

SUPPLEMENTAL POLYETHYLENE GLYCOL AND ENERGY INCREASE INTAKE OF MEDITERRANEAN SHRUBS BY SHEEP AND GOATS

DODATAK POLIETILENGLIKOLA I ENERGIJE POVEĆAVA KONZUMIRANJE SREDOZEMNIH GRMOVA ZA OVCE I KOZE

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SUMMARY

Maquis is an extensive shrubby vegetation type in the Mediterranean region that provides important habitat for wild and domestic herbivores. Although the majority of the shrubs are nutritious, virtually all contain secondary compounds that reduce their forage value.

In four trials, we examined the effect of polyethylene glycol (PEG) and a number of Mediterranean shrubs was offered species to sheep and goats. We offered 6 shrubs in trial 1, three shrubs in trial 2, two shrubs in trial 3, and one shrub in trial 4. Trials were conducted with 12 sheep and 12 goats (6 PEG vs. 6 control). In trial 1, goats ate more total shrub biomass than did sheep (60.7 vs. 45.9 ± 2.6 g/kg BW, respectively). There was a trend (P=0.08) towards 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 BW). In trial 2, both species of animals showed a numerical decrease in total shrub intake with or without supplemental PEG. Sheep receiving PEG ate more (P=0.002) total shrubs than did controls, but no such treatment effect was found for goats. In trial 3, supplemental PEG had a positive effect (P<0.001) on total shrub intake for both sheep and goats when only 2 shrubs (*Arbutus* and *Pistacia*) of lower palatability were offered. In trial 4, supplemental PEG had a marked positive effect (P<0.001) on intake of *Pistacia lentiscus* in both sheep and goats. PEG supplemented goats ate more *Pistacia lentiscus* (39.6 g/kg BW) than did PEG supplemented sheep (28.1 g/kg BW), whereas control sheep and goats ate similar amounts (12.2 and 15.3 g/kg BW, respectively). Our findings suggest that plant biochemical diversity plays a very important role in herbivore's diet selection, enabling animals to better meet their nutritional needs and avoid toxicity. In addition, as the number of shrubs in the diet decreased, the impact of PEG on intake of shrubs increased. PEG alone had a greater influence on sheep than on goats, and it had the greatest influence on both sheep and goats when only one or two foods were available.

Key Words: Mediterranean shrubs, biological diversity, sheep, goats, diet selection, secondary compounds, polyethylene glycol.

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INTRODUCTION

Natural pastures in the Adriatic part of Croatia are often dominated by evergreen shrubs (Rogosic et al., 2006). These traditional grazing areas are important sources of forage for livestock, particularly during the dry summer (Rogosic, 2000). Their use is often limited by condensed tannins (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 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 amino acids to the intestine (Mc Nabb et al., 1996), and reduce the impact of intestinal nematodes and nematode larvae (Hutchings et al. 2003). However, at too high concentrations they adversely affect forage intake and animal health and they can be toxic (Pritchard et al., 1992, Reid, 1995).

Condensed tannins bind and precipitate proteins in the rumen (Jones and Mangan, 1977), reduce protein degradation, and the absorption of amino acids 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 nutrient and toxins, which likely enables them to increase food intake and avoid toxicosis (Provenza, 1996).

Animals can also learn to ingest substances such as polyethylene glycol (PEG) that alleviate the adverse impacts of tannins (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). In Israel, PEG has been used successfully as a supplement to improve utilization of Mediterranean shrublands by sheep, goats and cattle (Silanikove et al., 1994; Silanikove et al., 1996a).

The objective of our study was to determine if supplemental PEG and energy (such as barley) affected intake of six species of shrubs by sheep and goats. We also determined if the effect of supplemental PEG was consistent when animals were offered different combinations of shrubs or a

single, less palatable shrub (*Pistacia lentiscus*). We hypothesized that intake of shrubs by both sheep and goats would increase with supplemental PEG and as the number of shrubs offered increased (Provenza et al., 2003).

MATERIALS AND METHODS

Shrubs

The Mediterranean maquis vegetation type includes 20 to 25 shrub species in Southern Croatia. The shrubs used in this study are dominant species of the *Myrto-Quercetum ilicis* plant community, the most common type of maquis vegetation in the Central part of the Adriatic coast. (Rogosic, 2000). They vary in palatability and in concentrations of secondary phytochemicals (Rogosic et al. 2006). In total, we conducted 8 trials with sheep and goats.

In the first trial, six 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). In the second trial three shrubs were offered: *Quercus*, *Arbutus* and *Pistacia*, while in the third trial *Arbutus* and *Pistacia* were offered. In the fourth trial only *Pistacia lentiscus* was offered. Shrubs were cut each week in the vicinity of the feeding trials. Shrub leaves and current season's growth (i.e., twigs) were clipped and ground to 1 cm length with a chipper, 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

The sheep (n=12) were crossbred wool-type 8 months of age, whereas the goats (n=12) were purebred Alpines 6 months of age. Each group was an equal mix of both sexes. Sheep weighed 23.0 kg and goats 21.2 kg at the beginning of the trial and 24.0 and 21.5 kg, at the end. All animals were raised on the same farm on the island of Brac (Central Dalmatia) and were adapted to the shrubby vegetation of the Mediterranean maquis. 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, 1994).

To establish a baseline, *ad libitum* intake of alfalfa was measured for 5 days. After the baseline was established, all animals had a 5-day preconditioning period where 25g of PEG (molecular weight = 3.350; Spectrum Chemicals, Los Angeles, Ca) mixed with 175 g of barley was given from 0800 to 0830, and all 6 shrubs were offered in individual feeding boxes, and continually replenished from 0830 to 1400. Shrub intake was monitored, and animals divided into two treatment groups (PEG and controls) based on total shrub intake, by ranking animals and using all odd ranks as one treatment. Animals remained in their respective treatment groups for all 4 trials. Throughout the experiments, animals had free access to trace mineral blocks and fresh water.

Feeding

All trials lasted 10 days, and ran consecutively. Sheep and goats in the PEG group received 20 g of PEG mixed with 200 g of barley and controls were not given barley. In all trials, animals were fed the PEG/barley mixture at 0800, and given the ground shrubs at 0830. All sheep and goats in the treatment groups ate all of the PEG-grain mixture within 30 minutes in all trials. Shrubs were fed (200 g) in individual boxes to each animal, and the amounts replenished as necessary during the day. Any uneaten amounts were weighed each afternoon at 1400.

During trial 1 alfalfa pellets were fed to all animals at 1400 and given at 50% of their baseline intake.

Statistical analysis

The total 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 (SAS 2000). The model included treatment (PEG vs. control), species of animal (i.e., goats vs. sheep), the

species x treatment interaction, with individual animals nested within treatment and species. The model also used days as a repeated measure with all other interactions included. All analyses on shrub intake were adjusted to body weight (g/kg BW).

RESULTS

Trial 1. Six shrubs offered to sheep and goats

Goats and sheep differed ($P=0.0008$) in total shrub consumption (Figure 1 and 2). Regardless of treatment, goats ate substantially more total shrub biomass than did sheep (60.7 vs. 45.9 ± 2.6 g/kg BW, respectively). There was a trend ($P=0.08$) towards 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 body weight).

In general, treatment and control animals showed a tendency for increasing intake from the first to the last day in trial 1. Even though sheep and goats differed in total amounts of shrubs eaten, the rank order of the amount eaten for each shrub species was essentially the same for sheep and goats. The mean amounts eaten of the shrubs across all treatments and animal species were: *Viburnum tinus* (15.6 g/kg), *Erica multiflora* (14.4 g/kg), *Arbutus unedo* (8.84 g/kg), *Quercus ilex* (8.67 g/kg), *Pistacia lentiscus* (5.4 g/kg) and *Juniperus phoeniceae* (0.46 g/kg).

Trial 2. Three shrubs offered to sheep and goats

When 3 shrubs were offered to sheep and goats, either with or without supplemental PEG, both treatment groups and both species of animals showed a numerical decrease in total shrub intake (Figure 1 and 2) from the previous trial. Sheep receiving PEG ate more ($P=0.002$) total shrubs than did controls, but no such treatment effect was found for goats (Figure 2). Regardless of treatment, sheep and goats ate almost the same amount of total shrubs (34.4 g/kg BW for sheep vs. 41.4 g/kg BW for goats). Sheep and goats preferred *Quercus ilex* (18.58 g/kg) and *Arbutus unedo* (15.95 g/kg) over *Pistacia lentiscus* (3.34 g/kg).

Trial 3. Two shrubs offered to sheep and goats

Supplemental PEG positively affected ($P < 0.001$) total shrub intake when only 2 shrubs (*Arbutus* and *Pistacia*) of lower palatability were offered (Figure 1 and 2). Both sheep and goats fed supplemental PEG ate numerically more *Pistacia lentiscus* (17.54 g/kg BW) compared to non-supplemented sheep or goats (7.57 g/kg BW).

Trial 4. One shrub (*Pistacia lentiscus*) offered to sheep and goats

Supplemental PEG had a marked positive effect ($P < 0.001$) on intake of *Pistacia lentiscus* in both sheep and goats (Figure 1 and 2). PEG-supplemented goats ate more *Pistacia lentiscus* (39.6 g/kg BW) than did PEG supplemented sheep (28.1 g/kg BW), whereas control sheep and goats ate similar amounts (12.2 and 15.3 g/kg BW, respectively).

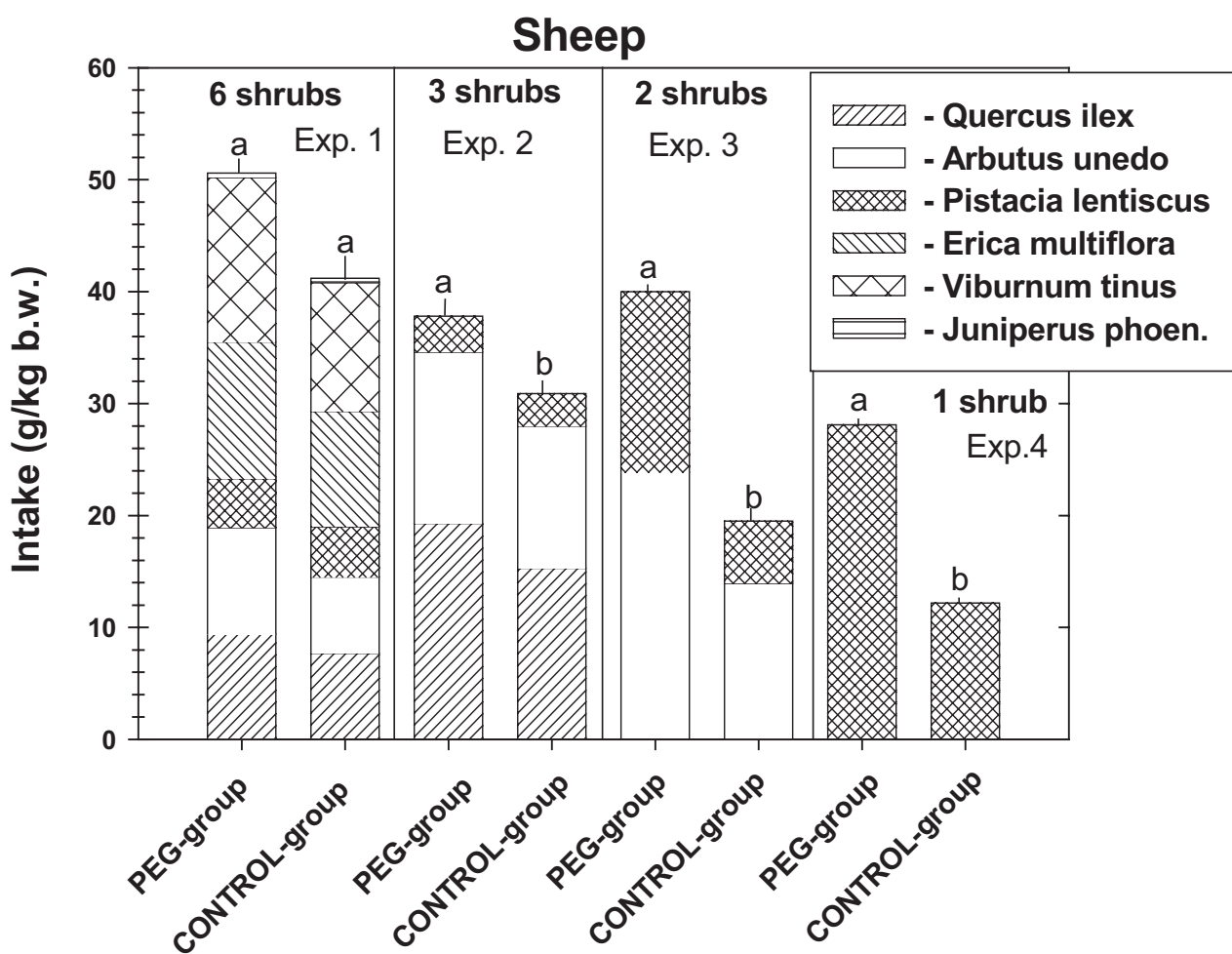
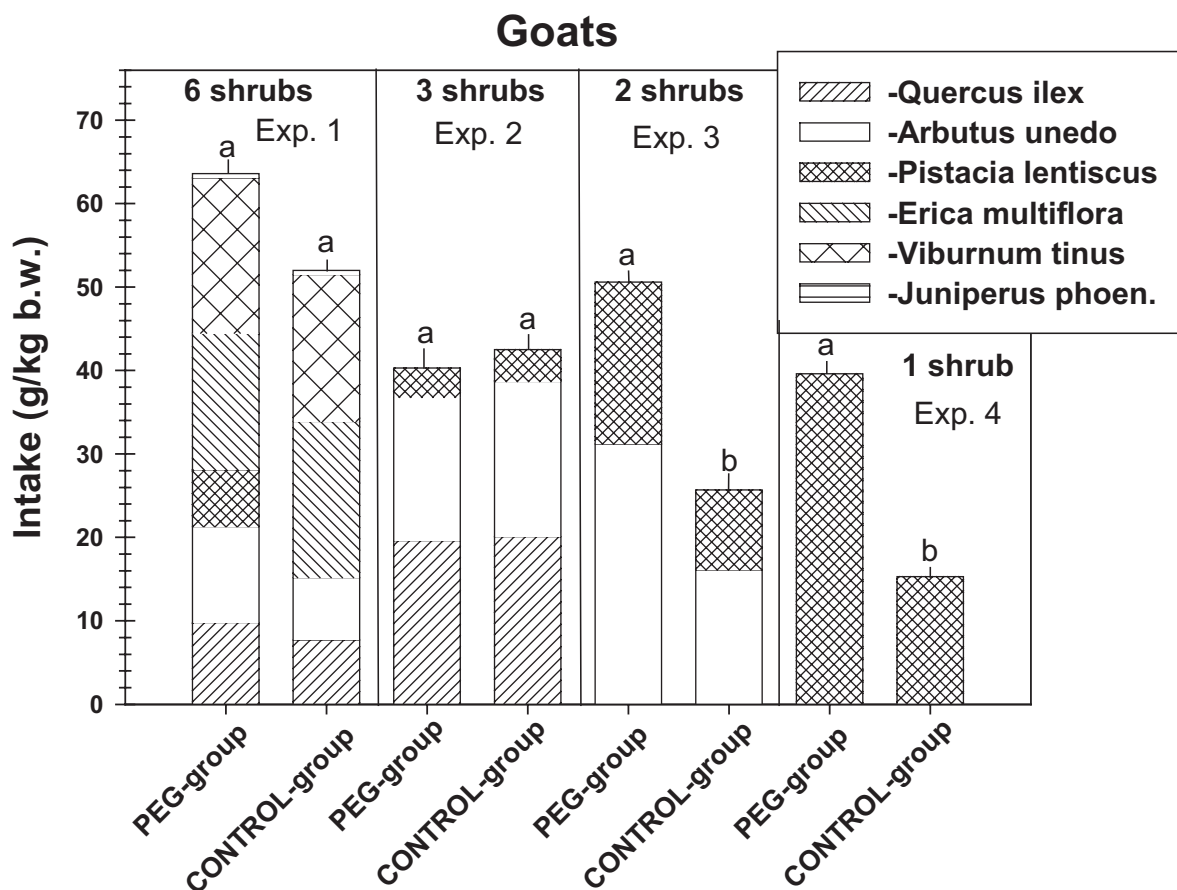


Figure 1. Trial 1, six shrubs offered together; Trial 2, three shrubs offered together; Trial 3, two shrubs offered together, and Trial 4, one shrub (*Pistacia lentiscus*) offered to sheep

Slika 1. Pokus 1, šest grmova ponuđeno zajedno; pokus 2, tri grma ponuđena zajedno; pokus 3, dva grma ponuđena zajedno, i pokus 4, jedan grm (*Pistacia lentiscus*) ponuđen ovcama



Figures 2. Trial 1, six shrubs offered together; Trial 2, three shrubs offered together; Trial 3, two shrubs offered together, and Trial 4, one shrub (*Pistacia lentiscus*) offered to goats

Slika 2. Pokus 1, šest grmova ponuđeno zajedno; pokus 2, tri grma ponuđena zajedno; pokus 3, dva grma ponuđena zajedno, i pokus 4, jedan grm (*Pistacia lentiscus*) ponuđen kozama

DISCUSSION

Importance of variety for small ruminants on Mediterranean rangelands

Total shrub intake increased as the number of shrubs offered increased. Although the 4 trials were not compared statistically, the results clearly showed that combinations of shrubs promoted greater intake in both goats and sheep. Likewise, sheep ate more when offered several foods (3 > 2 > 1) that contain complementary toxins (Villalba et al., 2004).

These results are consistent with the satiety hypothesis which contends diets and habitats that allow animals to select among alternatives enabling

individuals to better meet needs for nutrients and to better cope with toxins (Provenza et al., 2003). All plants contain toxins, and the amount of toxin an animal can ingest depends on the kinds and amounts of nutrients and toxins in the forages on offer. Nutrients and toxins both cause animals to satiate, and excesses of nutrients, nutrient imbalances, and toxins all limit food intake. Thus, individuals can better meet their needs for nutrients and regulate their intake of toxins when offered a variety of foods that differ in nutrients and toxins than when constrained to a single food, even if the food is "nutritionally balanced." Thus, feeding and grazing practices that allow producers to capitalize on biochemical diversity, as opposed merely to taxo-

onomic diversity, are likely to improve performance of the herd. Under this hypothetical framework, goats and sheep should eat small amounts of poorer quality feeds even though other palatable feeds are available for consumption (Provenza et al. 2003). Depending on prevailing conditions, livestock may be able to mix their diets in such a way as to provide sufficient energy and protein while reducing toxin loads.

The shrubs offered in this trial varied in kinds and amounts of secondary compounds, including tannins (*Arbutus unedo*, *Quercus ilex* and *Pistacia lentiscus*), terpenes (*Juniper phoenicea*) and iridoid glycosides and terpenes (*Viburnum tinus*) (Tomasini 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 toxins are not complementary, to strongly positive if nutrients and toxins are complementary (Provenza, 1996). Animals are likely to eat more if species differ in toxins, macronutrient concentrations, and flavors. Some toxins may be less aversive in plant communities containing high level of nutrients (macronutrient-rich species) needed for detoxication (nutrient-toxin complementary), or containing complementary toxins (toxin-toxin complementary; Rogosic 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 toxins and macronutrients. Understanding such relationships may assist in the design of grazing systems that increase productivity of shrub rangelands and herbivores.

The effect of PEG on forage intake of the Mediterranean shrubs

PEG 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 and goats (Titus et al., 2000, 2001) and cattle (Hanningan and McNeil,

1998). Nevertheless, it is very interesting that when sheep and goats were offered 6 shrubs, and when goats were offered 3 shrubs, PEG alone (both groups had barley) had no beneficial effect on intake. In general, as the number of shrubs in the diet decreased, the impact of PEG on intake of shrubs increased. PEG alone increased consumption of the 3-shrub mix by sheep. The effects of PEG and energy in trials 3 and 4 were confounded because controls had no supplement. Even so, the results of PEG and energy were substantial. When 3 species were offered to sheep (trial 2), and 2 or 1 species offered to goats, PEG and energy substantially increased intake of shrubs high in tannin (Figures 1 and 2).

Interactions among nutrients, toxins and PEG affected use of shrubs. For example, in trial 3 the relatively unpalatable shrub *Pistacia lentiscus* was not avoided when offered with another more palatable shrub (*Arbutus unedo*) even though animals were not forced to eat *Pistacia lentiscus*. Further, goats and sheep receiving PEG and energy in the 2-shrub trial ate much more of both shrubs than did control animals, indicating the effectiveness of PEG/barley in reducing the impacts of tannins from *Pistacia lentiscus* and *Arbutus unedo*.

Supplemental macronutrients increase intake of foods that contain toxins as diverse as lithium chloride (Wang and Provenza, 1996), terpenes (Banner et al., 2000), menthol (Illus and Jessop, 1996), and quebracho tannin (Villalba et al., 2002b). Consequently, the ability of herbivores to consume shrubs high in secondary compounds will depend on the interaction between the quantity and quality of nutrients available and the classes of toxins present in the mix of plant species.

Comparative responses of sheep and goats

In all 4 trials, goats ate more total shrubs than did sheep, suggesting they had a higher tolerance for secondary compounds. Further, supplemental PEG and energy only affected shrub intake by goats when either 2 or 1 high-tannin shrub(s) were offered. 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 toxic (i.e., tannin) effects is higher in goats than in sheep.

Goats typically eat more browse than sheep (Nefzoui et al., 1993), and utilize tannin-rich foods

better than do sheep. Food intake and dry matter digestibility of tannin-containing forages are often higher for goats than for sheep (Silanikove et al., 1996a), and goats often use protein more efficiently than sheep (Kronberg and Malechek, 1997). Difference in ruminal fermentation and adaptation of rumen microbes to tannins probably enables goats to more efficiently use tannin-rich foods. Ruminal degradation is a primary mechanism for neutralizing the anti-nutritional effect of tannins in goats (Silanikove et al., 1996b).

Some animals have also been adapted for 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 (Distel and Provenza, 1991), but goats secrete more saliva containing a higher level of nitrogen than do sheep. A 50% reduction in tannins in extrusa samples from the esophagus of goats consuming blackbrush, a tannin-containing shrub (Provenza and Malechek, 1984), also suggest that even though praline-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.

CONCLUSIONS

Maquis vegetation dominates rangelands throughout the Adriatic part of Croatia. 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.

Most Mediterranean shrubs contain large quantities of secondary compounds that limit intake and cause animals to eat a variety of foods. In our studies, as the number of shrub species in the diet decreased, so, too, did the intake. Indeed, when sheep and goats were offered 6 shrubs, and when goats were offered 3 shrubs, PEG alone had no beneficial effect on intake. Our findings suggest that biochemical diversity plays a very important role in

herbivore's diet selection, enabling animals to better meet their nutritional needs and avoid toxicity. Further analysis of secondary compounds in Mediterranean shrubs, grasses and forbs will allow managers to elucidate the complementarity among toxins and macronutrients and assist in the design of grazing systems that increase productivity of shrub rangelands and herbivores. Finally, PEG alone had a greater influence on sheep than on goats, and it had most influence on both sheep and goats when only one or two foods were available.

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SAŽETAK

Sredozemna makija je grmoliki tip vegetacije koja osigurava važno obitavalište za divlje i domaće životinje. Premda je većina grmova makije hranjiva, uglavnom je opterećena sekundarnim metabolitima koji im dodatno smanjuju hranjivu vrijednost.

U četiri pokusa proučavan je učinak polietilenglikola (PEG) i različitog broja sredozemnih grmova ponuđenih na konzumiranje ovčama i kozama. U prvom je pokusu ponuđeno 6, u drugom 3, u trećem 2 i u četvrtom jedan grm. Pokusi su provedeni s 12 ovaca i 12 koza (6 PEG-skupina i 6 kontrolna-skupina). U prvom pokusu koze su pojele više biomase grmova nego ovce (60.7 nasuprot 45.9 ± 2.6 g/kg BW). Utvrđen je trend ($P=0.08$) u pravcu pozitivnog učinka PEG-a na ukupno konzumiranje grmova, s time da su PEG-om prihranjivane životinje konzumirale ukupno više grmova nego kontrolna skupina životinja (56.7 nasuprot 50.0 ± 2.6 g/kg BW). U pokusu 2, obje vrste životinja pokazuju numeričko opadanje ukupno konzumiranih grmova sa ili bez prihranjivanja PEG-om. Ovce prihranjivane PEG-om pojele su više ($P=0.002$) ukupnih grmova nego kontrolna skupina, ali taj učinak nije utvrđen za koze. U pokusu 3, dodavani PEG imao je pozitivan učinak ($P<0.001$) na ukupno konzumiranje grmova za obje vrste životinja (ovce i koze) kada su im ponuđena samo 2 grma niže palatabilnosti (*Arbutus unedo* i *Pistacia lentiscus*). U pokusu 4, dodavani PEG imao je izrazito pozitivan učinak ($P<0.001$) na konzumiranje *Pistacia lentiscus* za ovce i koze. PEG-om prihranjivane koze pojele su više *Pistacia lentiscus* (39.6 g/kg BW) nego PEG-om prihranjivane ovce (28.1 g/kg BW), dok su kontrolne skupine ovaca i koza pojele približnu količinu (12.2 odnosno 15.3 g/kg BW). Naša istraživanja pokazuju da biokemijska raznolikost igra važnu ulogu u odabiru obroka za herbivore, omogućujući životinjama bolje zadovoljavanje hranidbenih potreba i izbjegavanje trovanja. Nadalje, kako se broj grmova u obroku smanjivao, učinak PEG-a na konzumiranje grmova se povećavao. PEG ima veći utjecaj na ovce nego na koze, a najveći utjecaj na ovce i koze ostvaruje kada im je ponuđen samo jedan ili dva grma.

Ključne riječi: Sredozemni grmovi, biološka raznolikost, ovce, koze, odabir obroka, sekundarni metaboliti, polietilen glikol.