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The effect of polyethylene glycol on intake of Mediterranean shrubs by sheep and goats

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ABSTRACT: Poor nutritional quality and high levels of secondary compounds can reduce consumption of Mediterranean shrubs by herbivores. In 2 sequential trials, we examined the effect of polyethylene glycol (PEG) and number of shrub species offered on daily intake of Mediterranean shrubs by 12 sheep and 12 goats. The PEG (25 g) was fed to experimental animals with barley. In trial 1 (6 shrubs), goats ate more (P = 0.0008) daily total shrub biomass than did sheep (60.7 vs. 45.9 ± 2.6 g/kg of BW). There was a trend (P = 0.08) toward a positive PEG effect on total shrub intake, with PEG-supplemented animals consuming more total shrubs than controls (56.7 vs. 50.0 ± 2.6 g/kg of BW). Trial 2 (using 3 shrubs) was a continuation of trial 1, except that animals were given less barley and treatment animals were given more PEG (50 g). Both sheep and goats showed a numerical decrease in total shrub intake from trial 1 to trial 2. Sheep receiving PEG ate more (P = 0.002) total shrubs than did controls, but no PEG effect was found for goats. Thus, PEG had a greater influence on sheep than goats when only 3 shrubs were offered, a result that may be related to the fact that fewer shrubs with complementary secondary compounds were offered and that goats appear to have a greater ability to consume and detoxify secondary compounds from Mediterranean shrubs. Overall, as the number and diversity of shrubs offered increased, supplemental PEG had less effect on increasing intake for both goats and sheep.

Key words: chaparral, diet selection, maquis, secondary compound, shrubland, tannin

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INTRODUCTION

Evergreen shrubs often dominate grazing lands in the Mediterranean basin, including the Adriatic littoral of Croatia (Rogosic 2000; Rogosic et al., 2006). Their use is often limited by secondary compounds, typically condensed tannins (Owen-Smith, 1993; Titus et al., 2001; Rogosic et al., 2007), which are phenolic compounds found in 80% of the Mediterranean vascular plants (Silanikove et al., 1994). At high concentrations tannins adversely affect intake and can be toxic (Pritchard et al., 1992). Tannin concentrations greater than 5% adversely affect forage intake and digestibility of Mediterranean shrubs such as *Quercus calliprinos*, *Pistacia lentiscus* (Perevolotsky et al., 1993), and *Ceratonia siliqua* (Silanikove et al., 1994, 1996a). Condensed tannins at too high concentrations bind and precipitate proteins in the rumen (Jones and Mangan, 1977), reduce protein degradation, and reduce the absorption of AA reaching the small intestine, resulting in low digestibility and voluntary intake. Small ruminants foraging in the Mediterranean maquis select diets from an array of species that vary in nutrients and secondary compounds, which has been hypothesized to enable them to increase food intake and avoid toxicosis (Freeland and Janzen, 1974).

Animals also can learn to ingest substances such as polyethylene glycol (**PEG**) that alleviate the adverse impacts of tannins (Provenza et al., 2000; Villalba and Provenza, 2001). Polyethylene glycol has a high affinity for binding condensed tannins and preventing the formation of tannin-protein complexes. Thus, PEG increases intake of high tannin forages by livestock by attenuating the negative effects of tannins (Silanikove et al., 1997; Titus et al., 2001; Villalba et al., 2002).

The objective of our study was to determine if supplemental PEG affected intake of 6 species of shrubs by sheep and goats. They vary in palatability and in concentrations of secondary phytochemicals (Rogosic et al., 2006).

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MATERIALS AND METHODS

IACUC[AU1: Please insert a statement verifying that all procedures involving animals were approved by your local animal care and use committee or its equivalent; if not, please give the authority under which the research was conducted and also state specifically which guidelines were used.]

Shrubs

Two trials were conducted at an experiment station 25 km from Split in the central part of the Croatian Adriatic coast (latitude 43°52′ N; longitude 16°23′ E). In the first trial, 6 shrubs were offered simultaneously: *Quercus ilex* L. (Fagaceae), *Erica multiflora* L. (Ericaceae), *Arbutus unedo* L. (Ericaceae), *Juniperus phoeniceae* L. (Cupressaceae), *Viburnum tinus* L. (Caprifoliaceae), and *Pistacia lentiscus* L. (Anacardiaceae). Trial 2 was a continuation of the first trial, but in this case 3 shrubs were offered with no change to the experimental groups: *Q. ilex*, *A. unedo*, and *P. lentiscus*. Shrubs were harvested each week from the vicinity of the feeding trials. Shrub leaves and the current season's growth (i.e., twigs), 10 cm long, were clipped and ground to 1-cm length with a chipper. Ground material was mixed for uniformity, placed in woven, polyethylene feed sacks, and stored at 4°C. Every day before the trial, sufficient bags of shrubs to feed the animals were removed from cold storage and offered to the animals.

Animals and Diets

Animals were housed individually (1.5 × 2 m pens) in covered stalls with wire mesh sides. The sheep (n = 12) were crossbred hair-type 8 mo of age, whereas the goats (n = 12) were purebred Alpines 6 mo of age. Each group was an equal mix of both sexes. Sheep weighed 23.0 kg of BW and goats 21.2 kg of BW at the beginning of the trial and 24.0 and 21.5 kg of BW, respectively, at the end. All animals were raised on the same farm on the island of Brac (central Dalmatia).

To reduce food neophobia, the experimental animals were offered a barley/PEG mix for 30 min/d for 5 d before the trials. Similarly, they were also offered each of the 6 shrubs for 120 min/d for 5 d before the trials commenced. Throughout the experiments, animals had free access to trace mineral blocks and fresh water. Before the experiments, baseline intake of alfalfa pellets was determined for each animal on d 1 to 5. After the baseline was established, all animals had a 5-d preconditioning period in which 25 g of PEG (molecular weight = 3,350; Spectrum Chemicals, Los Angeles, CA) mixed with 175 g of barley was given from 0800 to 0830 h, and all 6 shrubs were offered in individual feeding boxes, and continually replenished from 0830 to 1400 h. Shrub intake was monitored, and animals divided into 2 treatments groups (PEG and controls, n = 6) based on total shrub intake, by ranking animals and using all odd ranks as 1 treatment. This procedure balanced initial shrub acceptance across treatment groups and often reduces variability (McIntyre, 2005). Ranking, rather than simple random assignment, is often preferable to balance treatment groups and reduce unintentional bias (Dell and Clutter, 1972[AU2: "Dell and Clutter, 1972" is not in the reference list.]; Hurlbert, 1984).

Feeding

Trial 1 was 11 d long and trial 2 was 10 d in length, and the trials ran consecutively. From 0730 to 0800 h daily during trial 1, sheep and goats in the PEG group received 25 g of PEG and 175 g of barley; controls received 200 g of barley alone. During trial 2, control and experimental animals received 100 g of barley daily; experimental animals also received 50 g of PEG. All sheep and goats in the treatment groups ate all of the PEG-grain mixture within 30 min in all trials.

All animals were then fed 200 g of each shrub from 0800 to 1400 h. Additional shrub material was added as necessary every 30 min until 1400 h. Each day refused shrubs were collected and biomass consumption was calculated.

In trial 1, at 1400 h, animals were offered varying amounts of alfalfa pellets calculated to provide 70% of their maintenance energy requirements (including barley; INRA, 1989). During trial 2, the amount of alfalfa fed was reduced to 50% of baseline intake.

Nutritional Composition

The nutritional composition of the shrubs, including relative concentrations of tannins, was determined in conjunction with another study, and the detailed methods and results are reported there (Rogosic et al., 2006). Briefly, shrubs were analyzed for CP and fiber using standard analyses (Rogosic et al., 2006). Shrubs were also analyzed for tannins using colorimetric methods described by Waterman and Mole (1994). Tannin concentrations are expressed as a relative tannin index.

Statistical Analysis

The total daily amount consumed of all shrubs offered in each trial was used in the analysis because consumption of each shrub was not independent of the other choices. The experimental design for the PEG trials was a completely random design. Animals were a random factor in the mixed model analysis (Littell et al., 1998). The repeated measures model included treatment (PEG vs. control) and species of animal (i.e., goats vs. sheep). The only interaction included was treatment × species to avoid overfitting the model. Various covariance structures were examined, and Akaike's information criterion was used to determine best fit (SAS Inst. Inc., Cary, NC). For most analyses, the compound symmetry option was the best alternative. All analyses on shrub intake were adjusted for BW.

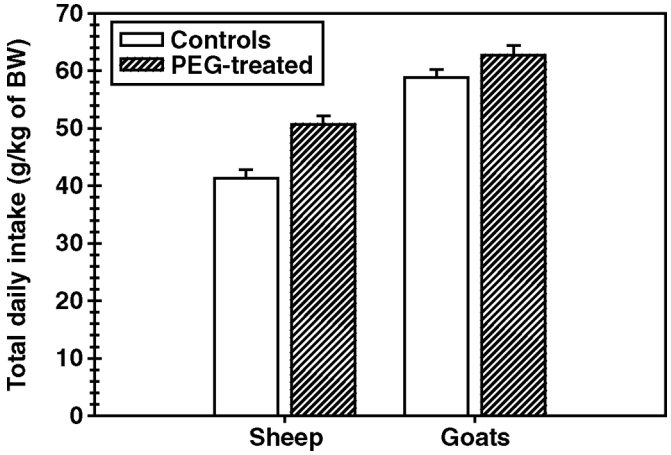

Figure 1. Total daily intake (g/kg of BW ± SD) of 6 Mediterranean shrubs offered concurrently to sheep and goats given polyethylene glycol (PEG) or control in trial 1. Goats and sheep differed ($P = 0.0008$) in total shrub consumption. The PEG-supplemented animals tended to consume more total shrubs than controls ($P = 0.08$).

RESULTS

Trial 1. Six Shrubs Offered to Sheep and Goats

The nutritional composition of the 6 shrubs varied greatly (Table 1). Overall, the CP content of all shrub leaves and current seasons growth was low (mean 6.4%) and ranged from 4.9% (*E. multiflora*) to 7.8% (*P. lentiscus*). Shrubs had high concentrations of cell wall constituents (NDF and ADF), particularly lignin (ADL). The high tannin indices for *P. lentiscus* (1.48), *A. unedo* (1.33), *E. multiflora* (0.98), *Q. ilex* (0.99), and *J. phoeniceae* (0.86) indicate high tannin concentrations.

Goats and sheep differed ($P = 0.0008$) in total shrub consumption (Figure 1); goats ate substantially more total shrub biomass than did sheep (60.7 vs. 45.9 ± 2.6 g/kg of BW). There was a trend toward a positive PEG effect on total shrub intake ($P = 0.08$), with PEG-supplemented animals consuming more total shrubs than controls (56.7 vs. 50.0 ± 2.6 g/kg of BW). There was also a day effect ($P = 0.001$), but no species × treatment interaction ($P = 0.47$). In general, treatment and control animals increased intake from the first to the last day in trial 1 (data not shown).

Even though sheep and goats differed in total amounts of shrubs eaten (Figure 2, trial 1 with 6 shrubs), the rank order of the amount eaten for each shrub species was essentially the same for sheep and goats. The mean ± SD amounts eaten of the shrubs across all treatments and animal species were *V. tinus* (15.6 ± 0.4 g/kg of BW), *E. multiflora* (14.4 ± 0.4 g/kg of BW), *A. unedo* (8.84 ± 0.3 g/kg of BW), *Q. il*ex (8.67 ± 0.3 g/kg of BW), *P. lentiscus* (5.4 ± 0.2 g/kg of BW), and *J. phoeniceae* (0.46 ± 0.04 g/kg of BW).

Trial 2. Three Shrubs Offered to Sheep and Goats

When 3 shrubs were offered to sheep and goats, with or without supplemental PEG, both treatment groups and both species of animals showed a numerical decrease in total shrub intake from the previous trial (Figure 2). There was a species × treatment interaction because sheep that received PEG ate more ($P = 0.002$) total shrubs than did controls, but no such treatment effect ($P = 0.47$) was found for goats (Figure 2). Regardless of treatment, sheep ate less shrubs than goats ($P = 0.02$; 34.4 ± 0.71 g/kg of BW for sheep vs. 41.4 ± 0.76 g/kg of BW for goats). Sheep and goats preferred *Q. ilex* (18.58 ± 0.3 g/kg of BW) and *A. unedo* (15.95 ± 0.2 g/kg of BW) to *P. lentiscus* (3.34 ± 0.08 g/kg of BW).

DISCUSSION

Importance of Variety for Small Ruminants on Mediterranean Rangelands

Total shrub intake increased as the number of shrubs offered increased. Although the 2 trials were

Table 1. Chemical composition (% of DM) of 6 Mediterranean shrub species fed to Croatian sheep and goats

	Species						
Item	*Arbutus unedo*	*Quercus ilex*	*Juniperus phoeniceae*	*Erica multiflora*	*Pistacia lentiscus*	*Viburnum tinus*	Average
DM	49.8	61.4	54.7	48.9	50.9	46.9	52.2
Ash	4.2	4.3	5.3	2.7	6.2	5.6	4.7
CP	5.6	7.4	5.6	4.9	7.8	7.2	6.4
Ether extract	6.3	3.3	8.9	8.6	3.2	9.4	6.6
Crude fiber	16.8	30.4	28.4	38.9	18.2	20.3	25.5
NDF	46.7	62.6	53.9	62.9	53.0	53.4	55.4
ADF	37.2	47.3	41.0	51.8	31.1	37.7	41.0
ADL	24.0	24.5	24.2	33.0	17.9	22.1	24.3
IVOMD[1]	43.0	35.7	35.5	27.2	32.2	43.4	36.2
ME (MJ/kg of DM)	6.54	5.40	5.38	4.14	4.84	6.57	5.27
Ca	1.47	1.43	2.04	0.45	1.59	1.30	1.36
P	0.08	0.07	0.09	0.06	0.10	0.09	0.08
Ca/P	18.4	20.4	22.6	7.5	15.9	14.4	16.6
Tannin index	1.33	0.99	0.86	0.98	1.48	ND	1.08

[1]In vitro OM digestibility.

not compared statistically, the results clearly showed that combinations of 6 shrubs promoted greater intake in goats and sheep (Figure 2). The shrubs offered in this trial varied in kinds and amounts of secondary compounds, including tannins (*Arbutus, Quercus*, and *Pistacia*), terpenes (*Juniper*), and iridoid glycosides and terpenes (*Viburnum tinus*; Tomassini et al., 1995). The efficacy of plant defenses varies with the mix of plants, and the chemical attributes of a single plant species must be considered within a larger context of the plant community (Bryant et al., 1991). In a mix of species, an animal's preference may range along a continuum from strongly aversive, if nutrients and secondary compounds are not complementary, to strongly positive if nutrients and secondary compounds are complementary (Provenza, 1996; Wang and Provenza, 1997**[AU3: "Wang and Provenza, 1997" is not in the reference list. 1996?]**). Animals are likely to eat more if species differ in secondary compounds, macronutrient concentrations, and flavors (Provenza et al., 2003). Some secondary compounds may be less aversive in plant communities containing high levels of nutrients (macronutrient-rich species) needed for detoxification (nutrient-secondary compound complementary; Villalba and Provenza, 1999) or containing complementary secondary compounds (Burritt and Provenza, 2000; Rogosic et al., 2003).

We often have failed to recognize that meaningful diversity requires species that are biochemically complementary, not just taxonomically different (Provenza et al., 2003). Therefore, it is important to identify complementary mixtures in Mediterranean rangelands. Analysis of secondary compounds in Mediterranean shrubs, grasses, and forbs will allow managers to elucidate the complementarity among secondary compounds and nutrients. Understanding such relationships may assist in the design of grazing systems that increase productivity of shrub rangelands and herbivores.

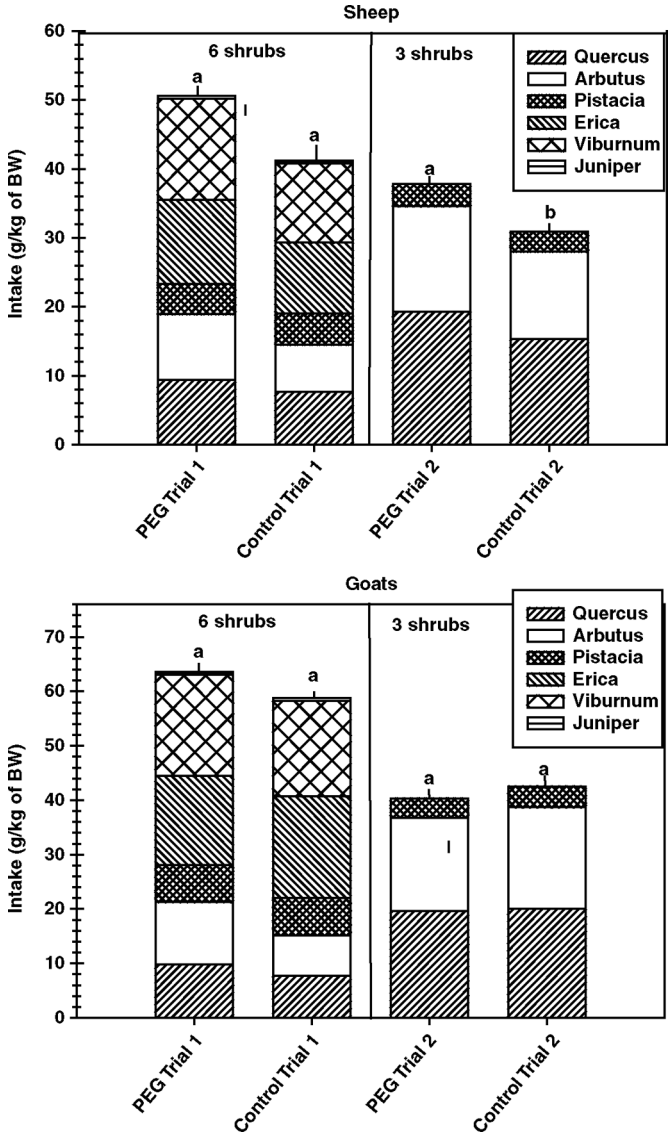

Figure 2. Total daily intake (g/kg of BW ± SD) of shrubs offered to sheep and goats. Trial 1: 6 shrubs offered together; trial 2: 3 shrubs offered together in sequential trials. [a,b]Different letters indicate that polyethylene glycol (PEG)-treated animals and controls differ ($P < 0.05$) within a trial.

Effect of PEG on Forage Intake of Tannin-rich Mediterranean Shrubs

Tannins (1.08%) can reduce intake and digestibility of forage (Butler, 1989). Depending on their structure and concentration, tannins can have adverse or beneficial effects on forage digestibility and intake. Some tannins reduce digestibility of protein, whereas others are toxic. Condensed tannins are found in 80% of the Mediterranean vascular plants (Silanikove et al., 1994). At low concentrations, some condensed tannins can improve nutrition for ruminants by reducing protein degradation in the rumen and increasing the flow of protein and essential AA to the intestine (McLean and Duncan, 2006). Other benefits include reduced impact of intestinal nematodes and nematode larvae (Waghorn, 1996). However, greater concentrations of condensed tannins have detrimental effects on animal performance (Pritchard et al., 1992). Tannin concentrations greater than 5% adversely affect forage intake and digestibility of Mediterranean shrubs such as *Quercus calliprinos*, *Pistacia lentiscus* (Perevolotsky et al., 1993), and *Ceratonia siliqua* (Silanikove et al., 1994, 1996a). Condensed tannins bind and precipitate proteins in the rumen (Jones and Mangan, 1977), reduce protein degradation, and reduce absorption of AA reaching the small intestine, resulting in low digestibility and voluntary intake.

Polyethylene glycol is a polymer that binds to tannins irreversibly over a wide range of pH, thus alleviating the negative effects of tannins (Landau et al., 2000). Supplemental PEG increases intake of tannin-containing plants by sheep, goats (Pritchard et al., 1988; Titus et al., 2000, 2001), and cattle (Hanningan and McNeill, 1998). Nevertheless, it is interesting that

when sheep and goats were offered 6 shrubs, PEG had only a slight beneficial effect on intake. The PEG alone increased consumption of the 3-shrub mix by sheep. The animals all had previous browsing experience with these shrubs in a free-grazing setting, and the previous experience probably influenced the choices made by individual animals during these trials (Distel and Provenza, 1991, 1994). The PEG had a greater influence on sheep than goats when only 3 shrubs were offered, a result that may be related to the fact that fewer shrubs with complementary secondary compounds were offered and that goats appear to have a greater ability to consume and detoxify secondary compounds from Mediterranean shrubs. Overall, as the number and diversity of shrubs offered increased, supplemental PEG had less effect on increasing intake for goats and sheep.

Comparative Responses of Sheep and Goats

In all trials, goats preferred to eat more total shrubs than did sheep, suggesting they had a greater tolerance for secondary compounds. Sheep, on the other hand, showed a positive response to PEG alone when 3 shrubs were fed in trial 2. This suggests that the threshold for effects of secondary compounds, particularly tannins in this study, is greater in goats than in sheep.

Goats typically browse more than sheep (Nefzoui et al., 1993; Perevolotsky et al., 1998) and utilize tannin-rich foods better than do sheep (Landau et al., 2000). Food intake and DM digestibility of tannin-containing forages are often greater for goats than for sheep (Silanikove et al., 1996a), and goats often use protein more efficiently than sheep (Kronberg and Malechek, 1997). Differences in ruminal fermentation and adaptation of rumen microbes to tannins also may enable goats to more efficiently use tannin-rich foods (Landau et al., 2000). Ruminal degradation is a primary mechanism for neutralizing the antinutritional effect of tannins in goats (Silanikove et al., 1996b). At low concentrations (i.e., <5%), condensed tannins may improve ruminant nutrition by reducing protein degradation in the rumen and increasing the flow of protein and essential AA to the intestine (McNabb et al., 1996), and tannins reduced impact of intestinal nematodes and nematode larvae (Waghorn, 1996; Hutchings et al., 2003).

Some animals also have adaptations such as production of proline-rich salivary proteins that bind tannins and minimize their potential adverse effects (Robbins et al., 1987). Sheep and goats do not produce proline-rich salivary proteins (Austin et al., 1989; Distel and Provenza, 1991), but goats secrete more saliva containing a greater concentration of nitrogen than do sheep (Domingue et al., 1991). A 50% reduction in tannins in extrusa samples from the esophagus of goats consuming blackbrush, a tannin-containing shrub (Provenza and Malechek, 1984), also suggests that even though proline-rich proteins may not be present in the saliva of goats, other salivary proteins contribute to forming complexes with tannins, thereby alleviating their negative effects.

Management Implications

Woody shrubs (maquis vegetation) dominate rangelands throughout the Mediterranean basin. Sheep and goats are an environmentally and economically sound alternative for using the forage potential of Mediterranean shrubs. Increasing use of these shrubs by livestock would likely enhance the production of grasses and forbs and create a more diverse mix of plants. Grazing by livestock also reduces the likelihood and the impacts of fires, common in these regions during summer.

Most Mediterranean shrubs contain large quantities of secondary compounds that limit intake and cause animals to eat a variety of foods. Goats consumed more total shrubs in each trial than did sheep. Even so, PEG supplementation had a greater effect on sheep intake than for goats, which may provide an additional management tool for producers raising sheep on Mediterranean shrublands.

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