

**FORAGING IN BIOCHEMICALLY HETEROGENOUS  
PLANT COMMUNITY: SECONDARY METABOLITES  
COMPLEMENTARITY****J. Rogošić****Summary**

Goats are primary consumers of the Mediterranean shrubby vegetation, and thereby shape the diversity, structure and dynamics of these extensive ecosystems. Low nutritional quality and high levels of secondary compounds can result in low consumption of Mediterranean shrubs by herbivory. Goats were allowed to choose between Mediterranean shrubs containing tannins and other Mediterranean shrubs containing tannins and saponins. Twelve goats offered tannin-containing shrubs consumed less biomass than animals offered shrubs containing tannins and saponin. Simultaneous consumption of shrubs that contain tannins and saponin may promote chemical interactions that inhibit toxin absorption from the intestinal tract. Besides complementary tannin and saponin interactions, biodiversity of Mediterranean shrubs also plays a positive role in increased biomass intake by goats.

Key words: Mediterranean shrubs, goats, tannin and saponin interaction, biodiversity

*Introduction*

Goats foraging on Mediterranean shrubs that contain secondary compounds (toxins) may consume a variety of foods to avoid toxicosis (Freeland and Janzen, 1974). Different toxins have different physiological effects on herbivores (Cheeke, 1998), and herbivores are equipped with various physiological mechanisms to counter toxins (McArthur et al., 1991). A varied diet should enhance goats ability to meet its nutritional needs when foraging on Mediterranean shrubs with secondary compounds provided 1) the toxins have different physiological effects, 2) the toxins are detoxified by independent metabolic pathways, and 3) the toxins do not interact with each other to become more toxic.

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Jozo Rogošić, Department of Ecology, Agronomy and Aquaculture, University of Zadar, Mihovila Pavlinovica bb. 23000 Zadar, Croatia

A varied diet with plant species containing different kinds of nutrients and toxins may increase food intake and animal production by enabling individuals to select plants that are biochemically complementary (Rogošić et. al., 2003).

Tannins and saponins are among the more common classes of phytochemicals used as defences by plants and can be expected to influence herbivore-feeding behaviour. Freeland et. al. (1985) suggested that herbivores may be able to overcome the toxic effects of tannins and saponins by simultaneously consuming both classes of chemicals.

Goats were provided with Mediterranean shrubs containing tannin and/or saponin in order to determine whether ingestion of condensed tannins in conjunction with saponin resulted in reduced toxic effects, manifested as increased shrub intake. The objective of this study was to determine if goats consuming shrubs with different toxins (tannin + tannin or tannin + saponin) would ingest more shrubs foliage than goats consuming a shrubs with a single toxin (tannin). We used five Mediterranean shrubs that contain tannins and/or saponins in different combinations. Also we studied how different combinations of the Mediterranean shrubs, which containing different amount of nutrient, toxins and flavor influence foliage intake and show how plant biochemical/biological diversity influences shrub intake by goats.

#### *Materials and methods*

The four most important shrubs of the Mediterranean maquis plant community *Orno-Quercetum ilicis* (Rogošić, 2000) have been chosen in these experiments. These shrubs included *Quercus ilex* L. (Fagaceae), *Arbutus unedo* L. (Ericaceae), *Pistacia lentiscus* L. (Anacardiaceae) and *Hedera helix* L. (Araliaceae).

Tannin analysis showed that *Pistacia lentiscus* had the highest tannin index (1.50), followed by *Arbutus unedo* (1.26) and *Quercus ilex* (0.96). Beside these shrubs are rich in tannins, they content other components, so for example genus *Quercus* contain a cyclic diterpene alcohol, and quinic acid, species *Arbutus unedo* contains arbutoside and ethyl gallate (which is ethyl 3,4,5-trihydroxy benzoate), while *Pistacia leutiscus* was rich in terpenes including alpha pinene, beta pinene, camphene, trans-caryophyllene, cubebene (or similarsesquiterpene), cadinene, and fatty acids (Rogošić and Clausen, unpublished).

The shrub *Hedera helix* is reported to contain a mixture of pentacyclic terpenoids (Burrows and Tyrl, 2001). These terpenoids are classed into

one of three groups: genins, monodesmosides ( $\alpha$ -hederin and  $\beta$ -hederin), and bidesmosides (hederacosides C and B). This complex mixture is often referred to saponins. Clinical signs of toxicity include vomiting, excess salivation, and diarrhea, indicating both liver and digestive tract irritation (Harshberger, 1920). *Hedera helix* has also been reported to cause abortions in pregnant sheep and goats. Saponins are generally bitter tasting. Saponins that have a triterpenoid aglycone may not be bitter, but may have a licorice taste as glucuronic acid replaces sugar in triterpenoids.

Experimental animals were Alpine meat goats (average body weight 24.17 kg) with a background of grazing on Mediterranean shrublands on the Croatian coast. Animals were placed into individual pens and maintained on alfalfa pellets and barley on the level of 50% energy diet. Prior to the experiment all animals (n=12 goats) 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. We did not change the groups during the experiments but between shrubs feeding (each trial) baseline was reestablished using alfalfa pellets and barley at a maintenance level for three days.

Each morning from 0800 to 0830 sheep and 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 6 days. We controlled food boxes each half of hour and in each empty boxes we additionally offered shrub biomass. Food refusal was collected and shrubs consumption was calculated. At the 1500 hours animals offered amounts of alfalfa pellets, including the former offered barley to the level of 50% energy diet. Throughout the experiments, animals had free access to trace mineral blocks and fresh water. We conducted eight trials at an experimental station 25 km far from Split in the Central part of the Adriatic seaside.

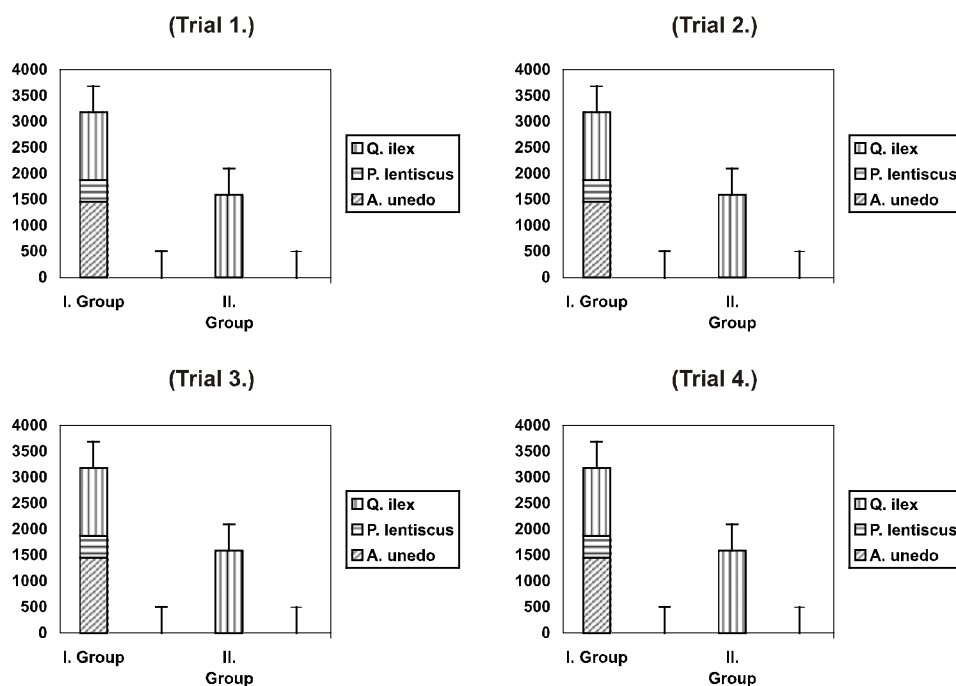
*Tannins and tannins.* The objective of Trial 1 was to determine if goats consuming three shrubs with the same toxin (tannin), but in different toxin concentration and different flavor would consume more biomass than goats offered one shrub containing a single toxin and flavor. We offered three shrubs *Quercus ilex*, *Arbutus unedo* and *Pistacia lentiscus* to the first group and only *Quercus ilex*, which has the lowest concentration of tannins to the second group.

*Tannins/Tannins and Saponins.* The objective was to determine if goats offered 3 shrubs with the same toxin, but in different amount of nutrients,

different tannins concentration and different flavor would consume more biomass than goats offered only one shrub (*Hedera helix*) containing a single toxin (saponins) and flavor.

FIGURE 1. – DIFFERENT COMBINATION OF HIGH-TANNIN SHRUBS (*QUERCUS ILEX*, *ARBUTUS UNEDO*, AND *PISTACIA LENTISCUS*) AND HIGH-SAPONIN SHRUB *HEDERA HELIX* SIMULTANEOUSLY OFFERED TO GOATS IN EXP.1 – 4.

SLIKA 1. – RAZLIČITE KOMBINACIJE GRMOVA S VISOKIM SADRŽAJEM TANINA (*QUERCUS ILEX*, *ARBUTUS UNEDO*, AND *PISTACIA LENTISCUS*) I GRMA *HEDERA HELIX* S VISOKIM SADRŽAJEM SAPONINA U POKUSIMA 1 – 4.



#### *Tannins/Tannins and Tannins + Saponins*

The objective of the trial 3 was to determine if goats offered 3 shrubs with the same toxin but in different flavor and tannins concentration would eat more than goats offered two shrubs containing different toxins – tannin + saponin and different flavor.

We offered three shrubs *Quercus ilex*, *Arbutus unedo* and *Pistacia lentiscus* to the first group and *Quercus ilex* + *Hedera helix* (tannins + saponins) to the second group.

*Tannins (3 shrubs) and tannins (3 shrubs) + saponin (1 shrub)*. The objective was to determine biochemical/biological interaction and influence of the saponin in *Hedera helix* on the biomass intake of the three other shrubs with different tannins concentration and different flavors. So, we offered three shrubs *Quercus ilex*, *Arbutus unedo* and *Pistacia lentiscus* to the first group and the same combination of shrubs + *Hedera helix* (3 tannins-rich shrubs + saponins) to the second group.

#### *Results and discussion*

Goats select diets from a different plant species that vary in nutrient, toxins and flavor. We offered the three shrubs to the first group and only one shrub *Quercus ilex* to the second group (Trial 1). There are significant differences between the first and the second group in regard to shrubs biomass intake. According to this graph we can conclude that goats prefer to eat a greater variety of shrubs that differ in toxin concentration and flavor than one shrub, notwithstanding the very different concentration of toxins and palatability between shrubs. As we can see on the second graph (Trial 2) the first group of goats which were offered three shrubs that contain tannins ate more biomass than the second group of goats which was offered only *Hedera helix* containing saponins.

Combination of shrubs that contain tannins with *Hedera helix* that contain saponins may enhance shrub intake because tannin and saponins chelate in the intestinal tract, thereby reducing the aversive effects of both compounds (Freeland et al., 1985). The second group of goats ate more biomass of the shrubs *Quercus* and *Hedera* (tannins + saponins) than the first group of goats that was offered a combination of the three shrubs *Arbutus*, *Quercus* and *Pistacia*, shrubs which are loaded with tannins. Tannins and saponins are one of the major groups of phytochemicals, which are widespread in Mediterranean shrubs. Goats provided with a choice between shrubs containing tannins and saponins consumed combinations of the shrubs that apparently avoided symptoms of toxicity associated with consumption of either toxin alone. This study supports Provenza's hypothesis (2003) that combining the toxins in diet selection might eliminate toxicity. The nullification of any toxic effects is obviously dependent on the relative proportions of the toxins ingested. Tannins, saponins and other phytochemicals are likely to chelate within the intestinal tract. Tannins and saponins chelate with one another in "in vitro" systems (Freeland et al., 1985). Intestinal bonding of tannin and saponin may attenuate toxic affects by reducing absorption of the toxin. Herbivores have

various mechanisms that prevent absorption or reduce the effect of ingested toxins. For example, induced microsomal enzymes can reduce (not eliminate) the effect of toxins that are absorbed. Polygastric (i.e., ruminant) animals may sometimes have an advantage over monogastric animals in regard to reductions in toxicity because of ruminal detoxification mechanisms. A dietary strategy using simultaneous consumption of plants containing chemical chelators (e.g. tannin) and plants containing other toxins may provide a mechanism for reducing both pre- and post- absorption toxicity. As we can see on the last graph the second group of goats ate more biomass than the first group which indicates that *Hedera helix* positively influenced the consumption of shrubs like *A. unedo*, *Q. ilex* and *P. lentiscus* containing tannins. It is obviously that combination of shrubs containing tannins with *Hedera helix* that contain saponins may enhance shrub intake, but likewise biochemical diversity has a positive influence on shrub intake.

### *Conclusion*

Tannins and saponins are very common classes of phytochemicals (secondary compounds) in Mediterranean shrubby species. Goats offered a choice of shrubs each containing a different toxin – tannins and saponins eat more forage than goats offered only one shrub. Goats offered rations of shrubs with tannins and saponins eat more biomass than sheep and goats offered shrubs with either tannins or saponins. Besides complementary tannin and saponin interactions, the variety of shrubs offered to goats is likely to be an important concern in effort to increase forage intake and animals performance on Mediterranean shrublands.

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### **ISPAŠA U BIOKEMIJSKI RAZNOVRSSNOJ BILJNOJ ZAJEDNICI: KOMPLEMENTARNOST SEKUNDARNIH METABOLITA**

#### **Sažetak**

Koze su primarni konzumenti sredozemne grmolike vegetacije i stoga oblikuju raznolikost, strukturu i dinamiku ekstenzivnih grmolikih ekosustava. Niska hranidbena vrijednost i visoki sadržaj sekundarnih metabolita najčešće rezultira niskom konzumacijom sredozemnih grmova od strane herbivora. Kozama je omogućen izbor između sredozemnih grmova koji sadrže tanine i druge skupine sredozemnih grmova koji sadrže tanine i saponine. Dvanaest koza kojima su ponuđeni grmovi bogati taninima manje su konzumirali biomase grmova nego koji druga skupina koza kojima su ponuđeni grmovi koji sadrže tanine i saponine. Istovremeno konzumiranje grmova koji sadrže tanine i saponine može se postići kemijska interakcija i tako spriječiti apsorpcija biljnih otrova iz intestinalnog trakta. Osim komplementarne interakcije između tanina i saponina, biološka raznolikost sredozemnih grmova igra važnu ulogu u povećanju konzumacije biomase grmova od strane koza.

Ključne riječi: Sredozemni grmovi, koze, tanini i saponini, interakcija, biološka raznolikost

Primljeno: 08.11.2007.