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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 × 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