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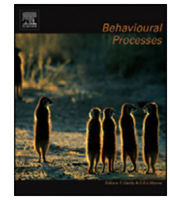

# Tannins and self-medication: Implications for sustainable parasite control in herbivores

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ABSTRACT

Animals adapt to the variability of the external environment and to their changing internal needs not only by generating homeostatic physiological responses, but also by operating in the external environment. In this study, we determined whether sheep with a gastrointestinal parasite infection increased intake of a low-quality food containing a natural antiparasitic agent (tannins) relative to non-parasitized sheep. Four groups of lambs ($n=8$ lambs/group) were assigned to a $2 \times 2$ factorial design with parasitic burden (P = parasites; NP = no parasites) and the offer of a supplement containing tannins (yes, no) as the main factors. Parasitized lambs ate more of the tannin-containing food than non-parasitized lambs for the first 12 days of the study, when parasite burdens were high, but differences became smaller and disappeared toward the end of the study when parasite burdens decreased. This result suggests the lambs detected the presence of internal parasites or associated symptoms and modified their ingestion of an antiparasitic agent as a function of need.



## 1. Introduction

Internal parasitism is a major disease problem in grazing livestock worldwide (Min and Hart, 2003; Waller, 2006). Parasitism is a pervasive challenge to host survival and reproduction (Hutchings et al., 2003), and failure to control gastrointestinal nematodes results in poor growth rates, ill thrift and death (Min et al., 2004). Nevertheless, controlling internal parasite infections is not easy, particularly in recent times. One of the most immediate threats to medicine is the rise of drug-resistant diseases (Plotkin, 2000). Programs to control gastrointestinal parasites based on chemotherapy are failing due to the increased prevalence of parasite resistance to current drugs (Pomroy et al., 2002; Min et al., 2004).

A potential low-cost and sustainable alternative to chemotherapy-based parasite control involves managing the foraging behavior of herbivores. Just as foraging behavior is influenced by nutrients (Provenza and Villalba, 2006), some responses may also be geared toward reducing disease (Lozano, 1998). If a mammal can evolutionarily or contemporarily learn to prefer foods because they raise fitness, it may also learn to seek and ingest other substances in the environment such as medicines, as they too raise fitness (Janzen, 1978). Little is known about the abilities of animals to self-medicate, and many of the observations are anecdotal and equivocal (Clayton and Wolfe, 1993; Lozano, 1998; Houston et al., 2001), but recent evidence suggests animals can self-medicate (Huffman, 2003; Villalba et al., 2006).

If so, medicinal plants could provide an important tool for rural communities where commercial antiparasitic agents are inaccessible or too expensive. More generally, consumers increasingly demand products that are both "clean" and "green" (Waller, 2006). Use of plant secondary metabolites (PSM) may be an effective way to treat animals for parasite infections in an "organic" production where use of synthetic anthelmintic drugs is restricted.

Considerable attention has been given recently to the antiparasitic properties of tannins, a class of polymerized phenolic compounds. Livestock feeding on plants with tannins such as sulla (*Hedysarum corarium*) and sericea lespedeza (*Lespedeza cuneata*) show lower nematode burdens and fecal egg counts, and higher body gains, than animals eating plants of similar quality without tannins (Niezen et al., 2002; Coop and Kyriazakis, 2001; Min and Hart, 2003; Min et al., 2004). The mode of action of tannins is primarily a direct anthelmintic effect (Athanasiadou et al., 2000), but tannins also increases the supply of bypass protein (Reed, 1995; Foley et al., 1999), which enhances immune responses to intestinal parasites (Niezen et al., 2002; Min and Hart, 2003).

This study determined the extent to which parasitized sheep modified their foraging behavior when offered foods with bioactive compounds (quebracho tannins) known to reduce parasitic burdens, even when those compounds were present in a food of very poor nutritional quality.

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## 2. Materials and methods

The study was conducted at the Green Canyon Ecology Center, located at Utah State University in Logan, according to procedures approved by the Utah State University Institutional Animal Care and Use Committee (Approval #1013).

### 2.1. Experimental design

#### 2.1.1. Basal diet

Lambs received a basal diet of beet pulp (95%) and alfalfa hay (5%) (3.5 Mcal digestible energy (DE)/kg; 9.8% crude protein (CP); NRC 1985) formulated to maximize the effects of quebracho tannin by providing a low concentration of CP. Condensed tannins form strong complexes with proteins (Makkar et al., 1987), which can potentially neutralize the effects of condensed tannins. Quebracho tannin is less effective at combating parasites when diets contain high (22% CP) as opposed to low-protein (9.7% CP) diets (Butter et al., 2000).

#### 2.1.2. Animals and parasite burdens

Lambs were selected from a group of 32 commercial crossbred lambs (4 months of age) grazing on irrigated grass-clover pastures where they acquired a natural worm burden. Fecal samples were taken from the rectum of all animals and analyzed for nematodes (eggs/g feces). After collection, feces were placed in a refrigerator at 4 °C and analyzed the same day. Fecal egg counts (FEC), which are used routinely as a reliable indicator of parasite infection in livestock (Niezen et al., 1995; Hutchings et al., 2003), were determined by a fecal flotation procedure (Hendrix and Robinson, 2006). Briefly, 1 g of feces was emulsified in 20 ml of flotation medium (a saturated solution of zinc sulfate) using a vial and strainer (Ovassay, Synbiotics Corp., San Diego, CA). Flotation medium was added to the vial until a meniscus formed. A glass coverslip was placed on top of the fluid and after 30 min the coverslip was placed on a microscope slide and all eggs under the coverslip were counted by the same observer using a compound microscope.

Lambs were stratified (from highest to lowest) according to initial FEC and sub-groups of four lambs were randomly assigned to four groups of lambs ($n = 8$ lambs/group). Thus, differences between groups due to initial FEC were balanced.

Half of the animals ($n = 16$ non-parasitized—NP) received an oral dose of the anthelmintic ivermectin (0.2 mg ivermectin/kg BW; Merial, Duluth, GA). Eight days later, the same lambs were dosed again with ivermectin. The remaining 16 untreated sheep comprised the parasitized group (*P*). Lambs were then assigned to a 2 × 2 factorial design that included parasite burden (*P* = parasites; NP = no parasites) and offer of quebracho tannin (yes, no). Thus, four groups were formed: Group 1 = NP + no tannin; Group 2 = NP + tannin; Group 3 = P + no tannin; and Group 4 = P + tannin.

Lambs were penned outdoors under a protective roof in individual, adjacent pens measuring 2.4 m × 3.6 m. To avoid cross-contamination, P and NP were penned at separate locations, but within those locations lambs in different treatments were randomly distributed in adjacent pens. Throughout the study, lambs had free access to trace mineral salt blocks and fresh water. Before exposures to the experimental feeds and diets, the lambs were given an adjustment period of 2 weeks, during which they received 350 g of rolled barley grain/animal/day and free access to alfalfa pellets. Lambs were weighed at the end of the study and average daily gains (ADG) were estimated. The initial average body weight (BW) of the 32 lambs was 36 ± 1 kg.

#### 2.1.3. Feeding protocol

From 08:00 to 12:00 every day lambs were offered 300 g of a supplement that did (lambs in Groups 2 and 4) or did not (Groups 1 and 3) contain quebracho tannins. At 12:00 refusals were collected and weighed, and supplement intake for each lamb was estimated. At 13:00 lambs received 2 kg of the basal diet and refusals were collected and weighed at 17:00, and basal diet intake was calculated. No other food was offered until the following day at 08:00. We began feeding the supplements on August 16, 2006 and ended on October 4, 2006 for a total of 49 days.

The supplement without tannin was grape pomace. The supplement with tannin contained (on an as-fed basis) grape pomace (70%) mixed with quebracho tannin (30%). Quebracho tannin is a powder and grape pomace was ground to a particle size <1 mm, which made the mix homogeneous. Quebracho tannin is a source of condensed tannin extracted from the South-American quebracho tree (*Aspidosperma quebracho*). Grape pomace is the low nutritional quality solid remains of grapes (skins, pulp, seeds, and stems of the fruit) after pressing for juice.

We estimated fecal egg counts prior to group formation (July 20, 2006), the day before we started feeding the supplements (August 15, 2006), during (August 28 and September 6, 2006) and at the end (October 4, 2006) of the study.

### 2.2. Statistical analyses

Data were analyzed as a 2 × 2 factorial design with two factors: (1) parasite burden (P and NP), and (2) quebracho tannin (yes or no). Day (intake) and sampling period (FEC) were the repeated measures in the analyses. When significant effects were detected ($P < 0.10$), differences among means were determined using LSD differences. Intake of the basal diet and of the supplements, FEC, and ADG were the response variables.

As intake of the supplement without tannin was substantially higher than intake of the supplement with tannin, intake of each of the supplements was also analyzed separately as a split-plot design with lambs nested within Group (*P*, NP with tannin or placebo) and day was the repeated measure.

Linear regression analyses were used to estimate the relationship between intake of the supplements (tannin and placebo) and FEC (Log transformed) in parasitized animals. Intake values and fecal egg counts selected for regressions were those displayed 12 days after the animals started to consume the supplements (August 28, 2006) and in the ensuing sampling periods (September 6 and October 4, 2006).

Analyses were computed using the MIXED procedure of SAS (SAS Inst., Inc. Cary, NC; Version 9.1 for Windows). The model diagnostics included testing for a normal distribution of the error residuals and homogeneity of treatment variance. Comparison of least square means was made by the LSMEANS statement using the DIFF option.

## 3. Results

### 3.1. Intake of supplements

Averaged across all days, lambs consumed more supplement without tannins (12.0 g/kg of metabolic body weight [$BW^{0.75}$]) than with tannins (2.3 g/kg $BW^{0.75}$; SEM = 1.0; $P < 0.001$; Fig. 1). Nevertheless, this pattern was influenced by the parasitic burdens of the lambs. Parasitized lambs consumed less grape pomace (placebo) than non-parasitized lambs throughout the study ($P < 0.05$; Fig. 1). In contrast, parasitized lambs consumed more tannin-containing supplement than non-parasitized lambs for the first 12 days of the study ($P = 0.01$), a period when FEC showed the greatest decline relative to initial values (see below; Fig. 2). Parasitized lambs continued to consume more tannin-containing supplement than non-parasitized lambs after 21 days of supplement offer, but the differences became less significant ($P = 0.14$), and they were not present when intakes of tannin-containing supplement were