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Seasonal changes in one seed juniper intake by sheep and goats in relation to dietary protein and plant secondary metabolites

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ABSTRACT

Plant secondary metabolites (PSM) and nutrients can influence patterns of feed intake of small ruminants. Their effects depend on the type and amounts of PSM and nutrients fed. We hypothesized that one-seed juniper intake of goats and sheep would change in response to seasonal variations of PSM concentrations and type and amount of CP fed. To test this hypothesis, we fed 12 does (46.7 ± 1.25 kg) and 12 ewes (74.9 ± 1.23 kg) freshly harvested one-seed juniper branches in summer, fall, winter, and spring. Animals also received isoenergetic diets (1.6% BW) with either addition of a high rumen degradable (RDP, 12.5% CP) or undegradable (RUP, 12.5% CP) protein source or with no addition of supplemental protein (Control, 5% CP). Juniper branches were offered in unrestricted amounts for 30 min prior to feeding treatment diets and short-term intake was determined for 10 d per season. One-seed juniper leaves contained a diverse mix of terpenes, phenolics, and condensed tannins which were positively correlated to each other ($P < 0.05$) and varied seasonally in concentration and composition ($P < 0.05$). Juniper intake was greater for goats than sheep ($P < 0.05$), and twice as high for animals fed high CP diets vs. control animals ($P < 0.05$). Juniper intake in the fall, when levels of plant secondary metabolites were highest, was 41, 58, and 52% less ($P < 0.05$) than in summer, winter, and spring, respectively. Addition of high RDP and RUP sources into diets induced different patterns of juniper intake across seasons, herbivores, and individual animals. In each diet treatment, a distinct subset of a few PSM explained 30–78% of overall variation in juniper intake of goats and sheep. This study suggests that increases in dietary protein levels can increase voluntary intake of one-seed juniper of small ruminants during seasons when PSM levels are low. Diets with protein sources of different degradability can potentially influence juniper intake of small ruminants differently, probably due to different nutrient–PSM associations that may result in different detoxification capabilities and post-ingestive experiences that influence individual juniper preference.

1. Introduction

One-seed juniper (*Juniperus monosperma* [Englem.] Sarg.) is a common rangeland woody plant in western North America that is lightly browsed opportunistically by small ruminants (Holechek et al., 1989). Low utilization of one-seed juniper can be attributed to toxic plant secondary metabolites (PSM) such as terpenes (Utsumi et al., 2006) and phenolics (Nunez-Hernandez et al., 1989) that appear to suppress voluntary intake of this otherwise nutritious plant (Gershenson and Croteau, 1991; Foley et al., 1999). Soluble phenolics (e.g. hydrolyzable

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tannins) and mono- and sesquiterpenes have bactericidal effects on rumen microorganisms (Schwartz et al., 1980a), reduce forage fermentation (Villalba et al., 2006), and can increase energy and protein requirements for PSM detoxification reactions (Illius and Jessop, 1995). In contrast, insoluble phenolics (condensed tannins) can bind dietary proteins and carbohydrates, decreasing nutrient absorption (Nunez-Hernandez et al., 1989; Makkar, 2003).

Various studies have investigated the effect of PSM on voluntary intake of *Juniperus* species by small ruminants (Schwartz et al., 1980a; Pritz et al., 1997; Animut et al., 2004) and the effect of seasonal PSM variation on pattern of juniper intake (Riddle et al., 1996), yet relatively less is known about interactions among juniper PSM and nutrients. Supplemental protein could mitigate the detrimental effects of phenolics and terpenes and enhance one-seed juniper intake. Goats fed diets relatively high in energy and protein (30% alfalfa, 17% cottonseed meal, 26% corn) consumed detectably more ashe juniper (*Juniperus ashei* Buchholz) and redberry juniper (*Juniperus pinchotti* Sudw.) during seasons when concentrations of monoterpenes were lowest (Riddle et al., 1996). Sheep and goats fed rumen-degradable protein supplements (55% alfalfa and 35% soybean meal) exhibited increased preference for sagebrush (*Artemisia tridentata* Nutt.), a shrub with high terpene concentration, and foods containing condensed tannins (Villalba et al., 2002a,b). Supplements fed in these studies were apparently able to partially offset detrimental effects of toxins (Villalba et al., 2002a,b). One-seed juniper synthesizes a unique suite of terpenoids, which individually can have significant effects on ruminants (Adams et al., 1981; Adams, 1994; Utsumi et al., 2006). Their collective effect on ruminant metabolism, however, is unknown. No prior studies have investigated the efficacy of protein feeding strategies in mitigating the deterrent effect of one-seed juniper PSM on voluntary intake of this plant by small ruminants.

Deterrent effects of one-seed juniper PSM may be related to their deleterious impacts on rumen microbial fermentation (Oh et al., 1967) and/or the sequestration of dietary energy and protein, which is diverted to sustain PSM bio-transformation in the liver (Illius and Jessop, 1995). Depending on which of these two processes is dominant, the type of protein fed (rumen degradable or undegradable protein) may be of critical importance. Although goats have greater enzymatic liver detoxification capabilities than sheep (Wisnewski et al., 1987), and presumably greater capacity to cope with juniper PSM (Campbell and Taylor, 2006), each species may respond differently to additions of rumen-degradable and undegradable protein sources in diet. No prior studies have examined these aspects of nutrient–PSM interactions in sheep and goats fed one-seed juniper.

We hypothesized that the short-term intake of one-seed juniper by sheep and goats would increase with increasing levels of dietary protein but would decrease with increasing concentration of PSM. Specific objectives were to evaluate patterns of juniper intake as a function of: (a) seasonal changes in PSM; and (b) addition of a rumen-degradable or undegradable protein source to diets.

2. Materials and methods

2.1. Animals, holding pens, and diet adaptation

Our study was conducted at the New Mexico State University Campus Farm in Las Cruces, NM. We used non-pregnant and non-lactating adult Western White Face ewes ($n = 12$; 74.9 ± 1.23 kg, mean ± SE) and Boer/Spanish commercial crossbred does ($n = 12$; 46.7 ± 1.25 kg). Animal handling procedures were approved by the New Mexico State University Institutional Animal Care and Use Committee.

Prior to and between feeding trials, sheep and goats were kept in separate groups in two 30 m × 12 m pens with a roofed bedding ground area with walls on the N-facing side and with free access to fresh water and mineralized salt blocks. Animals received a daily maintenance ration of sudangrass hay at 08:00 h. Two weeks prior to the first trial (July 2005), all animals were offered freshly harvested juniper branches twice per week. This preconditioning phase allowed naïve animals to become familiar with harvested juniper plant material. Shortly before each feeding trial, animals were placed in individual pens (2 m × 3 m) with a roofed bedding area, walls on the N-facing side, and free access to fresh water.

2.2. Feeding trials

Juniper feeding trials were carried out during 17 days in summer (July) and fall (October) of 2005, and winter (January) and spring (May) of 2006. The first seven days were to adapt animals to treatment diets and juniper feeding protocol. Intake of treatment diets and juniper were recorded during the last 10 days.

Animals were randomly assigned to one of three protein diet treatments (4 ewes and 4 does per treatment): (a) control (no protein added); (b) rumen undegradable protein (RUP) added; and (c) rumen degradable protein (RDP) added. Animals remained within a same diet treatment throughout the study. Diets were formulated with ground sudangrass hay (<1 cm particle size) completely mixed with other ingredients. The control diet contained 5% CP, whereas RDP and RUP diets contained 12.5% CP and included soybean, 36% by pass CP (Preston, 2000) and fish meal 60% by pass CP (Preston, 2000), respectively (Table 1). The three diets were isoenergetic and formulated to satisfy sheep and goat mineral and vitamin requirements (Table 1). Diets were fed in 25-l rubber feeders at a rate of 1.6% body weight from 13:00 to 17:00 h every day during the 17-d feeding trial. Intake was calculated as the difference between amount of food offered and refused.

2.3. Juniper feeding

Animals received juniper daily for 30 min starting at 08:00 h during each 17-d trial. The feeding sequence and temporal delay between juniper and treatment diet intake minimized the likelihood of inducing

Table 1
Ingredient composition of experimental diets.

Ingredient (g/kg)	Treatments[a]		
	CTRL	RUP	RDP
Sudan	712	707	714
Corn	233	117	10
Soybean meal 45%	–	–	231
Fish meal 60%	–	147	–
Mineral–Vitamin Premix[b]	55	29	45
ME (Mcal/kg)	2.0	2.0	2.0
CP (g/kg)	50	125	125

[a] Treatments were control (CTRL, no supplement) or rumen undegradable (RUP) or degradable (RDP) protein supplement.

[b] Mineral–Vitamin Premix composition: CTRL: Mineral oil 1.51%, Limestone 34.02%, Dicalcium Phosphate 33.51%, Salt 12.80%, Ammonium Sulfate 16.89%, EDDI 0.01%, Sodium Selenite 0.54%, Vitamin A (60,000 UI/g) 0.25%, Vitamin E (400 UI/g) 0.46%. RUP: Mineral oil 1.64%, Limestone 46.88%, Salt 22.27%, Ammonium Sulfate 26.49%, EDDI 0.01%, Sodium Selenite 1.17%, Vitamin A (60,000 UI/g) 0.54%, Vitamin E (400 UI/g) 1.00%. RDP: Mineral oil 1.60%, Limestone 42.52%, Dicalcium Phosphate 27.77%, Salt 13.98%, Ammonium Sulfate 12.62%, EDDI 0.01%, Sodium Selenite 0.65%, Vitamin A (60,000 UI/g) 0.30%, Vitamin E (400 UI/g) 0.55%.

aversions to treatment diets (Villalba et al., 2002c) and was intended to stimulate intake of juniper defended plant material (Mote et al., 2008; Papachristou et al., 2007). Juniper branches offered consisted of current year's growth and were ≤30 cm long and had ≤3 mm stem basal diameter. Juniper branches were harvested weekly in each season at the Corona Range and Livestock Research Center in central New Mexico. Harvested branches were placed in plastic bags and stored at 4 °C until used. This handling protocol has shown to prevent significant alterations of terpene profiles in one-seed juniper (Utsumi et al., 2006) and red cedar (*Juniperus virginiana*; Animut et al., 2004). Branches from same saplings were used on each trial day, to reduce plant-to-plant variation in terpenes that could affect intake (Utsumi et al., 2006).

Juniper branches were stapled at a 45° angle on artificial wooden stands to resemble the architecture of a young juniper tree (<1.2 m height). Stands consisted of a 5 cm × 5 cm wooden cylindrical pole (1.5 m height) mounted on a 0.5 m × 0.5 m wooden base secured to the ground and one side of the pen. Preliminary observations ensured that the total number of branches (12 ± 3) and the amount of fresh juniper offered (197 ± 3 g) on stands did not limit juniper intake during the 30 min browsing bouts. Stands with attached branches were weighed (±1 g) immediately before and after the 30-min bouts and juniper intake was determined from the difference. Juniper intake was adjusted for water loss by weighing 5 control stands with 10 branches each, which remained in a vacant pen for the duration of feeding bouts. Two composite daily samples from control stands were collected at the onset of bouts, frozen within 10 min at −80 °C until analysis of mono- and sesquiterpenes, total phenolics, condensed tannins, and DM.

2.4. Juniper chemical analysis

Terpenes were analyzed following a protocol described by Utsumi et al. (2006). Total phenolics and condensed tannins were determined by spectrophotometry following the Folin–Denis method and vanillin/HCl methods, respectively (Galyean, 1987). Samples from days 1, 5, and 10 were analyzed for nitrogen (CN-2000, LECO Corp., St. Joseph, MI) to estimate CP, and for NDF, and ADF (ANKOM 200 fiber analyzer, ANKOM Tech., Macedon, NY). Dry matter digestibility (DMD) was calculated as DMD = 88.9 − (0.799 × ADF) following Rohweder et al. (1978), and used to derive metabolizable energy (ME) following NRC guidelines (NRC, 1985).

2.5. Statistical analysis

The study followed a 2 × 3 × 4 factorial arrangement of 2 herbivore species, 3 treatment diets, and 4 seasons. Juniper and treatment diet intake (g/kg $W^{0.75}$) were analyzed with the MIXED procedure (SAS, 2004). Individual animal nested within diets and herbivore species was treated as random effect. Fixed effects were herbivore species, treatment diet, and season. Double and triple interactions among fixed factors were also included in the model. Because significant interactions existed between herbivore species and other main effects, reduced models by herbivore species were considered. Best fit of covariance structure (UN, CS, AR[1]) was determined prior to final analysis using the Bayesian Information Criterion (Littell et al., 1998). The Kenward–Rogers method of degrees of freedom was used (SAS, 2004). Intake of juniper and treatment diet was averaged across days within seasons before analysis.

Levels of total terpenes, specific mono- and sesquiterpenes, total phenolics, condensed tannins and DM, in addition to juniper CP, NDF, ADF, DMD, and ME, were analyzed following a completely randomized design with sampling days as replicates. Seasons were the independent factor included in the model. When significant *F* values ($P < 0.05$) were detected, LSD mean separation was conducted (alpha 5%).

Pearson's correlation coefficient was used to: (a) examine the relationship between the concentration of total phenolics, condensed tannins, and total terpenes in juniper across seasons; (b) evaluate potential relationships between juniper intake and NDF, ADF, and ME concentration; and (c) examine the relationship between relative juniper intake of individual ewes and does in summer (first season of juniper exposure) and the change in juniper intake during subsequent seasons (fall, winter, and spring). Relative juniper intake in summer was expressed as the level of initial juniper intake by individual ewes and does in the study, and reflected the difference between juniper intake of an individual and the average juniper intake for sheep or goats in that season. Values above or below zero indicate intake levels above or below average values. The subsequent change in juniper intake relative to the summer expressed the influence of prior juniper exposure and experience, and reflected the difference between juniper intake of each individual animal in the current season and juniper intake in the previous summer. Values above or below zero indicate an increase or decrease in juniper intake relative to summer values.

Stepwise regression was used to identify the smallest suite of PSM likely to explain patterns of juniper intake of sheep and goats within treatment diet groups following an approach previously used by Riddle et al. (1996). An alpha value of 0.1 was used to retain or remove explanatory variables during the stepwise procedure. Prior to analysis, juniper intake (g/kg $W^{0.75}$) was averaged across sheep and goats within diet groups. The explanatory variables tested were total phenolics, condensed tannins, total terpenes, and individual mono- and sesquiterpenes with significant seasonal variation (Tables 4 and 5). Preliminary selection of predictors was conducted to increase the ratio of observation points to predictors and avoid unstable models with biased estimates (Kenneth and Anderson, 2002). Separate regressions were conducted for sheep and goats in each of the three treatment diet groups. All analyses were conducted using SAS (2004).

3. Results

3.1. Juniper and supplement intake

Goats consumed more juniper than sheep (mean ± SEM: 1.72 ± 0.17 g/kg $W^{0.75}$ vs. 0.72 ± 0.08 g/kg $W^{0.75}$; herbivore effect $P < 0.001$; Table 2), and both goats and sheep responded differently to treatment diets across seasons (herbivore × treatment diet × season effect, $P = 0.006$; Figure 1; Table 2). Goats fed the RDP treatment diet consumed more juniper than goats fed RUP or control diets in summer ($P < 0.05$); whereas in winter, goats fed the RUP diet treatment consumed more juniper than goats fed RDP or control diets ($P < 0.05$). Sheep fed the RDP diet consumed more juniper than sheep fed the RUP or control diet in summer ($P < 0.05$) and RUP diet in winter ($P < 0.05$). Averaged across seasons, goats fed the control, RUP, and RDP diets consumed 0.97 ± 0.19, 1.97 ± 0.28, and 1.62 ± 0.33 g juniper/kg$^{0.75}$, respectively (treatment diet effect, $P = 0.018$; Table 2). Similarly, sheep fed the control, RUP, and RDP diets consumed 0.53 ± 0.10, 0.50 ± 0.13, and 1.15 ± 0.15 g juniper/kg$^{0.75}$, respectively (treatment diet effect, $P = 0.016$; Table 2). Across summer, fall, winter, and spring, juniper intake was 1.58 ± 0.36, 0.78 ± 0.14, 1.96 ± 0.23 and 1.75 ± 0.21 g/kg $W^{0.75}$ for goats (season effect, $P < 0.002$; Table 2), and 0.56 ± 0.13, 0.49 ± 0.08, 0.96 ± 0.12, and 0.87 ± 0.23 g/kg $W^{0.75}$ for sheep (season effect, $P < 0.002$; Table 2), respectively.

Intake of juniper in summer (first season of exposure) was related to intake patterns of individual animals in subsequent seasons. Fall, winter, and spring juniper

Table 2
Summary of probability values for the herbivore, treatment diet, and season effects on juniper intake by sheep and goats.

Factor	Full model	Herbivore	
		Goats	Sheep
Herbivore (H)	<0.001	NA	NA
Treatment diet (Trt)	0.002	0.018	0.016
H × Trt	0.006	NA	NA
Season (S)	<0.001	0.002	0.002
H × S	0.020	NA	NA
Trt × S	0.030	0.040	0.012
H × Trt × S	0.006	NA	NA

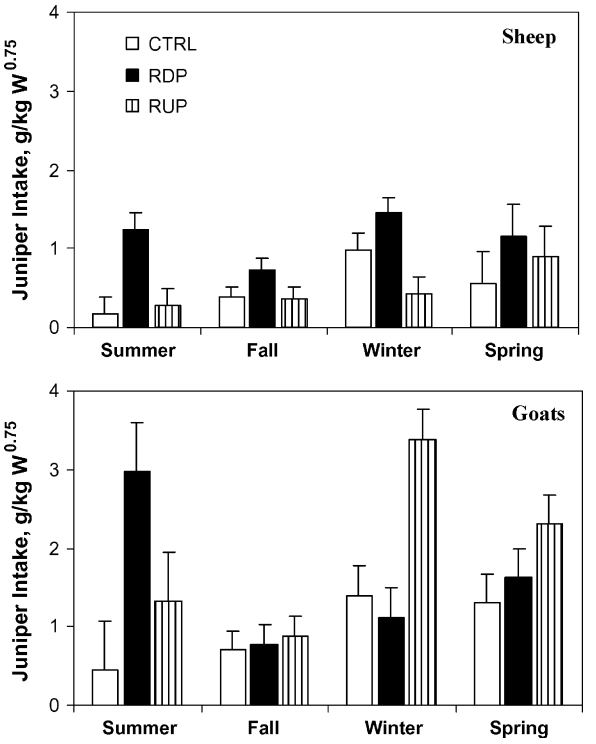

Fig. 1. Intake of juniper (Fresh basis) during 30-minute feeding bouts by sheep and goats fed diets with addition of rumen degradable (RDP, 12.5% CP) or undegradable (RUP, 12.5% CP) protein, or no addition of protein (CTRL, 5% CP). Bars denote SE of 4 ewes and 4 does per treatment.

intake was negatively correlated with juniper intake of the previous summer ($r = -0.66$ to -0.97, $P < 0.05$; Figure 2). Individual goats, and to a lesser extent sheep, which consumed high amounts of juniper during the first trial (summer), decreased juniper intake in following seasons (Figure 2), while the opposite was observed for animals that consumed low amounts of juniper during the first trial. Interestingly, individuals with juniper intake near the average during the first trial showed little or no change in subsequent juniper intake throughout the experiment (Figure 2). Goats and sheep in the RDP treatment diet exhibited greatest season to season variation and animal to animal variation in juniper intake (Figure 2).

Goats and sheep consumed almost all the basal treatment diet offered ($0.94 \pm 0.03\%$), but intake differed between herbivores ($P < 0.001$) and treatment diet ($P = 0.044$) throughout the study (herbivore × treatment diet effect, $P < 0.13$). Sheep in the control, RDP, and RUP groups consumed similar amounts of basal treatment diet, whereas goats tended to consume more feed when the RDP source was provided (Figure 3). Treatment diet intake also varied across seasons (season effect, $P = 0.002$); intake (g/kg $W^{0.75}$) was lowest in fall (40.14 ± 0.96) compared to summer (41.70 ± 0.89), winter (42.78 ± 0.89) and spring (41.75 ± 0.94).

3.2. Juniper chemistry

Dry matter content peaked in spring, was lowest in fall, and exhibited intermediate values in summer and winter (Table 3). Crude protein remained above 7% (DM basis) in summer, fall, and winter but was below 6% in the spring (Table 3). The NDF and ADF content tended to increase in summer and fall; consequently, juniper DMD and ME tended to decrease during these two seasons (Table 3).

Plant secondary metabolites in one-seed juniper leaves consisted of a mixture of phenolics and terpenes that varied in concentration seasonally (Table 3). Together, total phenolics and terpenes accounted for 8–10% of DM (Table 3), which resulted in ratios of juniper CP to total PSM consistently lower than 1 throughout the year (Table 3). Total terpene levels were positively correlated with total phenolics ($r = 0.36$; $P = 0.023$, $n = 40$) and condensed tannins ($r = 0.34$; $P = 0.033$, $n = 40$). A positive correlation was also detected between condensed tannins and total phenolics ($r = 0.50$; $P < 0.001$, $n = 40$). These three major classes of secondary metabolites co-varied from season to season (Table 3). In general, concentrations of total phenolics, condensed tannins, and total terpenes peaked in the fall, dropped in the summer, and exhibited intermediate values in winter and spring (Table 3).

Juniper terpene chemical profile was highly diverse and consisted of a rich mixture of 54 hydrocarbon and oxygenated mono- and sesquiterpenes, diterpenes, and three unknown compounds, which were tentatively classified as sesquiterpenes (Tables 4 and 5). Most of the individual mono- and sesquiterpenes also varied across

Table 3
Forage quality and secondary compounds of juniper leaves across seasons.

Parameter*	Seasons				*P*-value
	Summer	Fall	Winter	Spring	
DM (%)	51.16 ± 0.98[b]	47.16 ± 1.48[c]	53.60 ± 0.63[b]	61.37 ± 0.84[a]	<0.001
CP (%)	7.01 ± 0.19[a]	7.84 ± 0.31[a]	7.34 ± 0.33[a]	5.87 ± 0.56[b]	0.029
NDF (%)	37.24 ± 0.71	37.63 ± 1.26	35.01 ± 0.96	34.33 ± 0.93	0.113
ADF (%)	32.96 ± 0.43	33.39 ± 1.69	29.88 ± 1.68	29.96 ± 0.50	0.153
DMD (%)	62.57 ± 0.35	62.22 ± 1.45	65.03 ± 1.35	64.96 ± 0.40	0.153
ME (Mcal/kg)	2.21 ± 0.01	2.20 ± 0.05	2.29 ± 0.05	2.29 ± 0.02	0.153
Total phenolics (mg/g)	63.48 ± 0.86[c]	78.73 ± 1.61[a]	72.28 ± 1.75[b]	78.67 ± 0.95[a]	<0.001
Condensed tannins (mg/g)	42.68 ± 2.37[b]	59.18 ± 1.63[a]	42.76 ± 2.49[b]	46.87 ± 1.60[b]	<0.001
Total terpenes (mg/g)	16.95 ± 1.13[b]	23.64 ± 0.77[a]	20.04 ± 1.82[ab]	21.15 ± 1.21[a]	0.008

Values within rows with the same superscripts (a–c) do not differ significantly ($LSD_{0.05}$).

* Values are mean ± SE values of 3 and 10 samples for forage quality and secondary compounds, respectively.

Table 4
Seasonal concentrations of individual monoterpenes in one-seed juniper leaves.

Compound (mg/g)	Class	Seasons*				SE	*P*-value
		Summer	Fall	Winter	Spring		
alpha-Pinene	Monoterpene hydrocarbon	10.916[b]	15.365[a]	13.809[a]	14.254[a]	1.327	0.012
Limonene + beta-phellandrene	Monoterpene hydrocarbon	1.056[b]	1.435[a]	1.249[ab]	1.313[a]	0.109	0.030
3-Carene	Monoterpene hydrocarbon	0.558[b]	1.181[a]	0.478[b]	0.309[b]	0.180	0.000
Myrcene	Monoterpene hydrocarbon	0.351[b]	0.583[a]	0.423[b]	0.366[b]	0.046	0.001
beta-Pinene	Monoterpene hydrocarbon	0.166	0.195	0.183	0.173	0.041	0.876
Terpinolene	Monoterpene hydrocarbon	0.121[b]	0.215[a]	0.137[b]	0.122[b]	0.016	<0.001
alpha-Phellandrene	Monoterpene hydrocarbon	0.074[b]	0.144[a]	0.126[a]	0.121[a]	0.016	0.002
Bornyl acetate	Monoterpene ether acetate	0.073[b]	0.121[a]	0.076[b]	0.097[ab]	0.020	0.023
gamma-Terpinene	Monoterpene hydrocarbon	0.061[c]	0.102[a]	0.071[bc]	0.094[ab]	0.010	0.004
Camphene	Monoterpene hydrocarbon	0.060[b]	0.101[a]	0.071[b]	0.062[b]	0.007	<0.001
Tricyclene	Monoterpene hydrocarbon	0.032[b]	0.044[a]	0.038[ab]	0.037[ab]	0.004	0.034
Sabinene	Monoterpene hydrocarbon	0.022	0.011	0.032	0.018	0.020	0.086
cis-para-Menth-2-en-1-ol	Monoterpene alcohol	0.018[b]	0.034[a]	0.029[a]	0.029[a]	0.004	0.006
Verbenene	Monoterpene hydrocarbon	0.017	0.037	0.045	0.026	0.012	0.124
(e)-beta-Ocimene	Monoterpene hydrocarbon	0.017	0.027	0.024	0.027	0.010	0.718
alpha-Thujene	Monoterpene hydrocarbon	0.017[a]	0.013[ab]	0.008[ab]	0.005[b]	0.006	0.046
p-Cymene	Monoterpene hydrocarbon	0.015[b]	0.021[ab]	0.020[b]	0.027[a]	0.002	0.007
trans-Sabinene hydrate	Monoterpene alcohol	0.013[b]	0.031[a]	0.017[b]	0.012[b]	0.006	0.023
Terpin-4-ol	Monoterpene alcohol	0.012[b]	0.019[a]	0.018[a]	0.017[ab]	0.002	0.044
cis-Sabinene hydrate	Monoterpene alcohol	0.012	0.011	0.011	0.009	0.004	0.831
alpha-Terpinyl acetate	Monoterpene ether acetate	0.012	0.010	0.011	0.010	0.002	0.898
Camphor	Monoterpene ketone	0.009[b]	0.008[b]	0.028[a]	0.020[ab]	0.004	0.007
Verbenone	Monoterpene ketone	0.008[b]	0.015[a]	0.018[a]	0.017[a]	0.003	0.002
trans-para-Menth-2-en-ol	Monoterpene alcohol	0.007[b]	0.012[a]	0.011[ab]	0.014[a]	0.002	0.020
2-Carene	Monoterpene hydrocarbon	0.007[b]	0.009[b]	0.012[ab]	0.017[a]	0.004	0.016
alpha-Terpineol	Monoterpene alcohol	0.004[b]	0.018[a]	0.005[b]	0.003[b]	0.003	<0.001
alpha-Terpinene	Monoterpene hydrocarbon	0.004	0.005	0.005	0.005	0.001	0.506
Borneol	Monoterpene alcohol	0.004	0.004	0.006	0.004	0.001	0.530
para-Cymen-8-ol	Monoterpene alcohol	0.003	0.004	0.003	0.004	0.001	0.454
para-Mentha-2,4(8)-diene	Monoterpene hydrocarbon	0.002[b]	0.004[a]	0.002[b]	0.001[b]	0.001	0.028
Pinocarvone	Monoterpene ketone	0.002	0.002	0.002	0.002	0.001	0.954
trans-Carvyl acetate	Monoterpene ether acetate	0.001	0.003	0.002	0.002	0.001	0.260
(z)-beta-Ocimene	Monoterpene hydrocarbon	0.001[c]	0.002[bc]	0.004[a]	0.003[ab]	0.000	0.002
alpha-Campholenal	Monoterpene aldehyde	0.001	0.001	0.001	0.001	0.000	0.434
o-Cymene	Monoterpene hydrocarbon	0.0004[a]	0.0001[b]	0.0001[b]	0.0001[b]	0.000	<0.001

Values within rows with the same superscripts (a–c) do not differ significantly ($LSD_{0.05}$).
* Values are means of 10 samples per season.

Table 5
Seasonal concentration of individual sesqui- and diterpenes in one-seed juniper leaves.

Compound (mg/g)	Class	Seasons*				SE	*P*-value
		Summer	Fall	Winter	Spring		
Unknown 1	Oxygenated sesquiterpene	0.702 [c]	1.159 [a]	0.939 [b]	0.939 [b]	0.099	0.001
beta-Eudesmol	Sesquiterpene alcohol	0.490 [b]	0.478 [b]	0.418 [b]	0.709 [a]	0.095	0.013
alpha-Eudesmol	Sesquiterpene alcohol	0.461 [b]	0.464 [b]	0.400 [b]	0.678 [a]	0.092	0.014
8-alpha-Acetoxyelemol	Sesquiterpene ether acetate	0.328	0.395	0.374	0.445	0.049	0.250
8-alpha-11-Elemodiol	Sesquiterpene alcohol	0.229 [a]	0.214 [a]	0.043 [b]	0.036 [b]	0.093	0.019
Unknown 3	Oxygenated sesquiterpene	0.210 [b]	0.299 [a]	0.192 [b]	0.199 [b]	0.024	0.002
Germacrene B	Sesquiterpene hydrocarbon	0.138 [b]	0.209 [a]	0.137 [b]	0.176 [ab]	0.019	0.001
Abietal	Diterpene ketone	0.131	0.085	0.101	0.107	0.029	0.370
e-Caryophyllene	Sesquiterpene hydrocarbon	0.102	0.133	0.110	0.119	0.015	0.330
Elemol	Sesquiterpene alcohol	0.093 [b]	0.098 [b]	0.103 [b]	0.151 [a]	0.017	0.002
Unknown 2	Oxygenated sesquiterpene	0.074	0.098	0.082	0.092	0.008	0.057
alpha-Humulene	Sesquiterpene hydrocarbon	0.059	0.081	0.055	0.052	0.013	0.265
gamma-Eudesmol	Sesquiterpene alcohol	0.053 [b]	0.029 [b]	0.028 [b]	0.126 [a]	0.033	0.002
Manoyl oxide	Diterpene oxide	0.042	0.032	0.028	0.033	0.009	0.381
Germacrene D	Sesquiterpene hydrocarbon	0.020 [b]	0.031 [a]	0.020 [b]	0.020 [b]	0.002	< 0.001
e-Nerolidol	Sesquiterpene alcohol	0.014 [b]	0.019 [a]	0.017 [a]	0.018 [a]	0.001	0.012
alpha-Bulnesene	Sesquiterpene hydrocarbon	0.010 [b]	0.017 [a]	0.010 [b]	0.009 [b]	0.001	< 0.001
beta-Selinene	Sesquiterpene hydrocarbon	0.009	0.010	0.010	0.012	0.001	0.162
alpha-Selinene	Sesquiterpene hydrocarbon	0.009	0.010	0.008	0.010	0.002	0.423

Values within rows with the same superscripts (a–c) do not differ significantly ($LSD_{0.05}$).
* Values are means of 10 samples per season.

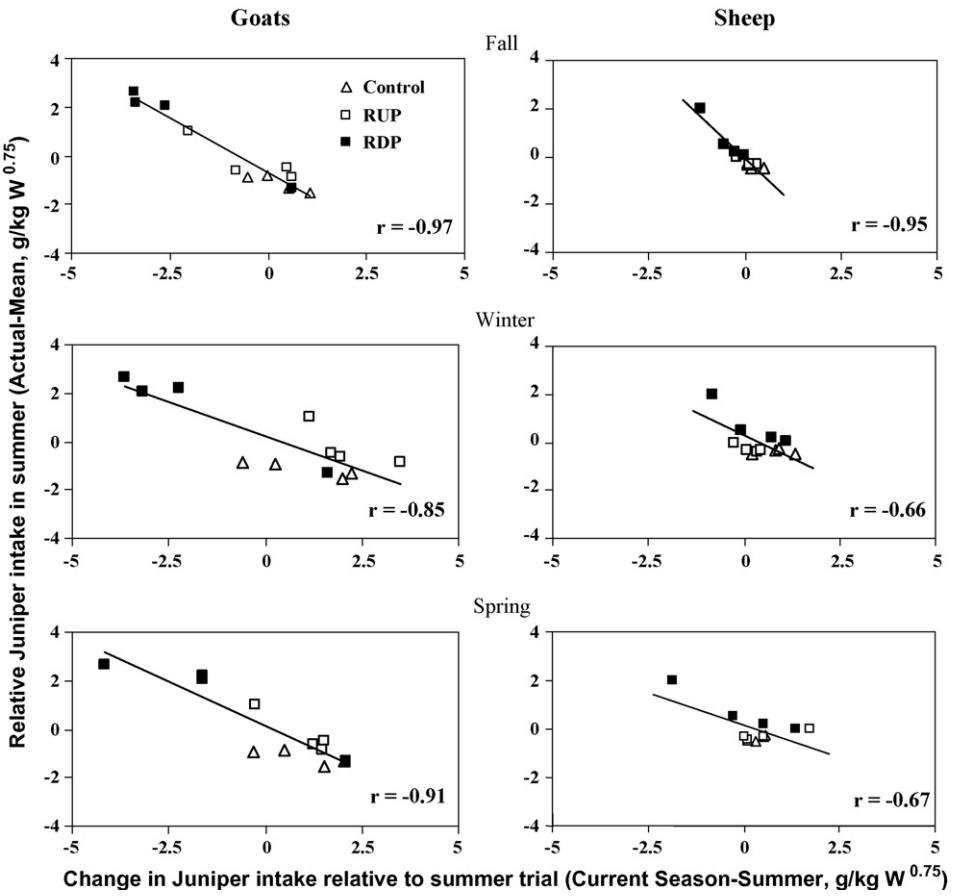

Fig. 2. Relative juniper intake (fresh basis) in summer and associated change in intake during the following fall, winter, and spring of individual sheep and goats fed diets with addition of rumen degradable (RDP, 12.5% CP) or undegradable (RUP, 12.5% CP) protein or no addition of protein (CTRL, 5% CP). The trend in juniper intake over all animals is represented by solid lines and the associated coefficient of determination (r), $n = 12$.

seasons (Tables 4 and 5). The general pattern of variation for most individual compounds followed that of total terpenes, with some exceptions. Concentrations were generally highest in fall, lowest in summer, and intermediate in winter and spring (Tables 4 and 5). Of the 20 monoterpene hydrocarbons identified, only four (beta-pinene, sabinene, verbenene, (e)-beta-ocimene, and alpha-terpinene) did not change across seasons, and two exhibited highest concentration in summer, in contrast to the general pattern for most terpenes (Table 4). Concentrations of three monoterpene alcohols (cis-sabinene hydrate, borneol, and p-cymen-8-ol) did not change across seasons, while the other five monoterpenes of this class peaked in fall and were lowest in summer as did most hydrogenated monoterpenes (Table 4). Monoterpenes with aldehyde or acetate oxygen groups did not change across seasons with the exception of bornyl acetate, which followed the general pattern of seasonal variation (Table 4). Pinocarvone was the only monoterpene ketone that did not change seasonally, the remaining two (verbenone and camphor) were highest in winter and lowest in summer (Table 4). Sesquiterpene hydrocarbons varied the least; only three of the seven compounds of this class (germacrene-B, germacrene-D, and alpha-bulnesene) varied seasonally and like most volatiles peaked in fall (Table 5). With the exception of 8-alpha-11-elemodiol which peaked in summer, the other five sesquiterpene alcohols (beta-eudesemol, alpha-eudesemol, gamma-eudesemol, elemol, and e-nerolidol) all peaked in the spring and exhibited lowest concentration in summer or winter (Table 5). The two diterpenes identified did not change across seasons (Table 5).

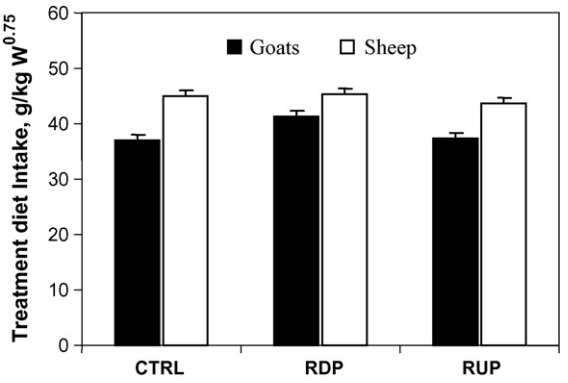

Fig. 3. Treatment diet intake by sheep and goats. CTRL: control, 5% CP), RDP: rumen degradable protein (12.5% CP), and RUP: rumen undegradable protein (12.5% CP). Bars represent SE of 4 ewes and 4 does per treatment.

3.3. Juniper intake in relation to juniper chemistry

No correlations between intake and CP content of juniper leaves were found for sheep or goats in any treatment diet ($P > 0.05$). Juniper intake (g/kg $W^{0.75}$) of sheep and goats in the control treatment group was negatively correlated with NDF (Goats: $r = -0.68$, $P = 0.016$, Sheep: $r = -0.62$, $P = 0.032$; $n = 12$) and ADF (Goats: $r = -0.72$, $P = 0.008$, Sheep: $r = -0.67$, $P = 0.016$; $n = 12$), but positively correlated with ME of juniper leaves (Goats: $r = 0.72$, $P = 0.008$, Sheep: $r = 0.67$, $P = 0.016$; $n = 12$). No correlations were found between juniper intake and NDF, ADF, or ME for sheep or goats fed RDP or RUP treatment diets ($P > 0.05$).

Thirty to 78% of the overall variation in juniper intake was explained by the concentration of a few individual juniper PSM (Table 6). These compounds explained a higher amount of variation in the intake for the RDP vs. the RUP or control treatment groups (Table 6). Ewes and does fed the control diet responded negatively to a few monoterpene hydrocarbons and sesquiterpene alcohols and positively to monoterpene ketones (Table 6). Condensed tannins were significant predictors of juniper intake of animals fed the RDP treatment diet and were negatively associated with juniper intake of both ewes and does (Table 6). Variation in juniper intake of sheep and goats fed RUP was explained by monoterpene hydrocarbons and sesquiterpene alcohols which were negatively related to intake (Table 6).

Individual mono- and sesquiterpenes associated with juniper intake varied with treatment diet and herbivore with a few exceptions. Regardless of treatment diet juniper intake was negatively related to sesquiterpenes, such as 8-alpha-11-elemodiol for goats fed control and sheep fed RUP, gamma-eudesemol for goats in RUP and sheep in RDP group, and beta-eudesemol for sheep in the control group (Table 6). Unknown 3 (tentatively identified as oxygenated sesquiterpene) was also negatively related to the amount of juniper consumed by goats fed RUP, and accounted for most of the variation in juniper intake of these animals (Table 6). Verbenone, a monoterpene ketone, was positively associated with juniper intake of sheep and goats in the control group, but was negatively related to juniper intake of goats fed RDP (Table 6). The monoterpene hydrocarbons limonene + beta-phellandrene were negatively associated with intake of sheep fed control and RDP (Table 6). The same negative relation was detected between the monoterpene hydrocarbon, alpha-thujene, and juniper intake of sheep and goats fed RUP (Table 6).

4. Discussion

4.1. Juniper intake in relation to treatment diets

Goats showed greater capacity to consume juniper and to increase juniper intake in response to protein level than did sheep. As predicted, juniper intake of sheep and goats was lowest in the fall, when concentration of secondary metabolites in juniper was highest. Addition of rumen degradable (RDP) and undegradable (RUP) protein sources into diets triggered differential patterns of juniper intake of goats across seasons. This differential pattern of juniper intake could be the result of different nutrient–secondary metabolite associations which may have affected detoxification capabilities and post-ingestive experiences thus influencing individual juniper preference.

Goats and sheep fed protein supplements consume higher quantities of plants or foods with high levels of terpenes (Villalba and Provenza, 2005; Campbell et al., 2007; Dziba et al., 2007). Supplemental protein enhances the oxidation of terpenes, phenolics, and xenobiotics in general, facilitating toxin clearance as oxidized compounds or as glucose or amino acid conjugates (Guengerich, 1995; Boyle et al., 2000; Parkinson, 2001). Illius and Jessop (1995) argued that enhanced levels of nutrients (amino acids and glucose) would lead to faster toxin clearance, which should result in increased intake levels of defended plants. In sheep, terpene absorption in rumen and post-absorption clearance rate are relatively rapid processes that occur in the order of minutes (Dziba et al., 2006). Thus, the increase in short-term juniper intake in this study could have been triggered by a positive effect of higher levels of dietary protein on detoxification rates. Increases in dietary protein and detoxification rates may have increased thresholds of PSM satiety (Provenza et al., 2003).

Alternatively, animals that received diets higher in protein in this study may have ingested higher quantities of juniper due to a hedonic shift in preference (Villalba and Provenza, 1997, 1999). This phenomenon has been demonstrated with foods of low palatability and even with foods containing phenolics or terpenes (Baraza et al., 2005; Villalba and Provenza, 2005). Villalba and Provenza (1997) demonstrated that protein-limited lambs (80% of requirements) increased ingestion of a low quality straw when the straw was paired with gradient infusions (0.23–0.69 g N/d) of degradable (casein) or escape (gluten) protein. Use of RDP may increase preference for a low quality food because it favors rumen fiber digestion and rumen clearance (Van Soest, 1994), hence minimizing rumen fill effects that restrict intake (Mertens, 1994). Furthermore, because terpenes could affect rumen microorganisms and slow down rumen clearance rates (Villalba et al., 2006), addition of RDP to diets may have improved both treatment diet and juniper intake by offsetting the detrimental effects of juniper terpenes. Use of RUP may also improve intake by indirectly enhancing fiber digestion and rumen clearance through recycled non-protein nitrogen (Egan and Moir, 1965; Egan, 1965; Weston, 1967).

This study was the first to document differential influence of diets containing RDP and RUP on juniper intake across seasons and among individual sheep and goats. This could be attributed to different post-ingestive experiences associated with juniper intake which may have affected subsequent levels of juniper ingestion. Individuals that exhibited highest juniper intake in the first period exhibited greater decreases in juniper ingestion levels in subsequent seasons, and vice versa. This pattern was associated with the type of protein fed; the greatest seasonal changes in intake and the largest variation among individuals were observed in animals fed the RDP treatment diet. Addition of RDP to diets could trigger a short-term increase in preference for juniper followed by longer term avoidance if the short-term beneficial stimulus discussed above is later offset by detrimental stimuli associated with a higher dose

Table 6
Stepwise regression of juniper intake (fresh; g/kg W $^{0.75}$) vs. concentrations of secondary metabolites in juniper leaves for goats and sheep fed diets with addition of rumen degradable (RDP), rumen undegradable (RUP) or no added protein (CTRL)[a].

Goats				Sheep			
Parameter	Class	Estimate ± SE	Partial R^2	Parameter	Class	Estimate ± SE	Partial R^2
CTRL							
Intercept		0.95 ± 0.23		Intercept		0.48 ± 0.18	
Verbenone	Monoterpene ketone	60.82 ± 12.06	0.25	Camphor	Monoterpene ketone	8.07 ± 3.15	0.26
Myrcene	Monoterpenes hydrocarbon	−1.70 ± 0.64	0.14	Verbenone	Monoterpene ketone	49.48 ± 12.30	0.13
alpha-Thujene	Monoterpenes hydrocarbon	−19.57 ± 6.79	0.08	beta-Eudesmol	Sesquiterpene alcohol	−0.49 ± 0.17	0.11
8-alpha-11-Elemodiol	Sesquiterpene alcohol	−0.74 ± 0.34	0.07	Limonene + beta-phellandrene	Monoterpene hydrocarbon	−0.42 ± 0.21	0.06
para-Mentha-2,4(8)-diene	Monoterpenes hydrocarbon	49.33 ± 30.62	0.03				
Model R^2			0.57	Model R^2			0.55
RDP							
Intercept		4.67 ± 0.57		Intercept		2.88 ± 0.40	
o-cymene	Monoterpene hydrocarbon	1665.26 ± 574.69	0.42	Condensed tannins	Phenolic	−0.02 ± 0.01	0.16
Condensed tannins	Phenolic	−0.05 ± 0.01	0.16	Limonene + beta-phellandrene	Monoterpene hydrocarbon	−1.41 ± 0.32	0.14
Verbenone	Monoterpene ketone	−113.20 ± 33.85	0.08	gamma-Eudesmol	Sesquiterpene alcohol	−2.42 ± 0.77	0.12
alpha-Phellandrene	Monoterpene hydrocarbon	−7.53 ± 2.83	0.06	trans-para-Menth-2-en-1-ol	Monoterpene alcohol	101.56 ± 18.44	0.10
trans-para-Menth-2-en-1-ol	Monoterpene alcohol	141.38 ± 30.95	0.05	o-Cymene	Monoterpene hydrocarbon	537.43 ± 301.77	0.06
(z)-beta-Ocimene	Monoterpene hydrocarbon	−107.43 ± 57.96	0.02	2-Carene	Monoterpene hydrocarbon	−13.80 ± 8.02	0.04
Model R^2			0.78	Model R^2			0.62
RUP							
Intercept		5.34 ± 0.60		Intercept		0.82 ± 0.10	
Unknown 3	Oxygenated sesquiterpene	−7.11 ± 2.11	0.25	alpha-Thujene	Monoterpene hydrocarbon	−27.91 ± 9.60	0.21
alpha-Thujene	Monoterpene hydrocarbon	−152.51 ± 37.15	0.17	8-alpha-11-Elemodiol	Sesquiterpene alcohol	−0.52 ± 0.24	0.09
gamma-Eudesmol	Sesquiterpene alcohol	−5.66 ± 2.42	0.08				
Model R^2			0.50	Model R^2			0.30

[a] All variables included in models are significant at a 10% alpha value; $n = 40$.

of phenolics and terpenes. Pritz et al. (1997) reported that goats that initially exhibited high and low juniper intake reversed to low and high consumers a few days later when redberry juniper was paired with a high protein food (19% CP) composed of ground alfalfa, soybean, and cotton seed meal, all of which provide highly degradable protein. In that study, oral gavages of juniper essential oils decreased juniper intake in kids compared to naïve controls. Thus, acquired preference for juniper when fed RDP could later result in induced avoidance if levels of toxin ingestion are sufficient to eventually inhibit rumen microbes (Oh et al., 1967; Nagy and Tengerdy, 1968; Schwartz et al., 1980a), depress rumen fermentation and digestion (Straka et al., 2003; Villalba et al., 2006), and impair liver metabolism (Pritz et al., 1997), all of which can condition food aversions (Provenza et al., 2003). Degradable protein could, therefore, induce temporary conditioned aversions that may outweigh short-term benefits of increased juniper intake. Inclusion of RUP, on the other hand, appeared to induce more conservative responses that appeared to allow animals to achieve better regulation of secondary compound intake, thus avoiding conditioned aversions.

Regardless of the type of protein added to diets, the level of protein had almost no effect on juniper intake in the fall when secondary metabolite levels were highest. Furthermore, juniper intake in this season appeared to depress diet intake. Increases in overall concentration of juniper phenolics and terpenes above a certain threshold appear to override any positive effects of feeding higher dietary protein levels to small ruminants.

4.2. Juniper intake in relation to plant nutrients and secondary metabolites

Juniper intake of sheep and goats fed the control diet was negatively correlated with NDF and positively correlated with ME of the juniper material. Conversely, no relationships between NDF, ME, and juniper intake were found in animals fed RDP or RUP diets. It is possible that animals in the control group may have responded to the fiber and energy content of juniper in an effort to compensate for diet-related deficits in energy and to offset bulk limitation. Forage quality of juniper may influence voluntary intake of energy-restricted and intake-limited animals. However, once this barrier is overcome with addition of protein to diets, intake limitations appear to be more closely associated with plant PSM.

Individual secondary compounds explained a larger proportion of the variation in juniper intake of goats and sheep fed RDP compared to animals in the other treatment groups. Animals fed the RDP diet treatment consumed on average almost twice as much juniper as their control counterparts. Therefore, higher sensitivity of control vs. RDP to juniper terpenes may have been a function of the absolute concentration of toxins in diets. In contrast, differences in sensitivity to terpenes between animals fed RDP vs. RUP may have been associated with protein–**tannin–terpene interactions. It is possible that RDP was more readily bound by juniper tannins than the escape protein in RUP. Therefore, animals fed RDP may have had relatively less protein available for terpene detoxification, making them more sensitive to seasonal changes in terpene concentration because of an antagonistic relationship of these two PSM classes. Tannins may have also depressed rumen fermentation, subsequent digestion, and overall nutrient uptake (McMahon et al., 2000; Makkar, 2003), making animals in the RDP group more sensitive to detrimental effects of specific terpenes.

The depressor effect of the most abundant juniper monoterpenes on herbivory has been well documented (Schwartz et al., 1980b; Riddle et al., 1996; Pritz et al., 1997). This study supports those findings and also suggests that minor (less abundant) mono- and oxygenated sesquiterpenes could also have important deterrent effects. In contrast, alpha-pinene, which accounts for approximately 65% of one-seed juniper volatiles, was not a significant predictor of juniper intake of sheep or goats in either supplemented group. Conversely, sesquiterpene alcohols that were present in lower concentrations were almost always negatively associated with juniper intake. The relationship between individual compounds and the amount of juniper consumed was affected by the type and amount of protein fed. Juniper intake of sheep and goats fed RUP was associated with a reduced set of individual terpenes that were always negatively related to the amount of juniper consumed. A relatively larger subset of volatiles, that were either negatively or positively associated with intake, explained a sizeable proportion of the variation in juniper consumption by animals in the RDP group.

Monoterpenes such as verbenone, p-mentha-2,4(8)-diene, camphor, o-cymene, and trans-para-menth-2-en-1-ol were all positively associated with juniper intake. The concentration of these compounds may have been negatively correlated with those having true deterrent effects (Riddle et al., 1996). Alternatively, some compounds may confer pleasant odors or flavors that may attract herbivores (Langenheim, 1994). Some of the compounds positively related with juniper intake in this study, such as camphor and cymene, were also positively associated with intake of ashe and redberry juniper of goats (Riddle et al., 1996).

Monoterpenes negatively related to juniper intake differed for sheep and goats and also differed among treatment diets. Myrcene explained lower juniper intake of goats in the control group, alpha-thujene was associated with lower intake in goats fed control and goats and sheep fed RUP, limonene + beta-phellandrene was related to juniper intake suppression in sheep fed control and RDP, verbenone, alpha-phellandrene and (z)-beta-ocimene were negatively associated with juniper intake in goats fed RDP, and 2-carene was related to a decrease in juniper intake in sheep fed RDP. These results suggest that the depressor effect of some monoterpenes could be protein- or herbivore-specific. Sheep and goats differ in liver detoxification capabilities (Wisnewski et al., 1987) and the effectiveness of RDP and RUP in neutralizing toxins may also differ.

Some of the compounds which were negatively related to juniper intake have also been associated with reduced intake of other plant species including juniper. Limonene and myrcene were negatively correlated with intake of

ashe and redberry juniper of goats (Riddle et al., 1996). Myrcene was reported to deter deer feeding (Vourc'h et al., 2002). Limonene was also present in a subset of tarbush (*Flourensia cernua* DC.) secondary metabolites that were negatively related to sheep, goat, and cattle herbivory (Estell et al., 1998a). Interestingly, neither limonene nor myrcene decreased intake of lambs when these compounds were individually tested (Estell et al., 1998b, 2002). This suggests that the deterrent effect of some terpenes may depend on the presence and concentration of other compounds in a mixture, due to the fact that specific terpenes may compete for a given pathway of detoxification (Pass and McLean, 2002).

Despite their relatively low concentration and inconsistent patterns, oxygenated sesquiterpenes also explained an important part of the variation in juniper intake. Influence of oxygenated sesquiterpenes varied between sheep and goats and diet groups. For example, 8-alpha-11-elemodiol was negatively related to juniper intake in goats fed control and sheep fed RUP, gamma-eudesmol was similarly related to intake in sheep fed RDP and goats fed RUP, and beta-eudesmol in sheep fed the control diet. One additional unknown oxygenated sesquiterpene (classified on the basis of its molecular weight and formula, mass spectra, and retention time) was also negatively related to juniper intake by goats fed RUP. Studies on the influence of oxygenated sesquiterpenes (e.g. alcohols) on sheep and goat herbivory are limited compared to those conducted on major monoterpenes. Confirmation of their deterrent properties will require further research.

5. Conclusions

Goats were more willing to consume one-seed juniper than sheep and exhibited greater capacity to increase juniper intake in response to increases in dietary protein during seasons when juniper exhibited lower PSM concentrations. An undesired side effect of increasing protein levels (particularly with RDP sources) could be the induction of temporary conditioned aversions. Supplementation schedules tailored to improve juniper intake could provide combinations of protein sources to avoid induced aversions with degradable proteins, if any.

Juniper phenolics and terpene concentrations were positively correlated across seasons and affected the manner in which tannins, terpenes, and proteins interacted and modified foraging behavior. Animals that were fed higher levels of RDP appeared more sensitive to variation in concentrations of juniper condensed tannins and oxygenated sesquiterpene alcohols and monoterpenes with important intake depressor effects.

Overall, results of this study suggest that one-seed juniper intake is largely affected by the amount and type of protein in the diet and by seasonal changes in the profile of plant secondary metabolites. This study also suggests that patterns of juniper intake cannot be predicted solely on the basis of plant secondary metabolites or nutrients. The manner in which animals experience secondary metabolites could be affected by level and type of dietary protein and therefore, the potential exists to manipulate voluntary intake.

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