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Short communication

Anthelmintic effect of three tannin-rich Mediterranean shrubs in naturally infected sheep

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ABSTRACT

Gastrointestinal nematodes are recognized as some of the most important causes of production losses in extensive sheep farming. Due to the current trends and need for finding ecologically acceptable alternatives to chemically produced anthelmintics, many research efforts are focusing on secondary plant metabolites that showed promising anthelmintic effects (e.g. tannins). In this study we investigated the effect of a mixture of three tannin-rich Mediterranean shrubs (*Arbutus unedo* L., *Pistacia lentiscus* L. and *Quercus ilex* L.) on the fecal egg counts (FEC) of nematodes in naturally infected adult sheep. A total of 28 sheep was randomly assigned to a treated and control groups ($n = 14$) and the effects of tannin-rich diet on FEC were evaluated throughout 30 days of study. No significant difference in FEC between the treated and the control group was noted at days 0, 23, and 30 of the study, suggesting that these three shrubs in combination do not exhibit a favorable antiparasitic effect for gastrointestinal nematodes in naturally infected sheep.

1. Introduction

Extensive sheep and goat farming is the dominant animal production system along the Adriatic coast of Croatia (Rogosic, 2000). In all grazing ruminants, gastrointestinal nematodes (GIN) are often implicated as a main cause for substantial production losses in extensive farming operations throughout this region, and worldwide (Sykes, 1994; Kostelic et al., 2008). Fecal excretion of nematode eggs into the environment during grazing or browsing is a major route for a wide spread contamination and fecooral infestation of host animals. Repeated use of chemically produced anthelmintics, recommended and prescribed by veterinarians, represents an effective treatment/control program for GIN. However, development of anthelmintic resistance in nematodes, together with the current trend for organic farming, have increased the demand for alternatives to chemoprophylaxis in order to reduce or exclude the use of anthelmintic drugs to control parasites (Jackson and Coop, 2000; Waller and Thamsborg, 2004). Hoste et al. (2012) reported that tannin-rich plants show an anthelmintic effect on various gastrointestinal nematodes by affecting different stages of the parasite life cycle. Several reports have demonstrated beneficial anthelmintic effects of some Mediterranean plants rich in tannins for decreasing nematode load in experimentally infected goats and sheep (Landau et al., 2010; Manolaraki et al., 2010).

However, negative effects of tannins on animal health are also well documented (Min et al., 2003a; Waghorn and McNabb, 2003). Also, in order to avoid plant

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Table 1
Content of condensed tannins in three investigated Mediterranean shrubs (mean ± SD; $n = 6$).

Shrubs	Condensed tannins (g/kg dry matter)
Arbutus unedo L.	1.939 ± 0.006
Pistacia lentiscus L.	1.922 ± 0.005
Quercus ilex L.	1.828 ± 0.082

toxicity, grazing ruminants are able to control intake of plants rich in secondary metabolites (Pfister et al., 1997). Rogosic et al. (2007) showed that biological/biochemical diversity among Mediterranean shrubs has a positive effect on the amount of consumed green biomass rich in tannins. Also, Juhnke et al. (2012) showed that lambs are able to learn about the antiparasitic effects of condensed tannins and consequently increase their preference for the tannin-rich feed.

The aim of this study was to determine the combined effect of three tannin-rich Mediterranean shrubs *Arbutus unedo* (strawberry tree), *Pistacia lentiscus* (mastic tree), and *Quercus ilex* (holly oak), on fecal nematode egg count (FEC) in sheep naturally infested with gastrointestinal nematodes.

2. Materials and methods

The research was conducted at the University of Zadar experimental station in Zemunik Donji. The research protocol was approved by the Ministry of Agriculture, Agency for Animal Care and Use Committee; No. UP/I-322-01/11-01/118, Republic of Croatia. Animals used in the research were sheep of local Croatian breed – Pramenka. The experiments lasted for 30 days total, and included 28 female animals between 3 and 4 year of age that originated from the same farm, with an average weight 39.09 ± 3.05 kg (mean ± SD). Prior to the experiment, sheep were randomly assigned to two groups ($n = 14$). Animals were housed individually in covered wire-net pens, 2 m × 2.5 m in size, with a free access to fresh water and a mineral block.

Mediterranean shrubs *Arbutus unedo* L. (Ericaceae), *Pistacia lentiscus* L. (Anacardiaceae) and *Quercus ilex* L. (Fagaceae) are species naturally occurring in this region and were selected for their high tannin concentration (Rogosic et al., 2006.). Shrubs were hand harvested at several locations in Zadar County area. Shrub leaves and current season's growth (i.e., twigs) were clipped and chopped to 1 cm in length with a chipper. Afterwards, all three shrubs were mixed together in equal portions (1:1:1) for daily rations, placed in woven, polyethylene feed sacks, and stored at 4 °C. Throughout the experiment, bags with shrubs were removed from the cold storage and offered to the animals on a daily basis. During the experiment, both groups of animals were fed each morning at 8:00 h with 200 g of barley and 7 g of Optigen®II (non-protein nitrogen source) per animal. At 8:30 h, each animal from the treated group received 200 g of shrub mixture, while those from the control group were fed with hay ad libitum. Animals from both groups were fed individually in their respective pens. Feed boxes in treated group were inspected every hour and if needed, additional amounts of fresh shrub mixture were added. Each day at 16:00 h feed boxes containing leftovers of shrub mixture were removed and sheep were offered hay ad libitum. Leftovers were weighed for each animal, and the total amounts of shrubs consumed were calculated for each sheep, expressed as g/kg BW.

Shrubs for determination of condensed tannin content were collected at the beginning of the experiment. The collected plant material was stored in portable cooler and transported to the laboratory. Samples of the supernatant of investigated shrubs and determination of condensed tannin was performed by method described by Makkar (2003). Results are presented as a mean of six replicates ± standard deviation.

On days 0, 23 and 30, individual fecal samples were collected manually from the rectum in the morning measurement to determine fecal egg counts (FEC), expressed as nematode eggs per gram of feces (EPG), according to the McMaster technique (MAFF, 1977).

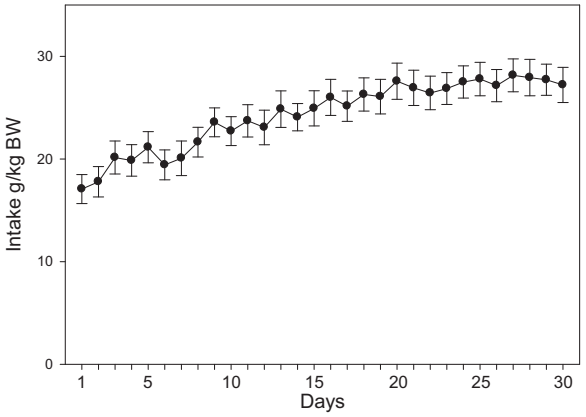

Fig. 1. Average daily green biomass intake (mean ± SEM) in sheep of treated group ($n = 14$) fed with a mixture of equal portions (1:1:1) of tannin-rich Mediterranean shrubs (*Arbutus unedo*, *Pistacia lentiscus*, *Quercus ilex*).

The experimental model included two treatments (treated vs. control). Comparisons between the groups were conducted only for the same days of the experiment (on days 0, 23 and 30). The EPG number was used as the dependent variable in the statistical data analysis between the groups. The Kruskal–Wallis one way analysis of variance on ranks was used for all analyses, and was performed by the SigmaPlot 11 statistical package (Systat Software). The level of the statistical significance was $P < 0.05$.

3. Results

The amounts of condensed tannins in three investigated shrubs are presented in Table 1. The average daily green biomass intake for the mixture of three tannin-rich Mediterranean shrubs throughout the experiment in the treated group was 24.29 ± 0.32 g/kg BW (mean ± SEM) (Fig. 1).

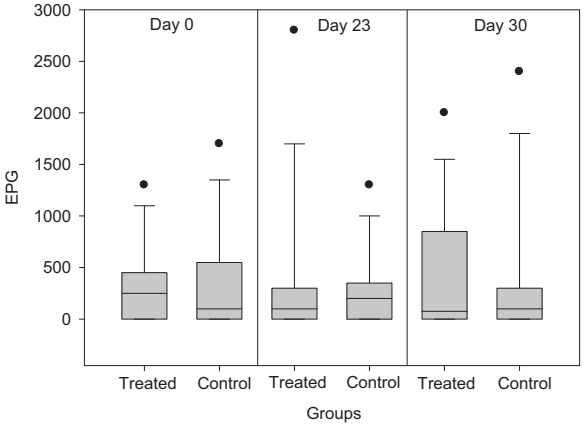

Fig. 2. Comparison of sheep fecal egg counts (FEC expressed as eggs per gram of feces – EPG) between the two groups on the same days of experiment. Treated group was fed with a mixture of equal portions (1:1:1) of tannin-rich Mediterranean shrubs (*Arbutus unedo*, *Pistacia lentiscus*, *Quercus ilex*), and the control group received feed without tannins ($n = 14$ per group). Results represent the median, 10th, 25th, 75th and 90th percentiles.