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SUPPLEMENTAL ACTIVATED CHARCOAL AND ENERGY INCREASE INTAKE OF MEDITERRANEAN SHRUBS BY SHEEP AND GOATS

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SUMMARY

Utilization of the Mediterranean shrubby vegetation is often limited by secondary compounds, such as terpenes, which at too high concentrations can adversely affect forage intake and animal health. Ingesting compounds such as activated charcoal and energy can ameliorate the negative effects of secondary compounds and enable animals to eat more shrubs. Thus, our objectives were to determine if supplemental charcoal, energy and numbers of shrub species offered influenced intake of shrubs by sheep and goats. We conducted three experiments each with 12 lambs and 12 kids (6 activated charcoal vs. 6 controls). In the first experiment, we initially offered three shrubs (Juniperus phoenicea, Helichrysum italicum and Juniperus oxicedrus),) then in the second one, two shrubs (Juniperus phoenicea and Helichrysum italicum), and finally one shrub (Juniperus phoenicea) in the third experiment. In all three experiments (Exp. 1, P<0.001; Exp. 2, P < 0.0003 and Exp. 3, P < 0.03), supplemental charcoal and energy had a positive effect on total shrub intake for both lambs and kids. Kids ate more shrubs than lambs did in all three experiments (P<0.01). Regardless of experiment, both species of animals showed a numerical decrease in total shrub intake, with or without supplemental charcoal and energy, as the number of shrub species on offer decreased. Our findings support the hypothesis that biochemical diversity plays an important role in diet selection, thus enabling animals to better meet their nutritional needs and avoid toxicity.

Key-words: activated charcoal, biodiversity, kids, lambs, diet selection, Mediterranean maquis, secondary compounds, terpenes

INTRODUCTION

Grazing lands in the Mediterranean Basin, including the Adriatic littoral of Croatia, are often dominated by evergreen shrubs. These types of vegetation cover over 1 million hectares in the Mediterranean area of Croatia and represent an important sources of forage for livestock, particularly during the dry summer (Rogosic et al., 2003). Utilization of Mediterranean shrublands is often limited by secondary compounds like terpenes, which adversary affect forage intake and animal health (Rogosic et al., 2007). Terpenoids are the largest group of plant secondary chemicals, with over 30,000 terpenes identified structurally. Terpenes have been found to decrease in vitro digestibility in ruminants, suggesting antimicrobial activity especially if the rumen microbial population is not adapted to the compounds (Schwartz et al., 1980). Nagy and Tengerdy (1968) reported sagebrush essential oil decreased bacterial numbers in deer rumen fluid, but observed no evidence of microbial adaptation. Actual effects of terpenes in vivo have been suggested to be less because of loss during mastication and rumination eructation of warm volatiles and/or absorption from the rumen. Newbold et al. (2004) reported that an essential oil mixture fed sheep caused no major effects on fermentation, rumen ammonia, protozoa numbers, or digestion, although they did observe reduction in sacco protein degradation. Secondary compounds in too high concentrations limit how much of any particular food an animal can be eaten, and there are ways animals can cope with secondary compounds (Villalba and Provenza, 2005). Activated charcoal (Ach) can increase use of shrubs rich in terpenes by adsorbing

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terpenoids, thereby reducing their negative effects. In addition, supplementing livestock with energy and protein can facilitate detoxification processes and increase intake of a food high in secondary compounds. Another strategy to reduce the effects of toxins is to eat a variety of plants that differ in their kinds and amounts of secondary compounds. The objective of our study was to determine if supplemental activated charcoal and barley (By) affected intake of three species of shrubs (*Juniperus phoenicea, Helichrysum italicum*, and *Juniperus oxycedrus)* by sheep and goats. We hypothesized that intake of shrubs by both sheep and goats would increase with supplemental activated charcoal as the number of shrubs offered increases.

MATERIAL AND METHODS

Shrubs

Three experiments were conducted at an experiment station 25 km from Split in the central part of the Croatian Adriatic coast. In the first experiment, three shrubs were offered (*Juniperus phoenicea, Helichrysum italicum*, and *Juniperus oxycedrus*), in the second one two shrubs *(Juniperus phoenicea, Helichrysum italicum),* while in the third experiment *Juniperus phoenicea* were offered. Shrubs were harvested each week from the vicinity of the feeding experiments. 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^{o}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 lambs (n=12) were crossbred hair-type 5 months of age, whereas the goats (n=12) were purebred Alpines 5 months of age. Each group was an equal mix of both sexes. Lambs weighed 22.0 kg and kids 20.2 kg at the beginning of the trial and 23.5 and 22.5 kg, respectively, for lambs and kids at the end. All animals were raised on the same farm on the island of Brac and were adapted to the shrubby vegetation of the Mediterranean maquis. To reduce neophobia, the experimental animals were offered a By/Ach mix for 30 minutes/day for 5 days before the trials. Similarly, they were also offered each of the 6 shrubs for 120 minutes/day for 5 days before the trials commenced. Throughout the experiments, animals had free access to trace mineral blocks and fresh water. 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 when 10 g of Ach mixed with 200 g By was given from 08:00 to 08:30. All 3 shrubs were offered in individual feeding boxes, and continually replenished from 08:30 to 14:00. Shrub intake was monitored, and animals divided into two treatment groups (Ach-treatment and By-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 3 experiments.

Feeding

All experiments lasted for 10 days, and the experiments ran consecutively. Lambs and kids in the Ach-group received 10 g of activated charcoal and 200 g of barley whereas controls were not given barley. In all the experiments, animals were fed the Ach/By mixture at 08:00, and given the ground shrubs at 08:30. All lambs and kids in the treatment groups ate all of the Ach-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 the all experiments 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 experiment was used in the analysis because consumption of each shrub was not independent of the other choices. The experimental design for the Ach experiments was a completely random design. Animals were a random factor in the mixed model analysis (SAS 2000). The model included treatment (Ach vs. By-control), species of animal (i.e., lambs vs. kids), 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 B.W.).

RESULTS

Experiment 1: Influence of activated charcoal on intake of 3 Mediterranean shrubs offered to lambs and kids

Kids and lambs differed in total shrub consumption ($P < 0.01$; Figure 1). Regardless the treatment, kids ate substantially more shrub than lambs did ($P = 0.002$; 37.9 ± 1.4 g/kg B.W. versus 28.2 ± 1.3 g/kg B.W., respectively). Ach-treated animals and controls did not differ in total shrub intake ($P = 0.39$; mean 37.5 ± 0.8 g/kg B.W.). There were no species x treatment or treatment x day interactions ($P > 0.50$), but there was a species x day interaction as both kids and lambs increased intake of the 3 shrubs over the period ($P = 0.004$). Even though lambs and kids differed in total amounts of shrubs eaten, the rank order of the amount eaten for each shrub species was essentially the same for both lambs and kids. The mean amounts eaten of the shrubs across all treatments and animal species were: *Juniperus oxycedrus* (17.2 g/kg B.W.), *Helichrysum italicum* (12.8 g/kg B.W.), and *Juniperus phoeniceae* (6.7 g/kg B.W.).

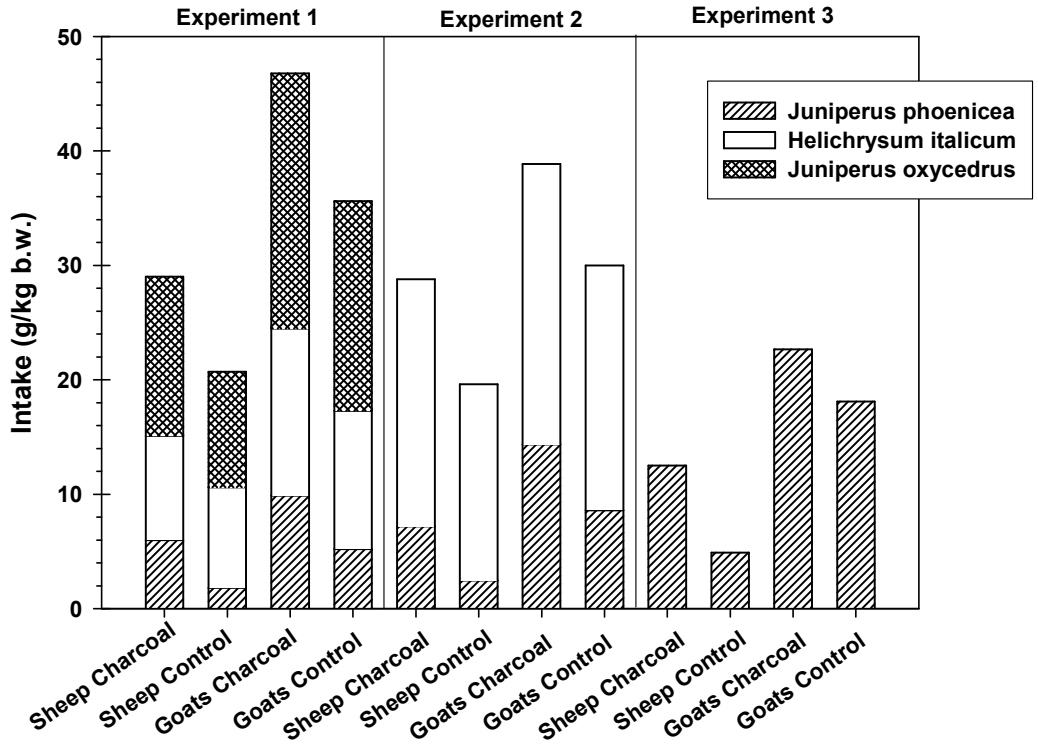

Figure 1. Differences in biomass consumption by kids and lambs supplemented or not with activated charcoal and barley when offered three (Experiment 1; $P < 0.01$), two (Experiment 2; $P=0.001$) or one species (Experiment 3; $P = 0.02$) of shrub
Slika 1. Razlike u konzumaciji biomase grmova za jariće i janjce kojima je dodatno ponuđen aktivni ugljen i ječam, te tri (pokus 1: $P < 0,01$), dva (pokus: 2; $P=0,001$) ili jedan grm (pokus: 3; $P = 0,02$)

Experiment 2. Influence of activated charcoal on intake of 2 Mediterranean shrubs offered to lambs and kids

Kids had a higher total intake when offered 2 shrubs than lambs did ($P = 0.0003$; 34.5 ± 0.9 g/kg B.W. versus 24.3 ± 0.9 g/kg B.W.; Figure 1). Lambs and kids fed charcoal ate more shrubs than the control animals did ($P=0.001$; 33.9 ± 0.8 g/kg B.W. versus 24.8 ± 1.1 g/kg B.W.). There were no species x

treatment or species x day interactions (P > 0.50), but there was a treatment x day interaction (P = 0.03) as Ach-treated animals increased intake of the 2 shrubs over the 10-day period more than the control animals.

Experiment 3. Influence of activated charcoal on intake of one shrub – *Juniperus phoenicea* offered to lambs and kids

There was a treatment x day (P = 0.03) interaction as Ach-treated animals, particularly lambs, increased intake of *Juniperus phoenicea* over time compared to control animals (Figure 1). There was also a species x day interaction (P = 0.02) as lambs increased intake over the period more than kids did even though lambs ate less *Juniperus phoenicea* biomass compared to kids.

DISCUSSION

Importance of Biological/biochemical variety for small ruminants on Mediterranean rangelands

Total shrub intake increased as the number of shrubs offered increased. Although the three experiments were not compared statistically, the results clearly showed that combinations of shrubs promoted greater intake in both kids and lambs. Likewise, lambs eat more when offered several foods (3 > 2 > 1) containing 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 enable 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 the kinds and amounts of nutrients and toxins in the forages on offer. Both nutrients and toxins 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 taxonomic 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. As the number of shrubs decreased from Experiment 1 through the Experiment 3, the concentration of terpenes in shrub biomass offered to animals increase, so the total shrub intake decreased numerically for both lambs and kids. In other words, these results clearly showed that combinations of more shrubs offered to animals promoted greater intake. These results are consistent with the satiety hypothesis (Provenza et al., 2003) that interactions among flavors, nutrients, and toxins (i.e., secondary compounds) lead to enhanced intakes, even of lower preference shrub such as *Juniperus phoenicea*. Our results suggest that interaction between macronutrients, activated charcoal, flavor and toxins influenced how lambs and kids mixed their diets and utilized shrubs. Also, supplemental macronutrients enhanced intake of food that contain terpenes and other secondary metabolites, as found in other studies (Rogosic et al, 2007). Likewise, different concentrations of nutrients in plant species may have different effects on selection of food by sheep and goats depending on the classes and concentration of toxins in the plant community. So, relationships among shrubs are likely to vary on a case-by-case basis depending on the biochemical composition. Chemical deterrents will be most effective in shrubs with low levels of nutrients needed to rectify imbalances created by a toxin,e.g. tannic acid-protein (Raubenheimer, 1992).

Effect of Activated charcoal on Intake of Mediterranean Terpenes Rich Shrubs

High-terpene shrubs and their extracts have been shown to negatively affect shrub intake, digestibility, and/or performance in various ecosystems (Rogosic et al, 2007). Likewise, kids and lambs exhibit

limited preference for terpene-laden shrubs (*Juniperus phoenicea, Helichrysum italicum*, and *Juniperus oxycedrus*) in the Mediterranean maquis ecosystem (Figure 1).
Activated charcoal can adsorb and decrease bioavailibility of phytochemicals in the gastrointestinal tract through the interaction of its positively charged surface with negatively charged toxins, allowing them to be excreted in the feces of browsing herbivores (Poage et al., 2000). Sheep and goats receiving 10 g of activated charcoal and 200 g of barley (energy) consumed more total biomass of high-terpene shrubs (Figure 1) when offered either three (*Juniperus phoenicea, Helichrysum italicum*, and *Juniperus oxycedrus;* Exp. 1), two (*Juniperus phoenicea,* and *Helichrysum italicum;* Exp. 2) or one shrub (*Juniperus phoenicea;* Exp. 3) species. Activated charcoal and energy presumably reduced the impact of terpenes on intake of the shrubs and substantially increased intake of shrubs high in terpenes.
Other studies with sagebrush (*Artemisia* sp.) in the U.S. have shown that supplemental energy impacted shrub intake when given in combination with activated charcoal (Villalba et al. 2002). We speculate that sagebrush contains higher and more toxic concentrations of sesquiterpene lactones (i.e., types of terpenoids) compared to Croatian Mediterranean shrubs, thus charcoal and energy interact for a greater impact on sagebrush intake. In this study, we attribute most of the treatment effect to activated charcoal and barley.
Interactions among nutrients, toxins and activated charcoal affected use of shrubs. For example, in all experiments the relatively unpalatable shrub *Juniperus phoenicea* was not avoided when offered with another more palatable shrub (*Juniperus oxycedrus* and *Helichrysum italicum)* even though animals were not forced to eat *Juniperus phoenicea*.

Comparative responses of lambs and kids

In all 3 experiments, kids ate more total shrubs than lambs did, suggesting they had a higher tolerance for secondary compounds. Kids typically eat more browse than lambs and utilize secondary compounds-rich foods better than lambs do (Landau et al., 2000). Food intake and dry matter digestibility of Mediterranean shrubs are often higher for kids than for lambs and goats often use protein more efficiently than sheep (Kronberg and Malechek, 1997).
In this study lambs and kids also responded similarly in several respects. In general in all 3 experiments, both lambs and kids increased intakes of shrub as each experiment progressed, indicating ongoing adaptation to the shrubs. Even though kids generally ingested higher levels of shrubs than lambs did in all three experiments. Kids and lambs both responded similarly to supplemental activated charcoal and energy (barley). Finally, as the number of shrub species offered decreased, intake of food decreased for both kids and lambs. In another similar trial, PEG had a greater influence on lambs than on kids when three Mediterranean shrubs were available and it had the most influence on both lambs and kids when only one or two foods was available (Rogosic et al., 2007).

CONCLUSION

Small ruminants are environmentally and economically sound alternative for using the forage potential of Mediterranean shrubs. Most Mediterranean shrubs contain large quantities of secondary compounds that limit intake and cause animals to eat a variety of foods.
The conducted researches showed that the number of shrubs in the diet decreased (diminution of biodiversity), the concentration of terpenes in diet probably increased, and activated charcoal and energy (barley) had a greater impact on shrub intake. Activated charcoal and energy supplement positively influenced shrub intake when lambs and kids were offered 3, 2 or 1 shrub species. Activated charcoal and energy (barley) had the same influence on lambs and kids throughout the experiments, and it had the most influence on both lambs and kids when shrub availability was reduced. 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.

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DODATAK AKTIVNOG UGLJENA I ENERGIJE POVEĆAVAJU KONZUMIRANJE SREDOZEMNIH GRMOVA ZA OVCE I KOZE

SAŽETAK

Iskorištavanje sredozemne grmolike vegetacije često je ograničeno sekundarnim metabolitima, kao što su terpeni, koji u visokim koncentracijama mogu štetno utjecati na konzumiranje biomase grmova i zdravlje životinja. Dodatnim uzimanjem spojeva kao što su aktivni ugljen i energija (ječam) može se ublažiti negativni učinak sekundarnih metabolita i omogućiti životinjama konzumiranje više količine grmova. Cilj istraživanja bio je utvrditi utječe li dodatno hranjenje s aktivnim ugljenom, energijom i različitim brojem grmolikih vrsta na konzumiranje grmova. Istraživanja su provedena u 3 pokusa, od kojih je svaki uključivao 12 janjaca i 12 jarića (6 aktivni ugljen nasuprot 6 kontrolna skupina). U prvom pokusu životinjama je ponuđeno 3 grma (Juniperus phoenicea, Helichrysum italicum i Juniperus oxicedrus), u drugom dva grma (Juniperus phoenicea i Helichrysum italicum) i u trećem samo jedan grm (Juniperus phoenicea). U sva tri pokusa (prvi: P<0,001; drugi: P < 0,0003 i treći: P < 0,03) dodavani aktivni ugljen i energija imali su pozitivni učinak na ukupno konzumiranje grmova u janjaca i jarića, s tim da su jarići konzumirali više biomase grmova nego janjci u sva tri pokusa (P<0,01). Bez obzira na provedene pokuse, obje vrste životinja pokazuju numeričko opadanje u ukupnoj konzumaciji grmova, s ili bez dodavanog ugljena i energije, kako je životinjama ponuđeni broj grmova smanjen. Naši zaključci podupiru hipotezu da biokemijska raznolikost sredozemnih grmova ima važnu ulogu u odabiru obroka, omogućujući životinjama bolje zadovoljavanje hranidbenih potreba i ujedno izbjegavanje trovanja sekundarnim metabolitima.

Ključne riječi: aktivni ugljen, biološka raznolikost, jarići, janjci, odabir obroka, sredozemna makija, sekundarni metaboliti, terpeni

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