4±1 °C – Refrigerated Temperature. Sampling periods include: Day 1, Day 22, Day 43, and Day 64.

Over the period of 64 days, samples were taken from each environment every 21 days and analyzed for microbial populations. Day 1 samples were placed in their treatment

Figure 1. Copy of ingredient label of tested beef snack stick produced and sold at *The Meat Market* at the Angelo State University Food Safety and Product Development Laboratory.


Figure 2. Graph representing thermal processing parameters of the batch of beef snack sticks sampled in the first trial of the shelf life project.

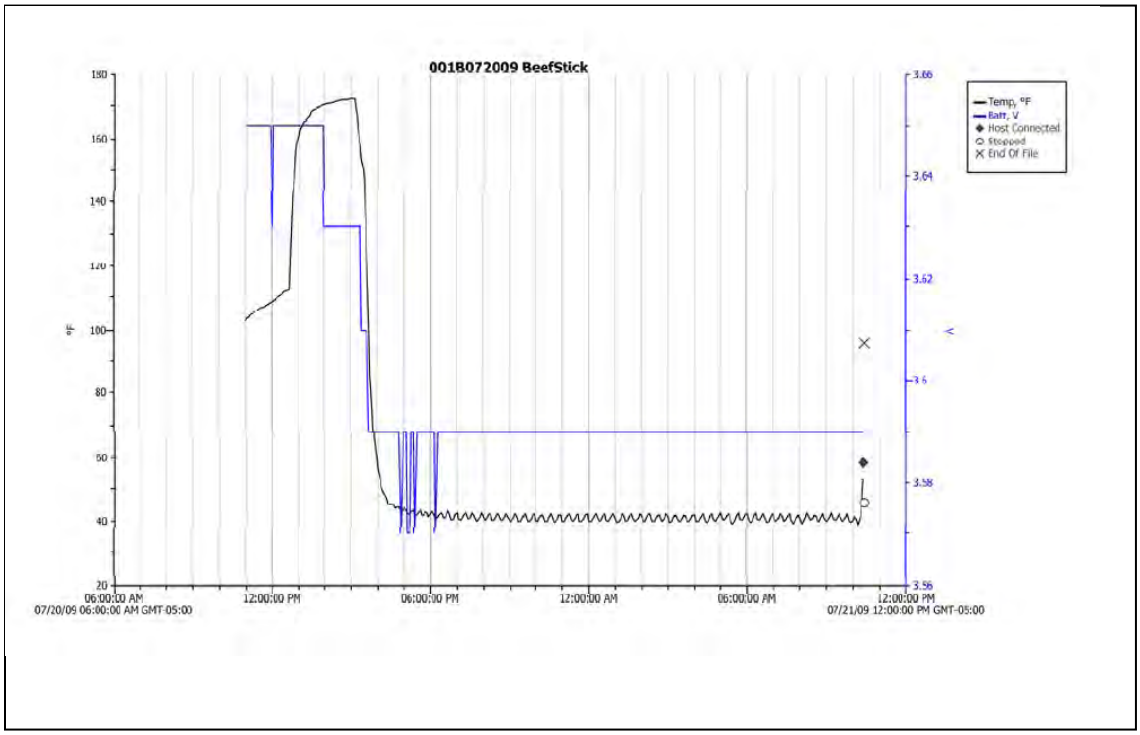
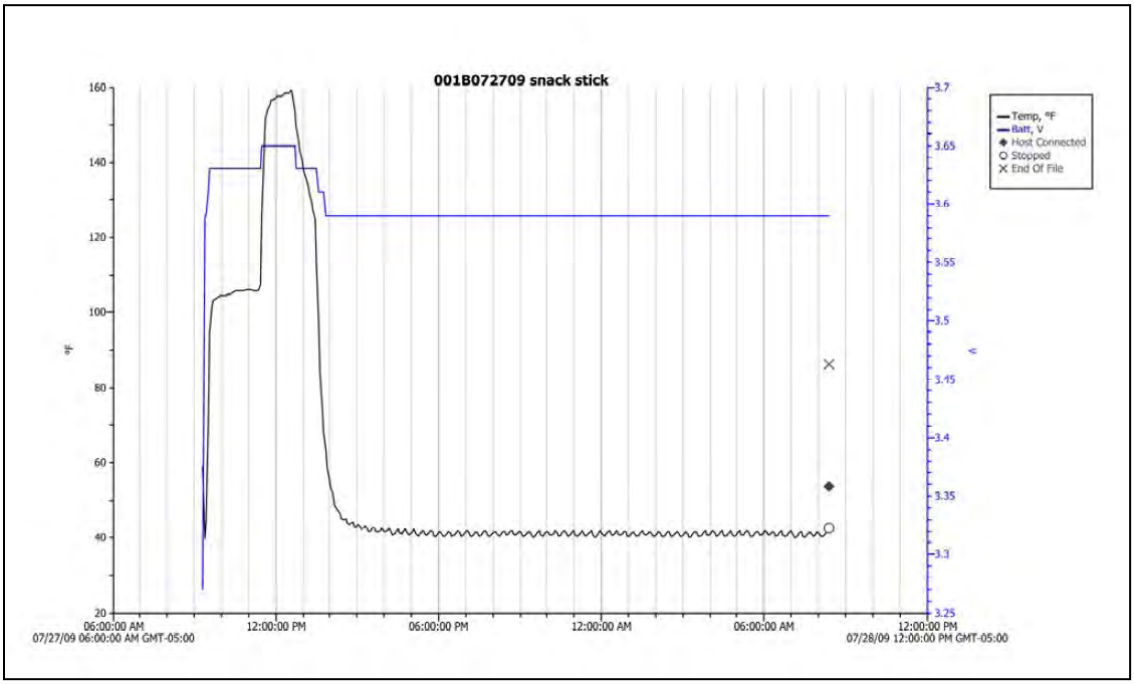


Figure 3. Graph representing thermal processing parameters of the batch of beef snack sticks sampled in the second trial of the shelf life project.




environments for approximately 1 hour in order to acclimate and accurately represent their treatment environments at time of sampling. A 10 g representative sample was mixed at a 1:10 ratio with sterile Buffered Peptone Water to create the sample homogenate. Serial dilutions were performed and plated. Total aerobic bacteria were enumerated using AOAC approved 3M™ Petrifilm™ Aerobic Count Plates[1] methods; the film was incubated at 37°C for 48 hours. *Pseudomonas* spp. was enumerated using a spread plate method on Pseudomonas F agar[3]; the Pseudomonas F agar plates were incubated at 37°C for 24 hours. Populations were converted using a $\log_{10}$ transformation appropriate for microbial analysis and analyzed using the PROC MIXED function of SAS Version 9.1.3 (Institute, Cory, NC). Differences in populations were considered significant if $P < 0.05$.

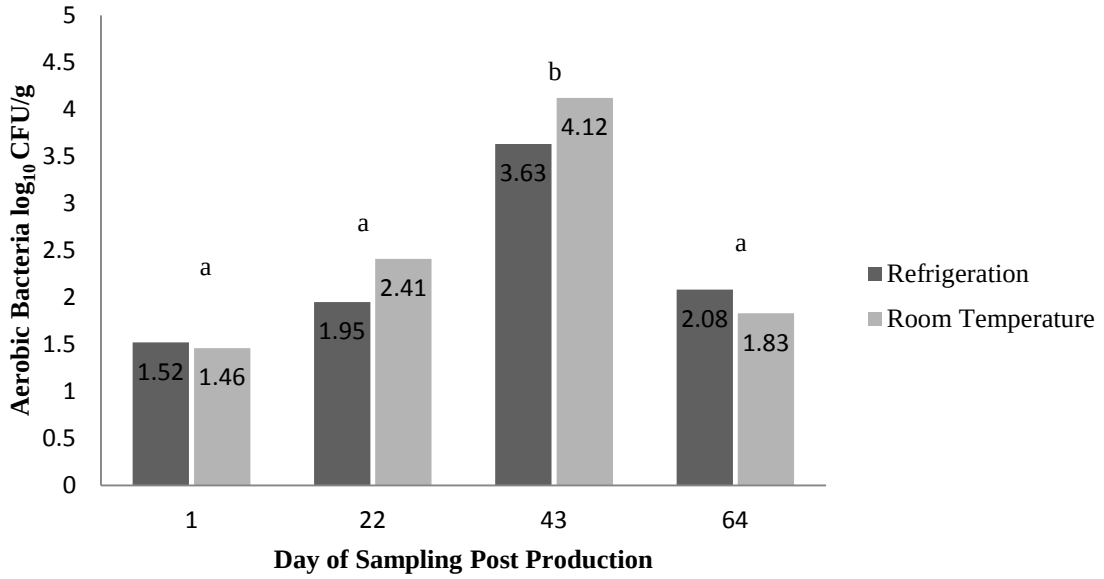
## RESULTS

The objective of this study was to measure shelf stability of a beef snack stick product in a retail environment at room temperature compared to product stored at refrigerated temperatures. Bacterial populations from four individual samples per treatment were averaged to gain an accurate representation of treatment effect.

### Aerobic Bacterial Population Results

A summary of aerobic bacterial populations can be seen in Figure 4. There was no treatment by sampling day interaction ($P = 0.72$). Storage temperature had no significant effect on aerobic bacterial populations ($P = 0.57$). However, sampling day did have a significant effect on populations ($P < 0.0001$). Aerobic bacterial populations from refrigerated samples from various days ranged from 1.52 to 3.63 $\log_{10}$ CFU/g. Aerobic bacterial populations from room temperature samples from various days ranged from 1.46 to 4.12 $\log_{10}$ CFU/g. Samples taken from day 43 of both the refrigeration and room temperature samples were significantly higher than those taken on days 1, 22 and 64 (Figure 4). This bacterial growth pattern is not unusual for

**Figure 4. Mean Aerobic Bacterial Populatoins Enumerated on 3M™ Petrifilm Aerobic Count Plates**




[a,b] Sampling days with different superscripts differ (P < 0.05).

bacterial populations found within a food matrix. All of these aerobic bacterial populations were either similar to or below those found by Angelidis and others in a survey of ready-to-eat foods[2]. The authors reported average aerobic bacterial levels of 3.85±1.80 $\log_{10}$ CFU/g in fifteen heat-treated meat products obtained from various retail outlets. As part of the current ASU study, two commercially available products (product A and B) were sampled to compare to the beef snack sticks produced at the FSPD. These products which have a standard shelf life of approximately 12 months were 9-10 months old at time of sampling. Commercial product A had 1.54 $\log_{10}$ aerobic CFU/g, while commercial product B had 2.62 $\log_{10}$ aerobic CFU/g. These levels were similar to those found in the tested product from the FSPD.

***Pseudomonas* spp. Population Results**

A summary of *Pseudomonas* spp. populations can be seen in Table 1. There was no treatment by sampling day interaction (P = 0.08). Storage temperature had no significant effect on aerobic bacterial populations (P = 0.13). Unlike aerobic populations, sampling day did not have a significant effect on *Pseudomonas* spp. populations (P = 0.08). Only two samples had *Pseudomonas* spp. levels which were high enough to be detected by the employed detection method. *Pseudomonas* spp. levels from refrigerated samples from various days were all below the detectable level of the employed detection and enumeration methods; while *Pseudomonas* spp. levels from room temperature samples from various days ranged from below detectable levels to 0.36 $\log_{10}$ CFU/g (Table 1). These results indicate very low levels of *Pseudomonas* spp. contamination. Commercial product A and B had levels of *Pseudomonas* spp. which were below the detectible limits of the detection method. These levels were similar to those found in the tested product from the FSPD.

**CONCLUSIONS AND IMPLICATIONS**

Bacterial population results for aerobic bacteria and *Pseudomonas* spp. of the tested beef sticks were within acceptable limits of the organisms in heat treated meat products. Even though there was a significant increase in aerobic bacterial populations at day 43, these

Table 1. Mean Pseudomonas spp. populations ($log_{10}$ CFU/g) enumerated on Pseudomonas F Agar.

| Sampling Day (post production) | Treatment | |
|---|---|---|
| | Refrigeration (4±1ºC) | Room Temperature (21±2ºC) |
| 1 | 0.00 | 0.00 |
| 22 | 0.00 | 0.00 |
| 43 | 0.00 | 0.36 |
| 64 | 0.00 | 0.00 |

Values which do not have superscripts do not differ (P > 0.05)

increased levels did not contribute to or decrease quality of the product. While not quantified by a sensory panel, organoleptic characteristics were evaluated at each sampling. No perceptible differences in color, odor or texture were observed between the refrigerated and room temperature stored products. No visible gas production was observed inside the vacuum sealed pouches. All of these are indicators of decreased quality or spoilage in processed meat products. The authors recognize that 64 days is not as long as the typical shelf life of similar products available in the food industry; however, 64 days is representative of the typical stocking time of the beef sticks in the FSPD. Additionally, the project is ongoing and additional samples from both trials will be sampled at a later date to give an indication of bacterial loads at extended storage times. Those results will be included in the MIR Progress Report and HACCP supporting documentation (Please see Future Publication section for more explanation). Preliminary results from this research project along with pH levels and water activity levels of the product indicate that refrigeration of the beef snack stick is not required for storage and retail display for time periods less than 64 days. Storage of the product at room temperature does not have adverse effects on bacterial populations found within the product. Results from future samples may indicate an extension on shelf life of the product.

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**EFFECTS OF ANTIOXIDANT APPLICATION AND RETAIL DISPLAY ON SENSORY, SHELF LIFE AND OXIDATIVE STABILITY OF BEEF STRIPLOIN STEAKS**

*Lee M. Batot, Jennie A. Canon, Kaci L. Foote, Mark F. Miller, Robert P. Cope, Loree A. Branham and Kirk W. Braden*

**ABSTRACT**

Beef strip-loin steaks were treated with two approved antioxidant formulations: organic citric acid (CIT) 0.3% and synthetic butylated hydroxyanisole / butylated hydroxytoluene combination (SYN) 0.08% and a control treatment (CON) of distilled water. Wholesale strip-loins (IMPS # 180) (n=32) at postmortem day 14, 21 and 28 strip-loins were removed from vacuum packages and fabricated into 2.54-cm thick steaks. Steaks (n=63) were randomly assigned to treatment groups to achieve (n=21) samples/treatment. After treatment steaks were over-wrapped with polyvinyl chloride film, and placed in simulated retail display for a five day period. There was no affects of treatment on any sensory attributes ($P > 0.05$). SYN treatment maintained lean color of strip-loin steaks ($P < 0.05$) while CON and CIT exhibited additional discoloration in DD 4 and 5 when compared to SYN within all postmortem aging periods. CON and CIT also contained additional browning in DD 3-5 ($P < 0.05$). SYN steaks exhibited higher a* values through DD periods ($P < 0.05$) when compared to either CON or CIT. SYN steaks exhibited higher oxymyoglobin level ($P = 0.03$) and reduced metmyoglobin levels ($P < 0.05$) throughout DD within all postmortem aging periods. CON and CIT exhibited increased TBA values on DD 1, 3 and 5 when compared to SYN steaks ($P < 0.05$). Results indicate that SYN antioxidant application to strip-loin steaks prolongs retail display shelf life by maintaining color and reducing rate of lipid oxidation without any negative sensory attributes.

**PRACTICAL APPLICATION**

Master bag packaging extends the storage of the meat product but does not provide any noticeable benefits once the product is placed on the retail shelf. This is a major aspect in which antioxidant application could be utilized to extend the shelf life of whole muscle products once removed from their protected master packaging environment. The use of antioxidant application in the case-ready consumer market could be of significant impact as the industry reverts back to a traditional overwrap packaging system. Master packaging systems could include an antioxidant into the case ready package to extend the needed case life characteristics.

**INTRODUCTION**

Overall the meat industries role is to provide a quality product to satisfy needs of the consumer in the worldwide marketplace (Resurreccion 2003). Depending on the region, over one-fourth to one third of the worldwide production of meat is lost every year because of deterioration and loss of product quality (Oussalah and others 2004). It has been estimated that average value deterioration is 3.7% for the entire meat department and 5.4 % for fresh meat (Williams and others 1992). Williams and others (1992) also stated that the U.S. industry stands to gain $175 million to $1 billion annually by increasing case life by 1-2 days. A percentage of this magnitude gives prime evidence why more research and development is needed for the preservation and maintenance of fresh retail meat products. Changing consumer demands in the U.S. have influenced the market for all types of meat. Due to these overall changes in demands, the meat products that are produced for sales and consumption must be of high standard quality and transportable to vast marketplaces located long distances away from production facilities. Meat is a highly perishable food; both oxidative and microbial processes are involved in meat spoilage (Camo and others 2008). Flavor, color and shelf life all are related to the oxidative state of meat. A major setback cited by supermarket meat managers is discoloration which cause beef retail products to be discounted or discarded depending on expiration date or shelf life of the product (Steiner and others 2001). When

muscle cuts are discolored on or before the sell by date, retail cuts must be marked down in price, faced, or repackaged (Smith and others 1993).

When products are re-handled or the form of original product is changed, there is usually a loss of quality and value along with the added chance of microbial contamination. The average consumer bases the purchase of a product predominantly on visual appearance of which color is the number one factor (Marth 1998). A bright cherry red color characterizes a freshly cut steak and is the desired visual characteristic of choice. Meat markets which still use over wrap packaging of products have an increased problem with oxidation and bacterial loads than compared to products packaged in a modified oxygen environment (Jeremiah and Gibson 1997). Oxidation is a chemical change of fat and muscle pigments that leads to rancidity, color changes and flavor deterioration. Over wrap is the most economically feasible method for retail display, but there is limited control of oxygen levels and other environmental factors associated with product deterioration. Lipid and protein oxidation is a critical point for red meat packaged under aerobic conditions, since it occurs at the same rate as discoloration and faster than microbial growth (Camo and others 2008). Past research has focused on the use of antioxidants in processed meat products for oxidative stability and overall shelf life. Research has not yet been conducted on the basis that defines effects of antioxidant application on color, oxidative stability and shelf life of whole muscle beef steaks over extended time in a retail environment.

## MATERIALS AND METHODS

*Treatments*

Experimental treatments consisted of two antioxidant formulations and a control to determine the overall effect of antioxidant application. Control group (CON) was applied with distilled deionized water. Antioxidant treatments consisted of 0.3% citric acid solution (CIT; 1 liter distilled deionized water + 3 grams of food grade citric acid) and 0.08% solution of butylated hydroxyanisole and butylated hydroxytoluene (SYN; 1 liter distilled deionized water + 0.4 g Butylated hydroxyanisole and 0.4 g butylated hydroxytoluene ) (Table.1). Citric acid (CIT) and SYN solutions were formulated in laboratory conditions to increase rate of crystal antioxidant absorption into the solution. These formulations were calculated once the level of uptake was determined to be 4 grams solution / striploin steak sample. All antioxidant treatments were formulated in accordance to guidelines set forth in CFR 184.0(b)(1). An automated belt line was utilized to transport each sample thru an atomization cabinet where application of treatment solution (CON, CIT and SYN) from a top mounted surface nozzle was applied while a constant level of pressure was maintained. A constant speed setting was used on the belt line to increase the rate of accuracy of treatment uptake. Weight samples were taken before and after application of each sample to determine if an increase of 4 grams was achieved.

*Sample Selection*

Wholesale beef striploin IMPS #180 (USDA 1996) were obtained from Cargill in Plainview, TX, vacuum packaged and transported to the Angelo State University Food Safety and Product Development Laboratory under refrigerated storage. Temperature recorders were utilized to ensure striploin temperatures maintained below 4°C during transport. Wholesale striploins were selected within a specified window of USDA quality and yield grade to minimize inherent variation. All selection criteria were evaluated by trained personnel according to USDA quality and yield grade guidelines (USDA 1989).

Table 1. Experimental treatment formulations applied to striploin steaks

| Treatment[a] | Formulation |
|---|---|
| Control (CON) | Distilled deionized water |
| Citric acid (CIT) | [b]0.3% citric acid + distilled deionized water |
| Butylated hydroxyanisole/ Butylated hydroxytoluene (SYN) | [c]0.08% Solution (BHA/ BHT) + distilled water |

[a] All treatments resulted in a uptake equaling 4.0 g of solution formulated based on accordance guidelines set forth in CFR 184.0(b)(1).
[b] Citric acid treatment (0.3% solution) 3 g citric acid/ 1 L distilled deionized water.
[c] Butylated hydroxyanisole/ Butylated hydroxytoluene treatment (0.08% solution) 0.4 g BHA 0.4 g BHT/ 1 L distilled deionized water.

*Sample Preparation*

Strip-loins were wet aged in vacuum package bags for 14, 21 and 28 days postmortem to simulate the approximate time beef remains in storage and transit prior to consumer availability. Sixteen wholesale strip-loins were utilized per trial. On days 14, 21 and 28 postmortem strip-loins were fabricated into four 2.54 cm thick steaks cut from the anterior end of each strip-loin. One steak of the total was randomly discarded to achieve equal representation within each of three treatments. Steaks (n = 63) were then randomly assigned to one of the three treatment groups (CON, CIT or SYN) to achieve n = 21 samples/treatment. Strip-loin steaks within each treatment were then trimmed down to 0.06 cm external fat thickness to maintain sample consistency. Within each treatment group (n= 21) steaks were randomly assigned to one of three display day (DD) ageing treatments (DD 1, DD 3 and DD 5) to achieve 7 steaks per DD ageing treatment. Within each DD ageing treatment 4 steaks were assigned to lipid oxidative stability analysis.

Three steaks were assigned to sensory analysis within each DD ageing treatment combination. Steaks for lipid oxidative were removed from the retail case based on DD ageing treatment. Sensory evaluation samples were removed from simulated retail display according to specified DD ageing treatment, vacuum packaged and stored at -0°C for subsequent sensory analysis.

*Simulated Retail Display*

Immediately post treatment application steaks were placed on a standard retail grade Styrofoam tray, coded with sample identification and overwrapped with polyvinyl chloride film (PVC) to mimic generally recognized retail presentation. Within 1 hr of treatment application, all steaks were placed in simulated retail display. Steaks were randomly placed in a Tyler retail display case (Model NM8, Tyler Refrigeration Corporation, Niles, MI) to mimic retail display conditions. Case temperature was maintained at 4°C and exposed to retail display lighting (Promolux Safe Spectrum T8 Platinum, Shawnigan Lake, BC, Canada) with illumination intensity maintained near 1900 lux. Simulated retail display was adapted from a previously published procedure (Braden and others 2007).

*Sensory Evaluation*

Prior to the start of evaluation, a sensory training session was conducted in reference to procedures of Cross and others (1978) so trainees would understand testing procedures and evaluation of sample meat products. Steaks were removed from frozen storage and placed under refrigeration 24 hr prior to sensory evaluation. Steaks were cooked on a George Forman electric clam shell style grill (Applica Consumer Products, Bedford Heights, OH) to an internal temperature of approximately 71°C to achieve a medium degree of doneness according to procedures outlined by

Kerth and others (2003). Steaks were then cut into 1-$cm^3$ cubes and stored in warming pans until entire panel was prepared. All samples were served to sensory panel warm within 15 minutes of cook time. At least six trained individuals were utilized for sensory evaluation during each panel. Apple juice, unsalted crackers and water was provided to each panelist to properly cleanse the pallet between receiving each sample. Steaks were analyzed for initial and sustained juiciness, initial and sustained tenderness, flavor intensity, off flavor and overall acceptability according to procedures of Cross and others (1978).

*Visual Color Evaluation*

Subjective color training was conducted prior to research to ensure no color vision deficiencies were present in the panel. During a five day display period steaks were evaluated daily by a trained panel, consisting of at least six members. The daily color evaluation was conducted within a one hour window for daily evaluation throughout each postmortem period for beef color, color uniformity, surface discoloration, and lean browning according to AMSA (1991) color panel evaluation guidelines.

*Objective Color Evaluation*

Commission Internationale de l'Eclairage (CIE) L* (muscle lightness), a* (muscle redness), b* (muscle yellowness), and reflectance spectra values were determined daily through the overwrap for display day from two random readings on each steak with a Hunter Miniscan XE Plus using illuminate D65 at 10° and a 3.5 cm aperture. Spectral reflectance values were determined and recorded every 10 nm over a range of 400-700 nm. Muscle chroma (color intensity/saturation), hue, angle (wavelength of light radiation red, yellow, green, blue and purple), myoglobin (fresh muscle pigment), oxymyoglobin (oxygenated muscle pigment), and metmyoglobin (brown oxidized muscle pigment) values were obtained utilizing equations as described by Hunt (1980) and Clydesdale (1991). Visual and instrumental color analysis protocols were utilized similar to those as presented by Braden and others (2007).

*Oxidative Stability Assessment*

Lipid oxidative stability was determined utilizing a thiobarbituric acid (TBA) reactive substance assay as detailed by Buege and Aust (1978). Lipid oxidative stability samples from display day 1, 3 and 5 were removed from frozen storage and a 10-g sample was homogenized with 30 mL of distilled water. Approximately 4 mL of homogenate was combined with 8 mL of trichloracetic/thiobarbituric acid reagent and 100µL of 10% butylatedhydroxyanisole. Samples were then incubated in a 99°C water bath for 15 minutes, allowed to cool in cold water (4°C) for 10 minutes and spun at 2000 x g for 10 minutes. The absorbance of the supernatant was then read against a blank containing like reagents at 531 nm. Malonaldehyde standard, utilizing 1,1,3,3-tetraethoxypropane and thiobarbituric acid, was used and thiobarbituric acid substances were reported as mg/10g of meat.

*Statistical Analysis*

Sensory and TBA data was analyzed as a completely randomized design using the general linear models procedures of SAS (SAS Inst. Inc., Cary, NC). Sensory and TBA data was included in the model with treatment as a fixed effect. DD 1, 3 and 5 visual and instrumental color data was analyzed for a completely randomized design, with a split plot repeated measures arrangement using the mixed models procedures as implemented in PROC MIXED (Littell and others 1996; SAS Inst. Inc., Cary, NC). Visual color, lean uniformity, lean discoloration, lean browning, L*, a*, b*, Chroma, hue, myoglobin, oxymyoglobin and metmyoglobin values were included in the model with treatment and display day and all two way interactions as fixed effects. Display day was analyzed as a repeated measure with steak as the subject of the repeated statement and based on AICC criteria an optimum covariance structure was selected (Littell and others 1996). Steak served as

experimental unit and significant ($P \leq 0.05$) treatment effect means were separated using Fisher's protected LSD.

## RESULTS AND DISCUSSION

*Color Measurements*

All subjective measurements taken by trained panelist deteriorated over time as steaks reached the extent of their display day periods throughout each postmortem aging period. Color score was evaluated for treatment x display day x postmortem aging ($P < 0.001$; Figure 1). When controlling for display day, there was no treatment x postmortem aging ($P = 0.51$) effect. A desired bright cherry red color was maintained by SYN treatment for a longer period of time when compared to CON and CIT, especially during display days 3, 4 and 5 of postmortem aging period 28 (Figure 2). For lean discoloration scores there was treatment main effect ($P = 0.01$) and for treatment x display day x postmortem aging ($P < 0.001$; Figure 3); but not for treatment x display day ($P = 0.11$) or treatment x postmortem aging ($P = 0.37$). SYN treatment exhibited less discoloration in later display day periods of postmortem aging periods 21 and 28 when evaluated by panelist. Lean browning was effected by treatment x postmortem aging x display day ($P < 0.001$). A lower level of browning was observed by panelist for steaks from SYN treatment throughout all three postmortem periods.

With increased postmortem and retail display day aging, all objective color attributes declined ($P < 0.05$). As these objective color measurements decreased there were varied rates of decline according to variable, treatments, treatments x display day, treatment x postmortem aging, and treatment x display day x postmortem aging combinations.

CIE L* values ($P < 0.001$), were slightly lower for SYN treatments when compared to CON and CIT treatments. No two way interaction for CIE L* values were seen for treatment x display day ($P = 0.74$) or treatment x postmortem aging ($P = 0.13$). There was an interaction for treatment affect on CIE a* values for treatment x postmortem aging ($P = 0.004$; Figure 4) but not for treatment x display day ($P = 0.52$). When comparing a* values between the three treatments during each postmortem period the SYN treatment had higher overall values, being more red when compared to the CON and CIT application. CIE b* values were similar to a* findings as interactions of treatment x postmortem aging ($P < 0.001$; Figure 5) along with treatment x display day ($P < 0.001$) values tended to be higher (more yellow) in steaks receiving SYN treatment. There were no interactions of display day x treatment ($P = 0.76$) or treatment x display day x postmortem aging ($P = 0.87$). Chroma values did show a positive treatment x postmortem aging affect ($P < 0.001$; Figure 6) where SYN treatment maintained higher levels of color saturation when compared to CON and CIT in all ageing periods. There were no interactions of treatment x display day ($P = 0.61$) or treatment x display day x postmortem aging ($P = 0.54$) when evaluating chroma. For Hue, there was only a positive treatment effect ($P < 0.001$) were SYN had lower hue values when compared to CON and CIT treatments. When evaluating myoglobin levels, there were interaction effects due to treatment x display day x postmortem aging ($P < 0.001$; Figure 7). Myoglobin (fresh muscle pigment) levels were maintained for longer periods of time by SYN treatment in the later of display days 3, 4 and 5. When samples were evaluated for oxymyoglobin content there was an effect due to treatment x postmortem aging x display day ($P = 0.011$; Figure 8). The oxymyoglobin levels were maintained higher for SYN treated steaks when comparing display days 4 and 5 throughout all three postmortem periods. Metmyoglobin levels did show a treatment effect ($P < 0.001$) were SYN did appear lower in value for oxidized myoglobin pigment. No two way or three way interactions for treatment had an effect ($P > 0.05$).

According to Mancini and Hunt (2005), meat color is the greatest quality factor that influences a meat purchasing decision made by consumers since it's an indicator of freshness and wholesomeness. As a result, nearly 15% of retail beef is discounted in price due to surface

Table 2. LS Means ± SE of Sensory Attributes of Striploin Steaks

| Attribute | Control | Citric Acid | Synthetic Acid |
|---|---|---|---|
| Average Initial Juiciness[a] | 5.85 ± 0.14 | 5.78 ± 0.14 | 5.72 ± 0.14 |
| Average Sustained Juiciness[b] | 5.56 ± 0.13 | 5.51 ± 0.13 | 5.43 ± 0.13 |
| Average Initial Tenderness[c] | 6.20 ± 0.24 | 5.70 ± 0.24 | 5.78 ± 0.24 |
| Average Sustained Tenderness[d] | 5.81 ± 0.14 | 5.66 ± 0.14 | 5.75 ± 0.14 |
| Average Flavor Intensity[e] | 5.88 ± 0.08 | 5.83 ± 0.08 | 5.75 ± 0.08 |
| Average Off Flavor[f] | 3.78 ± 0.04 | 3.75 ± 0.04 | 3.86 ± 0.04 |
| Average Overall Acceptability[g] | 5.65 ± 0.14 | 5.47 ± 0.14 | 5.47 ± 0.14 |

[a] (Initial Juiciness) 1=Extremely Dry, 8=Extremely Juicy

[b] (Sustained Juiciness) 1=Extremely Dry, 8=Extremely Juicy

[c] (Initial Tenderness) 1=Extremely Tough, 8=Extremely Tender

[d] (Sustained Tenderness) 1=Extremely Tough, 8=Extremely Tender

[e] (Flavor Intensity) 1=Extremely Bland, 8=Extremely Intense

[f] (Off Flavor) 1=Extreme Off Flavor, 4=None

[g] (Overall Acceptability) 1=Dislike Extremely, 8=Like Extremely

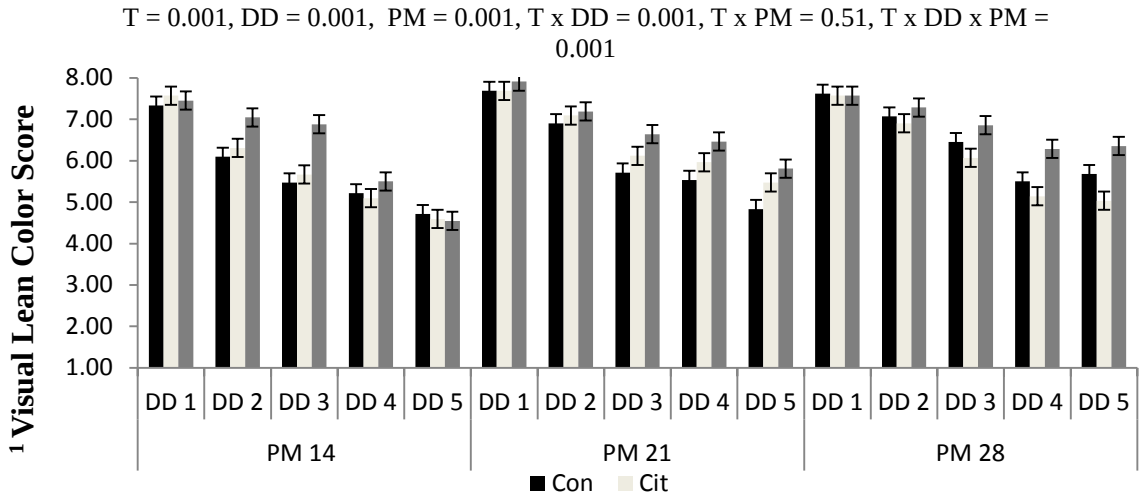


Figure 1. Least square means ± SEM for visual lean color of striploins by retail display day within postmortem aging days. Treatments consisted of control (CON), citric acid (CIT) and BHA/BHT (SYN); T = treatment, DD = days of simulated retail display and PM = days of postmortem aging [1]Lean color uniformity (1 = extremely dark red; 8 = extremely bright cherry-red)

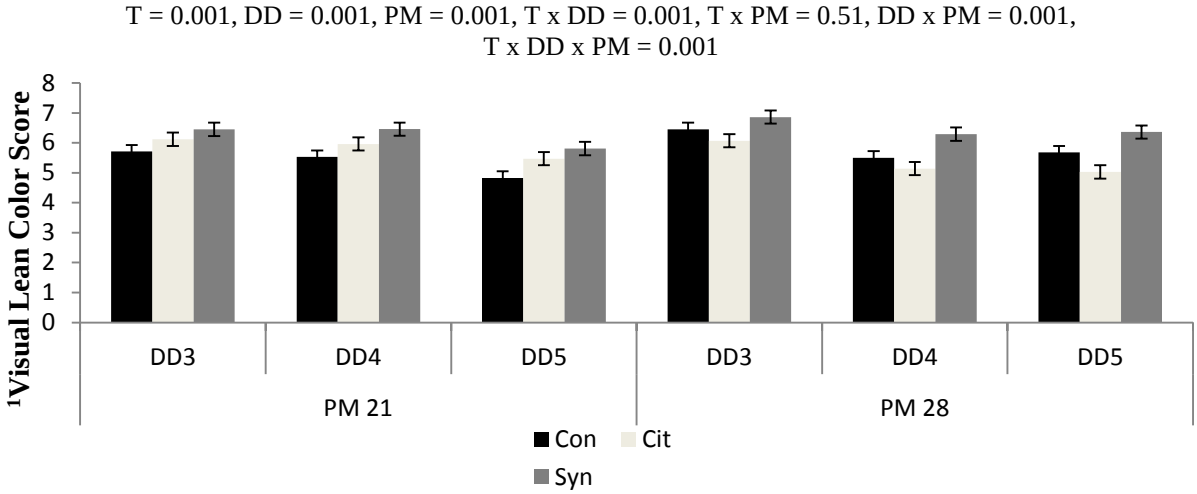


Figure 2. Least square means ± SEM for visual lean color of striploins by retail display day (3-5) within postmortem aging days (21 and 28). Treatments consisted of control (CON), citric acid (CIT) and BHA/BHT (SYN); T = treatment, DD = days of simulated retail display and PM = days of postmortem aging [1]Lean color uniformity (1 = extremely dark red; 8 = extremely bright cherry-red)

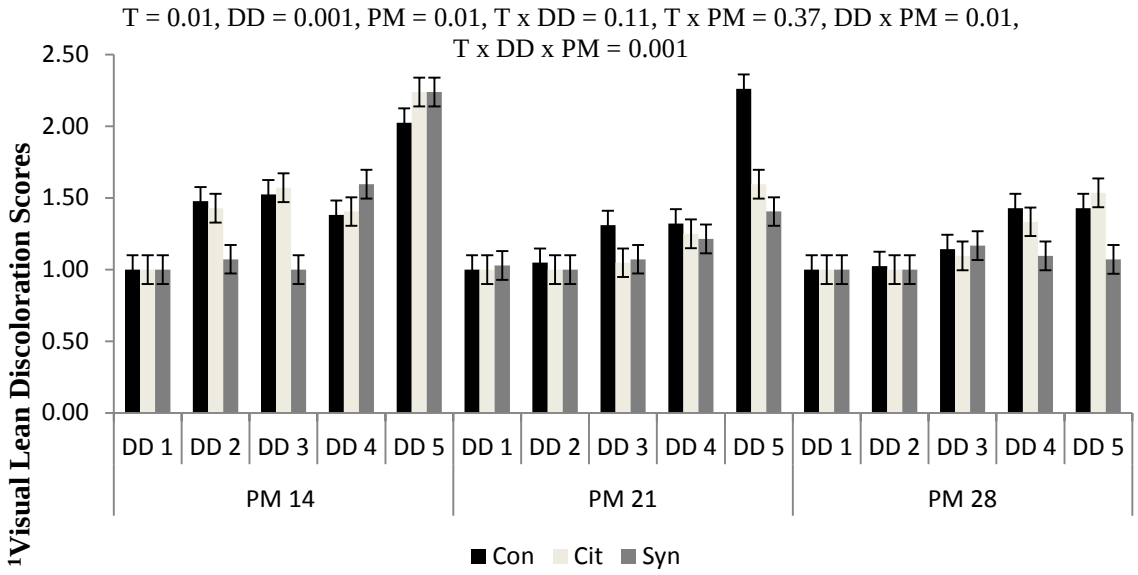


Figure 3. Least square means ± SEM for visual lean discoloration of striploins by retail display within postmortem aging days. Treatments consisted of control (CON), citric acid (CIT) and BHA/BHT (SYN); T = treatment, DD = days of simulated retail display and PM = days of postmortem aging; [1]Lean discoloration (1= 0%; 7= 100%)

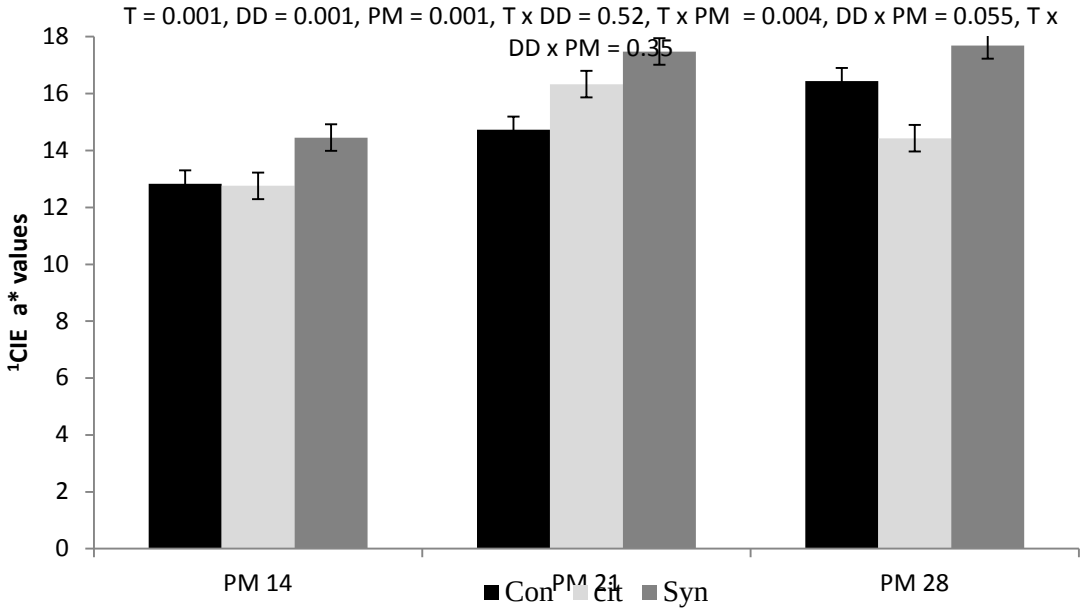


Figure 4. Least square means ± SEM for CIE a* instrumental values of striploin streaks by postmortem aging period. Treatments consisted of control (CON), citric acid (CIT) and BHA/BHT (SYN); T = treatment, DD = days of simulated retail display and PM = days of postmortem aging; [1]CIE a* value (positive = red, 0 = neutral, negative = green)

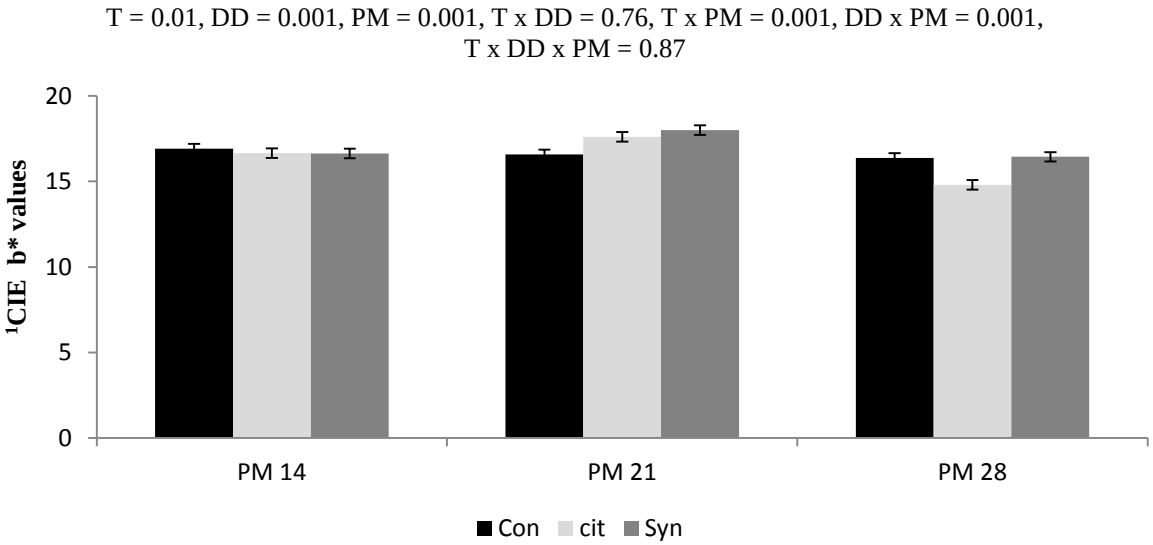


Figure 5. Least square means ± SEM for CIE b* instrumental values of striploin steaks by postmortem aging days. Treatments consisted of control (CON), citric acid (CIT) and BHA/BHT (SYN); T = treatment, DD = days of simulated retail display and PM = days of postmortem aging; [1]CIE b* Value (positive = yellow, 0 = neutral, negative = blue)

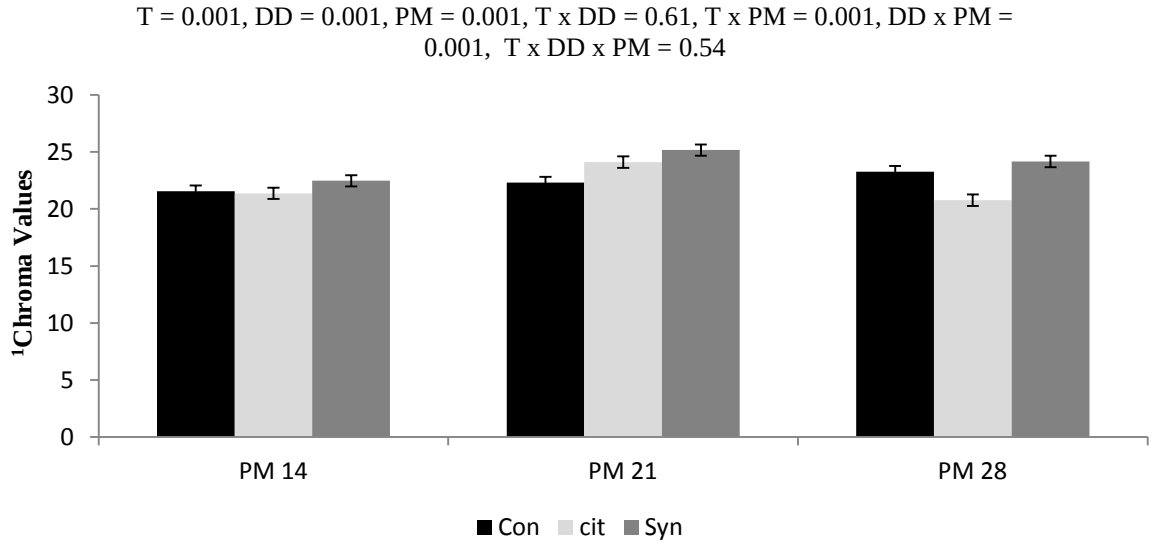


Figure 6. Least square means ± SEM for lean chroma instrumental values of striploin steaks by postmortem aging days. Treatments consisted of control (CON), citric acid (CIT) and BHA/BHT (SYN); T = treatment, DD = days of simulated retail display and PM = days of postmortem aging; [1]Lean Chroma Value (numerically increasing color saturation)

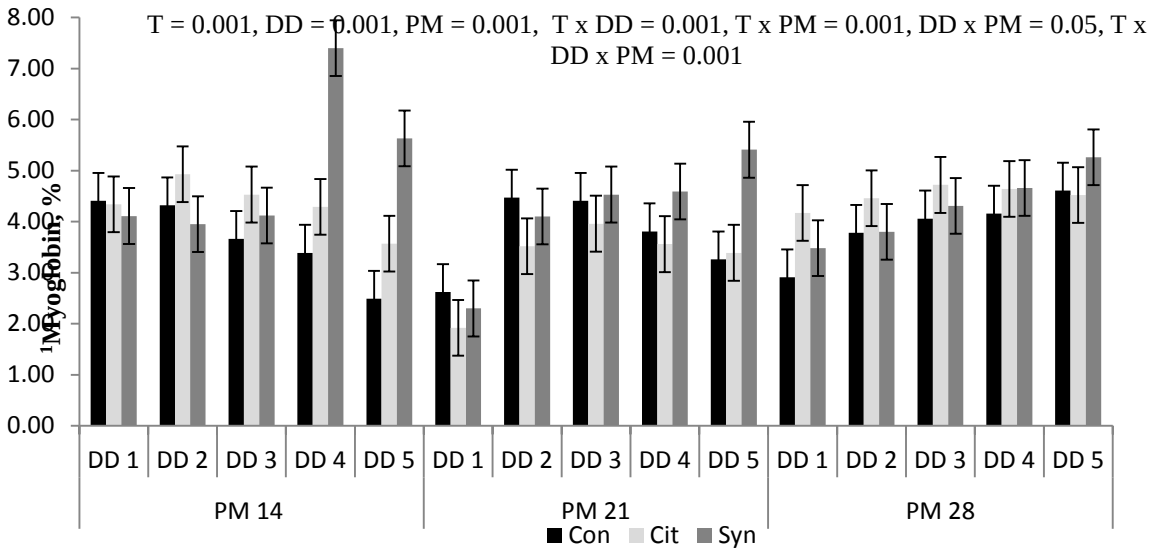


Figure 7. Least square means ± SEM percent myoglobin instrumental values of striploin steaks by retail display day within postmortem ageing days. Treatments consisted of control (CON), citric acid (CIT) and BHA/BHT (SYN); T = treatment, DD = days of simulated retail display and PM = days of postmortem aging; [1]Myoglobin percentage (fresh muscle pigment)

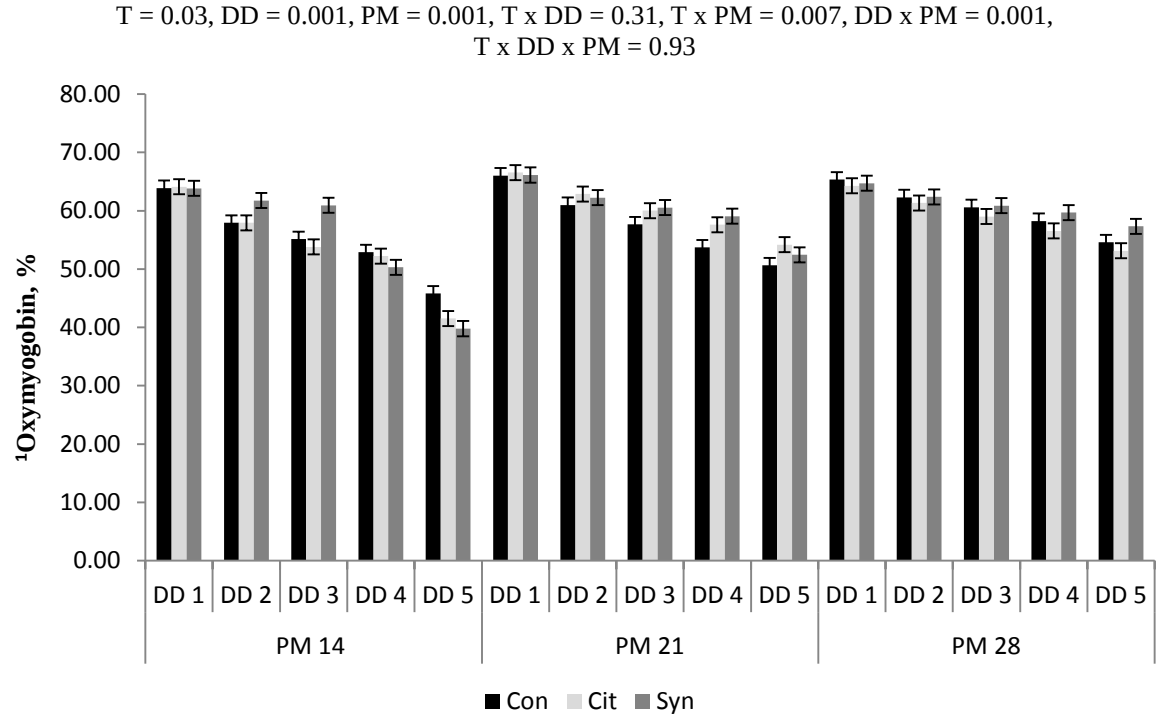


Figure 8. Least square means ± SEM for lean oxymyoglobin instrumental values of striploin steaks by retail display day within postmortem ageing days. Treatments consisted of control (CON), citric acid (CIT) and BHA/BHT (SYN); T = treatment, DD = days of simulated retail display and PM = days of postmortem aging; [1]Oxymyoglobin percentage (Oxygenated myoglobin pigment)

discoloration, which corresponds to annual revenue losses of $1 billion (Smith and others 1993). Visual color analysis provides evidence that is more comparable to consumer's perception of color than instrumental color analysis. The use of instrumental color analysis does provide scientific measurements that are widely accepted and comparable throughout many different criteria. Economic improvements associated with products that improve color life potential and stability has been sought after for many years.

The effect of SYN application on striploin steaks was increasingly evident in the advanced periods of display day within all three postmortem periods. CIT application exhibited less of an effect on color stability when compared to SYN treatment. CIT application actually increased discoloration scores and browning scores during subjective color analysis when compared to CON results. The effect of SYN application was visibly noticeable throughout the simulated retail display setting as redness and color uniformity was extended for an additional 1-2 days when compared to CON and CIT treatments. When analyzing each postmortem aging periods from display days 1 through 5 SYN treatment maintained visual color scores while CON and CIT had significant spikes on display days 3 through 5.

The effect of SYN treatment on striploin steaks on instrumental a* values showed the most significant trend in terms of meat redness as SYN maintained mean values of (16.54) compared to CON (14.66) and CIT (14.51).

*Sensory*

Sensory characteristics were evaluated on striploin steaks to compare treatment effects (Table 2). No affect of treatment on initial juiciness ($P = 0.815$), or sustained juiciness from treatment ($P = 0.768$) was evident from the panelist. There was also no affect from treatment on initial tenderness ($P = 0.281$) or sustained tenderness ($P = 0.769$). There was no affect of treatment on beef flavor intensity ($P = 0.463$), off flavor ($P = 0.158$) and overall acceptability ($P = 0.597$). Treatments of CON, CIT and SYN did not produce any detectable differences in relation to the sensory properties of overall striploin steaks.

Several different aspects must be considered when the addition of any chemical compound or additive is made to a meat or food product. It must not influence the flavor profile of the item in any negative way and also must not change the juiciness or tenderness properties. During the cooking process chemical reactions occur between fatty acids and amino acids and their degradation products provide a large number of compounds that can contribute to meat flavor (Wood and others 2004). Off odors and flavors are one of the most common negative sensory aspects developed during the aging process of meat products. As with a study conducted by Camo and others (2008), the direct addition of rosemary extract extended the fresh odor and color from 8 to 13 days when compared to the control that contained no extracts. As seen with the results from this study there was no significant difference for any attribute throughout the 5 day display period for all three treatments. As determine by Morrissey and others (1998) the typical shelf life of fresh meat is usually a time period of 3-5 days. Due to this information there were no major off flavors produced since each postmortem aging period only had five days in a retail case environment. All sensory characteristics in the current study were similar across treatments. Sebranek and others (2005) determined no unusual or uncharacteristic flavors were detected by panelist in a study were BHA/BHT was applied to pork sausage to determine its antioxidant effectiveness along with natural rosemary extract. Typical spices utilized in the production of pork sausage could have masked potential off flavors associated with synthetic antioxidants. In the present study, strip-loin steaks were not exposed to any spices or flavoring other than applied treatments of citric acid and BHA/BHT an acceptable determination could be made of sensory characteristics. Due to the very low concentration levels of antioxidants applied, citric acid 0.3% and BHA/BHT solution 0.08%, there were no detectible chemical flavors produced.

*Lipid Oxidation*

Thiobarbituric reactive substances (TBA) were dependent on treatment x display day ($P$ = 0.013; Figure 9). TBA levels increased with extended display day exposure for CON and CIT while SYN treatment maintained almost constant lower TBA values through display days 1 through 5. Since TBA is an indicator of lipid oxidation compounds this provides evidence that SYN treatment was not oxidizing as fast of a rate as other two treatments.

The typical oxidative deterioration of meat and meat products is caused by the degradation reactions of fats and pigments. Many of the oxidative processes that occurs in meat can lead to other organoleptic deterioration in taste, color and texture. Antioxidants, including vitamin E which is commonly utilized in feedstuffs is a primary lipid soluble antioxidant in biological systems and breaks the chain of lipid peroxidation in cell membranes and prevents the formation of lipid hydroperoxides (Halliwell 1987). As determined with results from Sebranek et al. (2005), utilizing synthetic antioxidants such as BHA/BHT combinations are effective in maintaining low thiobarbituric reactive substance (TBARS) values of pre-cooked meat products. The effects of selected antioxidants from this study on the oxidative stability of strip-loin steaks in a retail case environment are presented in (Figure 5). We found measures of lipid oxidation (TBARS) to increase with extended display day exposure and vacuum-packaged postmortem aging periods. TBARS increased in CON treatments and CIT treatments at an increased rate, when compared to SYN treatments especially in the later display day periods. As the data indicates SYN treatment had a relatively small increase from display day 1 through display day 5. This is a good indication that oxidation rates were reduced to a rate that could possibly increase the number of days before oxidative compounds are formed. Results from Sebranek and others (2005) determined that BHA/BHT treatments was the most effective method in keeping TBARS at or below the baseline value of 0.5 mg/kg for up to 11 days in pork sausage. Data from the current study on display day 5 SYN has a mean value of 0.11 mg/kg as compared to CON values of 0.32 mg/kg for striploin steaks.

## CONCLUSION

The overall quality and safety of a meat item in the consumer marketplace must be well accepted and supported in order for the product to be viable. Antioxidants including BHA and BHT are already utilized as food additives in many processed meat products that are widely consumed. An antioxidant application system that could improve the shelf life and color stability of beef retail cuts could increase the profitability of the meat industry greatly. Steaks applied with the SYN treatment did maintain visual and objective color over an extended display day period with a increased postmortem aging period. The rate of lipid oxidation was also decreased with SYN treatment which suggests that retail case life could be extended. Given the results of this study, BHA and BHT applied to the surface of whole muscle beef cuts appears to be particularly effective for extending the shelf life when compared to current practices. The use of antioxidant application in the case-ready consumer market could be very well utilized especially with the changes being made back to a traditional overwrap packaging system. Master packaging systems could include an antioxidant into the case ready package to extend desirable case life characteristics. This study examined the treatment effect only to a 5 DD. Future research could examine effects of extended periods of display. Since there is such wide range and forms of both synthetic and natural antioxidants available, other potential antioxidant formulation could be considered. The overall consumer acceptance is not yet known and needs to be further addressed before industry applications are implemented.

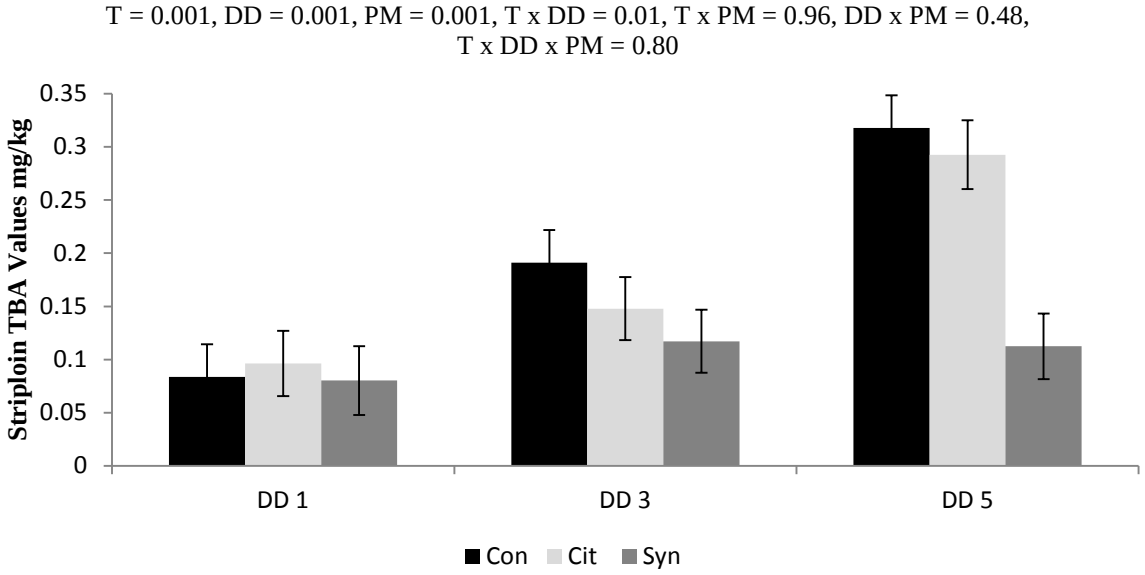


Figure 9. Least square means ± SEM for thiobarbituric reactive substances values of striploin steaks by retail display day. Treatments consisted of control (CON), citric acid (CIT) and BHA/BHT (SYN); T = treatment, DD = days of simulated retail display and PM = days of postmortem aging.

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**Acknowledgments**

Partially funded by the Angelo State University Meat and Food Science Program Dodge Jones Endowment.

**DEVELOPMENT OF A FUNCTIONAL MULTIVITAMIN MICROCAPSULE TO BE UTILIZED IN A READY-TO-EAT MEAT PRODUCT**

*Kaci L. Foote, Jennie A. Canon, Barbara Coty, Robert P. Cope, John J. Osterhout, Leslie D. Thompson, Loree A. Branham and Kirk W. Braden*

**ABSTRACT**

The objective of this study was to develop a multivitamin microcapsule to be utilized in a ready-to-eat (RTE) meat product. Commercial (COM) and laboratory (LAB) multivitamin microcapsules were implemented in a standard frankfurter formulation to produce a functional food. The control (CON) treatment consisted of the standard frankfurter formulation.Two trials (n = 20 / treatment / trial) were evaluated for sensory characteristics using a trained sensory panel and thiamine levels using high performance liquid chromatography (HPLC). While treatment did not have an effect on evaluated sensory characteristics ($P > 0.05$), an increase in display day (dd) aging increased cooking loss in Trial A. However, in Trial B, LAB and COM treatments had higher cooking loss ($P < 0.0001$) than CON; average initial juiciness scores increased depending on treatment ($P = 0.04$). Average initial and sustained tenderness in Trial B was dependent on dd with tenderness increasing throughout the aging intervals ($P < 0.0001$, 0.0002, respectively). Flavor intensity and off-flavor were not dependent on treatment or dd ($P > 0.05$) for both trials. In Trial A, overall acceptability was dependent on dd ($P = 0.0004$) with values ranging from a high in dd 1 ($7.12 \pm 0.10$) to a low in dd 16 ($6.47 \pm 0.10$). In Trial B, there were no differences in overall acceptability ($P > 0.05$). Thiamine levels were independent of trt, dd and trt x dd when analyzed by HPLC. Multivitamin microcapsule treatments did not have an effect on sensory characteristics when added to ready-to-eat meat products. Thus, multivitamin microcapsules may be added to frankfurter formulations to increase functional properties without adverse affects on sensorial properties.

**INTRODUCTION**

Microencapsulation is the technology of encapsulating solids, liquids, or gaseous substances into miniature capsules that have the ability to release the encapsulated ingredients in a specific/controlled environment. Microencapsulation is beneficial for food processors to incorporate food ingredients that would otherwise be volatile and not functional in a food system (Desai and Park, 2005). According to Desai and Park (2005), the food industry commonly uses microencapsulation to entrap core material for means of protection from degradation by reducing the reactivity from the outside environment. This is important when core materials need to be released at a further point in the processing of a product to mask flavor undertones and/or to be conducive to the products solution and overall acceptability (Desai and Park, 2005). Some food ingredients are extremely complex and are expected to fulfill properties of a food for the consumer that would be unachievable without encapsulation of these ingredients. Microencapsulation is applied to many areas of the food industry and continues to grow due to the advances that have been accomplished with these processes. Because of this, microencapsulation can possibly broaden the application array of food ingredients (Gouin, 2004). The manipulation of the substances within the microencapsulation process can provide a value added product with unmatched characteristics (Gouin, 2004).

The simplest form of a microcapsule consists of two layers, a wall and a core interior with or without a consistent shaped capsule (Gouin, 2004). Some microcapsules have the ability to contain many wall layers depending on the desired release and the composition of the core material (Pothakamury and Barbosa-Canovas, 1995).

The general objective of this project was to develop a multivitamin microcapsule as a meat additive that can withstand the physical, chemical, and thermal process of a commonly consumed ready-to eat (RTE) meat product such as frankfurters. Meat emulsions, i.e. frankfurters, are an optimal

food medium to apply the multivitamin microcapsule due to the large of amount of protein extraction that occurs in meat batter. This helps bind the food additive while also creating a uniform product. Some microencapsulation techniques that are utilized to develop microcapsules include spray drying, fluidized-bed coating, coacervation, liposome entrapment, and many others (Gibbs et al., 1999). . While many of these are used in the food industry, this literature review will solely focus on coacervation.

The method of release is also an important aspect of the microencapsulation process to be considered. Diffusing of the microcapsule into a fluid solution can cause quick release of the active ingredient. The release can be caused from biodegradation of the polymer in the food system into which it is placed. Another method of release is through osmotic pressure, this is achieved by allowing the wall material to be permeable to water resulting in the release of the core and by the swelling of the microcapsule from the application of heat that results in the release of the core ingredient (Pothakamury and Barbosa-Canovas, 1995). The above must be considered when manipulating the controlled release of an ingredient (Pothakamury and Barbosa-Canovas, 1995).

## MATERIALS AND METHODS

*Treatments*

Two trials were completed at the Angelo State University Food Safety and Product Development Laboratory in San Angelo, TX. Two experimental treatments and a control were utilized to determine the effects of microcapsule addition to a RTE meat product. The control group (CON, n = 20/trial) consisted of a standard frankfurter formulation (Table 1). The commercial treatment (COM, n = 20 / trial) consisted of a standard frankfurter formulation and a commercial multivitamin microcapsule (Table 2). The laboratory method (LAB, n = 20 / trial) consisted of a microcapsule developed at the Angelo State University Food Safety and Product Development Laboratory, which contained the same vitamin content as the COM microcapsule using the coacervation technique in the laboratory and a standard frankfurter formulation (Table 1).

The commercial microcapsule was donated from DSM Nutritional Products Inc. (Parsippany, NJ) and was utilized in the microcapsule treatments. The COM microcapsule is composed of the recommended daily intake without minerals of ascorbic acid, biotin, d-calcium pantothenate, niacinamide, pyridoxine hydrochloride, riboflavin, thiamine mononitrate, vitamin A palmitate, cyanocobalamin, cholecalciferol, tocopheryl acetate, phytoonadione, and maltodextrin as the carrier (Table 2).

*LAB Microcapsule Preparation*

Modified procedures from Jizomoto et al. (1993), Dong and Rogers (1993), and Green and Schleicher (1957) were utilized to produce the laboratory developed microcapsule. LAB microcapsules were prepared by incorporating 100 g of the vitamin premix mixed with vegetable oil at 100% of solution (100 mL of vegetable oil). This mixture was homogenized into an aqueous solution (10 g of gelatin in 340 mL distilled water) that was previously warmed to 50°C. After these two solutions were emulsified, a gum acacia solution (10 g of gum acacia in 340 mL distilled water) that was previously heated to 50°C was added into the gelatin/oil solution. Once all of these mixtures were homogenized, the mixture went through a washing step by adding it to 1500 mL distilled deionized $H_2O$. The pH was also adjusted using a 10% acetic acid solution to the pH of 4.0 then cooled using an ice bath until it reached 10°C or lower which accomplished initial capsule formation. After cooling, the pH was adjusted to 9 to 11 using 20% sodium hydroxide to facilitate further capsule hardening. The microcapsule solution was then placed in a separatory funnel for 1 h to separate the upper liquid from the supernatant. After

the majority of the supernatant was removed, the microcapsule slurry was partially dehydrated using a

Table 1. Frankfurter formulations for all treatments.

| **Ingredients** | **Amount (g)** |
|---|---|
| Beef Trim (90/10)[a] | 18144 |
| Pork Trim (50/50)[b] | 18144 |
| Ice[cc] | 3628 |
| Non-Fat Dry Milk | 1134 |
| Salt | 907 |
| Dextrose | 362 |
| Ginger | 48 |
| Ground Mustard | 51 |
| Garlic Powder | 24 |
| Ground Nutmeg | 48 |
| Ground White Pepper | 88 |
| Ground Coriander | 45 |
| Ground Paprika | 226 |
| Onion Powder | 24 |
| Mace | 45 |
| Black Pepper | 45 |
| Sodium Erythorbate | 16 |
| Sodium Nitrite | 88 |
| LAB/COM Microcapsule[d] | 48 |

[a] 90% lean skeletal muscle and 10% fat

[b] 50% lean skeletal muscle and 50% fat

[c] Water was used in Trial B

[d] Not used in CON treatment

Buchner funnel under a vacuum. The remaining microcapsules were then frozen overnight at 0°C to be

Table 2. DSM Nutritional Products (Parsippany, NJ) Vitamin Premix XR05415000 Formulation Sheet.

| **Active Ingredients** | **Declared Ingredient Level (mg/serving)** |
|---|---|
| Ascorbic Acid (Vitamin C) | 78.00 |
| Biotin (Vitamin H) | 0.35 |
| d-Calcium Pantothenate (Vitamin B5) | 12.50 |
| Niacinamide (Vitamin B3) | 22.10 |
| Pyridoxine Hydrochloride (Vitamin B6) | 2.70 |
| Riboflavin (Vitamin B2) | 1.96 |
| Thiamine Mononitrate (Vitamin B1) | 1.80 |
| Vitamin A Palmitate | 24.00 |
| Cyanocobalamin (Vitamin B12) | 0.78 |
| Cholecalciferol (Vitamin D3) | 4.80 |
| Tocopheryl Acetate (Vitamin E Acetate) | 69.00 |
| Phytonadione (Vitamin K1) | 1.92 |
| Maltodextrin (Carrier) | |

* Use Rate: 270 mg/serving

Lyophilized for 48 h. This resulted in a powder form of the microcapsules. To ensure the size of the capsules were more uniform, the powder was sifted and stored in a dry environment until the frankfurters were produced. Commercial (COM) and laboratory (LAB) treatment microcapsules were then incorporated into the frankfurter formulation at a rate of 270 mg / serving (44 g / treatment) (Table 2). All frankfurters (Control, Commercial, and Laboratory treatments) were produced at the Angelo State University Food Safety and Product Development Laboratory. An experimental unit (EU) was 680 g (8 frankfurters). Each treatment consisted of 20 EU or 13.6 kg of meat batter (n = 20 / trt / trial). The frankfurter batter was made, and the microcapsules were mixed after chopping.

*Sample Preparation*

The standard base frankfurter formulation utilized in all treatments is found in Table 1. Beef trimmings were 90% lean skeletal muscle (beef inside round) with 10% fat. Pork trimmings consisted of 50% lean skeletal muscle and 50% fat (jowl meat). All of the meat processing was conducted at the Food Safety and Product Development Laboratory at Angelo State University. Each treatment was prepared

under refrigerated temperatures (7°C). The beef and pork trim was separately ground through a 0.79 cm grinder plate. The beef trim was placed in the bowl chopper with the salt and half the water in the form of ice. This was mixed for approximately 3 min until the temperature reached 4 to 5°C or until the meat batter had a large amount of protein extraction. The pork trimmings, sodium nitrate, sodium erythorbate, seasonings were then added and chopped until the temperature reached 12 to 13°C. Each treatment had 13.6 kg of meat batter randomly partitioned out from the bulk batch of standard frankfurter formulation (Table 1) previously discussed. Once assigned, these treatments were mixed with the assigned microcapsule and vacuum stuffed. All of the meat batter was stuffed into 22-mm-diameter peel-able cellulose casings (DeWeid International, San Antonio, Texas). The frankfurters were labeled for treatment identification and smoked in a climate-controlled smokehouse (Alkar, DEC International Inc., Lodi, WI). The frankfurters were cooked until internal temperature reached 72°C with a cold shower applied once this temperature was reached to reduce potential shrinkage (Table 3). The frankfurters were cooled to 7°C or below. The fully cooked not shelf stable frankfurter was then vacuum packaged with 1 EU per package. Each package was placed in a retail type setting for 16 days that had a light intensity of 1900 lux (lx). All packages within each treatment were randomly assigned to aging treatments. Packages or EU within microcapsule treatments and within aging treatments were subjected to sensory analysis and laboratory thiamine analysis.

In Trial B, the temperature of the meat batter was not regulated in the same manner as Trial A where ice water was used. Trial B used only water due to the batter in Trial A staying at a low temperature. The temperature reached with adding just the water was increased but not over desired levels. The CON treatment was stuffed immediately after being taken out of the bowl chopper, while the COM and LAB microcapsules were mixed into the meat batter after the chopping before being stuffed in casing. The mixing increased the temperature of the meat batter, thus causing water loss in the cooking process (Alvarez et al., 2007).

*Simulated Retail Display*

Each package within a respective aging treatment was placed in a Tyler retail display case (Model NM8, Tyler Refrigeration Corporation, Niles, MI) at 4 to 7°C for 16 days on days and assigned to aging treatments of 1, 4, 8, 12, and 16 d. The retail display lighting (Promolux, Safe Spectrum t8 Platinum, Shawnigan Lake BC, Canada) was at a maintained intensity of ~1900 lx to simulate a retail setting. Sample ID was coded to maintain sample identification. Based on display day (dd) aging treatment, four EU from each treatment were removed from the retail coffin case at the appropriate assigned intervals. These packages were then each split into two different packages with each package having four frankfurters in them to be used for sensory and laboratory analysis. The separate packages were vacuum packaged and placed into a freezer at -20°C until subsequent analysis. The retail display research utilized the procedure according to Braden et al. (2007).

*Sensory Evaluation Panel*

Four EU from days 1, 4, 8, 12, and 16 were evaluated by a trained sensory panel. On the day of evaluation, the frankfurters were taken from the freezer to be defrosted in a cooler at 2 to 7°C and assigned a sample number to prevent panelist bias due to treatment. Frankfurters were placed in boiling water until the internal temperature reached 71°C. The samples were then cut into 1 cm slices using a grid cutting board. Uniform samples were placed in a labeled temperature maintained container and given to the trained panel to be analyzed. Trained panelists evaluated the samples on juiciness, tenderness, color, flavor, off-flavor, and overall acceptability (Table 4). These procedures were modified from the procedures of Cross et al. (1978). Each panelist was given unsalted crackers, apple juice, and

Table 3. Frankfurter smokehouse cycle time, dry-bulb, wet-bulb, relative humidity, dampers, and smoke application for each trial (Alkar).

| Step | Time (min) | Dry- Bulb (°C) | Wet- Bulb (°C) | Relative Humidity (%) | Dampers | Smoke |
|---|---|---|---|---|---|---|
| 1 | 5 | 43.3 | 37.8 | 68 | __ | |
| 2 | 5 | 43.3 | 0 | __ | Auto | |
| 3 | 30 | 48.8 | 0 | __ | Closed | On |
| 4 | 15 | 65.6 | 0 | __ | Auto | |
| 5 | 15 | 73.9 | 54.4 | 36 | Auto | |
| 6 | 10 | 82.2 | 73.9 | 68 | Auto | |
| 7 | 12 | __ | __ | __ | __ | |

Cooking time ~ 90 minutes

Table 5. Least square means ± SEM of sensory evaluation attributes of the control (CON), commercial (COM), and laboratory (LAB) treatments of frankfurters for Trial A.

| Attribute | Control | Commercial | Laboratory | *P* > F |
|---|---|---|---|---|
| Initial Juiciness[a] | 6.12 ± 0.11 | 6.27 ± 0.11 | 6.15 ± 0.11 | 0.6036 |
| Sustained Juiciness[b] | 6.42 ± 0.09 | 6.65 ± 0.09 | 6.50 ± 0.09 | 0.2098 |
| Initial Tenderness[c] | 6.15 ± 0.08 | 6.23 ± 0.08 | 6.11 ± 0.08 | 0.5855 |
| Sustained Tenderness[d] | 6.51 ± 0.08 | 6.51 ± 0.08 | 6.41 ± 0.08 | 0.6561 |
| Flavor Intensity[e] | 6.50 ± 0.08 | 6.49 ± 0.08 | 6.46 ± 0.08 | 0.9248 |
| Off Flavor[f] | 3.99 ± 0.01 | 3.99 ± 0.01 | 4.00 ± 0.01 | 0.3952 |
| Overall Acceptability[g] | 6.63 ± 0.08 | 6.83 ± 0.08 | 6.67 ± 0.08 | 0.1951 |

[xyz] Means within a row lacking a common superscript differ ($P < 0.05$)
[a] (Initial Juiciness) 1- Extremely Dry, 8- Extremely Juicy
[b] (Sustained Juiciness) 1-Extremely Dry, 8-Extremely Juicy
[c] (Initial Tenderness) 1-Extremely Tough, 8-Extremely Tough
[d] (Sustained Tenderness) 1-Extremely Tough, 8- Extremely Tough
[e] (Flavor Intensity) 1-Extremely Bland, 8- Extremely Intense
[f] (Off Flavor) 1- Extreme Off Flavor, 4- None
[g] (Overall Acceptability) 1- Dislike Extremely, 8- Like Extremely

Table 4. Scoring of sensory evaluation according to Cross et al. (1978).

| Juiciness | Tenderness | Flavor Intensity | Off Flavor | Overall Acceptability |
|---|---|---|---|---|
| 8- Extremely juicy | 8- Extremely tender | 8- Extremely intense | 4- None | 8- Like extremely |
| 7- Very juicy | 7- Very Tender | 7- Very intense | 3- Slight off flavor | 7- Like very much |
| 6- Moderately juicy | 6- Moderately tender | 6- Moderately intense | 2- Moderate off flavor | 6- Like moderately |
| 5- Slightly juicy | 5- Slightly tender | 5- Slightly intense | 1- Extreme off flavor | 5- Like slightly |
| 4- Slightly dry | 4- Slightly tough | 4- Slightly bland | | 4- Dislike slightly |
| 3- Moderately dry | 3- Moderately tough | 3- Moderately bland | | 3- Dislike moderately |
| 2- Very dry | 2- Very tough | 2- Very bland | | 2- Dislike very much |
| 1- Extremely dry | 1- Extremely tough | 1- Extremely bland | | 1- Dislike extremely |

water to cleanse the pallet between each sample. There were at least six panelists on each sensory evaluation panel.

*Laboratory Assay*

To determine if the addition of the multivitamin microcapsule had an effect on the overall vitamin content of the frankfurter in the final product, thiamine levels were determined. Vitamin $B_1$ (thiamine) has been shown to be less stabile than Vitamin $B_2$, but relatively stabile when compared to other water-soluble vitamins (Batifoulier et al., 2005). Thiamine is highly stabile in high acid solutions but tends to decrease in stability as a solution becomes more alkaline (Batifoulier et al., 2005). The frankfurters were at a somewhat neutral pH making the thiamine potentially susceptible to degradation. Analysis using High Performance Liquid Chromatography (HPLC) was utilized to determine the amount of thiamine in the samples. The purpose of testing the thiamine levels was to determine the stability of the microcapsules as a whole if they are to be used to produce a functional food. Thiamine was utilized as an indicator vitamin for the remaining vitamins because of its heat sensitivity characteristic.

*Chemicals and Reagent*

All samples were HPLC analyzed at the Angelo State University Biochemistry Laboratory in San Angelo, TX. Thiamine hydrochloride, HPLC grade methanol, potassium phosphate buffer, and trichloroacetic acid (TCA) were purchased from VWR International (Texas) and donated by the Department of Chemistry at Angelo State University. The stock solutions of thiamine HCl, trichloroacetic acid (TCA) (10%), and a potassium phosphate buffer (50 mM, pH 6) were prepared for the thiamine HPLC analysis.

*Chromatographic Conditions*

The HPLC system that was utilized was a Waters 2487 Dual λ Absorbance Detector .The 717 plus Autosampler (Waters, Milford, MA), and a 1525 Binary HPLC Pump (Waters, Milford, MA) was utilized with a reverse phase C18 (4.6 x 250 nm) column (Vydac).

Potassium phosphate buffer (50 mM, pH 6) in methanol (80/20 v/v) was the mobile phase that was prepared using dibasic and monobasic potassium phosphate and filtered to make the solvent HPLC grade. An isocratic method was utilized with an injection volume of 20 μL and a flow rate of 1 mL / min for 10 min.

Standards were made from a thiamine stock solution (1 mg thiamine HCl / 25 mL 10% TCA) to obtain a standard curve.

*Thiamine Extraction*

One gram of the frankfurter was added to 10 mL of the TCA (10%) solution. This mixture was homogenized using a Polytron homogenizer then placed on ice for 15 min. Each sample was centrifuged at 13000 rpm for 6 min at 10°C. The supernatant (1 mL) was placed in an epindorf tube and centrifuged at 12000 rpm for 5 min. All supernatant that could be removed was placed in a separate microcentrifuge tube to be centrifuged (12000 rpm for 5 min) again to obtain the most pure extracted vitamins possible. Each sample was diluted (1:5000) with the potassium phosphate buffer before the analysis.

Each treatment from the two trials had one sample from dd 1, 8, and 16 of each treatment from the two trials was sent to NP Analytical Laboratories (St. Louis, MI) commercial testing to be tested for thiamine levels utilizing an HPLC method (AOAC, 2000).

*Statistical Analysis*

Sensory scores and thiamine level data was analyzed as a completely randomized design using the general linear models procedures of SAS (SAS Inst. Inc., Cary, NC). Sensory scores and thiamine levels were included in the model as the dependent variables with treatment and display day as a fixed affects. Experimental units were 680 g of each treatment and significant ($P \leq 0.05$) treatment effect means were separated using Fisher's protected Least Significant Difference.

**RESULTS AND DISCUSSION**

*Sensory Evaluation*

In trial A, multivitamin microcapsule treatment and treatment by display day did not have any effect on cooking loss ($P = 0.7025$; 0.5194, respectively; Table 5). Cooking loss was, however, dependent on dd (Table 6). Display day four samples had a greater cooking loss of 2.17 ± 0.57 g compared to all other dd which range from 0.00 ± 0.57 to 0.33 ± 0.57 g ($P = 0.0417$). Treatment and treatment x dd did not affect the average initial juiciness ($P = 0.6036$; 0.4944, respectively). However, average initial juiciness was dependent on display day with scores lower on dd 4,8,12 and 16 when compared to dd1, thus decreasing as the aging interval increased ($P < 0.0019$; Table 6). There was no effect on the average sustained juiciness by treatment and treatment x dd ($P = 0.2098$; 0.3574, respectively). In Trial A, sustained juiciness was dependent on dd with dd 1 showing higher scores than all other display days ($P < 0.0001$). Average initial tenderness was not dependent on treatment and treatment x dd ($P = 0.5855$; 0.3310, respectively). Average initial tenderness was affected by display day ($P < 0.0001$). The initial tenderness decreased as the aging increased with the exception of dd 4 that did not fit this trend (Table 6). Treatment and treatment x dd did not have an effect on average sustained tenderness ($P = 0.6561$; 0.4043, respectively). Sustained tenderness was dependent on dd with dd 1 showing higher mean scores than all other display day ($P < 0.0001$). The average flavor intensity was dependent on dd ($P <0.0001$) with the least square means ranging

Table 6. Least square means ± SEM of sensory evaluation attributes of display day for frankfurters n Trial A.

| Attribute | 1 | 4 | 8 | 12 | 16 | *P* > F |
|---|---|---|---|---|---|---|
| Initial Juiciness[a] | 6.67 ± 0.14$^{y}$ | 5.93 ± 0.14$^{z}$ | 6.27 ± 0.14$^{z}$ | 6.14 ± 0.14$^{z}$ | 5.90 ± 0.14$^{z}$ | 0.0019 |
| Sustained Juiciness[b] | 7.05 ± 0.12$^{x}$ | 6.27 ± 0.12$^{yz}$ | 6.62 ± 0.12$^{z}$ | 6.41 ± 0.12$^{z}$ | 6.28 ± 0.12$^{z}$ | < 0.0001 |
| Initial Tenderness[c] | 6.64 ± 0.10$^{y}$ | 5.98 ± 0.10$^{z}$ | 6.244 ± 0.10$^{z}$ | 6.01 ±0.10$^{z}$ | 6.00 ± 0.10$^{z}$ | < 0.0001 |
| Sustained Tenderness[d] | 7.09 ± 0.10$^{x}$ | 6.29 ± 0.10$^{z}$ | 6.55 ± 0.10$^{y}$ | 6.29 ± 0.10$^{yz}$ | 6.18 ± 0.10$^{z}$ | < 0.0001 |
| Flavor Intensity[e] | 6.89 ± 0.10$^{x}$ | 6.24 ± 0.10$^{xy}$ | 6.62 ± 0.10$^{yz}$ | 6.44 ± 0.10$^{yz}$ | 6.23 ± 0.10$^{z}$ | 0.0002 |
| Off Flavor[f] | 4.00 ± 0.01 | 3.97 ± 0.01 | 4.0 ± 0.01 | 3.99 ± 0.01 | 4.00 ± 0.01 | 0.2435 |
| Overall Acceptability[g] | 7.11 ± 0.10$^{x}$ | 6.49 ± 0.10$^{yz}$ | 6.83 ± 0.10$^{xy}$ | 6.64 ± 0.10$^{z}$ | 6.47 ± 0.10$^{z}$ | 0.0004 |

from 6.89 ± 0.11 on dd 1 and 6.24 ± 0.11 on dd 4. Display days 1, 8, 12, and 16 decreased in flavor intensity scores
with the lowest score being on dd 4 (*P* = 0.0002). Treatment and treatment x dd did not have an effect on average flavor intensity (*P* = 0.9248; 0.7024, respectively).Treatment, dd, and treatment x dd did not have an effect on off flavors (*P* = 0.3952; 0.2435; 0.8077, respectively). Average overall acceptability was not dependent on treatment or treatment x dd (*P* = 0.1951, 0.6899, respectively). However, overall acceptability was dependent on dd (*P* = 0.0004; Table 7). As the aging interval increased, the overall acceptability decreased with the mean scores ranging from 7.12 ± 0.11 on dd 1 and 6.47 ± 0.11 on dd 16.

Cooking loss in trial B was not dependent on dd or treatment x dd (*P* = 0.6682; 0.6777, respectively). However, microcapsule treatment did have an effect on cooking loss (*P* < 0.0001). The LAB and COM treatments had higher levels of cooking loss with values of 20.9 ± 1.79 and 20.6 ± 1.79 g when compared to the CON treatment at a level of 2.20 ±1.82 g (Table 7). The microcapsules were mixed after chopping in the LAB and COM treatments that could have played a role in the amount of cooking loss by raising the temperature of the meat batter. According to Alvarez et al. (2007), the temperature of the meat batter had to be controlled due an increase in temperature can cause the surface tension decreases on the fat particles thus creating more surface area for the protein to coat. If there is not enough protein to coat the fat particles, the cooking process allows the fat to "expand and melt" out of the product relating to the amount of cooking loss (Alvarez et al., 2007). While the average initial juiciness was affected by the treatment (*P* = 0.0353), there was not an effect due to dd or treatment x dd (*P* = 0.1314; 0.2029, respectively). The COM and LAB treatments although having a higher amount of cooking loss had higher initial juiciness scores (7.20 ± 0.05, 7.17 ± 0.05) than the CON treatment. Treatment, dd, and treatment x dd did not affect the average sustained juiciness (*P* = 0.1458; 0.1817; 0.1578, respectively; Table 8). The average initial tenderness was not dependent on treatment or treatment x dd (*P* = 0.4234; 0.0791, respectively). However, average initial juiciness was dependent on display day (*P* < 0.0001). As the aging interval increased, the initial tenderness increased with the mean scored on dd 1 being 6.99 ± 0.07 to dd 16 with mean

Table 7. Least square means ± SEM of sensory evaluation attributes of the control (CON), commercial (COM), and laboratory (LAB) treatments of frankfurters for Trial B.

| Attribute | Control | Commercial | Laboratory | *P* > F |
|---|---|---|---|---|
| Initial Juiciness[a] | 7.00 ± 0.06[y] | 7.20 ± 0.05[z] | 7.16 ± 0.05[z] | 0.0353 |
| Sustained Juiciness[b] | 7.29 ± 0.06 | 7.45 ± 0.06 | 7.33 ± 0.06 | 0.1458 |
| Initial Tenderness[c] | 6.97 ± 0.04 | 7.02 ± 0.04 | 6.94 ± 0.04 | 0.4234 |
| Sustained Tenderness[d] | 7.26 ± 0.05 | 7.20 ± 0.05 | 7.17 ± 0.05 | 0.3739 |
| Flavor Intensity[e] | 6.84 ± 0.05 | 6.90 ± 0.05 | 6.84 ± 0.05 | 0.5978 |
| Off Flavor[f] | 3.97 ± 0.02 | 3.95 ± 0.02 | 3.94 ± 0.02 | 0.5533 |
| Overall Acceptability[g] | 6.93 ± 0.06 | 6.99 ± 0.06 | 6.85 ± 0.06 | 0.2046 |

[yz] Means within a row lacking a common superscript differ ($P < 0.05$)
[a] (Initial Juiciness) 1- Extremely Dry, 8- Extremely Juicy
[b] (Sustained Juiciness) 1-Extremely Dry, 8-Extremely Juicy
[c] (Initial Tenderness) 1-Extremely Tough, 8-Extremely Tough
[d] (Sustained Tenderness) 1-Extremely Tough, 8- Extremely Tough
[e] (Flavor Intensity) 1-Extremely Bland, 8- Extremely Intense
[f] (Off Flavor) 1- Extreme Off Flavor, 4- None
[g] (Overall Acceptability) 1- Dislike Extremely, 8- Like Extremely

Table 8. Least square means ± SEM of sensory evaluation attributes of display day for frankfurters in Trial B.

| **Attribute** | **1** | **4** | **8** | **12** | **16** | ***P* > F** |
|---|---|---|---|---|---|---|
| Initial Juiciness[a] | 6.98 ± 0.07[z] | 7.07 ± 0.07[y] | 7.20 ± 0.07[y] | 7.22 ± 0.07[y] | 7.15 ± 0.07[y] | 0.1314 |
| Sustained Juiciness[b] | 7.24 ± 0.07[z] | 6.27 ± 0.07[y] | 7.46 ± 0.07[y] | 7.36 ± 0.07[y] | 7.43 ± 0.07[y] | 0.1817 |
| Initial Tenderness[c] | 6.69 ± 0.05[z] | 6.88 ± 0.05[z] | 7.05 ± 0.05[z] | 6.01 ±0.10[z] | 6.00 ± 0.10[z] | <0.0001 |
| Sustained Tenderness[d] | 6.99 ± 0.06[z] | 7.11 ± 0.06[z] | 7.27 ± 0.06[y] | 7.37 ± 0.06[y] | 7.33 ± 0.06[y] | 0.0002 |
| Flavor Intensity[e] | 6.79 ± 0.06 | 6.89 ± 0.06 | 6.84 ± 0.06 | 6.82 ± 0.06 | 6.96 ± 0.06 | 0.2886 |
| Off Flavor[f] | 3.95 ± 0.02[y] | 4.00 ± 0.02[y] | 3.96 ± 0.02[y] | 3.95 ± 0.02[y] | 3.92 ± 0.02[z] | 0.2323 |
| Overall Acceptability[g] | 6.85 ± 0.07 | 6.90 ± 0.07 | 6.93 ± 0.07 | 6.98 ± 0.07 | 6.95 ± 0.07 | 0.7525 |

scores of 7.15 ± 0.07. Treatment and treatment x dd did not have an effect ($P$ = 0.3739; 0.0625, respectively), while the dd affected the average sustained tenderness ($P$ = 0.0002). The display day affect on average sustained juiciness was inconsistent with mean scores ranging from 7.46 ± 0.08 (dd 8) to 7.24 ± 0.08 (dd 1) ($P$ < 0.0001). In Trial B, flavor intensity, off- flavor and overall acceptability were not dependent on treatment, dd, or treatment x dd ($P$ > 0.05).

A study conducted by Kryitsi et al. (2011) showed that incorporating B complex vitamins in different cooked rice products, without any form of controlled release, had an effect on sensory characteristics. Flavor components such as metallic, bitter, and unpleasant characteristics were described with the addition of these vitamins (Kryitsi et al., 2011). This represents why the encapsulation technique can be useful when working with vitamins and other additives in food products. Microencapsulating multivitamins was shown to not have an effect on sensory characteristics in the standard frankfurter formulation, thus showing how encapsulation is important when applying vitamins to food products.

*HPLC Data*

The in-house laboratory analysis determined the amount of thiamine (mg/100g) in each EU. There was not an effect of trt, dd, or trt x dd in Trial A on the amount of thiamine present ($P$ = 0.1714, 0.7273, 0.8660, respectively; Table 9 and 10). In Trial B, thiamine levels were not affected by the trt, dd, or trt x dd ($P$ = 0.2742, 0.8593, 0.9440, respectively).

The commercial laboratory (NP Analytical Laboratories) samples were not statistically analyzed due to the small samples size (n=1/1, 8, 16 dd / trt / trial). However, when these results were evaluated there was a distinct difference in magnitude of values reported of the control when comparing the COM and LAB treatment results. This reported thiamine levels in COM frankfurter and CON frankfurters points to the potential effect of the treatments. These CON frankfurter thiamine levels also relate to previous literature from Tang et al. (2006) showing that the amount of thiamine in the CON treatment is closer to the published levels in this literature and by the USDA Nutrient Database (2002). The NP Analytical Laboratories data from Trial A and Trial B (Table 11) had differences between each treatment, which is contrary to the in-house HPLC method. Thus, data discrepancies could potentially be due to analysis methods used. The in-house HPLC thiamine extraction was not the AOAC (2000) method. The AOAC (2000) method includes an enzyme digestion step that was not utilized in the HPLC method (AOAC, 2000). Thiamine levels measured at the commercial laboratory were also measured by florescence method by converting the extracted thiamine to thiochrome to be measured with a fluorescence detector (AOAC, 2000). The levels of thiamine measured in the in-house HPLC laboratory were measured using absorbency, which demonstrated high amounts of thiamine that were not expected when looking at the USDA Nutrient Database (2002). Only using absorbance to measure the thiamine levels in the in-house HPLC method might not be specific enough, thus giving inflated thiamine readings. According to the USDA Nutrient Database (2002), pork and beef sausage contains 0.09 mg of thiamine / 26 g of meat. This is significantly smaller than that levels that were obtained using the in-house HPLC method in Trial A and B (Table 9 and 10). This can potentially be explained by the possibility that more vitamins were coming off the column at the same time as thiamine, which gave larger amount readings than were actually there. If the AOAC thiamine analysis method had been utilized, the thiamine data from HPLC method may have been a better representation of the actual amount of thiamine present. Samples (n= 1/ 1, 8, 16 dd / trt / trial) that were sent to NP Analytical Laboratories.

Table 9. Least square means ± SEM of thiamine levels (mg/100g) based on control (CON), commercial (COM), and laboratory (LAB) treatments and by display day of Trial A and Trial B in the in-house HPLC laboratory method.

| **Effect** | | **Trial A Thiamine (mg/100g)** | **Trial B Thiamine (mg/100g)** | **Trial A *P* > F** | **Trial B *P* > F** |
|---|---|---|---|---|---|
| Treatment | | | | | |
| | Control | 64.0 ± 0.03 | 63.0 ± 0.03 | 0.1714 | 0.2742 |
| | Commercial | 57.0 ± 0.03 | 57.0 ± 0.03 | 0.1714 | 0.2742 |
| | Laboratory | 62.0 ± 0.03 | 59.0 ± 0.03 | 0.1714 | 0.2742 |
| Display Day | | | | | |
| | 1 | 60.0 ± 0.03 | 57.0 ± 0.03 | 0.7273 | 0.8593 |
| | 4 | 58.0 ± 0.03 | 61.0 ± 0.03 | 0.7273 | 0.8593 |
| | 8 | 64.0 ± 0.03 | 59.0 ± 0.03 | 0.7273 | 0.8593 |
| | 12 | 63.0 ± 0.03 | 61.0 ± 0.03 | 0.7273 | 0.8593 |
| | 16 | 60.0 ± 0.03 | 58.0 ± 0.03 | 0.7273 | 0.8593 |

Table 10. Least square means ± SEM of thiamine levels mg/100g frankfurter) obtained by the in-house HPLC method (for control (CON), commercial (COM), and laboratory (LAB) frankfurter treatments.

| **Trial** | **Control** | **Commercial** | **Laboratory** | **Source** | ***P* > F** |
|---|---|---|---|---|---|
| Trial A | 64.0 ± 0.03 | 57.0 ± 0.03 | 62.0 ± 0.03 | trt | 0.1714 |
| | | | | dd | 0.7273 |
| | | | | trt x dd | 0.8660 |
| Trial B | 63.0 ± 0.03 | 57.0 ± 0.03 | 59.0 ± 0.03 | trt | 0.2742 |
| | | | | dd | 0.8593 |
| | | | | trt x dd | 0.9440 |

Table 11. Thiamine levels results of control (CON), commercial (COM), and laboratory (LAB) treatments from NP Analytical Laboratories (St. Louis, MI) on dd 1, 8, and 16 in Trial A and Trial B (n = 1 / trt / dd).

| Treatment | dd | Thiamine Levels (mg/100g) Trial A | Thiamine Levels (mg/100g) Trial B |
|---|---|---|---|
| CON | 1 | 0.16 | 0.16 |
| COM | 1 | 3.94 | 3.77 |
| LAB | 1 | 0.47 | 1.17 |
| CON | 8 | 0.16 | 0.17 |
| COM | 8 | 3.88 | 3.22 |
| LAB | 8 | 0.43 | 1.24 |
| CON | 16 | 0.15 | 0.17 |
| COM | 16 | 4.27 | 2.94 |
| LAB | 16 | 0.47 | 1.19 |

## IMPLICATIONS

A multivitamin microcapsule may be added to a frankfurter formulation to increase functional properties of the RTE meat food product. The addition of a microcapsule that is able to withstand chemical, thermal, and physical agitation will help broaden the use of the microencapsulation technique in the food industry. Although the production of a functional food is not a new idea, the implication of new food products could be widened and applied to the meat industry. Frankfurters are a largely consumed product by Americans and this alternative approach to a typical formulation could add another positive characteristic upon consumption. As the study did not accurately represent the in-house HPLC results to determine the different amounts of thiamine levels within each treatment, more studies can be conducted to change the method of thiamine extraction along with determining the remaining vitamins present in the cooked product. This study could also be applied to different RTE meat products not just specifically frankfurters.

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Sensory Evaluation

1. Frankfurters should have an internal temperature of 2 to 5°C before cooking. It is common to thaw food products before cooking at 2 to 5°C for 12 hours.
2. Take care and maintain sample identity throughout process by having labels with the product.
3. Pre-heat sample holding containers and pans by placing in the oven. Pans with separate suspended compartments can be utilized, with the addition of sand below to maintain temperature.
4. Boil a large pot of water to reheat the samples.
5. Internal temperature of each frankfurter should be taken in the geometric center and recorded. Temperatures should be in the range of 2 to 5°C.
6. Weigh each frankfurter in g before cooking and record.
7. Place each frankfurter in boiling water to reheat. The internal temperature of each frankfurter should be approximately 71°C.
8. Record weight and temperature of each steak recorded immediately after cooking utilizing same procedures as before cooking.
9. Slice each frankfurter into 1 cm pieces by using a premade cutting board to standardize the size of each sample.
10. Place all pieces of sample (excluding end pieces) in designated sample holding containers and maintain identity.
11. Panel room should be prepared before cooking to facilitate efficient panel time and minimize period after cooking until panel evaluations.
12. Panel set up and evaluations should be according to Cross et al., 1978.
13. Record all sensory data for analysis.

Laboratory Analysis

1. One gram of the frankfurter will be measured with a calibrated scale.
2. The 1 g sample will have 10 mL of trichloracetic acid (TCA) added to the tube.
3. The mixture will be homogenized using a polytron homogenizer.
4. This will then be placed on ice for 15 min.
5. The samples will be centrifuged at 13000 rpm for 6 min at 10°C.
6. The 1 mL of supernatant was transferred to an epidorf tube and centrifuged at 12000 rpm for 6 min two more times.
7. The samples were diluted to 1:5000
8. Place dilution in a 1 mL glass vial sample tube with polyethylene snap cap
9. The sample can then be put in the autosampler for analysis.

# DEVELOPMENT AND EVALUATION OF A QUICK SERVE RESTAURANT VALUE-ADDED BEEF PRODUCT

*Kari A. Mulle, Loree A. Branham, and Kirk W. Braden*

## ABSTRACT

Development of a quick serve restaurant value-added beef product is important to help meet the needs of consumers for a convenient, ready to eat, more nutritious product. While the poultry industry has advanced in the value-added product market, relatively little advancement has been done in the beef market. The objectives of this study included adding value to a traditionally underutilized meat cut, the *Semimembranosus* muscle, by developing a value-added bite sized breaded beef product, as well as, evaluating the flavor, overall acceptability and price willing to pay for this product from local consumers. This project was conducted at the Angelo State University Food Safety and Product Development Laboratory located in San Angelo, TX. *Semimembranosus* muscle was trimmed and cut into cubed pieces (approximately ¾ oz.) and randomly assigned to one of three treatment marinades: control, Cajun Marinade and Enhancement Marinade with tenderizer. After marination, beef cubes were par-cooked, chilled, pre-dusted, battered, breaded, par-fried, quick frozen and packaged in low oxygen modified atmosphere packaging. Flavor, overall acceptability and price willing to pay data was gathered from local consumers (n=108) at the San Angelo Stock Show and Rodeo in San Angelo, Texas. Demographic data gathered included ethnicity, age, gender, household income and how many times beef was consumed in the past month. There were no differences in scores of the three treatment marinades for flavor ($P = 0.17$), overall acceptability ($P = 0.53$) or price willing to pay ($P = 0.10$). When evaluating the potential effect of gender, a significant interaction was observed between treatment and gender class when looking at price willing to pay ($P = 0.05$) When controlling for gender class effect: there was no treatment effect within the female gender class ($P = 0.15$); however, males were willing to pay more for Cajun and Enhancement than the Control ($P = 0.01$). When comparing scores of the various age classes surveyed, marinade treatment had no significant effect; however, age class showed to have a significant effect on scores in flavor ($P = 0.001$), overall acceptability ($P = 0.009$) and price willing to pay ($P = 0.001$). This data will be used to further develop and refine a value-added beef product to be processed at Angelo State University Food Safety and Product Development Laboratory.

## INTRODUCTION

Meat is marketed in the United States in various forms and conveniences to suit the consumers' needs. Consumers today are demanding foods that are more convenient and healthy. The meat products they are demanding need to be more convenient, nutritious, visually attractive, cost efficient, flavorful, high quality and a safe product (Desmond et al. 2001). Murano et al. (2003) said "to be considered value-added, foods must offer the consumer some improvement in terms of quality or convenience over the traditional or previously available foods." These value-added products must be "economical and cost-effective" in order for the consumer to purchase these products. The poultry industry has already mastered the value-added products (The MeatSite 2011). The poultry industry currently produces chicken nuggets, popcorn chicken and several other value-added products. The poultry and seafood industries have muscles that are low in connective tissue resulting in a tender cut of muscle compared to beef cuts. Beef cuts that are utilized in value-added products are usually underutilized cuts. Adding value to underutilized products can be performed in several ways. "Edible coatings can improve the quality of fresh, frozen, and processed meat, poultry, and seafood products" by retarding moisture loss, reducing lipid oxidation and discoloration, sealing in flavors, functioning

as carriers of food additives, reducing oil uptake and enhancing product appearance by eliminating dripping (Gennadios et al., 1997). Marinades containing plant enzymes can breakdown the collagen and myofibrillar proteins within a muscle. Not only do the marinades provide a carrier for the enzymes but also a way to give flavor to these products (Calkins and Sullivan 2007). Consumers are demanding products with a variety of flavor profiles to help expand their options (DiUlio 2010). These techniques may enhance the use of beef in processed products similar to those currently produced from poultry and fish muscle (Desmond et al., 2001). Consumers want to enjoy a restaurant type dinner at home without having to go through the preparation steps. According to Deogade et al. (2008), family dynamics, rising incomes, changing food habits with preference for fast foods and heavy industrialization will greatly enhance the demand for fresh or frozen and nutritionally superior value added products. All of these motivating factors combine to make a U.S public open to trying new convenient products. Taking this into consideration the objective of this project was to develop a novel value-added beef product and measure consumer acceptance of this product.

## MATERIALS AND METHODS

Inside round (*Semimembranosus*) was trimmed of all heavy connective tissues and cubed into ¾-ounce pieces. Four pieces represented a serving size, approximately three ounces. Pieces were randomly assigned to three different marinade treatments. All three treatments contained the basic brine ingredients used across the industry; water, salt, sucrose, and phosphate. Full ingredient list for all three treatments is provided in Table 1. Each treatment was vacuum tumbled for twenty minutes to ensure marinade was evenly distributed across the product. Treatments were then par cooked using an impingement oven for approximately seven minutes. Internal temperature was taken post par cooking to make certain the internal temperature reached 145°F for food safety requirements as well as a quality measure to ensure the product did not have a red internal color after being fried. Each treatment was chilled for approximately 24 hours before breading. The breading process began with a pre-dust, applied to ensure proper adhesion of the batter. Next a batter was applied at a 2:1 ratio of water:batter mix. Lastly a corn and cracker mix breader was applied. The batter and breading steps took place twice to ensure proper coating of the product. All three treatments received the same pre-dust, batter and breading process. After the product had been through the batter and breading process each treatment was then par fried for two minutes at an oil temperature of 365°F to ensure the coating set. Each treatment was allowed to cool on racks then individually quick frozen (IQF) utilizing liquid nitrogen to ensure breading did not break off product in the freezer. Individually quick freezing is equivalent to the blast freezer or an IQF machine utilized in the industry.

The day of the sensory panel each treatment was kept frozen until it was final fried for two minutes. The final fry was accomplished using a home-grade commercially available deep fryer. Consumers were asked to fill out a survey that was constructed for ease including e basic demographic information that included gender, ethnicity, age, household income, and how many times they consumed beef within a month. Consumers were then asked to sample each treatment that was labeled A for the control treatment, B for the Cajun treatment and C for the Commercial Enhancement Marinade with Tenderizer so the consumer did not know the difference between samples. Consumers were asked to evaluate each sample on flavor, overall acceptability and price willing to pay. The survey contained a line with anchor points of extreme dislike to extreme like for flavor, overall acceptability and price willing to pay. Consumers were asked to place an X on the line anywhere in between or on one of the extremes (Figure 1). Numerical data was gathered by measuring each line in millimeters from starting anchor point to end anchor point that was 0-160mm. After the base was determined, numerical data was

Table 1. Ingredient list of three different treatment marinades for a novel value-added beef product

| Treatments | | |
|---|---|---|
| **Control** | **Cajun Marinade** | **Commercial Enhancement Marinade with 10% Tenderizer[a]** |
| Water 16 oz | Water 16 oz | Water 16 oz |
| Salt 1.6 oz | Salt 1.6 oz | Salt |
| Phosphate .8 oz | Phosphate .8 oz | Sodium Phosphate |
| Sucrose .05 oz | Sucrose .4 oz | Dextrose |
| | Red Pepper ½ tsp. | Bromelain |
| | Onion Powder ½ tsp | |
| | Black Pepper ½ tsp | |
| | Garlic Powder ½ tsp | |

**[a]AC Legg Blend Reference # 08-027-000 (Calera, AL)**

gathered by measuring from starting anchor point to the consumers mark on the line in millimeters. Consumers were also asked to rank their preference of the three products from most to least liked. Data was then entered into an excel spreadsheet. The Frequency and Mixed procedures of SAS were utilized for analysis (SAS Inst., Inc., Cary, NC).

**RESULTS**

One hundred and fourteen surveys were collected but only 108 were utilized in analysis. The six that are missing were missing data pertaining to the samples. Any surveys missing demographic data were still utilized in the analysis. Demographic summaries are presented in Figure 2. Out of the 108 surveys only 95 consumers indicated gender with 47.37% responding female (45) while the remaining 52.63% consumers indicated they were male (50). Eighty-eight prevent of consumers indicated they were Caucasian followed by 10% Hispanic. While looking at household income the majority of the consumers indicated, they fell between the $50,000-74,999 and greater than $99,999. While looking at age groups the majority of consumers were under the age of forty-five. When consumers were asked to indicate how many times they consume beef within a month (given the options 1, 2, 3 or 4 or greater), 93% of the consumers indicated they consumed beef four or greater times followed by 6% of the consumers indicating they consume beef 3 times a month.

When evaluating the main effect of marinade, there were no differences in scores of the three treatment for flavor with scores ranging between 96.18 and 105.73 on a scale of 1 to 160 ($P$ = 0.17), overall acceptability with scores ranging between 95.58 and 101.13 on a scale of 1 to 160 ($P$ = 0.53) or price willing to pay with values ranging between $5.06 to $5.17 on a scale ranging from $4.50 to $6.50($P$ = 0.10). When evaluating the potential effect of gender, the price willing to pay for a specific flavor was the only evaluated attribute that was dependent on gender ($P$ = 0.05). When controlling for gender class effect: there was no treatment effect within the female gender class ($P$ = 0.15, Figure 3); however, males were willing to pay more for Cajun and Enhancement than the Control ($P$ = 0.01, Figure 3). When comparing scores of the various age classes surveyed, marinade treatment had no significant effect; however, age class showed to have a significant main effect on overall scores in flavor ($P$ = 0.001, Figure 4), overall acceptability ($P$ = 0.009, Figure 5) and price willing to pay ($P$ = 0.001, Figure 6).

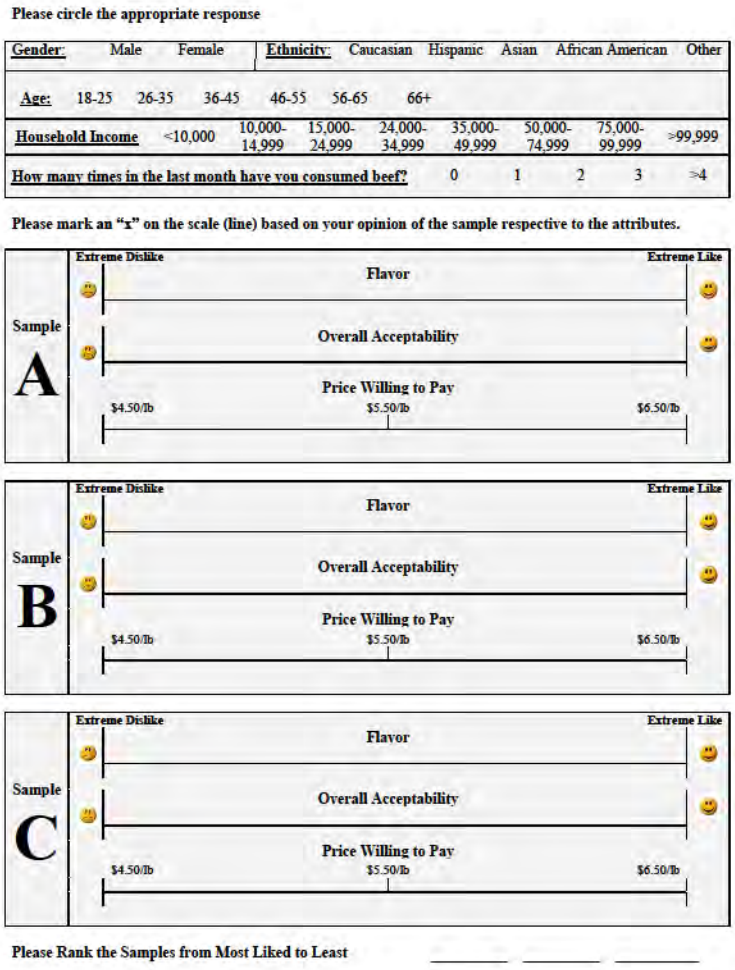


Figure 1. Diagram of actual survey questions consumers s were asked to fill out regarding a novel value-added beef product

## DISCUSSION AND IMPLICATIONS

The percent of each treatment ranked first from the consumer is as follows. Forty-seven percent of the consumers ranked the Cajun treatment first. Twenty-nine percent of the consumers ranked the Commercial Enhancement Marinade with Tenderizer first. The remaining 24% ranked the control treatment first. This corresponds with the verbal feedback received from the surveyed consumers. Consumers preferred the flavor of the Cajun marinade to all the other treatments but liked the tenderness of the enhancement marinade. The data collected in this project will be used to further develop and refine a value-added beef product. In some instances, both gender and age had an effect on flavor, acceptability and willingness to pay of the product**.** This information can be useful in determining which demographic to market the product toward**.** Further development of this product will take a further look into using various batters, breading and sensory panels.

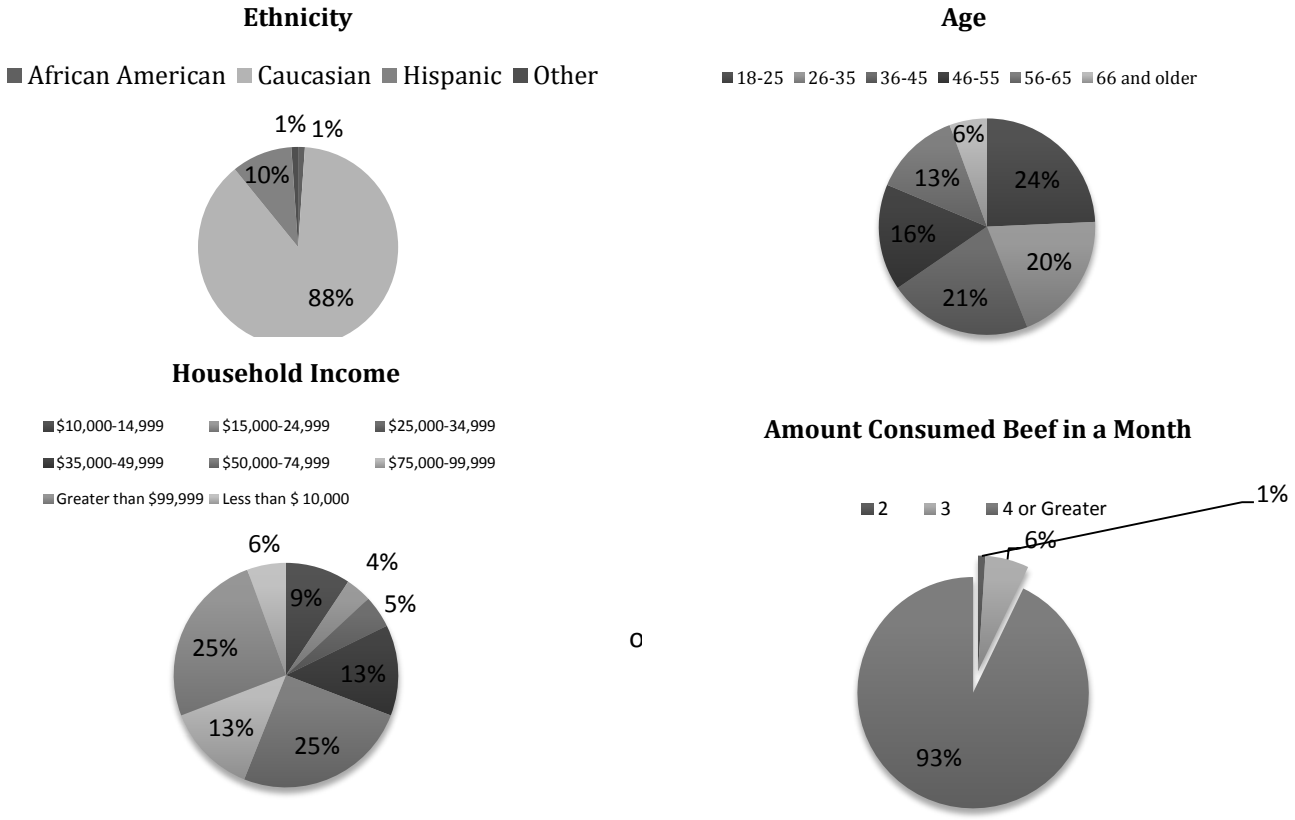


Figure 2. Demographic distribution of consumers who surveyed a novel value-added beef product n=108.

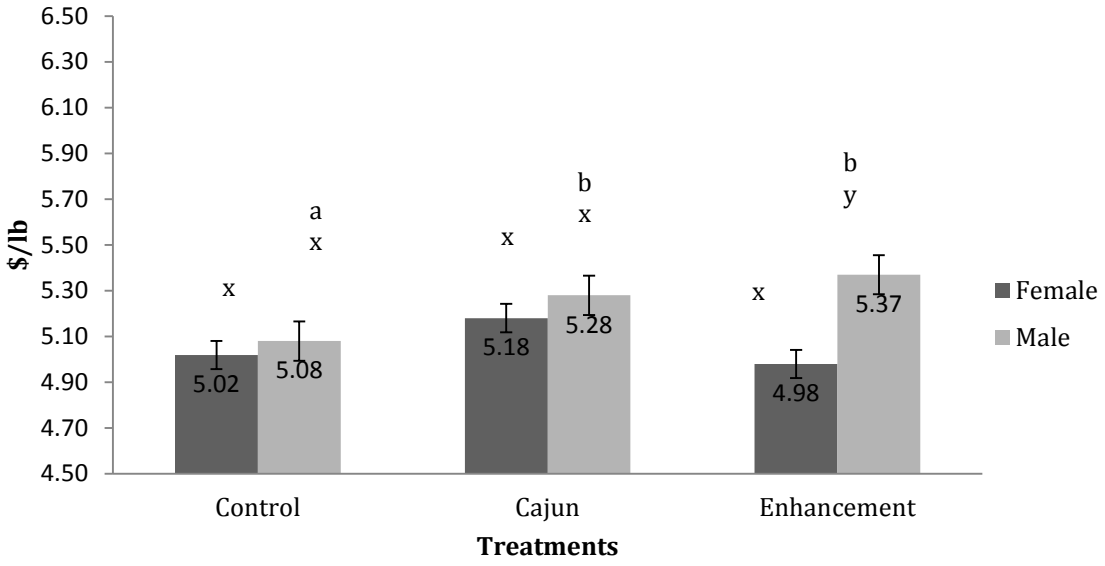


Figure 3. LS Mean Price Willing to Pay for Value-Added Beef Product Dependent on Gender (n=95)

[abc] treatment LS Means with no or similar superscripts, within a gender class do not differ (*P*>0.05)

[xy] gender LS Means with similar superscripts, within a treatment group do not differ (*P*>0.05)

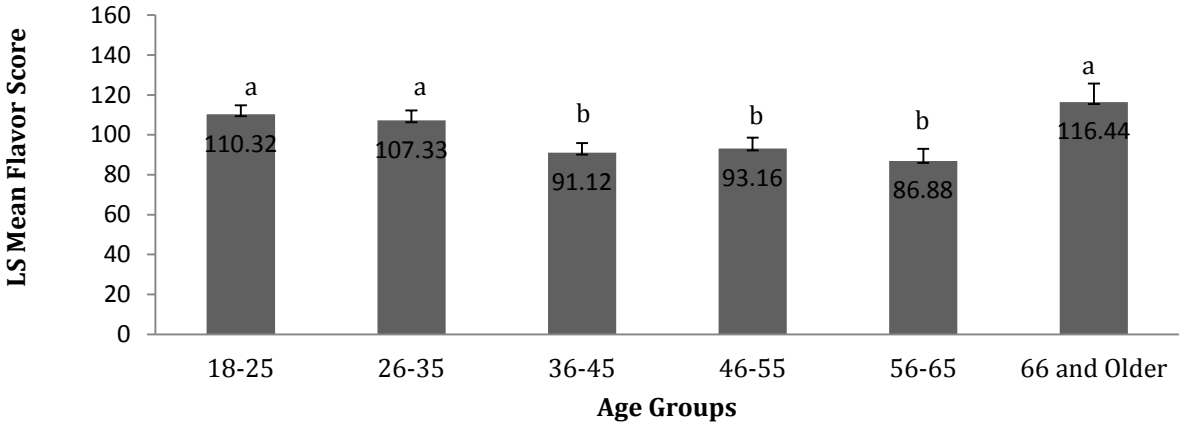


Figure 4. Flavor Score for Value –Added Beef Product Dependent on Age of Surveyed Consumer (n=108)

[ab] Age values with different superscripts differ (*P* < 0.05)

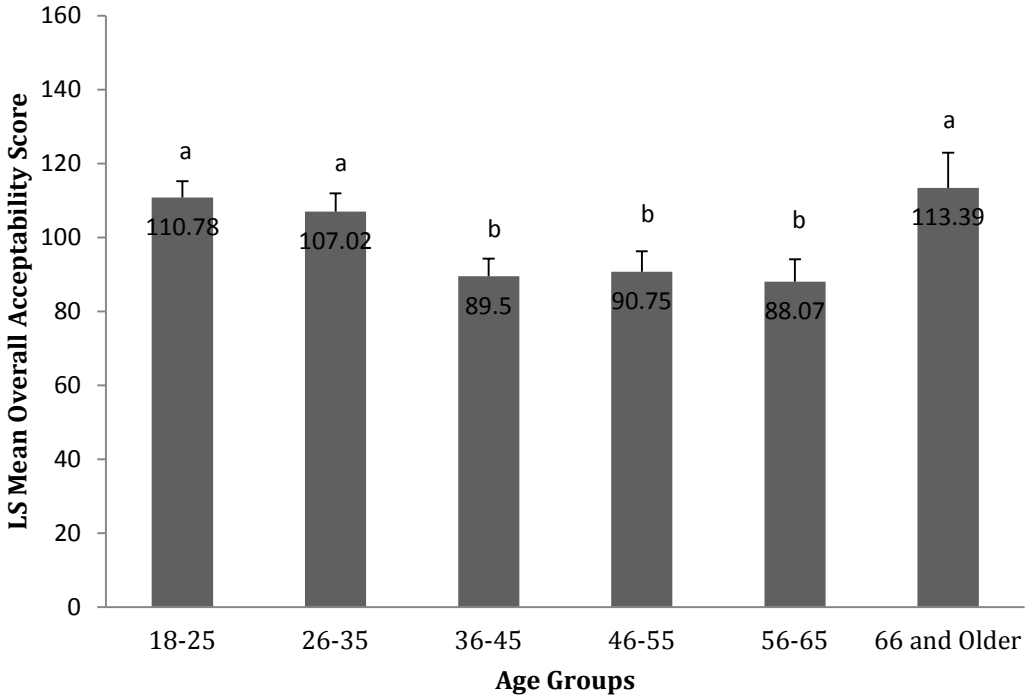


Figure 5. Overall Acceptability Score for Value-added Beef Product Dependent on Age of Surveyed Consumer (n=108)
[ab] Age values with different superscripts differ ($P < 0.05$)

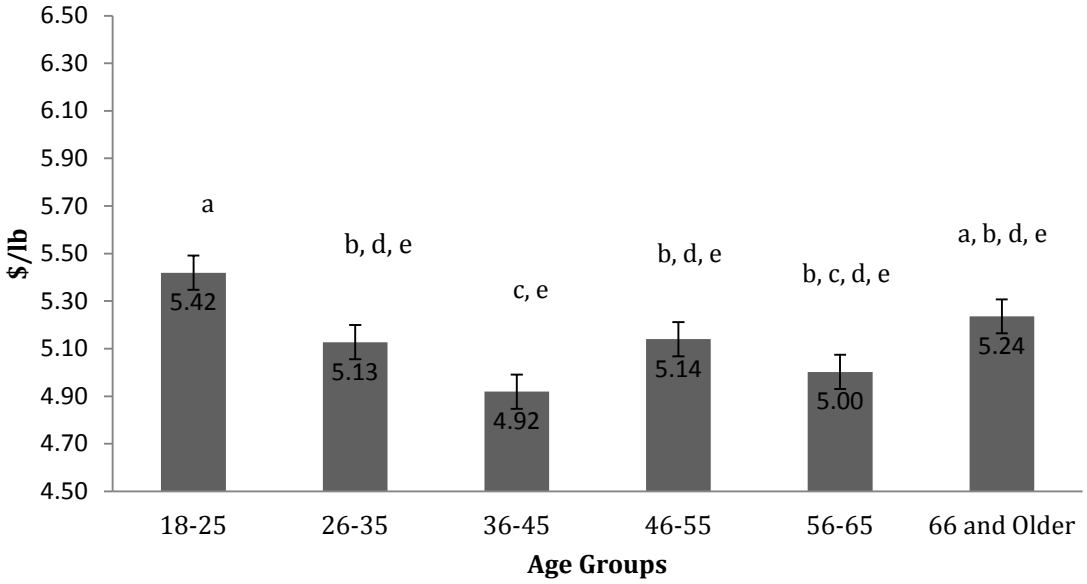


Figure 6. LS Mean Price Willing to Pay Score for Value-Added Beef Product Dependent on Age of Surveyed Consumer (n=108)
[abcde] Age Values with different superscripts differ ($P < 0.05$)

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# ANTIBIOTIC RESISTANCE IN WHITETAILED DEER AND RANGE CATTLE SHARING THE SAME RANGELAND

*Darci Owens, Loree Branham and Cody Scott*

## ABSTRACT

Little research has been done investigating wildlife as a potential source for antibiotic resistant bacteria. The objectives of this study were to evaluate antibiotic resistance levels in *Escherichia coli* isolated from whitetailed deer and their similarities and differences in susceptibility to antibiotics compared to *E. coli* isolates obtained from cattle on the same rangeland. Twenty fecal samples from deer and fourteen cattle fecal samples were aseptically collected during the months of November and December at the Angelo State University Management, Instruction & Research Center located in San Angelo, Texas. Samples were plated on MacConkey agar, enumerated and four typical *E. coli* colonies were isolated from each sample. For each isolate, minimum inhibitory concentrations of 15 antimicrobials of human and veterinary importance were determined using a microbroth dilution, 96-well, gram-negative Sensititre® plate. *E. coli* populations were not dependent on species of animal from which the fecal samples were collected ($P = 0.11$) One hundred twenty eight *E. coli* isolates were collected; 72 isolates were obtained from deer fecal samples, while 56 isolates came from cattle fecal samples. Of the 128 isolates analyzed, 71% were pansusceptible; while 28.91% of isolates were resistant to at least one antimicrobial and 16.41% were resistant to two or more antimicrobials. The most common resistance was to tetracycline, sulfisoxazole and ampicillin with 18.75 %, 9.38% and 8.59% of all isolates showing resistance to the respective drug. Resistance to antimicrobials including tetracycline, sulfisoxazole, ampicillin and nalidixic acid found in isolates from both cattle and deer suggest wildlife as well as domestic livestock can be sources of antimicrobial resistant pathogenic bacteria.

## INTRODUCTION

In addition to the traditional hazards foodborne bacteria originating from animals present, these pathogens are now showing the added threat of antibiotic resistance. The agriculture industry has been cited for an increase of antibiotic resistant infections in humans because of the large amount of antimicrobials administered to livestock. Research has shown antibiotic resistant *Escherichia coli* and *Campylobacter* bacterial infections increase in humans as the use of antibiotics for treatments of these infections in animals has increased (GAO 2004). Though antimicrobials are commonly administered therapeutically to prevent dissemination of disease, food animals are also often given subtherapeutic levels of certain antimicrobials over long periods of time for health benefits (NAHMS 1999). These growth promoters help to improve feed efficiencies and prevent infections that might occur as a result of raising a large number of animals in confinement (NAHMS 1999, USDA 2000). In spite of this, most of the pathogens demonstrating resistance cannot be traced back to animal sources (Bywater 2005). Estimates show that less than 10 percent of antibiotic drug-resistant pathogens in humans are directly associated with livestock production practices (Mathews 2001).

To solely target the agriculture industry for the increase in human antibiotic drug resistance would be incomplete. Subtherapeutic antimicrobials are administered at such low doses, it is unlikely the resistance problem in humans has arisen primarily from the use of growth promotants and more likely are additionally due to abuse by humans (Phillips et al. 2004). Some scientists believe the inappropriate or misuse of antibiotics administered to human patients further increases the number of antibiotic resistant infections (WHO 1997).

Therefore, it is believed modern medical practices have the principal role in the increase in antibiotic drug resistance (GAO 2004).

Though research has been performed in the feedlot prior to slaughter, cattle are susceptible to various forms of contamination before reaching the feedlot such as the possibility of contamination by wildlife species (Cray 2008). Analysis of fecal samples from cattle, sheep and goats grazing the same rangeland as white-tailed deer showed similar bacterial strains in the animals, as well as the water source they shared (Branham et al. 2005). This evidence suggests water sources may play a role in transferring bacterial species from one animal to another.

Though both the agriculture and medical industries are targeted, in reality, the cause of increased resistance is dynamic. Any animal or human can be a carrier of antibiotic resistant bacteria (Brashears et al. 2006). Resistant bacteria can be passed through the soil, water, sewage, contaminated produce, feedstuffs and even wildlife (Phillips et al. 2004). Wildlife is one area that has more recently been identified as a potential vehicle for antibiotic resistant pathogens (Cray 2008). However, little research has focused on the possibility of wildlife transferring antibiotic resistant bacterial strains to produce or livestock species sharing the same rangelands. Therefore, the objectives of this study were to establish baseline antibiotic susceptibility profiles and evaluate differences in resistance of *E. coli* isolates obtained from white-tailed deer and cattle grazing the same rangeland. In doing so, the potential role of wildlife was further established as a vector for antibiotic resistant *E. coli.*

## MATERIALS & METHODS

Sample Collection

Fecal samples from white-tailed deer and cattle grazing the same rangeland on the Angelo State University Management, Instruction and Research Center were collected (Figure 1). Samples were collected from 20 harvested deer of various gender and ages (Table 1) via direct rectal harvest during the months of November and December. Fourteen cattle samples were collected from individual fresh fecal pats. Cattle samples were taken only from fresh, physically isolated fecal pats to ascertain samples came from separate animals. All samples were placed in individually labeled sterile conical vials. Samples were collected using ascetic techniques, refrigerated at 4°C, transported to the Angelo State University Food Microbiology Laboratory and processed within 24 hours of collection.

Enumeration & Isolation

Upon arrival to the laboratory, all samples were serially diluted using Buffered Peptone Water and then plated and enumerated using MacConkey agar via spread plate method and incubated at 37°C for 24 h. After enumeration, four typical, well-isolated colonies from the most countable plate were re-isolated onto fresh MacConkey agar and incubated for 24 h at 37°C. Typical *E. coli* colonies are pink to red in color which allowed for differentiation from other bacteria.

Following incubation, colonies were individually transferred into 9 mL Brain-Heart Infusion (BHI) broth test tubes with a 10% glycerol solution and incubated at 37°C for 24-48 h. After incubation, the tubes were vortexed to redistribute the glycerol. A 1 mL portion of each culture was placed into a sterile cryogenic tube labeled with a unique code and stored in a -80°C freezer for further analysis.

Antibiotic Susceptibility

The frozen culture was removed from the freezer and allowed to sit at room temperature for 10 minutes. A portion of the culture was resuspended into sterile BHI broth and incubated for 24 h at 37°C. A 0.1 mL aliquot was plated onto a Tryptic Soy Agar petri-plate using the spread-plate method and incubated at 37°C for 24h. Three to five colonies were

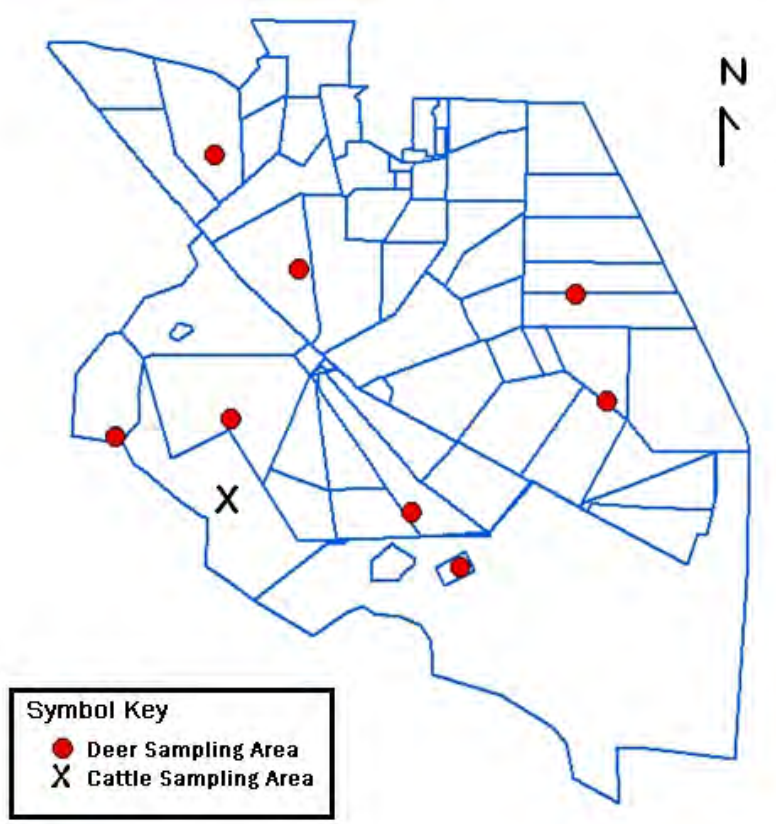


Figure 1. Map of cattle grazing areas and deer sampling locations located on the Angelo State University Management, Instruction and Research Center (Approx. 6,000 acres)

Table 1. Age class and gender of deer harvested and sampled on the Angelo State University Management, Instruction and Research Center

| Age (years) | Number of Males | Number of Females |
|---|---|---|
| 1.5 | 5 | 2 |
| 2.5 | 1 | 2 |
| 3.5 | 1 | 1 |
| 4.5 | 2 | 1 |
| 5.5 | 3 | - |
| 6.5 | - | 1 |
| 7.5 | - | 1 |

isolated and transferred to 5 mL sterile water test tubes and compared with a 0.5 McFarland standard turbidity. Following verification of turbidity, 10 micro liters of culture was transferred to a 10 mL Mueller-Hinton broth suspension. For each isolate, minimum inhibitory concentrations of 15 antimicrobials of human and veterinary importance were determined using a commercially available microbroth dilution 96-well gram-negative Sensititre® plate (Table 2).

All wells within the plate were inoculated with 50 micro liters per well and incubated for 18-24 hours at 36°C.

Minimum inhibitory concentrations were determined for each antimicrobial, and isolates were classified as resistant or susceptible using predetermined breakpoints. Descriptive statistics were generated using various procedures of SAS (Cory, NC; Version 9.3.1), and significant differences between populations were evaluated using a predetermined alpha of less than or equal to 0.05.

## RESULTS AND DISCUSSI0N

*E. coli* Population Results

Of the twenty deer fecal samples and fourteen cattle fecal samples collected and tested, 100% tested positive for *E. coli*. *E. coli* populations were not dependent on species of animal from which the fecal samples were collected ($P$ = 0.11, Figure 2). Alexander and others reported *E. coli* population levels from fecal samples taken from cattle consuming silage based diets at 5.32 log CFU swab$^{-1}$ (2008). Direct comparison to the current study is difficult due to differences in sample type; however populations do seem comparable when taking this into account.

Antimicrobial Drug Resistance Results

One hundred twenty eight *E. coli* isolates were collected; 72 isolates were obtained from deer fecal samples, while 56 isolates came from cattle fecal samples. Of the 128 isolates analyzed, 71.09% were pansusceptible (Figure3); while 28.91% of isolates were resistant to at least one antimicrobial and 16.41% were resistant to two or more antimicrobials. Two of the isolates were resistant to at least 9 different antimicrobials (Figure 3). The most common resistance was to tetracycline, sulfisoxazole and ampicillin with 18.75 %, 9.38% and 8.59% of all isolates exhibiting resistance to the respective antimicrobial.

Resistance by *E.* coli to similar antibiotics has been well documented over several decades. In 1966, *E. coli* isolates obtained from diseased humans and domestic animals showed the most resistance towards Tetracycline, Streptomycin and Sulphonamides (Smith et al. 1966). In a study conducted in a Texas feedlot, *E. coli* isolates collected from cattle exhibited resistant mechanisms toward Sulfisoxazole, Tetracycline, and Streptomycin following the administration of a subtherapeutic antimicrobial (Branham et al. 2007). With such slight variation of resistance patterns of *E. coli* compared to strains collected 40 years ago, changes in resistant *E. coli* is not thought be a rapid process and *E. coli* bacteria will continue to be treatable with certain types of antibiotics (Slocombe and Sutherland 1973).

Comparison of Isolates from Cattle and Deer

When comparing isolates obtained from cattle and deer samples similar pansusceptibility levels were observed with 69.64% of cattle isolates and 72.22% of deer isolates showing susceptibility to all antimicrobials tested (Figure 4). While overall susceptibility levels are similar, the authors did observe differences in the distribution of isolates which showed resistance to one or more antimicrobial. No isolates obtained from cattle were resistant to more than three antimicrobials, with 12.5%, 5.36% and 12.5% of the total 56 isolates showing resistance to one, two and three antimicrobials, respectively. Whereas, isolates coming from deer samples exhibited multi-drug resistance to as many as ten antimicrobials in one isolate's case. Specifically, 12.5%, 1.39% and 6.94% of the total 72 isolates showed resistance to one,

Table 2. Gram-Negative Antimicrobial Drug Concentration Range and Breakpoints.

| Antimicrobial Drug | Concentration (µg/mL) | MIC Breakpoint (µg/mL)* |
|---|---|---|
| Amikacin | 0.5-64 | ≥64 |
| Ampicillin | 1-32 | ≥32 |
| Amoxicillin/Clavulanic Acid | 1/0.5-32/16 | ≥32/16 |
| Ceftriaxone | 0.25-64 | ≥64 |
| Chloramphenicol | 2-32 | ≥32 |
| Ciprofloxacin | 0.015-4 | ≥4 |
| Trimethroprim/Sulfamethoxazole | 0.12/2.38-4/76 | ≥4/76 |
| Cefoxitin | 0.5-32 | ≥32 |
| Gentamicin | 0.25-16 | ≥16 |
| Kanamycin | 8-64 | ≥64 |
| Nalidixic Acid | 0.5-32 | ≥32 |
| Sulfisoxazole | 16-256 | ≥256 |
| Streptomycin | 32-64 | ≥64 |
| Tetracycline | 4-32 | ≥32 |
| Ceftiofur | 0.12-8 | ≥8 |

*MIC Breakpoint = Minimum Inhibitory Concentration Breakpoint, obtained from the Clinical Laboratory Standards Institute and the National Antimicrobial Resistance Monitoring System.

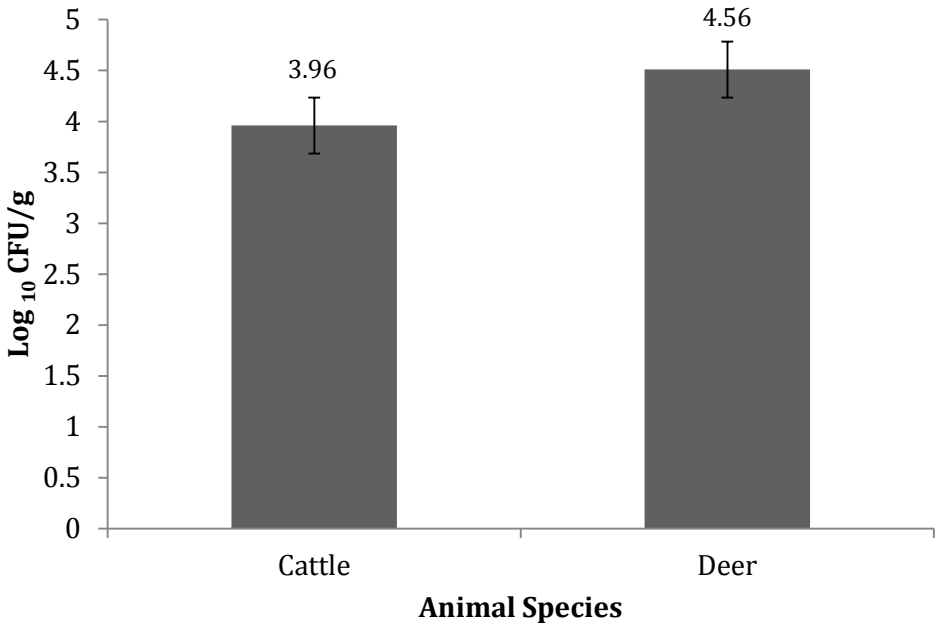


Figure 2. Average *E. coli* populations of fecal samples from deer and cattle grazing the same rangeland (*P* = 0.11, Standard Error bars are shown)

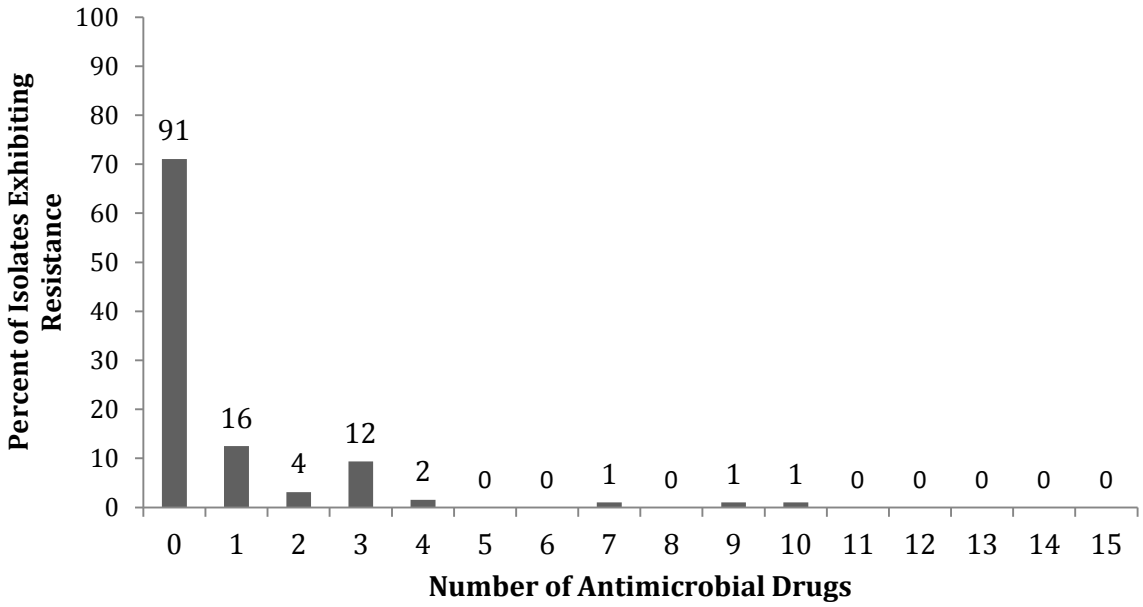


Figure3. Percent of total *E. coli* isolates collected from deer and cattle exhibiting resistance to various numbers of antimicrobials. (n=128).

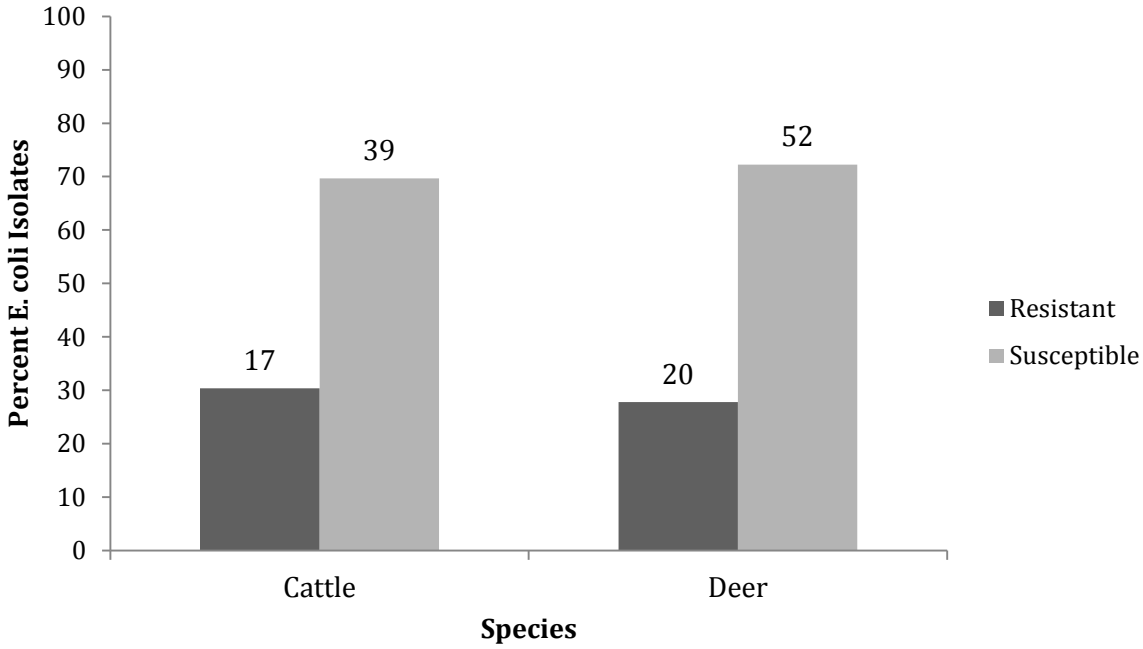


Figure 4 . Percent *E. coli* isolates isolated from cattle and deer which exhibit resistant to at least one tested antimicrobial

two and three antimicrobials, respectively. Resistance patters of isolates showing resistance to four or more antimicrobials are displayed in Table 3. These are of particular concern due to the unsanitary methods in which much wildlife meat is harvested. If these isolates were to contaminate meat product harvested from the animal and then that product not be cooked properly, a severe resistant infection could potentially result.

The most common resistance exhibited by *E. coli* isolates obtained from cattle samples was to tetracycline, sulfisoxazole and streptomycin with 25.00%, 14.29% and 12.50% of isolates exhibiting resistance to the respective antimicrobial (Figure 5). While the most common resistance exhibited by *E. coli* isolates obtained from deer samples was to tetracycline, cefoxitin and ampicillin with 13.89%, 12.50% and 12.50% of isolates exhibiting resistance to the respective antimicrobial (Figure 6).

## IMPLICATIONS

The resistance to antimicrobials including tetracycline, sulfisoxazole, ampicillin and nalidixic acid found in isolates from both cattle and deer suggest possible transference of bacteria between species or possibly transference of resistance factors between bacteria. Genetic typing of isolates would be required to make a definitive connection. Regardless of that final confirmation, this study did establish the presence of multi-drug resistant *E. coli* in wildlife that comes into contact with domestic livestock. Since the complete separation of domestic and wild animals is unrealistic, researchers must consider the contribution wildlife make on the presence of resistant bacteria when addressing the ever increasing problem of resistant infections in animal and human populations.

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Table 3. Most common multi-drug resistance patterns of *E. coli* isolates obtained from deer

| % Isolates Resistant (Frequency) | # of Drugs Resistant | Antimicrobials to Which Isolates Were Resistant |
|---|---|---|
| 0.78% (n=1) | 10 | Cefoxitin, Amikacin, Chloramphenicol, Amoxicillin/Clavulanic Acid, Ciprofloxacin, Nalidixic Acid, Ceftiofur, Sulfisoxazole, Kanamycin, Ampicillin |
| 0.78% (n=1) | 9 | Cefoxitin, Chloramphenicol, Tetracycline, Amoxicillin/Clavulanic Acid, Nalidixic Acid, Ceftiofur, Sulfisoxazole, Kanamycin, Ampicillin |
| 0.78% (n=1) | 7 | Cefoxitin, Amoxicillin/Clavulanic Acid, Chloramphenicol, Nalidixic Acid, Ceftiofur, Sulfisoxazole, Ampicillin |
| 0.78% (n=1) | 4 | Cefoxitin, Tetracycline, Amoxicillin/Clavulanic Acid, Ampicillin |
| 0.78% (n=1) | 4 | Cefoxitin, Tetracycline, Ampicillin, Streptomycin |

[a] All *E. coli* isolates obtained from cattle feces showed resistance to three or less antimicrobials

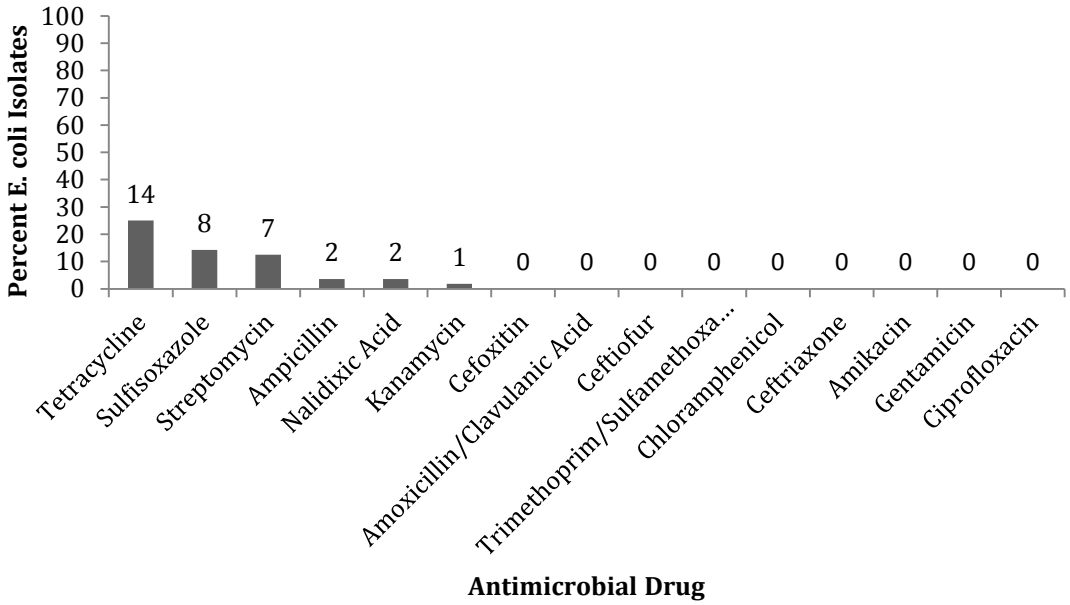


Figure 5. Cattle isolates showing resistance to various antimicrobial drugs (n=56)

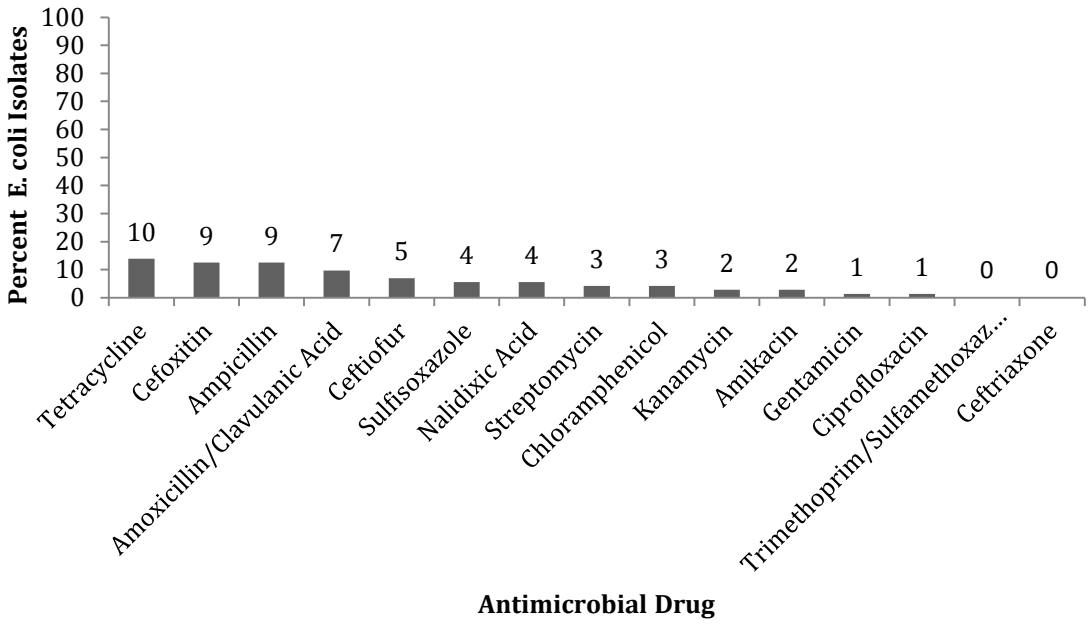


Figure 6. Deer isolates showing resistance to various antimicrobial drugs (n=72).

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# KNOWLEDGE & ATTITUDES REGARDING FOOD SAFETY IN THE HARVEST OF WILD GAME

*Darci M. Owens and Loree A. Branham*

## ABSTRACT

A survey of deer hunters in the San Angelo, TX area was conducted in the months of November and December 2007 to determine basic food safety knowledge in the harvest of wild game. Questionnaires included demographic information, the primary specie hunted, perceived knowledge of food safety, individuals' handling of gut-shot deer, tools used in the processing of wild game, temperature conditions and state of final product. Following completion of the survey, hunters were issued an informational pamphlet outlining proper harvesting and processing procedures. Results of the survey indicated most hunters surveyed were confident in their food safety knowledge with the majority selecting the numbers 6, 7 or 8 (21.79%, 23.08% and 19.23%, respectively) when asked to rate themselves on a scale of 1-10 with 1 knowing nothing about post-harvest food safety and 10 being very knowledgeable in post-harvest food safety. However, various other questions pointed out a negligence of time-temperature parameters with hunters claiming to leave carcasses hanging for extended hours in warm environments. Results of this study point out more efforts should focus on food safety education of wild game hunters, especially time-temperature constraints during processing in the field.

## INTRODUCTION

*Escherichia coli* is a rod-shaped, spore forming bacteria found naturally in the gastrointestinal tracts of man and animal. It may also be found on the hides and hair of animals and man (Jay et al. 2005). Once ingested by humans, *E. coli* illnesses cause symptoms such as bloody diarrhea, hemolytic uremic syndrome and in some cases death (CDC 2008). Any ingesta or fecal material that comes into contact with the carcass due to improper harvesting or processing techniques may contaminate the meat and often *E. coli* infections occur as a result (Elder 2000). Many hunters are unaware of the contamination hazards that may occur. Thus, proper sanitation must be executed to reduce the chance of a food-borne illness.

It is suggested venison is not a primary carrier of *E. coli* O157 (Fischer 2000). However, at least two cases of human illness due contaminated deer meat have been reported resulting from the mishandling of the venison product (Keene 1997, CDC 2002). One outbreak in 1995 occurred in an Oregon community where 6 people had confirmed illnesses and 5 other presumptive cases were identified. The cause of this outbreak was linked back to Black-tailed deer meat where the venison was dehydrated and processed for jerky. Pulsed-field gel electrophoresis (PFGE) testing identified *E. coli* O157:H7 as the contaminant in hide samples and uncooked meat from the deer, on the saw used in processing, as well as the jerky product suggesting deer may be a mode of transmission (Keene 1997). Another case involved a 7-year old-boy who consumed a large amount of undercooked venison steak. Following PFGE testing, dairy cows grazing the rangeland in proximity to the hunting site were believed to transmit the pathogen to the deer. Failure to cook the steaks to the proper internal temperature allowed for the pathogen to still be present in the food (CDC 2002). Recent outbreaks in ready-to-eat vegetables such as the 2006 California bagged spinach outbreak has directed attention to livestock and wildlife species as a source of contamination for produce. Epidemiologists identified the same strain of *E. coli* O157:H7 in environmental samples collected from the produce fields as well as in fecal matter from cattle and feral hogs in close proximity to the contaminated spinach fields. This evidence suggests livestock and wildlife can serve as a

medium for contamination in non-meat products as well (California Department of Health Services 2007).

Due to these outbreaks, wildlife is becoming a concern to public health. It is not known whether cattle are a source of contamination for deer and hogs or vice versa, even though cattle are often implicated in the harboring of *E. coli* O157:H7 (Sargeant 1999). A study conducted by Branham and others also identifies water as a possible source of contamination between animal species. Similar *E. coli* bacterial strains were found in deer and livestock grazing the same rangelands suggesting the pathogen has the ability to spread across species (Branham 2005). Deer are thought to be transient carriers of *E. coli* species and show the highest occurrence of the pathogen during the fall and winter months (Sargeant 1999). Since deer hunting season in Texas is limited to the months following the suggested shedding season of *E. coli*, extensive efforts should be taken to reduce contamination. In the handling of any raw meat product whether it is beef, pork, lamb, poultry or venison, safety and sanitation should be a top priority to prevent the incidence of a food-borne illness. Therefore, the objective of this study was to determine awareness of basic food safety knowledge and further educate area hunters on post-harvest food safety techniques.

## MATERIALS AND METHODS

Seventy-nine surveys were distributed to central-Texas hunters in the months of November and December to determine the amount of attention dedicated to food safety when in the field where wild game is harvested. Hunters were asked basic demographic questions including age, highest level of education attained, population of residence, ethnicity and occupation. To establish food safety awareness, hunters were asked to describe their knowledge of food safety and how often they sought information related to food safety practices. The survey asked hunters to describe their processing techniques in the field, their knowledge of the hazards associated with gut-shot deer and the likelihood of utilizing food safety information if it was supplied.

Further questions were used to determine risk of illness to humans by the contamination of improperly handled meat. Questions included the type of knife used to process the carcass, how to clean a gut-shot deer and whether or not the hunters are aware of the food safety risks in aging a gut-shot deer. Finally, participants were asked to describe their technique used to transport the game to a processor, or if they process themselves, and how the product is packaged. An informational brochure was distributed following completion of the survey outlining proper harvesting techniques and tips to reduce contamination, as provided by food safety professionals. Descriptive statistics were generated using various procedures of SAS 9.1.3 including PROCFreq and PROCMeans (SAS Institute, Cory, N.C.).

## RESULTS AND DISCUSSION

The majority of participants in this survey had attained at least a high school level of education with most having some college experience (Table 1). Due to the large percentage of participants claiming to hunt for both meat and trophy purposes, carcasses should be handled with caution and food safety techniques executed in all areas of processing (Table 2). Results of the survey indicated most hunters surveyed were confident in their food safety knowledge with the majority selecting the numbers 6, 7 or 8 (21.79%, 23.08% and 19.23%, respectively) when asked to rate themselves on a scale of 1-10 with 1 knowing nothing about post-harvest food safety and 10 being very knowledgeable in post-harvest food safety. Overall, hunters considered themselves attentive to food safety with only 1.28% (1) of hunters claiming to be "unconcerned" with post-harvest food safety techniques; 52.56% (41) considered themselves "careful" and 24.36% (19) were even more cautious considering themselves very "sanitary" when addressing food safety associated with their harvest.

Table 1. Education level of hunters surveyed about wild game food safety issues (n=79)

| Education Level | Frequency | Percent of total hunters surveyed |
|---|---|---|
| High School | 10 | 12.66% |
| Some College | 42 | 53.16% |
| Bachelor's Degree | 20 | 25.32% |
| Master's Degree | 6 | 7.59% |
| Doctorate Degree | 1 | 1.27% |

Table 2. Primary harvest objective of hunters surveyed about wild game food safety issues (n=79)

| Purpose for hunting | Frequency | Percent of total hunters surveyed |
|---|---|---|
| Trophy | 3 | 3.80% |
| Meat | 12 | 15.19% |
| Both | 64 | 81.01% |

The authors define a gut-shot deer as one that the bullet has penetrated the paunch and fill has come into contact with the meat or hide. Bacteria found in the ingesta can contaminate the carcass increasing the risk of a food-borne illness if the affected area is not properly sanitized (Carr et al. 2002). Of the 79 hunters surveyed, 74.68% (59) of participants claimed to be aware of food safety hazards in gut-shot deer; however, 47.44% (37) admitted they would allow a gut-shot deer to age overnight despite the hazards. This is one example of where food safety is compromised in the field.

Reduction of microbial loads is vital to maintaining food safety (Jay 2005). In this survey, most hunters claimed to initiate some kind of intervention following the incidence of a gut-shot animal including trimming the exposed meat, rinsing the carcass or cleaning the area with paper towels. Of those taking the survey, 100% of the respondents executed at least one intervention and 49.37% (39) stated they performed two or more procedures to increase cleanliness (Table 3).

Researches believe keeping the carcass in a controlled, refrigerated environment reduces bacterial replication (Carr et al. 2002). The authors of this study define proper refrigeration to be <41°F. Approximately 68.74% (53) hunters store their carcasses in a refrigerated environment if allowed to age for a period of time; however, of those, 23.53% (12) did not correctly associate the accurate refrigeration temperature. Allowing the carcass to cool and drain properly before aging helps reduce the water activity in the meat, further decreasing the ability for bacteria to grow. Approximately 81.33% (61) of respondents prop the chest cavity of the carcass open prior to aging. A clean dowel-rod or rib spreader should be used as a prop. Thirty-four (57.62%) participants claimed to use a tree branch or stick from the ground as a prop despite the risk of fecal contamination from the soil.

Participants were asked what major meat product their harvest is processed into. Steaks and sausage were most predominant at 77.22% (61) and 69.62% (55) respectively. However, fresh cuts of meat have a higher water activity allowing optimum conditions for microbial growth. Jerky has a reduced water activity prohibiting extensive microbial growth when processed correctly. Thirty-eight (97.43%) hunters who claimed some of their wild game was processed into jerky as a final product also said some of the meat was processed into

Table 3. Reported microbial intervention of gut-shot deer utilized by hunters surveyed about wild game food safety issues (n=79)

| Intervention Method | Frequency | Percent of total hunters surveyed |
|---|---|---|
| Rinse | 46 | 58.23% |
| Trim | 62 | 78.48% |
| Clean with paper towels | 9 | 11.39% |
| Two or more interventions | 39 | 49.37% |

steaks, sausage or a ground product which have a greater probability of harboring bacteria when processed incorrectly (Table 4).

Educating hunters of proper food safety techniques in post-harvest processing is critical to reduce the likelihood of contracting a food-borne illness. Most hunters claim to process their own game; however, 11.53% (9) of those surveyed allow an outside source to process their harvest into meat products increasing the risk of illness from mishandling. At the completion of the survey, over 96% of participants claimed they would be willing to initiate more food safety techniques into their practices if additional information was provided. This high acceptance rate leaves the door open for future distribution of food safety material. Information could easily be distributed when hunters are issued a license for the hunting season, online websites or hunting and outdoor magazines.

## IMPLICATIONS

This was a pilot study conducted by an undergraduate student to be used as a basis for a future research. It was determined that more efforts should focus on food safety practices in the field to ensure proper dressing of harvested game animals to reduce bacterial contamination. Most game meat is cooked to the proper internal temperature to reduce the "gamey" flavoring of wild animals. However, bacterial contamination is likely in the processing of wild game due to adverse weather conditions and unskilled professionals who harvest the animal. Thus, no short-cuts that may compromise human safety should be allowed while processing in the field.

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Table 4. Primary products produced from harvested wildlife of hunters surveyed about wild game food safety issues (n=79)

| Product | Frequency | Percent of total hunters surveyed |
|---|---|---|
| Jerky | 39 | 49.37 |
| Steaks | 61 | 77.22 |
| Sausage | 55 | 69.62 |
| Hamburger | 20 | 25.32 |


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# EFFECTIVENESS OF VARIOUS SPRAY TREATMENTS IN THE REMOVAL OF *ESCHERICHIA COLI* O157:H7 ON INOCULATED VENISON STEAKS

*Chance Hundley, Darci Owens and Loree Branham*

## ABSTRACT

A study was conducted to evaluate the effectiveness of various spray treatments of commercially available mouthwash , lactic acid, and sterile water on populations of *E. coli* O157:H7 on inoculated venison steaks. One white-tailed deer was harvested and processed for this study and individual muscles of the round were cut into 5cm x 12 cm x 2-3 cm steaks. The steaks were randomly allocated to four treatment groups including a control group and one of three sampling periods (3replicate steaks/trt/sampling period, n=36). All steaks were inoculated with a uniform high level of bacteria and allowed one hour for attachment in a controlled refrigerated environment. Steaks were then treated with the assigned solution. Treatments included: 1) control (no liquid treatment), 2) commercially available mouthwash (active ingredient Cetylpyridinium Chloride and Domiphen Bromide), 3) 2.5 % Lactic Acid, and 4) sterile distilled water. Liquid treatments were applied using a commercially available garden sprayer for 10 seconds on each side. At 0 (within 10 minutes of initial application), 1, and 4 hours post inoculation steaks were removed from refrigeration and sampled. A $50cm^2$ surface area was swabbed, serial dilutions carried out and samples plated on MacConkey agar. Following incubation at 37°C for 24 hours, colonies of *E. coli* O157:H7 were enumerated and recorded in $log_{10}CFU/50cm^2$. Values ranged from 5.38 to 6.88 log $CFU/cm^2$ with no significant differences due to treatments ($P = 0.33$). Results of this study indicate the various sprays had no effect on *Escherichia coli* O157:H7 populations on the venison steaks given the short application time. Further research is needed to determine if longer treatment times and stronger concentrations of commonly available products may help reduce bacteria's presence on meat harvested from wildlife.

## INTRODUCTION

*Escherichia coli* O157:H7 is considered a major foodborne pathogen and among the leading causes of food-borne illnesses in the United States (Riley et al. 1983, CDC 2002, CDC 2011). The O157:H7 serotype is a rod-shaped, gram-negative bacterium found in various sources including, contaminated raw ground red meat, unpasterurized milk products and contaminated water sources. Humans can become infected with the bacteria by consuming contaminated food. *E. coli* O157:H7 can cause severe symptoms of vomiting, nausea, cramping, bloody diarrhea, and kidney failure. The bacteria are most harmful to at-risk populations such as the young and the elderly due to their compromised immune systems (CDC 2008).

*E. coli* O157:H7 was first widely recognized in 1982 in an outbreak associated with contaminated hamburger meat (Riley et al. 1983). Latter studies showed *E. coli* O157:H7 to be present in foods previously considered safe, like fresh vegetables including leaf lettuce (Ackers et al. 1998). Contamination in produce may occur through the soil from manure or contaminated water and absorbed into the plant (Solomon et al. 2002). Recent reports link outbreaks due to contaminated fresh vegetables to area wildlife (Jay et al. 2006). Because these foods are considered ready-to-eat and do not require a cooking mechanism which would destroy most cells, humans consuming these foods could be at risk for contracting a food-borne illness.

Besides produce, *E. coli* O157:H7 has been isolated from numerous animal sources including cattle, sheep and pigs. One study found that both livestock and water sources are

carriers of the pathogen, but presence was limited to the month of September. The months of October, November and December showed no growth of *E. coli* O157:H7 which may be due to the seasonal shedding pattern of the bacteria (Branham et al. 2005). Another study determined O157:H7 numbers increased in warmer weather months (Kudva et al. 1996).

Though not found to be a common carrier of E. coli O157:H7, white-tailed deer have been found to be transient carriers of the bacteria. Out of 469 free-ranging deer sampled in 1997, only 3 deer were carriers of the pathogen in their feces but not in the meat (Fischer et al. 1997). However, there have been multiple illnesses linked to meat products from contaminated deer (Keene et al. 1997). In 2002, a child in Connecticut was infected with *E. coli* O157:H7 from contaminated venison. This was the first human illness to be linked to an *E. coli* infection from a white-tailed deer. One study determined the deer had acquired the bacteria in the pasture, but improper handling of the food led to the illness (Rabatsky-Ehr et al. 2002). Though the meat itself is not a natural carrier of *E. coli* O157:H7, it may become contaminated by unsanitary processing techniques following harvest or the environment in which the animals are raised.

Though carrier animals like cattle are not affected by the pathogen, once inside a human, this bacterium causes symptoms such as bloody diarrhea and Hemolytic Uremic Syndrome (HUS) which can lead to kidney failure (CDC 2008). Storage of carcasses at improper temperatures allows the bacteria to quickly replicate leading to increased possibility of contracting a food-borne illness. Many hunters are unaware of the risk of food-borne illnesses when harvesting and processing wild game. Proper care must be taken to ensure the meat remains in a safe condition and cooked thoroughly to prevent illness. There is some interest in identifying a safe, easy-to-apply, publically available solutions which could reduce pathogens on meat harvested from wildlife.

## MATERIALS AND METHODS

For this study, one white-tailed deer was harvested at the Angelo State University Management, Instruction and Research Center in San Angelo, TX. The deer was processed and individual muscles of the round were removed for sampling. Roasts were cut into 5 cm x 12 cm x 2-3 cm sample pieces, vacuum packaged and refrigerated for storage. The inoculant solution was made with *Escherichia coli* O157:H7 and sterile, buffered peptone water. All steaks were inoculated with a uniform high level of bacteria and allowed one hour for attachment in a controlled refrigerated environment. The steaks were randomly allocated to four treatment groups including a control group and one of three sampling periods (3 replicate steaks/trt/sampling period, n=36). Steaks were then treated with the assigned solution. Treatments included: 1) control (no liquid treatment), 2) commercially available mouthwash (active ingredient Cetylpyridinium Chloride and Domiphen Bromide), 3) 2.5 % Lactic Acid, and 4) sterile distilled water. Steaks were inoculated by immersing the meat in the pathogen solution with a pair of sterile tongs then placed on sterile, mesh racks and refrigerated for one hour at <41°F to allow for bacterial attachment.

Following refrigeration, the steaks were placed in a shallow pan and sprayed for ten seconds with a conventional garden sprayer with one of the three allotted liquid treatment approaches. Steaks allotted to the Control treatment received no liquid application. A 50$cm^2$ surface swab was taken of each product at 0(within 10 minutes of initial application), 1, and 4 hours. The swabs were aseptically placed into 9 mL tubes of sterile buffered peptone water and vortexed to allow for distribution. Serial dilutions were carried out to six dilutions and plated onto duplicate MacConkey agar petri-dishes at 100 microliters per plate using aseptic techniques. Extra steaks from the same deer were tested to confirm absence of naturally occurring generic *E.* coli and E*. coli* O157:H7. All plates were incubated at 37°C for 24 h.

Following incubation, plates were removed and enumerated to determine effectiveness of treatment methods. Colonies of *Escherichia coli* O157:H7 appear pink to red in color. Plates within the countable range of 25-250 colonies were considered for this study. After enumeration, all media was disposed of in Biohazard bags and autoclaved for sterility. Data was analyzed using the GLM procedure of SAS Version 9.1.3 (SAS Institute, Cory, N.C.). Significant differences were evaluated at $\alpha$ =0.05.

## RESULTS AND DISCUSSION

No significant differences between treatments, given the parameters, were indicated in this study. *Escherichia coli* O157:H7 populations ranged from 5.38 to 6.88 $\log_{10}$ CFU/cm$^2$ with no significant differences due to treatment ($P$ = 0.33, Figure 1) or due to sampling period ($P$ = 0.44, Figure 1). Results of this study indicate the applied various sprays at the given concentrations and application spray time have no effect on *Escherichia coli* O157:H7 populations on the venison steaks. Longer treatment times and stronger concentrations of sprays may help reduce bacterium's presence on the meat but further research is needed to confirm a pathogen response. Additionally stronger concentrations of certain treatments may cause an off-flavoring.

## IMPLICATIONS

Future studies may be more efficient in reducing bacterial loads in venison by increasing the concentration of lactic acid to >2.5%. Longer treatment times may be required to reduce numbers of *E. coli* O157:H7 colonies present in the meat. Many hunters today are not aware of the effects of pathogenic bacteria or how they can be present on the venison they consume. Therefore, we must continue to educate hunters on food safety issues to ensure they are cautious when handling deer meat in the field to reduce the risk of a food-borne illness.

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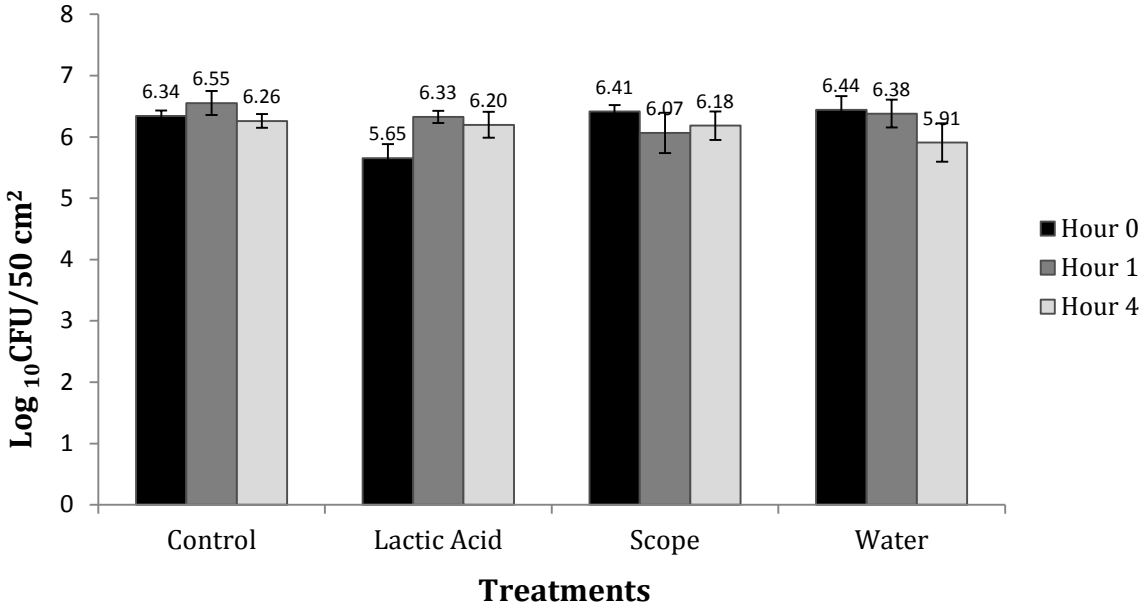


Figure 1. Escherichia coli 0157:H7 concentrations on inoculated venison across various treatments and sampling periods post inoculation

*(treatment x sampling period, *P*= 0.17; treatment, *P*= 0.33; sampling period, *P* = 0.44)


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# Agriculture Graduates

*Denotes graduate student

**1975**

Robert Glenn Burwick
(December 1974)
Tom Burson
Ronnie Edington
Alton Everett
Randy Gill
Rebecca Harris
Marcus McCellan
Donald Phelps
Horace B. Walker
Randy Bredemeyer
Thomas Jernigan
Guy Levey
Ricky Marks
Riley Sterling

**1976**

Bobbie Baldwin, Jr
Debra Beth Barker
Ricky Lane Childress
Warren Fay Dozier
Charles E. Fant
Ronald Edward Halfmann
Daniel Wayne Kujawski
John William Van Court
Donald Joe Wilde
James Carl Williams
Calvin Jackson
Johnny Wayne Todd
Malcolm F. Gerngross
Curtis Ben Cox, Jr.
Larry William Dean
Jimmie Lee Trojcak

**1977**

Thomas Lee Allen
Richard Ray Collett
William Stephen DeHay
Bernard Fuchs
Ernest Fred Groff
Charles Jackson Hughes
Sidney Truman Johnson
Kevin James May
Mark Louis Shepard
Virgil Neil Conner
Charles Bernard Halfman
Stephen Morris Hinshaw
Steven Hoelscher
James K Kiunga
Jerry Talley Jr.

**1978**

Paul Daniel Barnhill
Raymond W. Beam
Terry Lynn Blair
Tony Carl Frerich
James Keith Hood
Michael Fred Matthews
Jo Ann Snodgrass
Terry Lynn Stokes
Kenneth Wayne Straw
Jack M. Sykes
Jim William Wright
Danny David Daniels, Jr.
Darrell Gene Meyer
Dale Edward Neagle
Donald Harold Bunch
Vickie Patterson Hillger
John Lloyd Newman
John L. Seaton
Lee Edwin Warren
Galen Ray Weiershausen
Thomas Alton Williamson

**1979**

Shelia Elaine Allbright
Joe Bass Arnett
Calvin Dee Boatright, Jr.
Debra Ann Clouse
Andy Mike Eubanks
Ronald James Gill
Joel Wayne Holladay
Robert Benjie Jay
Brian Forrest Meeks
Randall Oein Pittman
David Lane Tunmire
Dennis Jay Uherik
Faron Almon Pfeiffer
Joe Don Roach
Tom Heath
Milford Logan
Bill Wilson

**1980**

Preston Elba Adams
John T. Bassinger
Howard Gene Callison
Mark Winn Dobbins
Dean William Eckert
Steven Neil Glass
David H. Masters, Jr.
Brian John May
Joseph Gregory McReynolds
Charles Richard Bradshaw
Bruce Deere
Michael Garza
Kelly Jean Gully
Brent Heinze
James Alton Kolb
Victor Roy Probandt
Jay Thomas Holstein
Mark Allan Mishnick
Gary Don Stokes
Tandy Sueann Wilmeth
Gary Lee Wilson

**1981**

Bruce Backland
Steve Cook
Kyle Christopher Hodges
Ricky Machen
Julie Ann McFarlin
Dennis Newton
Charles O'Connell
Brad Pierce
Rodolfo Diaz Ortiz
Elias C. Rodriquez
Pat H. Shannon
Vernon Elliot Sublett
Bernie Wallace
Brenda Kay York
Duane Allan Stryker
Mark Louis Shepard *
George Scott
Gerald Glen Risher
Ronald J. Gill *
Lee Edwin Warren*

**1982**

Randall Bankhead
Terry Lee Criner

Pat Dryden
David Fuessel
Stephen Kuhlmann
Mary Jean Owens
Danielle Rosser
Patty Dietrich Stokes
Arthur Wayne Striegler
Terry Waller
Michael Scott Wilburn
Mark Wylie Worthington
Robert Charles Surratt
Brian J. May*
Milburn Wright, Jr.*
Curtis Glenn Childress
Kelley Ann Collins
Craig Demere
Louie Grant Drennen
Timothy Clay McReynolds
Frank Pecina Jr. III
Vaden Aldridge*
Stephen Byrns*
Faron A. Pfeiffer*
Henry Gene Adams, Jr.
Alan Bossenger
Harlan Clay Nance
Ken Roy Pfluger
Paula Kay Saunders
Jeffery Kyle Wright

**1983**

Mike Barrera
Curtis Boos
Donald Loding Campbell
Dennis Vinson Cumbie
Edward Earwood
Kevin Hale
David Hayden
Joey Henderson, Jr.
Roberto Hernandez, Jr.
Dana Johnson
Johnny Murchison
Roy Musquiz, Jr.
Donald Reeh
Mark Swening
Alfred Vardeman
Brett Alan Williams
Bruce Backlund*
Yousef Bengharsa*
Will Walter Allison
Carol Cervenka
Mary Ann Kirk
Emily Elizabeth Moll
Barbara Pfieffer
Jack Renfro
Sandra Lynne Stewart
Mustafa Mohamed Mankusa*
Randall C. Ward*
Gary Lynn Bishop
James Robert Harris
Brand Mund

**1984**

James Hood*
Molly Baskett
Wilburn Baucom
Gideon Cheruiyot
Craig D. Cook
Charles Brett Cypert
Keith Floyd
Lisa Gabier
Danny Gunn
Timothy Fred Hardt
Taylor Dean Hayes
R.D. Hinojosa, III
Vanessa Lusby
Bill Pitman
Eddie Probandt
Bobby Rogers
Dee Ann Smart
Stephen Surratt
Wesley Joseph John Thee
Mike Thomas
Bradley Dean Thompson
James Wilde
Bruce Deere*
Robert R. Allen
Greg Browning
Leland Hunt, Jr.
Partick Pearce
Steve Sappington
David Wayne Schofield
Calvin Glenn Steward
Rodney Jay Winn
Gary Dewayne Callaway
Renee Michelle Evans
Rex C. Ewert
Hillie Hunter Hayes
Wesley Zane Hodges
Leland Wayne Key
Daniel Russell Koenig
William C. Kothmann
Roger Woods Lux
David Brent Sherrill
Joe David Sherrod
Todd Wade Swift

**1985**

Donald Blair
Randall Brown
Jimmy Fontenot
Randall Jenkins
Charlotte Klepac
Scott Porter
Stephen Wayne Reynolds
Pat Thomas
Wilton Weise
Ben Wilde
Darrell James Wilde
Rafael Suarez*
Johnny Murchison*
Sulaiman Awagi*
Jeff Hamilton
Jay Hawkins
Mark Ramirez
Danny Vann
David Bruce Fletcher
Ricard Kelly Gilbert
John Harvey Gronold
Cary Dean Hannsz
Stehpen Scott Mooney
Steven Reece Moore
Royce Lee Pyssen
George William Smith
James Curtis Turk

**1986**

Thomas Ray Allen, Jr.
William Banner
Scott Lamar Cauthen
Jonama Cox
Joe Branton Day
Carlos Gibbs
Greg Keith Hagel
Lee Strait Hitch
Wayne Carl Hofmann, Jr.
James Walter Keeton
James William Kothmann

Randy Dale Kruse
Mike T. Kyzar
Mark Randall Oates
Clay Yandell
Cary Don Baker
J.W. Carter, II
Jeffrey Duncan
Michael Wayne McDaniel
Wade McMurrary
Kaung-Huei Liu*
Stephen Ray Sappington*
Jeffery Kyle Wright*
Kevin Dale Barron
Carle Max Brandenberger
Stacy Todd Campbell
Eddie Frank Dusek
Kirk Lane Griffin
Gregory Irvin Hohensee
Regan Stuart Kirk
Bonnie Lou Mayer
Joao Livio Norberto
Steven Mark Quade
Eugene Bitner Roberts
Rocky Stewart Vinson
Raymond Roy Walston, Jr.
Jeffrey Craig Williams

**1987**

Leland J. Hunt*
Terry Brent Baucom
James Hubert Bell
Ella Marie Blair
Lonnie Randall Bolf
Jay Donivan Daniel
Samuel D. Fuhlendorf
Bradley Dwayne Fulton
Jeb Brant Henderson
Robert P. Hunter
Courtney Lance McNeely
Kathering Inez Pappas
Fernando Adlolfo Reyes
Darren Ray Richardson
Gregory Ray Schwertner
Maribel Alicia Tarango
Karl Tatsch
Rex Taylor
Roger Tinder
Jerry Don Vinson
Bryan A. Davis
Troy Lennon
Helio Paranagua, Jr.
Jimmy Fontenot*
Randall Jenkins*
Steve Moore*
Bennie Caly Edwards
Jan Hatler
Kraig Peel
Kenny Strube
Tracy Tippett

**1988**

Steve Kuhlman*
Nancy Benson
Scott Christopher Blanton
Kay Carrig
Michael Fanning
David Feldhoff
Karen Frey
Lee Higdon
Jeff Lewis
Wade Menges
Chris McReynolds
Monica Reining
Kathy Thompson
Ken Weidenfeller
Allan West
Cheryl Robinson
Dave Cleavinger*
Terry Criner*
Larry Herd
Trey Glen Morgan
C.W. Roberts

**1989**

Deborah Sue Divich*
Russell Stevens*
Clinton Calk
Browder Graves
Mark Gray
Kelly Griffin
William Mark Harris
Lester Everett Matthews
Kevin Pounds
Joseph Raff
Jacqueline Hermesmeyer
James Glen Miller
Noel Williams
David Carlisle
Amy Teagarden
Roddy C. Gordon II.
Novice Joe Moore
Russell Rogers
Frankie Sablan
Marck Todd Schafer
Barry Smith

**1990**

Adebanjo Adesoji
David Bohnert
Bill Head
James Horton
Clint Koenig
Kevin Owen
Cody Scott
Troy Seals
Jim Meredith*
Larry Herd*
Lee Clark
Ron Gillaspy
Bobby Herrington
Randy Houston
Ed Miller
Britton Lee Roman
Wiley Payne Rudasill
Trent Tankersley
Tom Underwood
Kevin Pfeiffer
Brad Spenrath

**1991**

Bryan Davis
Lynn Dye
Robert Charles Graff
Mike Harbour
Wendy Holman
Randall McCarty
Wade Menges*
Miguel Rendon*
Richard Shaver
Daryl Whitworth
Scott Grote
Frank Habecker
Gus Ward
Joe Raff*
John Clifford Fisher
John David Laxson
Billy Jay Ledet

Eldon Todd Love
Kevin P. Przilas
Kristi Lynn Stone
Todd Swift*

**1992**
Albert L. Booky*
Clinton Calk*
Barry Lee Cooper*
Cody Burk Scott*
Justin Amerine
Shawn Burns
Jeffrey William Cowan
Roy Kevin Downey
Sharilyn Sue Friesen
Justin Henefey
Jamie Carole Inman
Brett Johnson
Chad Seward
Robyn Sims
Timothy Smith
James Sullivan
William Alan Head*
Melissa Bollinger
Tim Lust
Keith Randall Shaffer
John David Whipple*
Pammy Lynn Millican
Timothy Sean Phy*
Todd Rossington
Debra Rozell
Todd Schafer*

**1993**
Bryan Davis*
James Weldon Faught
Randy Alan Gartman
Linda B. Naranjo
Steven Don Parker
Robert Pritz
Nikki Dawn Ramsey
Virginia Shannon Riley
Belinda Rivera
Kelly James Sanders
Mitchell Elton Wilmeth
Chad Coburn
Chris George
Kip Giles
Cody Hill
Kevin Shane Kelton
Kelly Gully*
David Laxson*
Jason Lee Bannowsky
Michael Brent Crawford
Robbie Glenn Eckhoff
Charles Estanol
Dawn Alicia Kleiber
James Clayton Richards
Dale Anthony Schwarts
Terri Bibb Webber

**1994**
Amber Bickham*
Terry Blair*
David Bohnert*
Marvin Dale Dunlap*
Russell Rogers*
Shannon Bennie Bannowsky
Michael T. Billingsley
Donna Cates
Melvin R. Davis
Darrell Dusek
William Todd Friend
Charles R. Hollingsworth
Gilbert Horton
Jason Victor Jones
Alyson Kay McDonald
Daniel Park
Micheal Salisbury
Roxanna Kate Schwinge
Allie Snider
Walker Walston
Keith Shaffer*
Thomas Bryan
Olen Burditt
Jeff Chisum
Janet Cox
Thomas Hughes
Jeremy Don McCollom
Michael Moore
Martin Weatherbee
Ed Miller*
Glen Allan Phillips*
Brain Harwell
Rebecca Haschke
Winston Herndon
Justin Marschall
Elizabeth McFadin
Allen Russell Morgan
Stacy Lane Morris
Chad Sims
Travis Kent Wier

**1995**
Marty Gibbs*
Kelly Sanders*
Katherine Allison
Ross Benson
Malcom Boger
Jimmy Caughron
Wade Cypert
Blain Ferris
Ramiro Guzman
Brian Hill
Brantely Hoelscher
Eddie Onofre
Fulton Pizzani
Rowdy Rea
Scott Smetana
Faron Sultemier
Make Zuniga III
Chad Coburn*
Mikel Harbour*
Homer Lee Higdon III*
Nancy Law*
Kimberly Ann Ball
Shelly Summerour
Philip Carter*
Ross Stultz*
Shelly Frazier Best
Kevin Duke
David Foster
Walden Hillert
Todd Holbrooks
Scott Hohensee
Thomas Franklin Kelso
Tara Mallett
James Murdoch
David Hershel White
Katerine Wurster

**1996**
Gibert Horton*
Wade Armke
Michelle Behrends
Billy Belew
Cain Cline

Jill Dice
Steven Hise
Thi Hoang Oanh Hoang
Billy Mac Howe III
Pam James
Shawn Nanny
Jeffrey Osbourn
Jody Osbourn
Brad Roeder
Cody Schoenfeld
Gwendolyn Sue Taff
Kathrine Valdez
Dan Vestal
Richelle Renee Wilson
Doug Bawcom*
Bryan Bendele
Todd Broncy
Trey Garmon
Pascual Hernandez*
Sara Lewis*
Abel Robles
Tim Sims
James Steen
Jason L. Denman
Christopher S. Herzog
John David Isenhower
Brook Dowell Matthews
Richard Minzenmayer*
Mike Salisbury*
Brady Weishuhn
Sandi Zimmerman

**1997**
Kim Ball*
George Trey Poage*
Rowdy Rea*
Ronnie Brewer
Kim Cox
Eddie Hall
Brandon Heiser
Bridget Mansell
Jerry McGinnis
Dac Pennick
Octavio Ramos
Christy Strube
Jodie Uptergrove
Shawn Uptergrove
Jeremy Blain Myers
Amy J. Pilmer
Peggy Simpson
John Barfield
Kevin Kuhlmann*
Bennett Tate Thoreson
Whitney Whitworth
Rebecca Young*

**1998**
Justin Alexander
Wade Armke*
Reace Bennett
Bryan Campbell
Richie Griffin
Lane Hughes
Pamela James*
Bridget Jones
Steven Jost
Charles Kneuper
Richard Lepard
Leesha Ligon
Jason McCoy
Rachel Pentecost
Andria Perales
Jennifer Rose
Geoffrey Scott
Adam Clay Warren
Casey White
William Wood
Jason Frost
Jeremy Brandon Hartgrove
Jay Holt
Robert Paul Law
Wesley Whitehead
Brain Shane Atzger
Parrish Braden
Rusty Fleeman
Daniel Kuntz
Thomas Randall Rakowitz
Stephen Wade

**1999**
Jennifer Bedell
William D. Burns
M'Liss Burrier
Amy Coburn*
Kim Cox*
Dewey Alan Drennan
Bronson Gobert
Eddie Hall*
Dale Ashton Harris
Brandon Heiser*
Brantely Heiser
Tessie Ingram
Shelby Johnston
Chris Lupton
Leslie Moczygemba
Fred Reyna
Karalina Rigsby
Anthony Sanchez
Kristina Schulze*
Peggy Simpson*
David Sirmons
Shelly Smith*
Julie Smithwick
David Stone
Wade Travis Tellinghuisen
Juan Vasquez
Clint Warren
Justin Weishuhn
Laurie Weishuhn
Karee May Wiggins
Jeriann Williams
Ashley Wilson
Grady Wilson
Brandon Zesch
Rhea Allen
Clint Matt Culp
Sarah Fitzgerald
Ronald Heineman
Ladd Hughes
Pamela Jetton
Kevin Shane Kelton*
Charles Seidensticker
Terry Sirmons
Whitney Whitworth*
Justin Clark
Sherry Hall
Mark Martinez
Todd O'Neil
Robert Allen Parry
Byron Wayne Pfeifer*
Robert Phillips*
Monica Swenson*
Michael Weckel
Cynthia Whitehead

**2000**
Maria Anzaldua

Brandon Asbill
Jamie Bass
Andrew Boomer
Garry Branham
Bret Breitenkamp
Bill Burnes
Cory Carroll
David Bradley Cook
DeAnna Crain
Dee Dusek
Kelly Edwards
Jason Frost*
Rachel Frost*
Brandon Green
Richard Griffin*
Beverly Gully
Tom Guthrie
Devin Hoover*
Caleb Kattner
Charles Kneuper*
Stacey Kotrla
Justin Lampier
David Lytle
Jerry McGinnis*
Zeno McMillan*
Alvaro Ruiz
Robert Ross Sims
Robert Steakley
Jared Taylor
Wynne Whiteworth
Lee Brinegar
Justin Douglas Dunlap
Kelly Hart
Matt McMillan*
Kari Ashcraft
Amanda Browder
Brock Fry
Jana Jackson
Quinn Johnston
Caylie McClure
Martin Schuh
Stephen Wade*
Charlie Wakefield
Chad Zibilski

**2001**

Jesus Becerra
Blake Belcher
Russell Dean Black
Joshua Blanek
Ben Brandon Brooks
Christopher Carey
Justin Collins
Marshall Davidson
Curry Dawson
Susan English
Heidi Ertresvaag
Caitlyn Felder
Becca Ferguson
Jeff Fiedler
Sarah Fitzgerald*
W.C. Foster
Curtis L. Garrett
Briana E. Harbaugh
Tami Harris
Ladd S. Hughes*
Jon Jennings
Will Kiker
Haley Jo Knutson
Eli Ornelas
Kari Pierce
Laura Rush
David Sirmons*
Craig Thomas
Jake Wagner
G.W. Yandle
Andy M. Laughlin*
James William Loveday
Deana L. Moore
Jason William Stewart
Jeffrey Wheeler
Justin Will Avery
Jeffrey Lane Berry
Corrie Maria Canava
Chad R. Ellis*
Brian Faris*
Patrick Fowlkes
Casey Ray Hayes
Jennifer Ann Heard
Shy L. Middleton
Fred Reyna*
Gary Alan Witt

**2002**

Casey Alexander
Garry Branham*
Loree Branham
Maria Terrie Carr
Cory Carroll*
Robert Cook
Justin Corzine
Bobby Deeds
Robert Diaz
Wayne Trey Dunson
Amy Heathcott
Jed Hruska*
Haden Keyser
Brandon Payne
Fabian Rodriguez
Eric Ross
Alicia Simpson*
Robert Steakley*
Jeff White
Ty Williams
Telitha Winge
Jason E. Entzminger
Rebecca Lynne Hill
Tessie Irene Ingram*
Kristopher Kaufmann
Charles H. Wakefield*
Lue F. Arn III
Bobbi Lee Blanek
Jessica Sue Boesen
Jason Brooks
John Lee Carr
Casey Dawn Carroll
Jessica Cobos
Melissa A. Cone
Derric Dustin Crowe
Justin Matthew Duyck
James Robert Ellison
Cameron Wade Everton
Richard H. Fohn
Brock Fry*
Brandi Lane Loftis
Fernando Martinez
Heidi Erin McIntyre*
Norberto Mendoza, Jr.
Jerrod K. Pitcock
Joe Martin Self, Jr.
Scott Jeffery Talley*
Krista Renee Tydlaska
Cassidy Watson
Wynne Rae Whitworth*
Dara Alyssa Wilde
John Zertuche

**2003**

Jessica Renee Atchley
James Ray Bilbrey, Jr.
Kevin Jacob Drennan
Brent J. Dugas*
Daniel C. Dusek*
William C. Foster*
Jessica Y. Gomez
Phoebe Ann Harrell
Justin Waid Jackson
Will Kiker*
Haley Jo Knutson*
Lacy Darlene Mercer
Christopher Merren
Jonathan Wayne Meurin
Michael J. Pentecost
David D. Powell
Mark Ray Sheets
Benjamin D. Taylor*
Carrie A. Taylor
Lena Alison Williams
Spencer W. Wyatt
Paul G. Yandle*
Lessa Ann Bullock
James M. Clark
Andrew M. Hart*
Sean M. Kendrick
Kristy Ann Melton
James Paul Skipworth
Sonya M. Washington
Audrey Akers
Courtney Allen
Leslie Ann Fangman
Dustin Gragg
Mary Elizabeth Guerra
Lauren Hahn
Jeremy Haynes
John Allan Henkhaus
Bryan Jennings
Jessica Kiker
Jason McDaniel
Trenton Stephens
Brian Stevens
Leticia Stogner
Cody York

**2004**

Leslie Alexander
Levi Babb
William Travis Bond
Michael Burrows
Shannon Counts
Mia Dues
Kyle Ellis
Krystal Farmer
Blake Gentry
Jenai Gill
Scarlett Lampier
John Henry Leifeste
Cathleen Moore
Chris Moore
Elliott Parks
Jamin Phipps
Kimberly Terrell
Jessica Williams
Loree Branham*
Robert Cook*
Daniel de Carvalho*
Bobby Deeds*
Wayne Dunson*
Shy L. Middleton*
Brandon Payne*
Marc Tucker*
Daniel Woolley*
Jon Austin
John Craig
Tara Basse
Ned Dunbar
Jonathan Ellison
Nathaniel McMillan
Michael Lackey*
Norberto Mendoza*

**2005**

Jon Calcote
Kevin Corzine
Jarrod Cook
Rodney Henderson
Cliff Kinnibrugh
Clint LeMay
Teresa Lovett
Rayle Taylor (Self)
Kayla Niehues
Corey Owens
Andrea Payan
Chase Ratliff
Andy Sandbothe
Levi Wilhite
Andrea Shields
Brandon Asbill*
Will Hartnett*
John Kellermeier*
Michael Thornton*
Lauren Hahn*
Joe Self*
Tammy Vretis*
Tyler Bybee
Ross Copeland
Blake Franke
Aaron Hart
Jeremy Hasty
Tabitha Lloyd
Darby Makloski
Morgan McCutchen
Alfredo Munoz
Thomas Parks
Dustin Ratliff
Dixie Simpson
Heath Stoerner
Cole Wadsworth
Trevor Watson
Ty Wheeler
Billy Krasowsky
Jessica Cobos*
Matthew Dahlberg*
Curry Dawson*
Steven Sturtz*
Lacy Vinson*

**2006**

Jon Bean
Cody Bundick
Jessica Burrus
Crystal Clayton
Destiny Dartez
Travis Downs
Clay Evans
Luke Everett
Dusty Gressett
Trey Hale
Justin Harlin
Jacob Harrison
Chance Hundley
Samson Jackson
Cole Jacoby
Kale Jones
Jake Johnson

Dustin Knowles
Drew McEachern
John McEachern
Matthew Menchaca
Douglas Miller
Anthony Munoz
Kasey Murphy
Bartley Murray
Blake Payne
Travis Pitcock
Braden Riha
William Ritter
Heather Rogers
Mario Saenz, Jr.
Thomas Schenkel
Sam Schiwart
Elizabeth Schulze
Victor Schulze
Chase Settle
Anthony Soliz
Jamie Steen
Elizabeth Stubsjoen
Shelley Talley
Aaron Taylor
Josh Thweatt
Justin Trimble
Rodney Weiser
Ryan Wells
Cole Wilkins
Evan Wilson
Kyle Youngblood
Blake Coates*
Tim Dietz*
Ned Dunbar*
Shelley Gunter*
Andrea Payan*
Jamin Phipps*
Natalie Sato*
Don Skiles*
Dustin Yates*

2007
Brandon Aery
Tiffany Barr
Thomas Bloodworth
Clinton Caudle
Kyle Cheatle
Katy Churchill
Matthew Coffman
Warren Day
Holly Ellis
Chad Evans
Tyler Frey
Jonathan Gilbert
Blake Hinckley
Carrie Koennecke
Elliott Love
Rick Luna
Valarie Marshall
Russell Massey
Juan Munoz
Kole Murchison
Darci Owens
William Renfro
Cooper Riley
Alan Sandbothe
Quisto Settle
Tyler Springer
Heather Stout
Cody Strube
Trey Weishuhn
Sarah West
Randy Whitlock
Ryan Yates
Ross Copeland*
Wade Day*
Chad George*
Teresa Lovett*
Alfredo Munoz*
Corey Owens*
Raelye Taylor Self*

2008
Justin Ahlers
Jess Anderson
Robin Anderson
Leo Batot
Chad Behrends
Brycce Burdick
Kellen Cave
Chris Connor
Kegan Crouch
Wesley Crouch
Tyler Davidson
Danielle DeFrain
Ashley Denton
Lyle Durst
Colin Elmore
Thomas Epting
Kari Galm
Aaron Gillespie
Clay Hale
Brandon Halfmann
Christopher Hart
Jared Hicks
Dustin Klein
Barrett Koennecke
Will Lindsey
Manzy Lowry
Kimberly Menchaca
West Moreland
Bradley Morgan
Casey Mund
Cody Pape
Joseph Petrowski
Shawn Reininger
Travis Rose
Michele Sanchez
Blake Scandolari
Eugene Schmidt
Jared Schniers
Laura Sleutel
Amanda Stallings
Kendall Tidwell
Kory Ware
Lauren Watts
Cody Bundick*
Jessica Burrus*
Kimberly Terrell (Dickinson) *
Chance Hundley*
John McEachern*
Drew McEachern*
Matthew Menchaca*
Anthony Munoz*
Sam Schiwart*
Chase Settle*
Shannon Wilber*

2009
Amanda Anderson
Chance Anderson
Sheree Beauclair
Rebecca Bielefeild
Wesley Bistline
Kayla Brooks
Scott Bryan
Hope Bynum

Clay Calfee
Jennie Canon
Westleigh Carter
Melissa Clary
Rianne Decker
Kristine Durkay
Kaci Foote
Ruben (Angel) Garcia
Zane Graves
Dana Hagemann
Laci Halfmann
Sadie Halfmann
Gary Henson
Derek Hinkson
Tyler Hinrich
Kari Hudspeth
Joe Key
Skylar Owens Kyzar
Sarah Lange
Sarah (Katie) Lee
Raymundo Lopez
Allison Lux
Josh McGinty
Jaron Nevins
Dawson Owens
Alex Pehl
Russ Pfeiffer
Barrett Phillips
Jaydon Pollei
Fisher Pyburn
Gabriel Rodriguez
Melissa Sanchez
Ashley Swan
Ross Teichelman
Tiffany Trevino
Tom Valliant
Russ Vanechek
Chase Weishuhn
Wade Williams
Chris Wolfenbarger
Ashlee Woods
Jeremy Yancy
Blake Hinckley*
Tiffany (Barr) Moorman*
Darci Owens*
Valarie (Marshall) Whitney*
Matthew Coffman*
Cassie (Campbell) Pfeiffer*
Amanda Turner*

2010

Worth (Ty) Allen
Cody Bahlman
Matthew Barker
Jimmy Levon Barragan
Zach Borroum
Molly Butler
Kyle Cook (interdisciplinary)
Barbara Coty
Abel Cuellar
Henry Dissler
Travis Dowell
Lane Flowers
Haley Forehand
Kendra Franke
Kevin Furlow
Sammy Gibson
Jesse Greene
Katie Greer
Jerred Griffith
Jake Halfmann
Lura Hayes
Jade Hughes
Garnet Jackson
James Jackson
Sam Janca
Katelyn Keyes
Leslie Langinais
Tiffany Martinez
Michael McCammon
Jeffrey McCuan
Anna McKinney
Ivana Milovanovic
Sara Mitchell
Elizabeth Mullins
Coty Nelson
Chance Payne
Morgan Plate
Travis Roberts
Gabriel Rodriguez
Blake Rotan
Jeremy Rychlik
Justin Smith
Josh Smith
Justin Strube
Cooper Swening
Terrica Taylor
Cari Thompson
Adriane Thomas
Britni Tjaden
Heather Tschirhart
Jason White
Gaylon Wilmeth
Clayton Wilson
Clayton Wooldridge
Melissa Yamma
Jess Anderson*
Leo Batot*
Kellen Cave*
Kris Ede*
Dustin Klein*
Kari Mulle*
Landon Pyle*
Kendall Tidwell*
Dustin Vick*
Lauren Watts*

2011

Sonja Blumentritt
Shannon Cunningham
Houston Dobbins
Jake Dodds
Lane Flowers
Garrett Haney
Cole Herring
Marcus Hinojosa
Sam Janca
Shawn Justiss
Charlena Lemaster
Bliss Long
Justin McGriff
Kristin Newman
Jeanette Redmon
Drew Reed
Kristyn Schultz
Hunter Stuart
Ridge Suttuth
Rachel Werbelow
Aaron Woods
Jacob Zachary
Angel Garcia*
Kayla Brooks*
Sarah Lange*
Kaci Foote*
Jaron Nevins*
Brittni Kayczk*
Jess Yeamen

*a*

RESEARCH

PROJECT

*of*

ANGELO

STATE

UNIVERSITY