

FEEDING SPECIFIC AMINO ACIDS TO INCREASE REDBERRY JUNIPER
CONSUMPTION BY GOATS

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FEEDING SPECIFIC AMINO ACIDS TO INCREASE REDBERRY JUNIPER
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ABSTRACT

Redberry juniper (*Juniperus pinchotii* Sudw.) is a chemically defended, invasive brush species in western states. Previous research has demonstrated that goats supplemented with escape protein sources will consume a higher volume of juniper. In this study I attempted to increase the intake of juniper by recently weaned, Boer-Spanish cross goats (n = 30) by randomly placing them into four treatments and a control, and supplementing each treatment group with one of the following amino acids: arginine, proline, leucine, and glutamine. Treatments did not have a significant effect on juniper intake ($P > 0.05$). All goats increased intake over time ($P > 0.05$). Goats exhibited significant weight gain over the course of the study ($P < 0.05$). Blood serum metabolites were within normal limits. Future studies on escape protein supplementation may provide a better understanding of small ruminant physiology in relationship to intake of juniper.

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INTRODUCTION

Redberry juniper (*Juniperus pinchotii* Sudw) is an invasive, problematic plant to livestock producers and range managers of west and central Texas, especially in the Rolling Plains and Edwards Plateau regions (Johnson et al. 1999). It is an evergreen, coniferous, basal-sprouting shrub that avoids herbivory and survives under a wide variety of growing conditions. It is also known locally as cedar or redberry cedar. Prior to the development of the range livestock industry in western Texas, redberry juniper populations were restricted primarily to rocky outcrops and north facing slopes that provided protection from intense grass fires (Ueckert 1997). Because of overgrazing and fire avoidance practices of European settlers in the early 1800s, redberry juniper has encroached upon open grasslands, presenting a problem of reduced forage quality and availability to grazing animals (Dye et al. 1995, Nelle 1997). Redberry juniper infestation also apparently reduces water yield, water quality, and wildlife habitat (Hicks and Dugas 1998, Ueckert et al. 2001).

Conventional methods of control and removal including bulldozing, chaining, burning, and herbicide applications vary in success rates and can be costly, although may improve net range productivity (Johnson et al. 1999). The costs of conventional control methods can vary dramatically depending on availability of contractors, fuel price, equipment rental costs, and perhaps most importantly, the density of trees to be removed (Johnsen and Dalen 1984). Grazing management and the use of goats for invasive species control offers an economical alternative for range stewards to conventional methods of

control (Walker 1995, Frost and Launchbaugh 2003). Junipers contain monoterpenoids, which serve as chemical defense mechanisms to discourage herbivory (Riddle et al. 1996, Pritz et al. 1997). Two areas of research have shown promise in finding viable ways to increase the tolerance of goats to monoterpenoids and increase the amount of juniper consumed by goats. Goats can be conditioned to increase consumption of undesirable plants (Walker 1995). Feeding redberry juniper to goats at weaning will increase juniper consumption on pasture (Bisson et al. 2001, Ellis et al. 2005, Dunson et al. 2007). Once released on pasture, goats continued to consume juniper throughout the year (Dietz et al. 2010). Goats can also be supplemented with escape protein to increase consumption of redberry juniper apparently because escape proteins provide the substrates for conjugation and excretion of monoterpenoids (George et al. 2010).

The objective of this study was to determine if specific amino acids that escape rumen degradation will improve juniper consumption. Corn dried distillers dried grains (DDG) has a unique amino acid profile compared with other protein supplements, e.g. cottonseed meal, including higher concentrations of leucine, aspartic acid, glutamine, and proline. If a specific amino acid can be identified, which can improve juniper consumption and reduce hepatic insult, it may be possible to supplement goats with that amino acid to increase their juniper intake.

OBJECTIVES

The objectives of this study were to:

- 1.) Determine the efficacy of supplementing specific amino acids to increase redberry juniper consumption by goats.
- 2.) Evaluate the ability of specific amino acid supplements to decrease hepatotoxicity caused by consumption of redberry juniper by goats.

LITERATURE REVIEW

Juniper Control Methods

Redberry juniper is a widespread, invasive brush species that reduces biodiversity and reduces the capacity of rangeland to support wildlife and livestock. It has become a serious invasive plant problem over vast ha of the Rolling Plains and Edwards Plateau regions of Texas (Ansley et al. 1995). Apparently, the invasion of several species of juniper on grassland savanna was triggered by introduction and overgrazing of domestic livestock by European settlers in the late 1800s, the reduced role of fire, and mild and wet climate conditions around the turn of the 20th century (Miller and Rose 1999). Encroachment of woody plants in Texas over the last 100 years has reportedly altered the hydrologic functioning of rangelands with the infestation of brush lowering water yields compared with grassland savannas (Ueckert et al. 2001, Wu et al. 2001). The simultaneous increase in the human population in Texas, thus demand for water, has brought increased attention to brush control as a method of increasing or at least maintaining water supplies (Olenick et al. 2004). Other species of juniper, such as Ashe juniper (*Juniperus ashei* Buchh.), are also increasing in density in the Edwards Plateau of Texas while Western juniper (*Juniper occidentalis* subsp. *occidentalis* Hook.) are encroaching into aspen (*Populus tremuloides* Michx.) communities in the northwest Great Basin (Hicks and Dugas 1998; Wall et al. 2001).

Control of mature juniper stands is typically very difficult. Several methods are currently used with varying success rates in controlling redberry juniper infestations on rangelands. Redberry juniper can be mechanically controlled through chaining, grubbing or root plowing, chemically controlled with herbicide application, burned using prescribed fire,

or biologically controlled with goats. Chemical application on mature juniper trees can be cost prohibitive and is not always an effective tool in inducing mortality in mature stands (Ansley et al. 1995). Prescribed fire can be hazardous because of the volatility of juniper oils, length of time partially burned juniper wood burns, and the potential for juniper to ignite fire spots up to 1,000 ft away (Owens et al. 1998). Prescribed burning is thought to be optimal at seven-year intervals under most conditions (Johnson et al. 1999) and is typically recommended on an 8 to 10-year cycle (Ueckert 1997). Climate conditions and burn bans may make prescribed burning an inaccessible management tool for ranchers and rangeland managers. Prescribed burning may be limited by available fuel load to carry the fire (Steuter and Wright 1983). Fire may be a more effective tool on species like ashe juniper that do not resprout after complete topkill (Noel and Fowler 2007). Mature redberry juniper, however, is not easily controlled by prescribed burning because it aggressively resprouts after top removal (Smith et al. 1975, Steuter and Britton 1983). Redberry juniper can be controlled with a combination of top removal and chemical application; most effectively a 2% solution of Picloram applied after resprouting (Tunnell and Mitchell 2001).

Control of redberry juniper using two-way chaining followed by prescribed burning was effective on very shallow range sites in the Texas Rolling Plains and increased range productivity and net revenues over a 30-year period (Johnson et al. 1999), however soil disturbances caused by chaining can potentially cause reinvasion of woody species by dispersal of the seedbed (Owens and Schliesing 1995). Two-way chaining is dependent on soil moisture so that the junipers are removed below the basal bud zone rather than being broken off above ground (Scifres 1976).

Recent research has investigated the efficacy of goats in controlling invasive redberry juniper on rangelands. Although juniper is chemically defended by essential oils, e.g. monoterpenoids, goats have shown the ability to control brush species that are generally less palatable to other species of livestock (Dietz et al 2010). Typically intake of juniper is inhibited because of monoterpenes produced by the plant. Essential oils produced by juniper inhibit intake in by: alteration of the rumen microbial population, (Calsamiglia et al. 2007), hepatopathy (Pritz et al. 1997, Bisson et al. 2001), and aversive post-ingestive feedback (Pritz et al. 1997, Provenza 1995, Riddle et al. 1996). Goats can be utilized more effectively as a tool for redberry juniper control on rangelands if the physiological mechanisms inhibiting intake are better understood and managed appropriately (Pritz et al. 1997). A study on browsing effects on ashe juniper demonstrated that at high levels of browsing pressure, goat utilization of juniper is sufficient to slow its encroachment and alter its growth form (Fuhlendorf et al. 1997).

Goats can be used in combination with other methods of juniper control. For instance, fire can be used to topkill redberry juniper followed by goat browsing of resprouts for further control. Juniper seedlings and regrowth contain lower levels of monoterpenes than mature juniper growth; thus, increasing the likelihood of consumption (Owens et al. 1998).

Escape Protein Supplementation and Monoterpene Detoxification

Goats can adapt to monoterpenoids through exposure at weaning and through enhanced ability of the liver to metabolize the monoterpenoids found in the plant (George et al. 2010). Rumen function does not appear to have an effect on monoterpene degradation (Dunson et al. 2007). Biotransformation of xenobiotic compounds are generally divided into two phases:

phase one, including oxidation, reduction, and hydrolysis, and phase two, which encompasses conjugation reactions (Nebbia 2001). Monoterpenes are partially oxidized and then conjugated. Once in the gastrointestinal tract, monoterpenes are absorbed in small amounts through the rumen and in larger amounts in the small intestine. Monoterpenoids are then carried by the hepatic portal system to the liver for detoxification by cytochrome P-450 dependent multifunctional oxidase enzyme systems (MFOs) and several conjugation enzymes. Mixed-function oxidase enzymes add molecular groups that break structural bonds to convert lipophilic terpenes into more polar, water soluble compounds that can be conjugated with other compounds and then excreted in the urine (Bidlack et al. 1986, Nebbia 2001).

Studies have suggested that supplementing goats with protein, especially escape protein, may increase the ability of goats to metabolize monoterpenoids by the liver and increase their consumption of redberry juniper (Campbell et al. 2007, George et al. 2010). In particular, it has been suggested that protein supplements containing high levels of glucogenic amino acids that escape rumen degradation may provide the substrates needed to conjugate monoterpenoids so that they may be excreted through the urine (George et al. 2010). Distiller's dried grains appear to be particularly high in the glucogenic amino acids glutamine and proline in comparison to reports of these amino acids in the rumen microbial population (Storm and Oskov 1983, O'Mara et al. 1997). When goats were offered DDG as a protein supplement, they consumed more redberry juniper (George et al. 2010).

MATERIALS AND METHODS

All procedures were approved by the Animal Care and Use Committee of Angelo State University prior to the commencement of the study.

The research took place at the Angelo State Management Instruction and Research (MIR) Center. In this experiment, recently weaned Boer-Spanish cross goats ($n = 30$, initial BW = 26.7 kg) were randomly placed into 4 treatments and 1 control group (6 goats/treatment or control) per feeding period (4 periods). Each treatment group was administered one of the four following amino acids as a supplement: arginine, glutamine, leucine or proline. All of the goats were purchased from the same source and were of mixed gender, comprised of 28 castrated males and 2 females. Prior to the commencement of the study the goats were administered the same preventative care, including subcutaneous vaccination for *Clostridium perfringens* type C&D and oral administration of Ivomec 0.08% for the treatment of internal parasites. One male goat died after the first pen adjustment period due to a urethral blockage caused by uroliths.

Goats were weighed prior to being randomly assigned a treatment in each period. Goats were then separated into individual pens (1 m \times 1.5 m), and allotted 7 days for pen adjustment. A soybean meal grain-based diet was fed at a rate of 2.5% BW daily to all animals to meet each animal's maintenance requirements during the pre-conditioning and testing phases (NRC 2007). Fresh water and calcium and phosphorus mineral with trace elements were provided *ad libitum* throughout the trial. The base diet was formulated to meet the animals' requirements with digestible energy comprising 2.6%, crude fiber comprising 16.3%, and crude protein comprising 13.0% (Table 1).

Table 1. Maintenance diet ingredients and weight (kg) composition of feed.

Ingredients	As fed	DM
Corn grain	340.2	299.4
Alfalfa pellets	158.8	142.9
Cottonseed hulls	136.1	123.8
Soybean meal	102.1	94.9
Soybean hulls	117.9	107.0
Cane molasses	29.5	22.1
Mineral premix ¹	22.7	22.7
Total	805.2	717.9

¹Active drug ingredient: lasalocid. Guaranteed analysis: CP (min) 48%, calcium (min) 17.5% (max) 19%, salt (min) 18.1% (max) 20.6%, Manganese (min) 1075ppm, Zinc (min) 1780ppm, Copper (max) 0.00ppm, Selenium (min) 3.95ppm, Vitamin A (min) 18.4IU/kg, Vitamin D 6.1 IU/kg, Vitamin E 101.8 IU/kg.

After the pen adjustment period and prior to the first and second feeding period, blood samples were taken via jugular veinapuncture, allowed to clot, placed in a centrifuge to separate serum, and stored at -80°C. Blood sampling was repeated on day 10 of each of the first two feeding periods. The samples were submitted to the Texas Veterinary Medical Diagnostic Laboratory in College Station, Texas and analyzed for serum aspartate transferase (AST), gamma glutamyltransferase (GGT), blood serum urea nitrogen (SUN) and creatinine.

Each juniper feeding period lasted 10 days and goats were naive to redberry juniper and their respective amino acid supplement prior to the first pen-adjustment pre-period phase. In the first 3 periods each goat within each treatment received an oral bolus of 1.5 g of a specific amino acid; arginine, glutamine, proline (glucogenic amino acids) or leucine (non-glucogenic), contained in a dissolvable 3.2-mL porcine gelatin capsule (Torpac Inc. in Fairfield, New Jersey) 30 min prior to being offered juniper each day.

The amino acids were obtained from Vitacost.com, an online wholesale nutritional supplement store. The arginine and glutamine supplements are manufactured by Vitacost (formerly Nutraceutical Life Sciences) of Lexington, Kentucky while the proline and leucine supplements are manufactured by Source Naturals of Santa Cruz, California. After the first two days of the first feeding period, it was decided that a different method of amino acid administration would need to be used, as the goats had extreme difficulty swallowing the gelatin capsules. The goats then received the amino acids in the form of a water-based drench administered with an esophageal tube. Individual doses of the amino acid drench were prepared each day prior to dosing. The control group was offered fresh juniper daily without

a supplement. In the fourth feeding period, the amino acid dose was increased to 3.0g per head per day, with all other procedures remaining the same.

Fresh juniper was offered for each day for 30 min after administration of the amino acid supplement and refusals were weighed to estimate intake. Initially, goats were offered 50 g of juniper per head during each feeding bout. If any goats consumed the entire amount of juniper offered, the amount fed was increased by 50 g/day until refusals were noted. Between each period, the goats were allowed one week of rest and rumen flora readjustment and received the maintenance diet only.

The period was then repeated three more times with different goats receiving different amino acids during each period, so that each goat received each of the four amino acids during the entire trial. Redberry juniper leaves and small pliable stems were harvested from randomly selected trees from the Texas AgriLife Experiment Station in Sonora, Texas, composited, and stored at 4°C. Three, randomly selected 100-g samples of juniper were dried in an oven at for 24 hr at 60°C, ground to 1-mm (Wiley Mill) and submitted for analysis to the Texas Agrilife Research Cener in Stephenville, Texas. Samples were analyzed for CP and condensed tannin concentrations.

STATISTICAL ANALYSIS

The experimental design of this study was a modified Latin square, where each individual goat received each amino acid during 10-day feeding periods. Each feeding period was separated by a 7-day adjustment period. Because 4 amino acids were used, 4 separate 10-day feeding periods were utilized. Goats allocated to the control group, served as the control in each feeding period, and did not receive any amino acids. Juniper and basal diet intake, and serum metabolite concentrations were analyzed using repeated measures analysis of variance within each feeding period and across all feeding periods. Individual goats nested within treatments and periods served as the experimental unit and day of collection as the repeated measure. Means were separated using Tukey's least significant difference when $P \leq 0.05$). Data was analyzed using the statistical package JMP (SAS 2007).

RESULTS

Dosing goats with a specific amino acid did not improve ($P > 0.05$) juniper consumption (Table 2). The hypothesis that dosing with glucogenic amino acids (arginine, glutamine, proline) would improve juniper consumption was rejected. Juniper intake was similar ($P > 0.05$) among goats across feeding periods (Table 3). Average daily intake of juniper varied ($P < 0.05$) across days of feeding (Figure 1). The treatment by day and treatment by period interactions were not significant ($P > 0.05$). For the fourth period, the amount of amino acid each goat received was doubled, however intake of juniper remained similar ($P > 0.05$) among treatments (Table 3).

Weight gain was not significant within period 1 ($P > 0.05$, Table 4). Over all four periods, goats in the arginine treatment group had an average weight gain of 28.5 kg, the glutamine treatment group had an average weight gain of 30.0 kg, the leucine treatment group had an average weight gain of 30.0 kg, while the proline treatment group had an average weight gain of 30.0 kg, and the control group had an average weight gain of 28.0 kg.

The basal diet intake was similar among treatments and days across all four feeding periods (Figure 2).

Table 2. Average juniper consumption ($\text{g}\cdot\text{kg}^{-1}$ BW) across the four treatments. Intake data is pooled across the four 10-day feeding periods.

Treatment	Juniper Intake	SEM
Arginine	3.3	0.3
Glutamine	3.1	0.3
Proline	3.3	0.3
Leucine	3.2	0.3
Control	3.3	0.3

n = 29

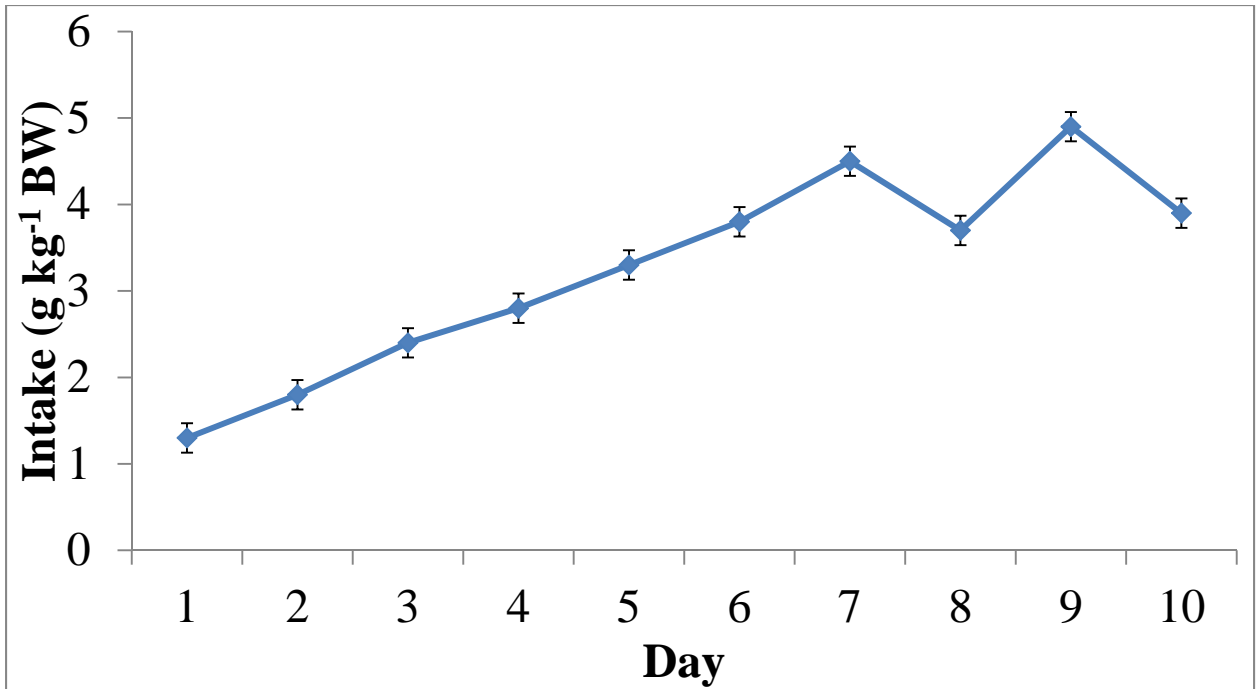


Figure 1. Average intake (g·kg⁻¹ BW) for juniper across the 10 days of feeding. Data is pooled across the four 10-day feeding periods and across the five treatments.

Table 3. Average juniper consumption ($\text{g}\cdot\text{kg}^{-1}$ BW) across the four feeding periods. Each of the four feeding periods lasted 10 days.

Treatment	Period			
	1	2	3	4
Arginine	2.7	3.6	3.1	4.1
Glutamine	2.4	3.6	3.2	4.3
Proline	3.6	3.6	2.6	3.5
Leucine	2.5	2.2	3.9	4.0
Control	2.6	2.3	3.8	4.4

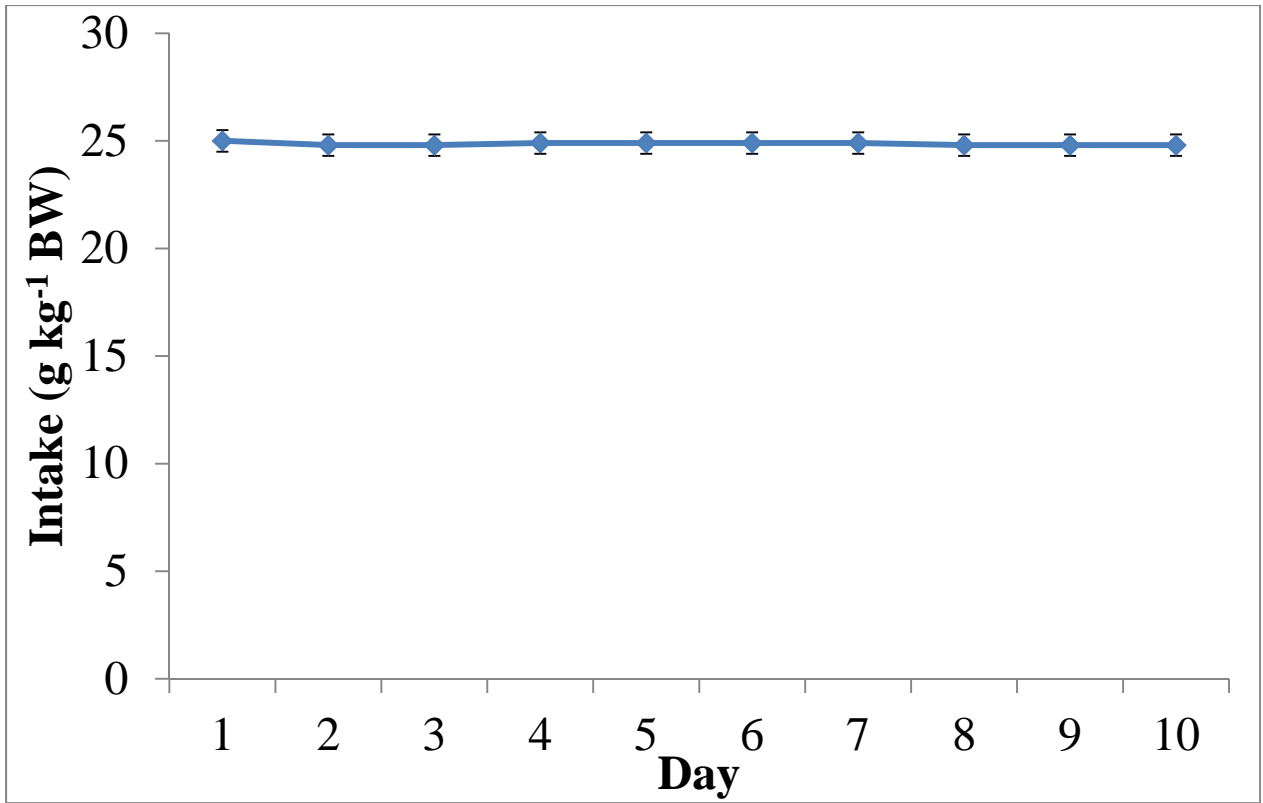


Figure 2. Average intake (g·kg⁻¹ BW) for the basal diet across the 10 days of feeding. Data is pooled across the four 10-day feeding periods and across the five treatments.

Table 4. Mean weight change (kg) for feeding period 1 by treatment.

	Initial	Final
Arginine	25.9	25.4
Glutamine	29.6	28.3
Leucine	25.9	25.3
Proline	26.5	24.7
Control	25.7	25.3

There were no differences among serum chemistry results between treatments ($P > 0.05$, Table 5). None of the average blood chemistry values were outside the normal physiological range except for initial creatinine. A significant difference existed between initial and final blood draws ($P < 0.05$) for total serum protein, glucose, blood urea nitrogen, and gamma glutamyltransferase but remained within normal range for healthy goats.

Table 5. Mean initial and final blood chemistry analysis at the beginning and end of the first feeding period.

		Initial	Final	Reference Range ^f
Arginine ^d	TSP	6.5	6.3	4.8-8.8 (g/dl)
	Glucose	80.8	75.6	26-126 (mg/dl)
	SUN	20.6	18.0	17-31 (mg/dl)
	Creatinine	0.7	0.7	0.4-1.2 (mg/ml)
	AST	92.1	90.5	32-152 (U/l)
	GGT	45.7	48.0	<319 (U/l)
	Glutamine ^d	TSP	6.6	6.5
Glucose		96.5	75.1	26-126 (mg/dl)
SUN		21.2	17.8	17-31 (mg/dl)
Creatinine		0.6	0.8	0.4-1.2 (mg/ml)
AST		85.3	95.1	32-152 (U/l)
GGT		55.8	59.0	<319 (U/l)
Leucine ^d		TSP	6.4	6.2
	Glucose	86.6	67.2	26-126 (mg/dl)
	SUN	54.3	51.9	17-31 (mg/dl)
	Creatinine	8.0	0.7	0.4-1.2 (mg/ml)
	AST	89.1	89.1	32-152 (U/l)
	GGT	44.9	46.1	<319 (U/l)
	Proline ^d	TSP	6.4	5.9
Glucose		76.8	69.6	26-126 (mg/dl)
SUN		19.5	17.1	17-31 (mg/dl)
Creatinine		0.7	0.7	0.4-1.2 (mg/ml)
AST		85.8	81.2	32-152 (U/l)
GGT		43.1	43.4	<319 (U/l)
Control ^d		TSP	6.5	6.1
	Glucose	96.6	75.1	26-126 (mg/dl)
	SUN	20.6	17.3	17-31 (mg/dl)
	Creatinine	0.7	0.7	0.4-1.2 (mg/ml)
	AST	80.3	76.3	32-152 (U/l)
	GGT	50.1	52.6	<319 (U/l)

^d No significant difference in blood chemistries between treatments ($P > 0.05$)

^f Texas Veterinary Medical Diagnostics Laboratory, College Station, Texas

DISCUSSION

Results of this study indicate that dosing goats with free form amino acids at a rate of 1.5 g/day per head or at a rate of 3.0 g/day does not have a significant effect on juniper consumption. Juniper consumption was similar among all periods ($P > 0.05$) and treatments ($P > 0.05$). It is possible that the amino acids administered were not at a significant level to escape microbial degradation. Because of goat rumen physiology, there are no established dietary requirement levels for proline, glutamine, arginine and leucine for goats (Owens and Zinn 1988). Also, the amino acids were not administered in a rumen protected form, which may reduce their potential in reaching the small intestine for absorption. Rumen protected amino acid supplements have been used in the dairy industry to improve dairy performance and promoted amino acid utilization in lactating cows (Wang et al. 2010). There is also not an established value for the rate of passage and metabolism for either juniper or escape protein, so it is unknown whether the timing of the supplement administration has any effect on liver metabolism and detoxification of monoterpene compounds found in the juniper (Owens and Zinn 1988).

Others have illustrated that protein supplementation increased juniper consumption by goats (Campbell et al. 2007). In addition, George et al. (2010) illustrated that protein sources that consist of amino acids that escape rumen digestion improve juniper consumption. It remains unclear as to why dosing goats with specific amino acids in this study did not improve juniper consumption. Goats were fed a basal diet to meet maintenance requirements, including protein (Table 1) (NRC 2007). In addition, juniper provides some protein as well. Thus, rumen microbial requirements for N should have been met, allowing

some amino acids to escape rumen degradation. Analysis of the juniper samples revealed average extractable crude tannins to be 3.8 %, while the average percent of protein bound crude tannin was 1.3%, the average percent fiber bound crude tannin was 0.25%, and the average percent total crude tannin was 5.37%. Average percent crude protein in the juniper sample was 6.9%. Weight gain was significant ($P < 0.05$) across the study. Some studies have shown weight loss in goats when individually penned and fed juniper (Scott, pers. comm. April 2012). Dosing with supplemental amino acids may have contributed to the weight gain observed in this study.

Based on blood chemistry analysis from Periods 1 and 2, goats on the control diet did not consume enough juniper to induce hepatopathy or renal insult. All results except for the initial creatinine in the leucine treatment group were within normal limits. Probably the value for the initial creatinine in the leucine group was elevated because the serum chemistry from the goat that perished because of urolith blockage was included in the initial blood chemistry data. Creatinine is a waste product generated from muscle metabolism and filtered from the blood by the kidneys to be excreted in the urine. Serum concentrations of creatinine can be used to detect renal insufficiency or disease. Urolith blockage causing prevention of urination would consequently cause a buildup of creatinine in the blood stream because the kidneys would not be able to filter sufficient amounts of the waste product. A significant difference ($P < 0.05$) was found between initial and final blood draws across treatments. Most chemistry values decreased, suggesting that liver metabolism of monoterpenes and kidney excretion of conjugated monoterpenes decreased as the goats consumed juniper. The amino acid supplements may not have been escaping the rumen in significant amounts to

provide substrates for conjugation and consequently increase the rate of liver metabolism of monoterpene compounds.

All goats, regardless of treatment group, increased intake daily from initial exposure to juniper and through the 10-day feeding periods. This observation agrees with others (Bisson et al. 2001, Ellis et al 2005, Dunson et al. 2007) that goats will increase consumption of juniper when exposed to the plant at weaning.

IMPLICATIONS

While the results of this study failed to indicate a significant relationship between juniper consumption and supplementing glutamine, proline, arginine or leucine at 1.5 g or 3.0 g/head per day, there may be future applications in the supplementation of amino acids to ruminants as detoxifying agents. The study did indicate a change in liver and kidney function over the course of the first two feeding periods. Studying the effect of rumen-protected amino acids dosed in significant quantities to reach the small intestine for absorption may provide new and useful information on detoxification mechanisms employed by the liver. A better understanding of small ruminant physiology in relationship to intake of chemically defended plants will assist range managers in effectively utilizing goats as an economical tool for managing invasive brush species.

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