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Effects of adding protein, condensed tannins, and polyethylene glycol to diets of sheep and goats fed one-seed juniper and low quality roughage

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ABSTRACT

The biochemical mechanisms that limit voluntary intake of one-seed juniper by browsing ruminants are not well understood. Twelve Rambouillet ewes (78 ± 2.3 kg BW) and 12 Boer-Spanish does (54 ± 1.4 kg BW) were used in a split-plot sequence design to investigate the effects of adding protein, quebracho tannins (QTs), and polyethylene glycol (PEG) on one-seed juniper intake and preference and on ruminal VFA and plasma AA concentrations. Animals received sudangrass hay and isoenergetic basal diets (4 does and 4 ewes/diet) with either rumen degradable (RDP) or rumen undegradable (RUP) protein (12.5% CP) or no additional protein (control, 5% CP) during four 15-d periods. The control, RDP, and RUP diets were considered the main plot, whereas the four supplement treatments were analyzed as the split-plot effects. Period 1 allowed for adaptation to basal diets and served as a baseline phase. In periods 2–4, animals were offered juniper leaves and twigs (period 2), juniper plus QT (10% of basal diet; period 3), and juniper plus QT plus PEG (50 g/animal; period 4). Juniper intake by sheep and goats was not affected ($P > 0.88$) by RDP or RUP when animals were first exposed to juniper, but marginally increased ($P < 0.10$) in period 4 (QT + PEG) regardless of the basal diet. Prior exposure to juniper did not affect ($P = 0.61$) the preference ratio for juniper, but goats had higher preference ratio for juniper ($P < 0.01$) when receiving PEG (period 4). Concentrations of total VFA tended to increase in sheep ($P = 0.10$) and goats ($P = 0.14$) fed protein supplements and molar proportions shifted toward acetate for goats fed RDP and RUP ($P = 0.07$) and to butyrate for sheep fed RDP ($P = 0.01$). Initial juniper exposure (period 2) elevated concentrations of acetate, propionate, and butyrate ($P < 0.01$), but the effect was extinguished in periods 3 and 4 with addition of QT ($P < 0.05$). Supplementation with PEG transiently mitigated the depressor effect of QT on acetate, propionate, and butyrate concentration at 12 h post juniper feeding ($P < 0.01$). RDP in goats and to a greater extent RUP in sheep increased plasma concentrations of various AA, especially the branched chained Val, Ile, and Leu ($P < 0.05$). Plasma concentrations of several AA, including Met, Cyst, Glu, Gly, Gln, Asn, Thr, Ser, and Phe, decreased with ingestion of juniper (period 2) and juniper plus QT (period 3). Concentrations of some AA that were depressed by ingestion of juniper and QT were partially restored with supplemental PEG ($P < 0.05$). Protein, terpenes, tannins, and PEG interacted to influence rumen VFA and plasma AA, which were related to intake of juniper and basal diets.

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1. Introduction

One seed juniper (*Juniperus monosperma* [Engelm.] Sarg.) is frequently dominant on New Mexico rangelands but rarely consumed in large amounts by livestock (Nunez-Hernandez et al., 1989). Small ruminants may self-limit juniper intake to avoid the detrimental effects of a number of terpenoids contained in its foliage (Adams et al., 1981; Utsumi et al., 2006, 2009) which exhibit antibacterial properties (Nagy and Tengerdy, 1968) that can depress rumen fermentation (Villalba et al., 2006) and increase the demand of nutrients needed to meet the metabolic costs of detoxification processes (Illius and Jessop, 1995). The increasing interest in using small ruminants as a tool to control one seed juniper encroachment (Utsumi et al., 2010) requires a better understanding of the factors that limit voluntary intake of this browse species.

Specific terpenes present in one seed juniper foliage can boost cellulolytic bacteria activity and increase concentrations of rumen VFA (especially acetate) while other less abundant terpenoids can have the opposite effect (Utsumi et al., 2006; Broudiscou et al., 2007). Less extensively degraded terpenes (possibly the more oxygenated compounds; Schwartz et al., 1980) may be the ones associated with negative metabolic effects and depression of voluntary intake. These terpernoids could indirectly limit AA availability by inducing an increase in AA required during phases I and II of terpene detoxification which involve maintaining acid–base balance disrupted by acidic products and conjugation of oxidized terpenes (Foley et al., 1995; Illius and Jessop, 1995; Lamb et al., 2001; Parkinson, 2001; Sorensen et al., 2005). Stimulation of intake and hepatic oxidative metabolism (elevated P-450 enzyme activities) with increased dietary protein has been well documented in sheep exposed to other xenobiotics (Thomford and Dziuk, 1988). If elevated plasma AA enhance hepatic oxidation of terpenes (Swick, 1984; Guengerich, 1995), additional dietary protein may improve plasma AA status and partially offset the costs of terpene detoxification. This could be the underlying mechanism responsible for increased voluntary consumption of terpene-containing plants in animals that receive protein supplements (Villalba et al., 2002a; Villalba and Provenza, 2005; Campbell et al., 2007; Utsumi et al., 2009). However, the relationships between added dietary protein, plasma AA, and one seed juniper intake have not been studied.

Juniper-dominated rangelands frequently support a variety of browse species including oaks (*Quercus* sp.) which are often selected by small ruminants despite containing relatively high levels of condensed tannins (CT, Davis et al., 1975; Villena and Pfister, 1990; Riddle et al., 1999). Biochemical interactions among juniper terpenes and CT can also influence intake, but the mechanism regulating this interaction is still unclear (Utsumi et al., 2009). A high dose of CT depresses rumen fermentation due to precipitation of proteins, and to a lesser extent soluble carbohydrates, ultimately decreasing protein availability and substrates for microbial digestion (McMahon et al., 2000; Silanikove et al., 2001; Makkar, 2003). Condensed tannin-protein binding could neutralize the positive effects of protein supplements on juniper voluntary intake (Utsumi et al., 2009). Polyethylene glycol (PEG) improves rumen function of animals that feed on tannin-rich browse (Landau et al., 2000; Silanikove et al., 2001; Makkar, 2003) by preventing protein-tannin binding (Bhatta et al., 2002; Villalba et al., 2002b; Ben Salem et al., 2005). Thus, feeding PEG may increase juniper intake of ruminants browsing rangelands containing both one seed juniper and co-occurring species rich in CT but little is known about PEG's effects on voluntary intake of diets that contain both terpenoids and CT.

Our objectives were to determine how one-seed juniper intake and preference, rumen fermentation parameters, and plasma AA are affected by rumen degradable or undegradable protein (RDP or RUP) alone or with CT and PEG in sheep and goat diets. We tested predictions from two sets of hypotheses related to: (a) juniper intake and preference, and (b) rumen fermentation parameters and plasma AA dynamics. The first set of hypotheses predicted that: (1) adding RDP or RUP would increase one seed juniper intake and preference, (2) CT plus RDP or RUP would depress one seed juniper intake and preference, and (3) providing PEG with diets containing CT plus RDP or RUP would restore the positive influence of added protein on one seed juniper intake and preference. The second set of hypotheses predicted that: (1) ingestion of one seed juniper would alter rumen fermentation and decrease plasma AA, (2) additional dietary protein would restore rumen function and plasma AA levels, (3) adding CT to the diet would neutralize the positive effects of protein, and (4) adding PEG to the diet would neutralize CT and restore the beneficial effects of protein on rumen function and plasma AA levels.

2. Materials and methods

2.1. Experimental design

The study was conducted at the New Mexico State University Campus Livestock Facility during spring 2007. Animal handling and experiment protocols used in this study were approved by the Institutional Animal Care and Use Committee at New Mexico State University. The experimental design consisted of a split-plot sequence of four experimental periods in which ewes and does previously adapted to a basal diet of sudangrass hay and ground corn alone or with RUP or RDP (period 1) were then supplemented with one-seed juniper (period 2), juniper and quebracho tannins (period 3), and juniper, quebracho tannins, and PEG (period 4, Table 1). Given the animal number constraints and the importance of order in which nutrients, plant secondary metabolites (PSM), and nutraceuticals (i.e., PEG) were fed, this experiment sequence was selected for the treatment design (Villalba et al., 2002c, 2004, 2006).

2.2. Animals, holding pens, and basal diets

Twelve non-pregnant and non-lactating adult Boer-Spanish does (54 ± 1.4 kg BW) and 12 Rambouillet ewes (78 ± 2.3 kg BW) with prior juniper intake records (Utsumi et al., 2009) were used. All animals received sudangrass hay daily and had not consumed juniper during the seven months prior to the onset of this study. After the conditioning phase, animals were placed in individual pens ($2\,m \times 3\,m$) with a roofed bedding area and free access to fresh water. Animals were assigned to 3 treatment groups (4 does and 4 ewes/group) balanced for individual juniper intake measured in previous feeding trials (Utsumi et al., 2009). Animals remained in the same treatment group throughout the study.

In addition to roughage (see feeding protocol below), each treatment group received ground corn and: (1) no added protein (control), (2) added

Table 1
Experiment protocol.

Experiment period	Trial	Procedure	Duration	Feeding protocol[a]	Measurements
(1)	1	Initial familiarization with experimental diets	15 d	• Noon: Control, RUP, and RDP • PM: Sudangrass	Intake, rumen fermentation and pH, Plasma AA
	2	Juniper preference in animals without prior exposure to juniper	3 d	• AM: Choice of juniper and Control, RDP or RUP • PM: Sudangrass	Intake and juniper preference
(2)	3	Exposure to juniper	15 d	• AM: Juniper • Noon: Control, RDP, and RUP • PM: Sudangrass	Intake, rumen fermentation and pH, Plasma AA
	4	Juniper preference in animals with prior exposure to juniper	3 d	• AM: Choice of juniper and Control, RDP or RUP • PM: Sudangrass	Intake and juniper preference
(3)	5	Exposure to juniper + condensed tannins	15 d	• AM: Juniper • Noon: Control, RDP, or RUP + QT • PM: Sudangrass	Intake, rumen fermentation and pH, Plasma AA
	6	Juniper preference following exposure to juniper and condensed tannins	3 d	• AM: Choice of juniper and Control, RDP or RUP + QT • PM: Sudangrass	Intake and juniper preference
(4)	7	Exposure to juniper + condensed tannins + PEG	15 d	• AM: Juniper • Noon: Control, RDP, or RUP + QT • PM: PEG and Sudangrass	Intake, rumen fermentation and pH, Plasma AA
	8	Juniper preference following exposure to condensed tannins + PEG	3 d	• AM: Choice of juniper and Control, RDP or RUP + QT • PM: PEG and Sudangrass	Intake and juniper preference

[a] AM (07:00–10:00), Noon (10:00–13:00), and PM (13:00–16:00), indicate the time of day when each food was offered. Juniper consisted of thawed chopped one-seed juniper leaves and twigs. Control diet: no protein added (5% CP), RDP diet: ruminal degradable protein added as soybean meal (12.5% CP); RUP diet: ruminally undegradable protein added as fishmeal (12.5% CP). QT: quebracho tannin extract fed at 10% (DM basis) of diets. PEG: 50 g polyethylene glycol 6000. Sudangrass: ground sudangrass hay.

Table 2
Diets fed to treatment groups.

Ingredient, g/kg (DM basis)	Control[a]	RUP	RDP
Periods 1 and 2			
Ground sudangrass hay	512	528	528
Ground corn	333	206	138
Soybean meal 45%	–	–	189
Fish meal 60%	–	137	–
Finely ground wheat straw (<5 mm)	100	100	100
Mineral–vitamin premix[b]	55	29	45
Periods 3 and 4			
Ground sudangrass hay	512	528	528
Ground corn	333	206	138
Soybean meal 45%	–	–	189
Fish meal 60%	–	137	–
Quebracho tannin[a] (powder)	100	100	100
Mineral–vitamin premix[b]	55	29	45
TDN[c], %	55	55	55
ME[c], Mcal/kg	1.99	1.99	1.99
NEm[c], Mcal/kg	1.28	1.28	1.28
NEg[c], Mcal/kg	0.78	0.78	0.78
CP[d], g/kg	50	125	125
RDP, % CP	62.2	44.8	65.9
RUP, % CP	37.6	55.2	34.1
CP/ME ratio, g/Mcal	28	70	70

[a] Control: no added protein; RUP (rumen undegradable protein): with fish meal added; RDP (rumen degradable protein): with soybean meal added.
[b] Mineral–Vitamin Premix composition: Control: Ca 19%, P 6%, Na 5%, S 4%, I 11 ppm, Se 3 ppm, Vit A 76001 IU/kg. RUP: Ca 16%, Na 9%, S 6%, I 23 ppm, Se 7 ppm, Vit A 163,493 IU/kg, Vit E 1245 IU/kg. RDP: Ca 21%, P 5%, Na 5%, S 3%, I 13 ppm, Se 4 ppm, Vit A 90,291 IU/kg, Vit E 688 IU/kg.
[c] TDN, ME, NEm, NEg values were based on NRC (1985).
[d] Calculated as 6.25 × % N (CN-2000, LECO Corp., St. Joseph, MI).

soybean meal (RDP), or (3) added fish meal (RUP). Basal diets were isoenergetic and only varied in amount and degradability of protein (Table 2). During periods 1 and 2, basal diets contained 10% finely ground wheat straw (<5 mm) (Table 2) which was replaced with quebracho tannin (QT) powder in periods 3 and 4 (Table 2). Wheat straw was included to simulate the physical properties of QT powder while maintaining energy and protein concentrations within treatments comparable across periods (Table 2).

2.3. Juniper plant material

One-seed juniper was hand-harvested at the Corona Range and Livestock Research Center, located 321 km from the experimental facility. Harvest was conducted at three different times during the fall (early November), 2 months before the study. Current season's leaves and twigs from juniper saplings (<1 m tall) were clipped, placed in polyethylene bags and frozen within 5 h of collection to avoid excessive volatilization of terpenes (Utsumi et al., 2006). Saplings within this size class are the suggested target of programs that use small ruminants to control one-seed juniper (Utsumi et al., 2010). Frozen juniper material was later chopped into 1–2-cm segments, mixed for uniformity, placed in plastic bags, and returned to the freezer. During the study, bags with juniper were removed from the freezer daily, thawed, and fed to animals. Samples of juniper material fed were composited by week ($n = 9$), dried at 60 °C for 48 h, ground to <1 mm particle size, and analyzed for nutritive value. The juniper fed was 50.6 ± 1.1% DM (AOAC, 1990), 7.4 ± 0.2% CP (AOAC, 1990), 36.6 ± 0.6% NDF (Van Soest et al., 1991), 32.1 ± 0.9% ADF (Goering and Van Soest, 1970), and 63.3 ± 0.7% in vitro true digestibility (Goering and Van Soest, 1970). The terpenoid profile of the juniper material fed was determined on 21 composite samples (7 composite samples per harvesting batch) as described in Utsumi et al. (2006). This analysis (data not shown) revealed a highly diverse terpene profile composed of a mixture of 53 mono- and sesquiterpenes, diterpenes, and 2 unknown compounds in the juniper fed (GCMS analysis; Tellez et al., 1997).

2.4. Condensed tannins and polyethylene glycol

Condensed tannins in the form of QT powder (74.8% CT; Chemtan Co., Inc., Exeter, NH) were mixed into the basal diet (10%, DM basis; Table 2) based on data suggesting this concentration of CT alters intake and preference of sheep and goats (Villalba et al., 2002b). Polyethylene glycol (molecular weight 6000, Sigma Aldrich, St. Louis, MO) was used in period 4 (50 g per animal daily; Table 1). This molecular weight has been reported to permanently inactivate tannins in feedstuffs at near neutral pH (i.e., rumen pH) (Makkar, 2003), and this dose has been shown to neutralize CT and increase digestion and intake of several tannin-rich shrubs by sheep and goats (Makkar, 2003).

2.5. Experimental protocol

The same feeding and sampling protocol was used in periods 1 to 4. Each period consisted of a 15 day intake experiment followed by a 3 day preference test and intake was calculated as the difference between amounts offered and rejected (Table 1). During the first 15 d of periods 2 through 4, animals were individually fed juniper leaves and twigs daily from 07:00 to 10:00 h at 10% above previous day's intake (no juniper was offered during the first 15 days of period 1). Juniper refusals were collected and weighed. Animals were fed corn or corn + RDP or RUP (without or with QT in periods 2 and 3, respectively) (Table 1) at 10% above previous day's intake at 10:00 h. At 13:00 h, refusals were collected and weighed (Setra Super IITM, Setra Systems, Inc., Boxborough, MA). In period 4, 50 g of PEG 6000 mixed with 50 g of ground corn were fed at 13:00 h immediately after refusals of the previous feeding phase were removed. Animals consumed PEG completely within 2 min of being fed.

All animals were fed ground sudangrass hay (<1 cm particle size) ad libitum for 3 h after basal diet refusals were removed. Sudangrass refusals were collected at 16:00 h and weighed and no other food was offered until the next day.

On the last 3 days of each period, preference for juniper was examined. All animals received a choice of the basal diet and juniper from 08:00 to 09:00 h. Refusals were collected and weighed, and preference for juniper was calculated as [(juniper consumed/total intake)/(juniper offered/total feed offered)]. At 10:00 h, animals were allowed ad libitum access to sudangrass for 3 h.

2.6. Rumen fluid and blood sampling analyses

Jugular blood samples were collected at 07:00, 10:00, 13:00, 16:00, and 19:00 h. Sampling times coincided with 0 (immediately before feeding), 3, 6, 9, and 12 h after feeding juniper in periods 2–4. Samples were collected in 10-mL 15% EDTA-coated vacuum tubes (Kendall, Ontario, CA), centrifuged at 1500 × g for 25 min at 10 °C (Sorvall RT6000, Thermo Electron Corp., Asheville, NC), plasma was decanted, transferred with pipettes into 7-mL plastic vials, and frozen at −20 °C until analysis. Solid-phase extraction of AA was conducted using a commercial kit (EZ:FAAST; Phenomenex, Torrence, CA) as described by Waggoner et al. (2009). Plasma AA were analyzed with a gas chromatograph/flame ionization detector (CP-3800, Varian, Walnut Creek, CA). The injection protocol was 2 μL injection volume, ZB-AAA 10 m × 0.25 mm i.d. column, split ratio 1:15, injector temp. 250 °C, detector temp. 320 °C, temp. program: 110–320 °C at 32 °C/min, and helium (1.5 mL/min) carrier gas. The detection limit for all analyses was 1 μM (EZ:FAAST, Phenomenex, Torrence, CA) and the inter- and intra-assay, respectively, CV were: alanine (Ala) = 3 and 6, glycine (Gly) = 3 and 7, valine (Val) = 7 and 9, leucine (Leu) = 2 and 7, isoleucine (Ile) = 4 and 8, threonine (Thr) = 8 and 14, serine (Ser) = 16 and 16, proline (Pro) = 4 and 5, asparagine (Asn) = 14 and 16, aspartic acid (Asp) = 14 and 17, methionine (Met) = 9 and 16, glutamic acid (Glu) = 13 and 17, phenylalanine (Phe) = 12 and 14, cysteine (Cys) = 21 and 21, glutamine (Gln) = 21 and 24, lysine (Lys) = 13 and 19, histidine (His) = 20 and 20, tyrosine (Tyr) = 14 and 21, and tryptophan (Trp) = 14 and 17%.

Rumen fluid was collected at 07:00, 13:00, and 19:00 h, coinciding with 0 (immediately before feeding), 6, and 12 h after feeding juniper in periods 2–4. Rumen fluid (200 mL) was collected with a stomach tube and a vacuum pump, pH was measured immediately with portable meter (glass electrode), and then fluid was acidified with 1 mL of 10 N HCl. Acidified samples were centrifuged at 27,000 × g for 10 min at 10 °C, and the supernatant was separated, placed in sealed bags, and frozen at −20 °C until analysis. Following procedures outlined by May and Galyean (1996), an aliquot was mixed with meta-phosphoric

acid/2-ethyl butyric acid in a 5:1 ratio, centrifuged for 10 min, and transferred into an autosampler vial for analysis on a gas chromatograph with flame ionization detector (Star 3400, Varian Inc., Walnut Creek, CA). The injection protocol was 0.5 μL injection volume, a capillary column (30 m × 0.32 mm i.d.; Alltech), injector temp. 250 °C, detector temp. 250 °C temp. program: 90 °C for 1 min, increase to 140 °C at 6 °C/min, increase to 240 °C at 25 °C/min and 240 °C for 1 min, and helium (2 mL/min) carrier gas. Detection limits were: acetate = 0.024, propionate = 0.012, butyrate = 0.008, iso-butyrate, valerate, and iso-valerate = 0.007 mM). Inter- and intra-assay, respectively, CV were: acetate and propionate = 2 and 5, butyrate, isobutyrate, and valerate = 1 and 4, and isovalerate = 1 and 3%.

2.7. Statistical analysis

Intake and juniper preference responses were analyzed using the MIXED procedure of SAS 9.1.3 (2004; SAS Institute Inc., Cary, NC). Animals nested within basal diet (control, RDP, and RUP) × animal species (sheep and goats) was the whole plot factor, and period or day was the subplot factor. Juniper preference ratio was averaged across days before analyses and tested with a model including basal diet (Control, RDP, and RUP) × animal species (sheep and goats) as a whole plot factor, and period as the subplot (repeated measure) factor. Intake data were expressed on a metabolic body size basis ($BW^{0.75}$) to correct for body size differences among animals prior to preference analyses.

Rumen fermentation and plasma AA responses were also analyzed using the MIXED procedure of SAS 9.1.3 (2004; SAS Institute Inc., Cary, NC). Rumen VFA, pH, and plasma AA were analyzed separately for sheep and goats, with animals nested within treatment diet (Control, RDP, and RUP) as the whole plot factor. Plasma AA, rumen VFA, and pH were pooled across sampling hours for analyses. Rumen VFA and pH were also analyzed across sampling times (h).

When significant F values were detected ($P \leq 0.05$), mean separation was conducted using LSD ($\alpha = 0.05$); F tests with $P \geq 0.05$ and $P \leq 0.10$ were interpreted as indicating a tendency toward statistical significance. Fit for best covariance structure was tested (Littell et al., 1998). Kenward–Rogers method was used to compute degrees of freedom (Schaalje et al., 2001).

3. Results

3.1. Intake of basal diets, sudangrass, and juniper

Variation in basal diet intake was greater across periods than between species, protein sources (Table 3) or from day to day within each period. Basal diet intake of sheep and goats dropped ($P < 0.01$) during periods 2 and 3 when juniper and juniper + QT were offered, but was partially restored when juniper + QT was followed by PEG ingestion in period 4. Averaged across basal diets, animal species, and days, animals consumed 37, 31, 20, and 25 ± 1.5 g/kg $BW^{0.75}$ of basal diet in periods 1–4, respectively. Sheep consumed more ($P < 0.01$) basal diet than goats throughout the study, but absolute differences between animal species decreased ($P < 0.03$) when juniper + QT (without or with PEG) was fed in periods 3 and 4, respectively. Rumen degradable protein was associated with greater ($P < 0.01$) basal diet intake in sheep and goats in period 3 and period 4. No basal diet × animal species × day interaction ($P > 0.07$) was detected for basal diet intake during any period, but basal diet intake of sheep and goats varied ($P < 0.01$) across days within periods. In period 3, a basal diet × day interaction ($P = 0.01$) indicated a sharp basal diet intake decrease after initial exposure to QT followed by a slight intake recovery for sheep and goats fed RDP.

Sudangrass intake also exhibited significant variation ($P < 0.01$) across periods (Table 3). Averaged across basal diets, animal species, and days, animals consumed 18, 11, 19, and 17 ± 1.0 g/kg $BW^{0.75}$ of sudangrass in periods 1–4, respectively. Sudangrass intake by sheep and goats did not differ ($P > 0.48$) during periods 1–3, but sheep consumed more ($P < 0.01$) sudangrass than did goats in period 4. During period 1, sheep fed RDP or RUP had increased ($P = 0.03$) sudangrass intake and both sheep and goats fed RUP consumed more ($P < 0.01$) sudangrass than those fed RDP in periods 3 and 4 (Table 3). No animal species × basal diet × day interaction ($P > 0.71$) was detected for sudangrass intake in any period. Regardless of basal diet, intake of sudangrass by sheep or goats varied ($P < 0.01$) among days in each period, but variations were least noticeable in period 2.

Juniper intake by sheep and goats also varied ($P < 0.01$) among periods (Table 3) and across days ($P < 0.05$) within periods, regardless of basal diet. Averaged across basal diets, animal species and days, animals consumed 5, 4, and 11 ± 0.7 g/kg $BW^{0.75}$ of juniper in periods 2–4, respectively. Greater ($P < 0.01$) detected in period 4 when juniper intake was expressed as percent of total intake. Juniper intake was 10, 10, and 20 ± 1.3% of total intake in periods 2–4, respectively. In period 2, juniper intake did not differ ($P = 0.49$) between sheep and goats or among basal diets ($P = 0.88$), but varied ($P < 0.01$) across days. In period 2, goats exhibited a cyclic (1–3 d) juniper intake pattern while sheep increased intake as the period progressed, which explains the animal species × day interaction ($P < 0.01$) detected in period 2. In period 3, goats fed RDP tended to consume more ($P = 0.07$) juniper than controls (7 vs. 2 ± 1.1 g/kg $BW^{0.75}$) and goats fed RUP exhibited slightly greater ($P = 0.09$) juniper intake than controls (4 vs. 2 ± 1.1 g/kg $BW^{0.75}$). Juniper intake by both animal species increased ($P < 0.01$) during the last few days of period 3 but the increase was greater ($P < 0.03$) for RUP and RDP than control. No basal diet × animal species × day interaction ($P = 0.60$) was detected for juniper intake in period 3. In period 4, PEG tended ($P = 0.08$) to stimulate juniper intake in sheep and goats fed RDP or RUP (12 ± 1.2 g/kg $BW^{0.75}$) compared to controls (8 ± 1.2 g/kg $BW^{0.75}$). An animal species × day interaction ($P = 0.02$) during period 4 reflected a consistent tendency of sheep to increase juniper intake over time, whereas goats exhibited a much more erratic pattern of juniper intake across days. No basal diet × animal species × day interaction ($P = 0.33$) was detected for juniper intake in period 4.

3.2. Juniper preference

No basal diet × animal species × period interaction ($P = 0.75$) was observed for juniper preference ratio. Juniper preference ratio was not affected ($P = 0.47$) by basal diets or prior exposure to juniper ($P = 0.61$), but differed ($P < 0.01$) between sheep and goats. Sheep consistently avoided juniper and instead selected the alternative basal diet with (periods 3 and 4) or without QT (periods 1 and 2), when offered the choice goats, however, showed preferential consumption of juniper ($P < 0.01$) over basal diets

Table 3
Feed intake of sheep and goats throughout experiment periods.

Variable	Treatment groups[a]												SEM[c]	*P* Value[d]		
	CTRL				RDP				RUP							
	Period[b]				Period				Period							
	1	2	3	4	1	2	3	4	1	2	3	4		Trt	Pd	Trt x Pd
Sheep																
Ingredient, g/kg^{-075}																
Juniper	NA[e]	3.3	3.8	9.0	NA	5.1	4.2	10.5	NA	4.8	5.2	12.9	1.4	0.494	<0.0001	0.020
Basal diet	40.6	36.9	21.3	24.1	49.5	39.4	27.5	36.3	45.1	40.0	22.5	26.0	3.1	0.255	<0.0001	<0.0001
Sudangrass	14.7	10.6	17.3	18.2	19.3	10.4	17.4	16.4	21.4	12.5	20.9	20.9	1.4	0.182	<0.0001	<0.0001
Total	55.3	50.8	42.4	51.2	68.8	54.9	49.0	63.3	66.4	57.3	48.5	59.9	4.4	0.311	<0.0001	0.001
Nutrients																
CP	2.9	2.7	2.3	2.9	7.3	5.9	4.8	6.3	6.9	6.1	4.4	5.4	0.4	0.003	<0.0001	<0.0001
ME, Mcal/kg$^{-0.75}$	0.102	0.095	0.078	0.096	0.127	0.103	0.091	0.119	0.122	0.107	0.089	0.112	0.008	0.302	<0.0001	0.001
Goats																
Ingredient, g/kg^{-075}																
Juniper	NA[e]	5.6	2.0	7.9	NA	4.6	6.6	13.0	NA	5.0	3.8	11.7	1.3	0.267	<0.0001	<0.0001
Basal diet	28.2	23.5	15.7	19.7	32.2	28.0	20.4	29.8	24.6	21.0	13.1	16.8	3.1	0.171	<0.0001	<0.0001
Sudangrass	18.4	13.3	19.6	16.3	16.1	9.8	17.0	12.6	19.5	12.2	20.1	17.0	1.2	0.130	<0.0001	0.054
Total	46.6	42.4	37.3	43.8	48.4	42.3	44.1	55.4	44.2	38.2	37.0	45.5	3.6	0.432	<0.0001	<0.0001
Nutrients																
CP	2.5	2.4	2.1	2.5	5.0	4.4	4.0	5.4	4.2	3.7	3.1	4.0	0.4	0.003	<0.0001	<0.0001
ME, Mcal/kg$^{-0.75}$	0.085	0.079	0.067	0.082	0.089	0.079	0.082	0.105	0.080	0.071	0.067	0.085	0.007	0.365	<0.0001	<0.0001

[a] Basal diets: no protein added (Control, 5% CP) or soybean meal (RDP, 12.5% CP) or fish meal (RUP, 12.5% CP) added.
[b] From periods (Pd) 1 to 4, sheep had no exposure (period 1) or were exposed to juniper only (period 2), juniper and quebracho tannins (period 3) and juniper, quebracho tannins and PEG (period 4).
[c] Standard error of means of 4 sheep or goats per treatment diet group.
[d] *P* value for the ANOVA *F* test of treatment diet, periods, and its interaction.
[e] Not applicable.

containing QT in period 4. Averaged across basal diets, juniper preference ratio in periods 1–4 were 0.0, 0.1, 0.3, 0.4 ± 0.1 for sheep and 0.1, 0.1, 0.4, 1.4 ± 0.1 for goats, respectively.

3.3. Rumen pH and volatile fatty acids

Rumen pH in sheep tended to decrease from period 1 to period 2 and to recover in periods 3 and 4 regardless of treatment diets (Table 4). In goats, rumen pH decreased from period 1 to period 2 before increasing in periods 3 and 4 in animals fed control and RDP diets (Table 5). This pattern of pH change was likely associated with variations in VFA concentrations, as total VFA, acetate, propionate, and butyrate concentrations in sheep (Table 4) and goats (Table 5) peaked in period 2, and then decreased in periods 3 and 4. In contrast, concentrations of the branched chain VFA, isobutyrate and isovalerate, were lower in period 2 for both species and recovered in period 3 and 4 in goats but not in sheep (Tables 4 and 5). In goats, valerate concentrations tended to be lower in period 1 vs. later periods (Table 5). In sheep, valerate concentrations differed across periods for the RUP-containing diet (Table 4).

Basal diets influenced concentrations of VFA and rumen pH in sheep and goats differently. Concentrations of total VFA and acetate tended to be greater in goats fed RUP and RDP than controls ($P < 0.14$; Table 5), whereas sheep fed RDP had greater butyrate ($P < 0.05$) and tended to have greater total VFA concentrations ($P < 0.10$) than controls (Table 4). Averaged across periods, concentrations of total VFA and acetate in goats fed control, RDP, and RUP were 60.9, 63.4, and 65.3 mM (SEM = 1.5), and 46.4, 47.9, and 50.8 mM (SEM = 1.3), respectively. Mean concentrations of total VFA and butyrate in sheep fed control, RDP, and RUP diets were 68.2, 73.8, and 67.0 mM (SEM = 2.2) and 5.6, 6.7, and 5.3 mM (SEM = 0.3), respectively. No basal diet effects were detected for propionate concentration in either sheep (Table 4) or goats (Table 5). Rumen fluid of sheep and goats fed the RDP diet contained greater concentrations of isobutyrate and isovalerate than those fed RUP or control ($P < 0.05$) diets (Tables 4 and 5). Compared to animals fed the control diet, valerate concentrations tended to be greater for goats fed RDP and RUP in all periods (Table 5) and greater for sheep fed RDP in periods 1–3 ($P < 0.05$) and sheep fed RUP in periods 2 and 4 ($P < 0.05$; Table 4). With the exception of valerate in sheep, total and individual VFA concentrations were not affected by interactions between basal diet and period for either species (Tables 4 and 5).

In period 1, concentrations of total VFA, acetate, propionate, and butyrate were lowest at 12 h ($P < 0.01$) for both species. In periods 2 and 3, concentrations of total VFA, acetate, and butyrate were not different at 0, 6, or 12 h after feeding juniper ($P > 0.10$). Conversely, propionate concentration in periods 2 and 3 peaked 12 h after animals ingested juniper ($P < 0.01$). In period 4, total VFA, acetate, propionate, and butyrate decreased at 6 h, but recovered and exhibited a delayed peak at 12 h ($P < 0.01$). In period 1, rumen pH tended to decrease at the 6 and 12 h sampling ($P = 0.09$). During periods 2–4, rumen pH exhibited a transient decrease 6 h after juniper ingestion but returned to original values 12 h post juniper ingestion ($P < 0.01$). In periods 1–3, time patterns in rumen pH, total VFA, and molar proportions of acetate, propionate, and butyrate did not differ by species ($P > 0.21$) or treatment diets ($P > 0.16$); however, in period 4, concentration of propionate tended to be greater for sheep ($P = 0.08$) and total VFA and acetate greater for goats at 12 h ($P < 0.03$), and for goats fed RUP than counterparts fed control or RDP ($P < 0.01$).

Table 4
Sheep ruminal pH and VFA in relation to basal diets with or without added protein before or after being fed juniper, quebracho tannins, and polyethylene glycol.[a]

Variable	Treatment diet[b]												SEM[d]	*P* value[e]		
	Control				RDP				RUP							
	Period[c]				Period				Period							
	1	2	3	4	1	2	3	4	1	2	3	4		BD[f]	Pd[g]	BD × Pd
pH	6.52	6.45	6.57	6.66	6.48	6.46	6.53	6.64	6.62	6.56	6.68	6.66	0.08	0.17	0.09	0.98
VFA, mM																
Total	65.56	78.29	68.91	59.94	64.88	89.36	77.12	63.97	66.49	73.26	65.82	62.38	4.63	0.10	<0.01	0.60
Acetate	48.21	58.91	49.52	43.70	45.65	66.00	56.14	46.65	48.74	53.53	49.14	46.10	3.72	0.26	<0.01	0.49
Propionate	9.89	11.71	12.68	10.12	10.02	13.30	12.39	9.89	10.19	11.92	10.37	9.80	0.90	0.38	0.01	0.70
Butyrate	5.82	6.43	5.43	4.86	6.71	7.90	6.63	5.36	5.52	6.01	4.85	4.95	0.45	0.01	<0.01	0.69
Isobutyrate	0.54	0.37	0.36	0.34	0.86	0.69	0.58	0.68	0.67	0.58	0.41	0.44	0.06	<0.01	<0.01	0.53
Valerate	0.32	0.30	0.33	0.37	0.47	0.48	0.48	0.41	0.37	0.47	0.33	0.42	0.02	<0.01	0.37	0.01
Isovalerate	0.77	0.57	0.59	0.54	1.17	0.99	0.89	0.99	0.99	0.75	0.71	0.68	0.07	<0.01	<0.01	0.87

[a] Values are average mean for 12 ewes (4/treatment) sampled on day 15 of periods 1–4 at sampling times equivalent to 0, 6, and 12 h post supplement feeding.
[b] Diet treatments had no protein added (Control, 5% CP) or soybean meal (RDP, 12.5% CP) or fish meal (RUP, 12.5% CP) added.
[c] From periods 1 to 4, sheep had no exposure (period 1) or were exposed to juniper only (period 2), juniper and quebracho tannins (period 3) and juniper, quebracho tannins and PEG (period 4).
[d] Standard error of means of 4 sheep per treatment diet group.
[e] *P* value for the ANOVA *F* test of treatment diet, periods, and its interaction.
[f] BD indicates basal diet (Control, RDP, or RUP).
[g] Pd indicates period (1; 2; 3; or 4).

Table 5
Goat ruminal pH and VFA in relation to basal diets with or without added protein before or after being fed juniper, quebracho tannins, and polyethylene glycol.[a]

Variable	Treatment diet[b]												SEM[d]	*P* value[e]		
	Control				RDP				RUP							
	Period[c]				Period				Period							
	1	2	3	4	1	2	3	4	1	2	3	4		BD[f]	Pd[g]	BD × Pd
pH	6.49	6.33	6.70	6.64	6.45	6.27	6.62	6.63	6.47	6.33	6.76	6.59	0.06	0.69	<0.01	0.02
VFA, mM																
Total	61.03	75.14	54.42	52.96	62.99	80.26	57.33	53.18	62.52	79.88	54.13	64.71	3.93	0.14	<0.01	0.65
Acetate	47.26	57.69	40.37	40.08	46.88	63.31	41.56	39.67	48.47	63.71	40.66	50.25	3.12	0.07	<0.01	0.56
Propionate	7.67	11.34	8.78	7.83	9.30	11.03	9.38	7.41	8.49	10.74	8.65	8.84	0.85	0.81	<0.01	0.74
Butyrate	4.79	5.06	3.91	3.74	4.94	4.69	4.36	4.44	4.10	4.28	3.27	4.11	0.36	0.20	0.01	0.34
Isobutyrate	0.46	0.30	0.46	0.44	0.64	0.39	0.67	0.65	0.50	0.35	0.54	0.51	0.05	0.01	<0.01	0.73
Valerate	0.23	0.27	0.28	0.30	0.31	0.29	0.37	0.32	0.23	0.31	0.29	0.33	0.03	0.06	0.07	0.29
Isovalerate	0.61	0.48	0.62	0.57	0.92	0.55	0.98	0.70	0.73	0.50	0.73	0.67	0.06	<0.01	<0.01	0.21

[a] Values are mean for 12 does (4/treatment) on day 15 of periods 1–4 at sampling times equivalent to 0, 6, and 12 h post supplement feeding.
[b] Diet treatments had no protein added (Control, 5% CP) or soybean meal (RDP, 12.5% CP) or fish meal (RUP, 12.5% CP) added.
[c] From periods 1 to 4,goats had no exposure (period 1) or were exposed to juniper only (period 2), juniper and quebracho tannins (period 3) and juniper, quebracho tannins and PEG (period 4).
[d] Standard error of means of 4 goats per supplementation group.
[e] *P* value for the ANOVA *F* test of supplement, periods, and its interaction.
[f] BD indicates basal diet (Control, RDP, or RUP).
[g] Pd indicates period (1; 2; 3; or 4).

3.4. Plasma AA

Several essential and nonessential AA concentrations varied among basal diet treatments and periods in both species. Concentrations of Met, Asp, Glu, and Asn decreased from period 1 to 4 in both sheep (Table 6) and goats (Table 7). Concentration of Cys, Thr, Ser, and Gly decreased from period 1 to periods 2 and 3, and increased in period 4 in both sheep (Table 6) and goats (Table 7). Concentration of Phe also decreased from period 1 to periods 2 and 3 but did not rebound in period 4 in either sheep (Table 6) or goats (Table 7). The concentration of Gln was more erratic across periods and peaked in period 3 in both sheep (Table 6) and goats (Table 7). Concentrations of Val, Leu, Tyr, and Trp in goat plasma also decreased from period 1 to periods 3 and 4 (Table 7). Plasma Lys and His concentrations in sheep were greater in period 3 than in other periods (Table 6). The concentration of His exhibited a similar pattern across periods in goats, albeit marginally detectable (Table 7).

Sheep fed RDP and RUP had greater concentrations of the branched chain amino acids (BCAA) Val, Leu, and Ile than control sheep in period 3 ($P < 0.05$), and sheep fed RUP had greater concentrations of BCAA than those fed control or RDP in period 4 ($P < 0.05$; Table 6). Plasma Thr differed across periods in sheep similarly to BCAA (Table 6). During periods 1 and 2, goats that received RDP had greater concentrations of Val and Leu than control goats ($P < 0.05$), but their concentrations did not differ from controls in periods 3 and 4 ($P > 0.05$) (Table 7). Goats supplemented with RDP also had elevated concentrations of Ile compared to control goats ($P < 0.05$); however, concentrations of Ile did not differ across periods as did other BCAA (Table 7). Goats fed RUP also exhibited higher plasma concentrations of Val, Leu, and Ile than controls ($P < 0.05$), although differences were only significant in periods 1–3 (Table 7). Plasma Trp and Asp were greater for goats fed RDP and RUP than controls ($P < 0.05$) in period 1, but did not differ in periods 2–4 (Table 7). Plasma Ser of goats fed RDP was greater than other treatment diets in period 1, but not in periods 2, 3, or 4 (Table 7). Plasma Glu concentration was higher for goats fed RUP in period 1, but lower for goats fed RDP in periods 2 and 3 and greater for goats fed RDP and RUP in period 4 (Table 7). In sheep (Table 6), His and Ala concentrations were greater for RUP and RDP groups than for controls ($P < 0.05$), and Met was greater for RUP than RDP or controls ($P < 0.05$). In goats (Table 7), RUP, and to a lesser extent RDP, increased concentrations of plasma Gly over controls ($P < 0.05$) and goats fed RDP had greater concentrations of Asn than controls ($P < 0.05$).

4. Discussion

4.1. Juniper intake and preference

Contrary to our first prediction, adding RDP and RUP to sheep and goat diets in this study did not stimulate intake or alter preference for one-seed juniper. Both sheep and goats exhibited low preference ratio for juniper regardless of type of protein added. Lack of response to protein treatments may be due to incomplete rumen microbial adaptation to terpenes (Oh et al., 1967), a lack of induction of terpene detoxification (Boyle and McLean, 2004), and/or (most likely) because high concentrations of terpenes, phenolics and condensed tannins in juniper fall material overshadowed any beneficial effects of protein (Utsumi et al., 2009). The fall-harvested juniper material used in this experiment contained a diverse array of hydrocarbon and oxygenated monoterpenes, sesquiterpenes, and di-terpenes. Many of these individual compounds, including those present at relatively low concentration such as α-thujene, α-phellandrene, β-eudesmol, γ-eudesmol, limonene, and β-phellandrene have been reported to reduce juniper intake by sheep and goats receiving RDP and RUP protein supplements (Utsumi et al., 2009). Thus,

Table 6
Sheep plasma AA in relation to basal diets with or without added protein before or after being fed juniper, quebracho tannins, and polyethylene glycol.[a]

AA, μM	Supplement treatment[b]												SEM[d]	*P* value[e]		
	Control				RDP				RUP							
	Period[c]				Period				Period							
	1	2	3	4	1	2	3	4	1	2	3	4		BD[f]	Pd[g]	BD × Pd
EAA[h]																
Lys	144.7	115.3	126.7	92.3	124.2	103.1	171.4	111.4	148.3	191.8	173.8	129.1	19.1	0.10	0.01	0.07
Met	18.1	15.0	11.3	10.6	13.9	12.0	13.4	12.1	18.3	18.3	16.2	14.8	1.2	0.02	<0.01	0.07
Cys	3.8	3.6	1.9	3.7	4.3	2.6	3.0	4.5	4.0	2.5	2.3	3.5	0.5	0.33	<0.01	0.33
His	68.5	67.1	92.1	72.7	72.2	82.8	125.6	91.0	83.0	93.9	110.9	84.2	8.6	0.05	<0.01	0.52
Val	176.8	147.4	126.6	153.1	161.6	152.6	184.5	156.7	168.0	191.2	190.8	214.0	15.4	0.06	0.80	0.03
Ile	67.8	59.6	58.2	59.5	63.7	63.9	77.0	66.0	67.6	74.9	74.2	85.0	5.2	0.03	0.69	0.07
Leu	104.2	90.5	82.5	88.6	89.0	88.5	109.7	95.9	106.7	111.6	112.8	118.4	7.6	0.03	0.78	0.09
Phe	49.3	46.6	33.8	31.1	50.7	38.5	41.7	38.4	60.4	50.2	42.2	38.2	3.8	0.21	<0.01	0.10
Tyr	58.6	55.5	53.1	50.2	61.4	49.1	64.7	58.1	69.7	64.6	57.6	58.1	6.6	0.49	0.28	0.40
Thr	111.9	93.4	54.6	72.6	136.1	100.6	81.0	70.6	102.7	115.3	81.2	105.0	9.3	0.16	<0.01	0.01
Trp	47.6	45.9	40.5	39.4	49.6	39.1	56.7	45.6	60.8	48.7	51.4	52.6	5.2	0.13	0.10	0.24
NEAA[h]																
Asp	4.2	3.8	3.0	2.3	3.5	2.6	3.4	2.7	4.5	3.3	3.4	3.1	0.5	0.59	0.02	0.52
Ser	73.6	58.7	33.1	54.2	93.5	67.6	42.7	70.1	76.4	66.8	28.1	57.9	6.4	0.06	<0.01	0.74
Glu	203.0	161.9	108.9	86.9	199.8	153.0	108.7	106.8	208.3	172.7	110.2	103.0	12.5	0.63	<0.01	0.93
Pro	95.2	86.6	81.9	85.9	88.6	83.3	103.2	98.1	102.4	102.7	99.3	108.5	6.7	0.06	0.61	0.28
Gly	356.0	342.8	451.9	387.5	358.2	366.6	491.1	497.8	430.0	352.8	364.6	364.9	39.1	0.40	0.01	0.11
Ala	214.9	214.5	206.3	169.0	201.2	195.4	205.2	231.8	260.0	237.5	244.6	213.3	16.6	0.01	0.51	0.20
Asn	44.6	41.2	37.3	35.5	52.9	38.4	48.4	42.0	49.4	46.2	41.9	41.8	3.5	0.17	0.01	0.13
Gln	222.4	238.9	285.5	225.1	249.7	204.4	328.1	281.4	263.0	264.2	298.0	238.8	21.9	0.36	<0.01	0.24
Total	2201.1	1998.1	1973.0	1797.1	2194.7	1945.9	2382.2	2171.4	2407.0	2361.3	2209.6	2135.0	126.8	0.10	0.04	0.13

[a] Values are mean values of 12 ewes (4/treatment) sampled on day 14 of periods 1–4 at sampling times equivalent to 0, 3, 6, 9 and 12 h post supplement feeding.
[b] No additional protein (Control, 5% CP) or soybean meal (RDP, 12.5% CP) or fish meal (RUP, 12.5% CP) added.
[c] From periods 1 to 4, sheep had no exposure (period 1) or were exposed to juniper only (period 2), juniper and quebracho tannins (period 3) and juniper, quebracho tannins, and PEG (period 4).
[d] Standard error of means for 4 sheep per supplementation group.
[e] *P* value for the ANOVA *F* test of supplement, periods and its interaction.
[f] BD indicates basal diet (Control, RDP, or RUP).
[g] Pd indicates period (1; 2; 3; or 4).
[h] EAA, essential amino acids; NEAA, non-essential amino acids.

concentration of these compounds in juniper collected in the fall may explain the lack of response to protein supplements and potential associative effects of juniper on the intake of companion feed ingredients. Juniper ingestion in this study was accompanied by reduced intake of both basal diet and sudangrass.

Addition of CT through QT supplementation depressed voluntary intake of basal diets but increased intake of sudangrass hay and juniper of both sheep and goats which was contrary to our second prediction. Both sheep and goats exhibited greater preference ratio for juniper when offered the choice between juniper and a basal diet containing QT. Rather than the predicted antagonism between the two types of PSMs, these results support the concept of diet complementarity (Villalba et al., 2004). The daily meal sequence (juniper in the morning and quebracho tannins at noon) and the high level of QT fed in this study (10% QT) may have been responsible for low levels of juniper intake in animals during initial days of period 3. Mote et al. (2007a) reported that lambs offered a meal of QT followed by a meal of terpenes (camphor, 1,8-cineole, and *p*-cymene) delivered in a high protein diet (12.6% CP) consumed more of the feed containing terpenes than when exposed to same feeds in reverse sequence, as in this study. Mote et al. (2007b) also found that feeding a restricted amount of tannins appeared to maximize the positive effect of high protein foods on terpene–tannin mixing, possibly because low tannin doses neutralize terpenes (Provenza, 2008) or improve by-pass nitrogen and amino acid status (Waghorn et al., 1987) to help detoxify terpenes (Illius and Jessop, 1995).

Adding PEG partially offset the negative effects of tannins on intake of basal diets, especially in animals receiving RDP. Juniper intake increased from 10 to 20% when sheep and goats received PEG, with greater effects for RDP and RUP than the control treatment in both species. Thus, our third prediction was supported by this PEG-induced increase in juniper intake and the strong preference for juniper exhibited by goats during period 4. Although this increasing trend in juniper consumption could be influenced by an animal's tendency to gradually adapt to PSMs (such as terpenes) over time, PEG may have also suppressed CT-protein binding (Makkar, 2003), thereby increasing availability of AA to sustain terpene detoxification (Illius and Jessop, 1995). Inactivating reactive tannins via PEG may have enhanced the effects of dietary protein on juniper intake of sheep and goats, to the extent that goats preferred juniper to alternative foods that were high in protein and tannins when PEG was also fed. Thus, goats consuming target plants containing arrays of CT and terpenes may benefit

Table 7
Goat plasma AA in relation to basal diets with or without added protein before or after being fed juniper, quebracho tannins, and polyethylene glycol.[a]

AA, μM	Treatment diets[b]												SEM[d]	*P* value[e]		
	Control				RDP				RUP							
	Period[c]				Period				Period							
	1	2	3	4	1	2	3	4	1	2	3	4		BD[f]	Pd[g]	S × Pd
EAA[h]																
Lys	141.0	110.7	127.8	113.8	164.7	163.5	149.2	127.2	138.5	168.4	175.8	139.2	16.9	0.15	0.15	0.30
Met	18.6	16.5	14.1	13.3	21.0	20.5	14.8	13.1	19.1	18.9	15.0	12.4	1.2	0.33	<0.01	0.59
Cys	3.6	4.2	2.2	4.6	4.8	2.7	2.1	5.4	3.9	2.5	1.9	4.4	0.5	0.34	<0.01	0.14
His	70.1	64.1	91.4	75.5	82.5	92.4	96.1	80.4	83.7	69.0	87.9	75.0	8.9	0.17	0.11	0.73
Val	194.4	179.5	173.4	226.2	255.4	246.9	203.4	205.8	214.7	211.6	184.3	176.1	14.1	<0.01	0.04	0.05
Ile	69.6	67.6	72.3	82.3	89.4	87.2	78.4	85.3	79.7	81.4	78.7	74.5	4.5	<0.01	0.73	0.21
Leu	105.5	99.4	93.8	113.4	133.0	127.3	106.7	103.4	121.8	115.1	95.6	90.4	6.6	0.01	<0.01	0.05
Phe	50.0	46.0	33.4	29.5	60.2	42.9	31.3	31.9	57.5	43.0	32.8	28.3	2.8	0.74	<0.01	0.15
Tyr	50.4	47.7	46.0	44.1	60.1	47.0	45.2	46.4	57.5	44.4	45.3	45.4	4.5	0.81	0.01	0.79
Thr	65.0	61.5	39.1	53.3	83.9	78.2	44.2	51.8	67.3	77.8	41.2	46.2	5.8	0.19	<0.01	0.32
Trp	41.9	43.1	40.0	36.7	53.5	39.1	39.8	41.5	54.8	33.3	42.2	38.1	4.9	0.85	<0.01	0.02
NEAA[h]																
Asp	1.9	2.9	2.4	2.1	3.3	2.4	2.1	2.5	3.5	2.4	2.3	2.0	0.3	0.57	0.02	0.01
Ser	48.2	41.6	13.7	35.4	64.3	46.8	15.5	34.5	44.7	52.6	15.0	37.9	4.1	0.39	<0.01	0.02
Glu	134.5	131.6	99.5	58.5	138.3	96.0	94.2	76.3	165.9	128.1	91.2	84.5	9.6	0.18	<0.01	0.01
Pro	102.0	95.3	88.2	104.2	119.8	116.9	95.0	98.7	109.0	113.8	99.7	99.2	7.2	0.13	0.05	0.52
Gly	488.1	434.1	431.4	425.3	489.8	520.2	392.9	561.6	524.8	502.5	478.2	623.4	36.0	0.05	0.01	0.07
Ala	209.9	187.3	193.4	193.1	203.5	209.4	182.6	223.7	230.0	211.9	202.4	199.0	22.4	0.78	0.61	0.74
Asn	38.7	38.7	34.6	35.5	58.4	44.8	39.0	37.9	45.1	45.5	35.2	34.5	3.0	0.01	<0.01	0.09
Gln	245.2	218.7	269.8	211.6	270.2	271.3	282.7	244.7	253.3	257.8	295.1	248.5	20.8	0.12	0.05	0.95
Total	2183.4	1976.6	1951.9	1948.0	2519.3	2393.2	2006.5	2179.9	2398.1	2292.0	2111.1	2144.1	127.5	0.04	0.02	0.86

[a] Values are mean values of 12 does (4/treatment) sampled on day 14 of periods 1–4 at sampling times equivalent to 0, 3, 6, 9 and 12 h post supplement feeding.
[b] No additional protein (Control, 5% CP) or soybean meal (RDP, 12.5% CP) or fish meal (RUP, 12.5% CP) added.
[c] Goats had no prior exposure (period 1) or were exposed to juniper alone (period 2), juniper and quebracho tannins (period 3) and juniper, quebracho tannins, and PEG (period 4).
[d] Standard error of means for 4 goats per supplementation group.
[e] *P* value for the ANOVA *F* test of supplement, periods and its interaction.
[f] BD indicates basal diet (Control, RDP, or RUP).
[g] Pd indicates period (1; 2; 3; or 4).
[h] EAA, essential amino acids; NEAA, non-essential amino acids.

from minimizing dietary CT through addition of PEG and/or supplemental protein (Mote et al., 2007b; Provenza, 2008).

Interestingly, sheep gradually increased juniper intake over time, particularly when PEG was fed in period 4. Goats on the other hand, exhibited more cyclical patterns of juniper intake, particularly during initial exposure to juniper in period 2 and when PEG was fed in period 4. As selective grazers, sheep may consistently avoid consumption of PSM-containing plants. Conversely, goats generally show higher diet breath and may rapidly increase consumption of PSM containing plants based on their capacity to cope with and detoxify PSMs (Provenza et al., 2003). As reported in recent pharmacokinetic studies, terpene detoxification and final clearance from the body is described as a time dependent process (Dziba et al., 2006) and the cyclical juniper intake patterns detected in goats may have reflected cycles of PSM detoxification and satiety (Foley et al., 1999; Dziba et al., 2006). Thus, findings from this study appear to support the hypothesis that allelochemical satiety causes cyclical patterns of ingestion of terpenes and other absorbable allelochemicals (Provenza et al., 2003).

Overall patterns of intake observed in this experiment are consistent with previous studies reporting a depressor effect of terpenes on overall feed intake (Villalba et al., 2006) and beneficial effects of supplemental PEG on intake of feed containing high levels of rumen degradable protein and CT (Provenza et al., 2000; Villalba et al., 2002b).

4.2. Rumen fermentation

Basal diet intake patterns resulted in differences in VFA concentrations and rumen pH throughout the study. Overall, greater dietary protein elevated concentrations of total and all individual VFA except propionate (Tables 4 and 5), partially supporting the first prediction of our second hypothesis. Protein supplementation may have affected VFA concentrations by stimulating microbial fermentation of sudangrass basal diet either directly with ruminal AA when soybean meal was fed or indirectly via recycled nitrogen when fishmeal was fed (Van Soest, 1994).

Contrary to our expectations and despite a decrease in feed intake, initial exposure of sheep and goats to juniper in period 2 increased the concentration of acetate, propionate, butyrate, and total VFA; this effect was marginally greater for animals fed RDP (Tables 4 and 5). Broudiscou et al. (2007) reported that α-pinene, which can account for up to 65% of one-seed juniper terpenes (Utsumi et al., 2009), more than doubled (243%) the amount of hexoses fermented by goat rumen microorganisms. Broudiscou et al. (2007) also reported that camphene, limonene, myrcene,

β-ocimene, sabinene, and γ-terpinene, which together account over 11% of one-seed juniper terpenes in this study (data not shown), depressed by approximately half (56%) the amount of hexoses fermented. High dietary α-pinene concentration may offset the negative effects of less abundant mono- and sesquiterpenes and ultimately increase VFA concentrations.

The initial positive effect of juniper ingestion on VFA concentrations (regardless of type of protein fed) in period 2 disappeared when QT was added to diets in period 3 (Tables 4 and 5), partially supporting our prediction, but the depressing effect of QT was only transiently restored after PEG was offered in period 4. Antagonistic effects of CT on rumen fermentation are typically due to precipitation of proteins, and to a lesser extent, soluble carbohydrates, ultimately decreasing substrates for microbial digestion (McMahon et al., 2000; Silanikove et al., 2001; Makkar, 2003). When PEG was fed, CT appeared to be neutralized and acetate, propionate, and total VFA concentrations increased within a few hours after PEG ingestion, particularly in animals fed the RUP diet. Feeding PEG in period 4 (45–60 d of feeding on juniper) improved concentrations of VFA only momentarily, possibly because greater juniper intake during this last period (20% of diet) exceeded the rumen microbial detoxification capacity or alternatively, because of deleterious ruminal effects due to long-term exposure to terpenes (Cardozo et al., 2004). Total VFA production in this study appeared to decrease as a function of time. Sheep, and to a lesser extent goats, showed decreased levels of total VFA production after 60 days of receiving one-seed juniper.

4.3. Plasma AA

As predicted, feeding diets with additional protein increased plasma concentrations of various essential and nonessential plasma AA (Tables 6 and 7), which may have allowed animals to consume more juniper as the trial progressed. Plasma concentrations of a number of amino acids declined after animals began consuming juniper in period 2, and remained at low levels thereafter (Tables 6 and 7) even after basal diet intake recovered in period 4 (Table 3). These results suggest a close relationship between plasma AA concentrations and juniper ingestion. Adding RDP and especially RUP to diets increased concentrations of plasma AA (Tables 6 and 7), likely due to greater flow to the small intestine and absorption of AA (Theurer et al., 1966; Titgemeyer et al., 1989; Merchen and Titgemeyer, 1992). Although protein supplements increased plasma AA levels in period 1, ingestion of juniper in periods 2–4 was associated with a decline in plasma AA levels regardless of the type of protein supplement (Tables 6 and 7). Ingestion of terpenes may deplete certain AA used for conjugation and excretion of terpenes (Boyle et al., 1999, 2000; Lamb et al., 2001) or for maintaining acid–base balance (Foley et al., 1995; Illius and Jessop, 1995).

Goats and sheep in this study exhibited reduced levels of Glu, Met, Gly, Ser, Asp, and Gln after ingesting juniper (Tables 6 and 7). Plasma levels of Glu, Met, and Asp did not recover in period 4 despite a recovery in crude protein intake (Table 3). This pattern agrees with previous evidence suggesting detoxification of terpenes and a number of xenobiotics could be associated with depletion of AA in plasma in marsupials and other small mammalian species (Boyle et al., 1999, 2000; Lamb et al., 2001).

Restoration of acid–base balance via bicarbonate and ammonia systems has been identified as an important sink of AA depletion caused by terpene detoxification (Foley et al., 1995). Hydrogen carbonate and ammonia acceptors, derived mainly from glutamine, neutralize proton loads (H+) of acidic conjugates to form CO_2 and ammonium ions (NH_4^+) which are eliminated via lungs (CO_2) and urine (NH_4^+) or recycled as urea (Jessop and Illius, 1997; Foley et al., 1995). In small ruminants, ammonium losses may decline if urea recycling increases, which may help to maintain nitrogen balance when fed low protein diets with one-seed juniper (Nunez-Hernandez et al., 1989). Maintenance of acid–base homeostasis may explain the decline in concentration of plasma Gln of sheep and goats after period 2 (Tables 6 and 7), which is consistent with studies showing plasma glutamine decreases by 25% and total AA by 19% after endotoxin challenges (Vesali et al., 2005).

Ingestion of juniper plus CT in period 3 may have further decreased plasma AA (Tables 6 and 7) due to interactions of terpene detoxification and CT–protein binding (McMahon et al., 2000; Silanikove et al., 2001). Supplemental PEG fed during period 4, on the other hand, should have limited CT–protein bonding (Silanikove et al., 2001), increasing availability of AA for absorption. This may have spared protein and permitted animals to cope with greater juniper terpene intake during period 4. Plasma levels of some AA such as Met, Asp, Glu, and Asn exhibited sharp declines with increased juniper intake in period 4, while others such as Cys, Thr, Ser, and Gly (possibly those not involved in terpene detoxification) reverted to pre-CT concentrations.

Studies addressing the metabolic fate of AA in ruminants in relation to ingestion or infusion of toxins are limited to a few examples with endotoxins of bacterial origin (Waggoner et al., 2009) or plant toxins such as mimosine in *Leucaena leucocephala* (Reis et al., 1999). No previous research has examined the influence of one-seed juniper intake on the fate of AA in small ruminants. This study suggests that one-seed juniper ingestion diminishes plasma concentrations of some AA such as Met, Asp, Glu, and Asn and that additional dietary protein can enhance an animal's capacity to cope with terpenes and improve voluntary intake of one-seed juniper. Supplements containing Cys, Met, sulfate, and antioxidants that elevate glutathione synthesis and activity successfully reduced toxicosis of bitterweed (*Hymenoxys odorata*, a forb with high concentration of sesquiterpene lactones) in sheep (Kim et al., 1982; Calhoun et al., 1989; Post and Bailey, 1992). Protein supplements fed to goats and/or sheep have been reported to increase voluntary intake of plants containing mono- and sesquiterpenes such as bitterweed (Post and Bailey, 1992), sagebrush (*Artemisia tridentata*; Villalba et al., 2002a), redberry juniper (*Juniperus pinchotii*; Campbell et al., 2007), and one-seed juniper (Utsumi et al., 2009). This study suggests those responses may be due to increased uptake and depletion of specific AA that help offset post-absorptive metabolic costs of terpene detoxification.

5. Conclusions

Adding RDP or RUP to sheep and goat diets increased rumen fermentation end-products but did not stimulate intake or change preference of one seed juniper harvested in fall. High seasonal PSM levels (Utsumi et al., 2009) or lack of short term rumen adaptation to plant tepenoids may be responsible for this result. Adding CT induced a slight increase in one seed juniper intake, although animals continued to avoid this browse species. Addition of PEG doubled juniper intake and had largest effects on goats that also received additional dietary protein; goats receiving diets with added protein and PEG exhibited a strong preference for juniper when offered the choice between this browse species and an alternative high CT feed.

Negative effects of terpenes did not appear to be due to deleterious effects on microbes since addition of juniper to sheep and goat diets was associated with increased rumen fermentation end-products. Plasma concentrations of a number of AA declined after animals began consuming juniper and in a few cases (Glu, Met, Asp) were not restored to pre-juniper ingestion levels despite the addition of dietary protein and PEG. Plasma concentrations of several other AA were restored to some degree by adding protein and PEG to sheep and goat diets. We argue that previously observed increased voluntary intake of one-seed juniper by sheep and goats that received supplemental protein may be due to the apparent role of added protein in offsetting the loss of plasma AA to terpene detoxification processes. Our results suggest that supplements containing PEG + protein could be a powerful tool to boost intake of one seed juniper by goats, even during the fall when juniper PSM levels are highest.

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