Increased intake of *Juniperus phoenicea* L. by supplementation with barley and Optigen® in sheep

**Tomislav Šarić, Jozo Rogošić, Suzana Tkačić, Ivan Župan, Zoran Šikić**

**Odjel za ekologiju, agronomiju i akvakulturu, Sveučilište u Zadru, Zadar, Croatia**

**Abstract**

Dry littoral grasslands and rocky ground pastures throughout the Mediterranean basin are a significant source of forage for small ruminants and as such associated with traditional grazing practices. During the last decade, dissemination of terpene-rich Mediterranean shrubs, *Juniperus phoenicea* L., onto pastures in Adriatic region of Croatia have caused reduction in forage production and plant diversity. In order to facilitate the intake of this new plant by small ruminants, we investigated the effects of barley and Optigen® (a source of controlled-release non-protein nitrogen) mixture to increase consumption of *J. phoenicea* by sheep. The preliminary results suggest that barley alone, or in combination with Optigen®, enhance the intake of *J. phoenicea* by sheep. Furthermore, this model can be used as an environmentally safe and economically affordable approach to reduce the abundance of less palatable *J. phoenicea* in the environment and to increase growth of alternate, better quality forage (grasses and forbs) on Mediterranean pastures.

**Introduction**

Dry Mediterranean grasslands and rocky ground pastures occupy over 30% of the agricultural land along the Adriatic coastline of Croatia and represent important source of forage for small ruminants in extensive farming operations (Rogosic, 2000). During the last decade, Phoenicean juniper (*Juniperus phoenicea* L.), an integral part of the Mediterranean maquis plant community, became an increasing component of many pastures in Mediterranean parts of Croatia and Bosnia/Herzegovina. Dissemination of species from genus *Juniperus* onto pastures is causing reduced favorable forage production and plant diversity, degraded wildlife habitat, and reduced availability of water (Hamilton and Ueckert, 2004). Low utilization of *J. phoenicea* by grazing quality can be attributed to the low nutritional quality and its toxic plant secondary metabolites (PSM), mainly terpenes and phenolics, which appear to suppress voluntary intake of this shrub (Rogosic et al. 2006). Although *J. phoenicea* is known to contain terpenes (Salido et al., 2002), a detailed chemical analyses reported that the main PSM constituents are α-pinene, α-phellandrene, γ-terpinyl acetate, D-3-carene, and myrcene (Tomassini et al., 1995., Rezzi et al., 2001).

Previous studies have described how high protein and high energy feed supplementation can mitigate the detrimental effects of phenolics and terpene compounds, facilitate detoxification processes, and increase the overall intake of food with high content of secondary compounds (Riddle et al., 1996.; Villalba et al., 2002.). It was also reported that combination of activated charcoal and high energy feed can substantially increase the intake of *J. phoenicea* by goats (Rogosic et al., 2009). Optigen® is a commercially available source of controlled-release non-protein nitrogen (NPN) in the rumen of livestock that was used in this study as a high-protein resource. The compound is composed of urea tablets coated with vegetable oil, that were developed and produced by Alltech Inc. Lexington, KY, in 2005 (Garcia-Gonzalez et al., 2007). Its main effect is in slowing down the NH3 released from urea, resulting with more urea being converted into microbial proteins and therefore supplying additional protein to the host animals (Calsamiglia et al., 2008).

The aim of this study was to evaluate the role of supplemental energy-rich macronutrient (barley) and non-protein nitrogen source (Optigen®) on intake of terpene-rich Mediterranean shrub *Juniperus phoenicea* L. by sheep.

**Materials and methods**

The experiment was conducted on an experimental station Zemunik Donji in October 2012, about 20 km from Zadar, Croatia (lat 44°06’ N, long 15°23’ E). Research protocols for the study were approved by the Ministry of Agriculture, Agency for Animal Care and Use Committee, No. UPY-322-01/11-01/118, Republic of Croatia.

A total of 18 female sheep of the endogenous Croatian breed Pramenka were used in the study; they were all 2 years of age and of the same weight (mean body weight ± SE = 28.3 ± 2.4 kg). Prior to the experiments, a baseline intake values were established for each animal by feeding them alfalfa pellets for 5 days. Afterwards, all animals were preconditioned to *J. phoenicea* for 5 days by offering *J. phoenicea* for 120 min/day. The animals were randomly assigned into three groups composed of 6 animals in individual pens, with free access to trace mineral blocks and fresh water.

*J. phoenicea* was collected during September 2012 from the area surrounding the experimental station. Shrub leaves and current season growth (i.e., twigs) were clipped and ground to 1 cm length with a chipper, mixed for uniformity, placed in woven, polyethylene feed bags, and stored at 4°C for a latter use. Every day, the allocated feeding bags of shrubs were removed from the cold storage and offered to the sheep as a feeding ration.

At 08:00 h daily, second group was offered 100g of barley and the third group was offered 100 g of barley and 7 g of Optigen®. Supplemented barley contained 8.1% of crude proteins and provided 3.43 Mcal of ME/kg. At 08:30 h all groups were offered 300g of *J. phoenicea* in feeding boxes. At 14:00h feed refusal was weighed, intake of *J. phoenicea*, was calculated, and all animals were fed 50% of their daily baseline intake with hay and alfalfa pellets.
until the next day. The procedure was repeated for the following 7 days.

Statistical analyses

The model included three treatments (G1, without supplemental feed; G2, with barley; and G3, with barley and Optigen®) with individual animals nested within treatments and repeated measures over the 7 days. The total daily intake of *J. phoenicea* was used as the dependent variable in the analysis. A two-way RM ANOVA was used for all analyses, and means of the groups were compared between groups through using Tukey’s test. The significance level was set at P<0.05. All analyses on shrub intake were adjusted for body weight (BW) (g/kg body weight) and performed with the SigmaPlot 11 statistical package (Systat Software).

Results and discussion

The lowest intake of shrub biomass *J. phoenicea* by sheep was recorded in control Group 1, when animals were not supplemented with barley and Optigen® (2.5±0.23 g/kg BW), in comparison to Group 2 (5.01±0.39 g/kg BW) and Group 3 (8.15±0.3 g/kg BW) in which supplemental barley and supplemental barley plus Optigen® was offered, respectively (Figure 1) (P<0.001). Likewise, a significant difference was noticed between Group 2 and Group 3 (P<0.05). Statistically significant difference in average daily intake of *J. phoenicea* is presented in Figure 2. (P<0.05). It is interesting to note that the average daily intake of *Juniperus phoenicea* L. in barley-supplemented group and in without-supplemented group is similar with the only exception at the first and third day of treatment, probably due to the limited group size and individual variability within groups. Our results indicate that adding barley as a source of energy to small ruminants can substantially increase intake of *J. phoenicea*, a shrub rich in plant secondary metabolites, especially terpene. Similar studies with sagebrush (*Artemisia* sp.) in the US have shown that supplementation with energy-rich feed-stuff has impacted shrub intake as well (Banner et al., 2000; Villalba et al., 2002). These results are consistent with the satiety hypothesis stating that interactions among flavors, nutrients, and toxins (i.e., PSMs) lead to enhanced intakes, even shrub of lower preference such as *Juniperus phoenicea*, or *Pistacia lentiscus* (Provenza et al., 2003). By adding Optigen® to barley, a further increase in feed intake was even more pronounced. Sheep receiving high-energy source coupled with Optigen® consumed significantly more shrub in comparison to control animals, indicating the effectiveness of high-energy and non-protein nitrogen source in reducing the impacts of terpenes from *J. phoenicea*. Optigen® supplementation in this study supports the findings of Villalba et al. (2002), which reported an increased intake of plants rich in secondary metabolites when adding a high protein meal supplement. Overall, nitrogen supplementation improves the digestibility of forages that contain condensed tannins (Petersen and Hill, 1991). Thus, increased nitrogen retention on low-nitrogen diets, or increased availability of nitrogen in high-protein diets is likely to offset the metabolic costs of secondary metabolite detoxification (McArthur and Sanson, 1993).

Conclusions

Our findings suggest that interaction between high-energy feed such as barley coupled with controlled-release urea products, such as Optigen®, can increase the intake of terpenes-rich shrub *J. phoenicea* by sheep. With sustained heavy grazing by small ruminants on the grasslands and rocky ground pastures, more palatable plants (high-quality forage plants) are replaced by a succession of plants and shrubs that tend to be increasingly low in palatability, lower in quality, and more toxic. Our results support findings of other authors that supplemental macronutrients have positive effect on intake of Mediterranean shrubs rich in plant secondary metabolites. Increasing intake of these shrubs by small ruminants would likely enhance the
production of better-quality grasses and forbs, and create a more diverse mix of plants in a given pasture.

Feed additives such as Optigen® could be further used as economically sound alternative to mitigate the effect, and to reduce the abundance of secondary compounds-rich shrubs. Over time, that can result in an enhanced growth of better-quality forbs and grasses in the ground layers, and create a more biological diverse plant community in pastures. Increased biological diversity would eventually result in greater variability in herbivore foraging behavior on Mediterranean pastures.

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