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IMPORTANCE OF BIOLOGICAL/BIOCHEMICAL DIVERSITY IN FORAGING ON MEDITERRANEAN RANGELANDS

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Summary

Vast arrays of the Mediterranean plants contain secondary compounds that are potentially toxic to herbivore. The herbivore's challenge is to acquire sufficient nutrients to evade starvation and produce viable offspring and yet avoid the consumption of lethal doses of secondary compounds (toxins). Animals possess several adaptations that enabling them to better meets their nutritional needs and avoid toxicity. These adaptations consist of some innate avoidance pattern and mechanism that alter the hedonic value of postingestive plant qualities (flavour) based on postingestive experiences (e.g. nutritional benefits or gastrointestinal distress). Plants become more palatable when their consumption has positive postingestive consequences and their palatability decrease when consumption results in gastrointestinal malaise. Understanding the role of plant secondary compounds in controlling plant-herbivores interactions is important for managing plant and animals populations in Mediterranean grazed ecosystems. This paper discuss ways in which plant secondary compounds alter the grazing behaviour of mammalian herbivores. Then, the focus will switch to ways in which herbivores protect themselves from over ingestion of phytotoxins.

Key words: Mediterranean shrubs, phytotoxins, complementarity, biodiversity, and sheep.

Introduction

Mediterranean shrubby vegetation (maquis and garrigues) covers over 1 million hectares in the Mediterranean Croatia (Rogosic et al., 2000; Rogosic et al. 2006) and about 100 million hectares in the entire Mediterranean basin (Le Houerou, 1980). These traditional grazing areas are a critical resource for animal forage in the Mediterranean Region, particularly during the dry summer (Devendra 1990; Rogosic, 2000).

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However, utilization of the Mediterranean shrubs is often limited by secondary compounds such as tannins (Perevolotsky et al. 1993; Silanikove et al. 1994; Silanikove et al. 1996; Rogosic et al. 2006a), terpenes (Banner et al., 2000; Villalba et al., 2002a; Rogosic et al. 2006b), and saponnins (Rogosic et al., 2003), which at too high concentrations can adversely affect forage intake and animal health. Level of plant defensive compounds are major determinants of herbivores production and defoliation patterns of Mediterranean maquis plant communities. Understanding how plant secondary compounds affect herbivores and how herbivores respond to these compounds is critical to our understanding of the role of herbivores in Mediterranean grazed ecosystems.

Materials and Methods

Study shrubs and animals

Three experiments were conducted at a research station in village of Postire on the island of Brac (Central Dalmatia). The experiments were conducted during spring (May - July, 2005). We studied five dominant shrubs of the Mediterranean holly oak maquis vegetation (Rogosic, 2000) on the island of Brac, which is mainly cover with this type of vegetation. The investigated shrubs are: *Quercus ilex* L. (Fagaceae), *Quercus pubescens* L. (Fagaceae), *Arbutus unedo* L. (Ericaceae), *Pistacia lentiscus* L. (Anacardiaceae) and *Hedera helix* L. (Araliaceae). All shrubs offered to sheep were hand-harvested each week in the areas 2 km around the research station. Leaves and one-year old twigs, about 10 cm long, were clipped and placed in bags. Within an hour the plant material was ground to 1 cm length with a chipper, mixed for uniformity, placed in woven, polyethylene feed sacks, and refrigerated at 4^{o}C. Every morning before the trials, bags of shrubs to be fed that day were removed from cold storage and offered immediately to animals. The experimental sheep (n=12, mean weight 27.21 kg) were a local breed that is a cross between the Croatian breeds “Pramenka” and “Wunterberg”. Animals had an approximately equal mix of both sexes. All experimental animals were raised on the same farm, with a background of grazing on Mediterranean maquis vegetation on the island of Brac, and the previous experience probably influenced the choices made by individual animals during these trials (Distel and Provenza 1994). Throughout the experiments, animals were individually penned (1.5 x 2 m pens) and had free access to trace mineral blocks and fresh water.

Conditioning

Prior to the experiments baseline intake of alfalfa pellets was determined for each animal on days 1 to 5. After baseline was established, all animals (n=12 sheep) were offered all 5 shrubs from 9:00 to 13:00 for 5 days. Shrub intake was monitored, and the study was balanced by dividing the animals equally into treatment groups based on total shrub intake. Treatment groups were unaltered during the trials, but between shrubs feeding (each Exp.) baseline was reestablished using alfalfa pellets and barley at a maintance level for three days. Each morning from 0800 to 0830 goats received 100 g barley. Then, from 0900 to 1500 hours all animals were fed with 200 g each shrubs biomass in separate food boxes daily for 10 days. We controlled food boxes each half of hour and in each empty boxes we additional offered shrub biomass. At 1500 hr, feed refusal was weighed and shrub consumption was calculated, and sheep were fed 550g of alfalfa pellets, including 100 g barley offered in the morning that is roughly 50% of energy diet.

Investigated of shrubs with consisted secondary compounds

Three experiments were designed using different combination of high-tannin shrubs as there are: *Quercus ilex, Quercus pubescens, Arbutus unedo* and*, Pistacia lentiscus* and high-saponin shrub *Hedera helix*. Combination of shrubs that contain tannins with *Hedera helix* that contain saponins may enhance shrub intake because tannins and saponins chelate in the intestinal tract, thereby reducing the aversive effects of both compounds (Freeland, et al., 1985; Rogosic et al., 2003). Tannin analysis showed that *Pistacia lentiscus* had the highest tannin index (1.50), followed by *Arbutus unedo* (1.26), *Quercus pubescens* (1.05) and *Quercus ilex* (0.96). Other secondary compounds in these shrubs include a cyclic diterpene alcohol and quinic acid (*Quercus ilex)*, arbutoside and ethyl gallate (ethyl 3,4,5-trihydroxy benzoate) *(Arbutus unedo)*, and terpenes (including α-pinene, β-pinene, camphene, trans-caryophyllene, cubebene [or a similar sesquiterpene], and cadinene) and fatty acids *(Pistacia lentiscus)* (Rogosic and Clausen, unpublished data). *Hedera helix* is reported to contain a mixture of pentacylic terpenoids (Burrows and Tyrl, 2001) classified as genins, monodesmosides (α-hederin and β-hederin), or bidesmosides (hederacosides C and B). This complex mixture is often referred to as saponins.

Statistical Analyses. Data were analyzed using analysis of variance (SAS, 2000), and means were separated using least significance differences ($P < 0.05$).

Results and Discussion

Plant secondary compounds as grazing deterrents

As most of Mediterranean shrubs contain secondary compounds (Rogosic et al., 2006), it is common for small ruminanrs browsing on Mediterranean shrublands to include small amounts of phytotoxins in their diets, but the norm is that selection of plants or plants parts is inversely related to their content of phytochemicals (Provenza et al., 1990; Freeland, 1991). Therefore, it is evident that animals possess grazing mechanisms to limit their exposure to phytotoxins (Pfister, et al, 1997). By understanding these grazing mechanisms we can predict ways in which the biochemical structure of plants influences their probability of being eaten. Successful antiherbivore strategies either decrease the survival and reproduction of the herbivore (Foley and McArthur, 1994). Not all chemicals are equally effective at deterring herbivory, therefore herbivores should be able to eat more foods with different kinds of toxins because they produce different effects in the body and they are detoxified by different mechanisms (Freeland and Janzen, 1974). To date, however, little is known about how different toxins interact to influence food intake.

Phytotoxins complementarity

Animals foraging on Mediterranean shrublands must eat a variety of plant species that contain different kinds of toxins to obtain nutrients need and avoid toxicosis. When lambs choose between foods that contain either amygdalin or lithium chloride, they eat more than lambs offered a food that contains only one of these toxins; the same is true with nitrate and oxalate (Burritt and Provenza, 2000). Mule deer also consumed more sagebrush (*Artemisia tridentata*) and juniper (*Juniperus osterosperma*) (plants high in different kinds of terpenes) than when consuming sagebrush or juniper alone (Smith, 1959). Phytochemicals such as tannins and terpenes apparently differ in their physiological effects and/or are detoxified by different means. Tannins and saponins are two of the major groups of phytochemicals in Mediterranean shrubs (Rogosic et al., 2003). Sheep provided the mixture of two tannin-rich shrubs (*Arbutus unedo,* and *Pistacia lentiscus*), three tannin-rich shrubs (*Quercus ilex, Pistacia lentiscus*, and *Arbutus unedo*) and four tannin-rich shrubs (*Quercus pubescens, Quercus ilex, pistacia lentiscus*, and *Arbutus unedo*) consumed significantly less foliage than those offered shrubs in mixture with *Hedera helix* (Exp. 1 – Exp. 3). Sheep offered two, three or four shrubs containing different classes of secondary compounds (tannins and saponins) consumed more foliage than sheep offered two, three or four shrubs which all contained only tannins. Sheep provided with a choice between shrubs containing tannins and saponins were able to consume combinations of the shrubs that may have reduced aversive feedbacks associated with consumption of either toxin alone. Complementary interactions between shrubs containing tannins and saponins further support the idea that biochemical diversity (higher number of shrubs species in the diet) has a positive influence on shrub intake, enabling animals to better meet nutritional needs and avoid toxicity (Provenza et al., 2003). Tannins, saponins, and other allelochemicals may form complexes within the intestinal tract, given that they form chelation complexes in "in vitro" systems (Freeland et al., 1985). Intestinal binding of tannins with saponins may minimize toxic effects by reducing absorption. Simultaneous consumption of plants containing chemical chelators (e.g., tannins) and those containing other toxins may provide a mechanism for reducing both pre- and post- absorption toxicity. If herbivores use phytochemical interactions as a means of neutralizing toxins, this mechanism could be very important regarding management of ruminants on Mediterranean rangelands.

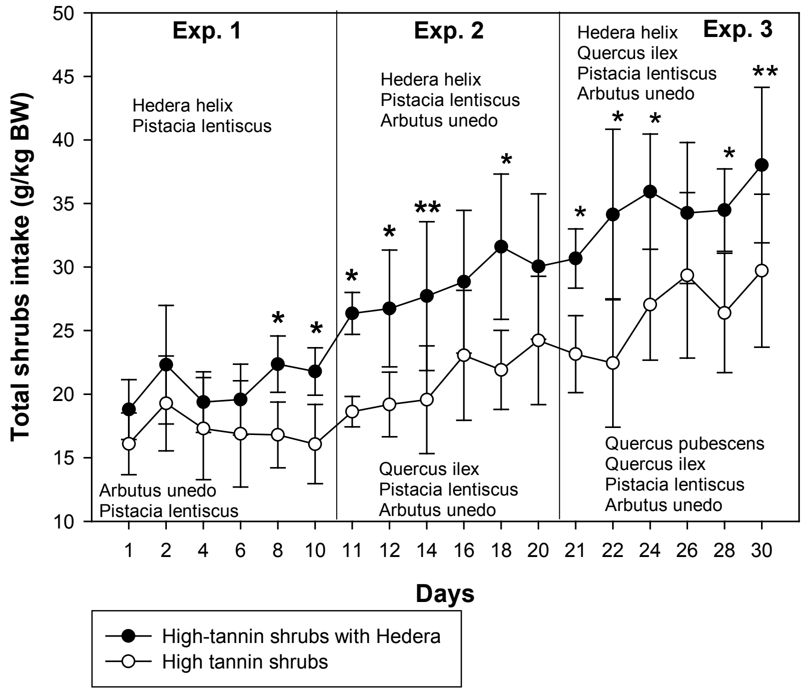
Importance of variety for small ruminants on Mediterranean rangelands

Total shrub intake increased numerically as the number of shrubs increased across the Exp. 1, 2, and 3 (Figure 1). Although the 3 experiments were not compared statistically, the results clearly showed that combinations of more shrubs promoted greater intake. These results are consistent with observations that a greater number of shrub species in the diet increases intake (Smith, 1959; Rogosic et al., 2006a; 2006b).

As noted above, these results clearly showed that combinations of more shrubs and especially complementary interaction between phytotoxins, nutrient and flavours promoted greater intake by herbivore (Provenza et al., 2003; Rogosic 2006a; 2006b). 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" (Villalba et al., 2002a). Likewise, different concentrations of nutrients in plant species may have different effects on selection of food by herbivores depending on the classes and concentration of phytotoxins in the plant community. So, relationships among shrubs are likely to vary on a case-by-case basis depending on the biochemical composition.

Figure 1. Sheep intake (total daily amount in g/kg BW ± SE) of different combinations of high-tannin shrubs (*Quercus ilex, Quercus pubescens, Arbutus unedo,* and *Pistacia lentiscus*) with or without high-saponin shrub *Hedera helix*.

Slika 1. Ovce konzumiraju (ukupna dnevna količina u g/kg tjelesne težine ± SE) različite kombinacije grmova s visokim sadržajem tanina (*Quercus ilex, Quercus pubescens, Arbutus unedo,* and *Pistacia lentiscus)* s ili bez grma (*Hedera helix*) s visokim sadržajem saponina



The variety of shrubs species composed in maquis plant community may make it difficult for herbivores to determine which combinations of shrubs will be chosen, to meet nutritional meet, minimize intake of toxins, optimise interactions between nutrients and toxins or recognize complementarity or aversion between toxins and toxins. While much more be learned about this behaviour (Belovsky and Schmitz, 1994), it is obviously that herbivores learn about the postingestive consequences of antagonistic and complementary interactions between nutrients/toxins and toxins/toxins.

Conclusions

Plants produce diverse mixtures of biochemicals that provide herbivores with nutrients for survival and reproduction and secondary metabolites that can both cause toxicity and yield health benefits. Just, a biochemical diversity of plant community enable herbivores to eat combination of foods that are complementary, and perform better when offered a mixture of plant species rather than single species. Here we discuss how complementary interaction among tannins and saponins containing in five dominant shrubs of the Mediterranean maquis plant community in different combinations of diet and how different number of shrubs (biodiversity) offered to sheep affect foliage intake of those shrubs.

Our results suggest that complementary interaction between phytochemicals (tannins and saponins) may influence how herbivores mix their diets and use food resources.

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VAŽNOST BIOLOŠKE/BIOKEMIJSKE RAZNOLIKOSTI PRI ISPAŠI NA SREDOZEMNIM PRIRODNIM PAŠNJACIMA

Sažetak

Velika većina sredozemnih biljaka sadrži sekundarne metabolite koji su potencijalni otrovi za herbivore. Ispašom na prirodnim pašnjacima životinje nastoje osigurati dovoljnu količinu hranjivih tvari kako bi zadovoljile hranidbene potrebe s jedne strane, te izbjegle konzumiranje letalnih doza sekundarnih metabolita (otrova) s druge strane. Životinje posjeduju nekoliko prilagodbi koje im omogućavaju da bolje zadovolje svoje hranidbene potrebe, a izbjegnu trovanje. Te prilagodbe uključuju urođeni instinkt, ali i cijeli mehanizam koji postingestivno mijenja hranidbenu vrijednost biljke (miris, okus). Postingestivni učinak hrane temelji se na iskustvu životinje, tj. da li je pojedena hrana prouzročila pozitivni hranidbeni učinak ili pak gastrointestinalne smetnje. Biljke postaju prikladnije za jelo (palatabilnije) kada njihovo konzumiranje rezultira pozitivnim postingestijalnim učincima i manje prikladne za jelo (palatabilnost opada) kada njihovo konzumiranje rezultira gastrointestinalnom mučninom. Razumijevanje uloge biljnih sekundarnih metabolita u kontroliranju interakcije između biljaka i životinja je važno u gospodarenju biljnim i životinjskim populacijama u sredozemnim pašnjačkim ekosustavima. U ovom se radu raspravlja kako biljni sekundarni metaboliti mijenjaju hranidbeno ponašanje ovaca koje se hrane sredozemnim grmovima. Isto se tako govori o načinima kako se herbivore u prirodi zaštićuju od prekomjernog uzimanja biljnih otrova.

Ključne riječi: Sredozemni grmovi, biljni otrovi, komplementarnost, biološka raznolikost, ovce

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