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Self-Medicative Behavior of Sheep Experiencing Gastrointestinal Nematode Infections and the Postingestive Effects of Tannis

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SELF-MEDICATIVE BEHAVIOR OF SHEEP EXPERIENCING

GASTROINTESTINAL NEMATODE INFECTIONS AND

THE POSTINGESTIVE EFFECTS OF TANNINS

by

Larry D. Lisonbee

A thesis submitted in partial fulfillment
of the requirements for the degree

of

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in

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Approved:

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ABSTRACT

Self-Medicative Behavior of Sheep Experiencing

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by

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Utah State University, 2008

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Diet selection and self-medication are fundamental to the survival of all species. The abilities to choose healthy foods in response to past consequences are basic elements of evolution. This study explores self-medication regarding tannins both as a medication and as a dietary challenge. In the first study, sheep with natural parasite infections were offered a low-quality supplement containing a dose of tannins considered to be therapeutic (medicine), while the control infected lambs received the same supplement without tannins (placebo). This study included a group of parasite-free lambs. The parasitized lambs ate more of the tannin-containing supplement than non-parasitized lambs for the first 12 d of the study, when parasite burdens were high, but differences became smaller and disappeared towards the end of the study when parasite burdens decreased. This result indicated lambs ability to detect the presence of internal parasites and to learn to ingest tannin when followed by relief from parasite burdens.

In the second study, lambs grazed on pastures with forages containing saponins (alfalfa), alkaloids (endophyte-infected tall fescue) and tannins (birdsfoot trefoil). I observed the foraging behavior of groups of lambs after intra-ruminal infusions of tannins (treatment). Lambs that first received intraruminal infusions of tannins and then were offered 2-way choices between varieties of forages with high and low concentrations of either saponins, alkaloids, or tannins increased their preference for the high-saponin variety of alfalfa and the high-alkaloid variety of tall fescue relative to lambs not infused with tannins (controls). Lambs infused with tannins and offered choices among the 3 forages with high concentrations of secondary compounds also manifested higher preference for the high-alkaloid variety of tall fescue than control lambs. In contrast, lambs infused with tannin reduced their preference for the high-tannin variety of birdsfoot trefoil.

This research has implications for many situations where forages with secondary compounds are available but underutilized. Landscapes where such forages are available could be used effectively with little or no harm to livestock if medicinal forages or supplements are made available and offered as alternatives so animals can learn about their benefits of chemical complementarities among different plant species.

(70 pages)

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Larry Lisonbee

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CHAPTER 1

INTRODUCTION

Natural landscapes are diverse mixes of plants that occur in patches reflecting a history of use in concert with particular soil, precipitation, and temperature regimes. For plants, diversity is the rule – for species, phenologies, growth forms, and biochemistries. Regarding the latter, plants are nutrition centers and pharmacies with vast arrays of primary (nutrients) and secondary (pharmaceuticals) compounds useful in animal nutrition and health (Engel, 2002). Regrettably, we have neither understood nor valued diversity in agriculture, as evident in our persistent attempts to simplify ecological systems to maximize yields of crops and pastures.

Simplifying agriculture to facilitate livestock production, coupled with a view of plant secondary metabolites (PSM) as toxins, has resulted in selecting for a biochemical balance in forages favoring primary compounds and nearly eliminating PSM. To increase intake of single-plant diets, one must reduce PSM as they limit how much of a food humans and livestock can consume (Provenza, 2003). The outcome is energy- and protein-dense monocultures low in the PSM. The alternative, which we have not pursued, is offering animals a variety of foods that differ in primary and secondary metabolites, thereby enabling them and us to obtain a much greater array of nutrition and health benefits from nature's bounty.

Interactions among PSM can lead to complementarities wherein eating a variety of foods is beneficial (Tilman, 1982; Provenza, 2003). Outside of a few studies, however, we know little about which PSM are biochemically complementary. When sheep choose between foods that contain either amygdaline or lithium chloride, they eat more than sheep offered a food with only one of these compounds; the same is true for nitrate and oxalate (Burritt and Provenza, 2000), and for tannins and the alkaloids gramine and ergotamine (Lyman et al., 2007).

Sheep eat more when offered foods with terpenes, tannins, and oxalates than when offered foods with only one or two of these PSC (Villalba et al., 2004). Sheep eat more food with terpenes when they first eat food with tannins (Mote et al., 2008). Cattle steadily decrease time eating endophyte-infected tall fescue when they grazed tall fescue first for 30 minutes and then birdsfoot trefoil and/or alfalfa alone for 60 minutes; however, when the sequence is reversed they foraged actively on trefoil and/or alfalfa and fescue throughout the 90-minute meal (Lyman et al., 2007). These patterns of foraging are analogous with birdsfoot trefoil/alfalfa and high-alkaloid reed canarygrass (Lyman et al., 2007).

Tannins are highly reactive chemical compounds increasingly recognized as important in health and nutrition, though historically they were thought by agriculturalists and ecologists alike to adversely affect herbivores. Eating plants high in tannin is a natural way for herbivores to reduce levels of internal parasites (Min and Hart, 2003), and tannin alleviates bloat by binding to proteins in the rumen (Waghorn, 1990). Tannin can also enhance nutrition by providing high-quality protein to the small intestines by binding to degradable protein in the rumen, making the protein unavailable for digestion and absorption until it reaches the more acidic abomasums (Barry et al., 2001). This high-quality-protein-bypass effect enhances immune responses and increases resistance to gastrointestinal nematodes (Niezen et al., 2002, Min et al., 2004). Tannin form complexes, mainly through hydrophobic/hydrogen interactions, with a wide variety of compounds including protein (Jones and Mangan, 1977), polysaccharides, nucleic acids, minerals (Jones and Mangan, 1977), alkaloids (Okuda et al., 1982) and saponins (Freeland et al., 1985).

In the first study reported below I determined if lambs would ingest low-quality food containing tannin (a natural antiparasitic agent) when challenged with a gastrointestinal parasitic burden and whether their intake was greater than that of non-parasitized lambs. I also determined if through food selection and intake lambs burdened with parasites were able to affect the viability of parasites (measured indirectly by fecal egg counts) and thus manage their overall health. I hypothesize that if herbivores learn to consume plants high in secondary compounds such as tannins to self-medicate for parasites; this could lead to management programs that seed "medicinal" plant species strategically in the environment, allowing herbivores to combat parasites by themselves.

In the second study, I determined whether tannins influence forage selection by sheep offered plants that varied in the concentration of saponins (alfalfa*, Medicago sativa)*, tannins (birdsfoot trefoil, *Lotus corniculatus*), and alkaloids (tall fescue, *Lolium arundinaceum*). As tannins bind to and likely deactivate the negative effects of alkaloids (Okuda et al., 1982) and saponins (Freeland et al., 1985) and birdsfoot trefoil contains tannins which could enhance the negative effects of the infused tannins, I hypothesized that: sheep receiving intraruminal infusions of tannins would 1) preferentially graze high-alkaloid varieties of tall fescue and high-saponin varieties of alfalfa and 2) avoid high-tannin varieties of birdsfoot trefoil. In general, determining enhancing knowledge of the ability of animals to use PSM for nutrition and health will be useful for animal production systems – on organic land in particular – that seek to minimize the negative effects of secondary compounds in diverse pastures and maximize their beneficial effects on animal, plant and soil health (Provenza, 2008).

REFERENCES

Barry, T.N., McNeill, D.M., McNabb, W.C., 2001. Plant secondary compounds: their impact on nutritive value and upon animal production. In: Procedures of the XIX International Grass Conference, Sao Paulo, Brazil, pp. 445-452.

Burritt, E.A., Provenza F.D., 2000. Role of toxins in intake of varied diets by sheep. Journal Chemical Ecology 26,1991-2005.

Engel, C. 2002. Wild Health. Houghton Mifflin Co., Boston, New York, NY.

Freeland, W.J., Calcott, P.H., Anderson, L.R., 1985. Tannins and saponin: interaction in herbivore diets. Biochemical System Ecology 13,189-193.

Jones, W.T., Mangan, J.L., 1977. Complexes of condensed tannins of sainfoin (*Onobrychis viciifolia Scop.*) with fraction-1 leaf protein and with submaxillary mucoprotein, and their reversal by polyethylene-glycol and pH. Journal of Science Food and Agriculture 28,126-136.

Lyman, T., Provenza, F.D., Villalba J.J., 2007. Sheep foraging behavior in response to interactions among alkaloids, tannins and saponins: Implications for chemical and taxonomic diversity in pasture ecosystems. Journal of the Science of Food and Agriculture, (In Press).

Min, B.R., Hart, S.P., 2003. Tannins for suppression of internal parasites. Journal of Animal Science 81,E102-E109

Min, B.R., Pomroy, W.E., Hart, S.P., Sahlu T., 2004. The effect of short-term consumption of a forage containing condensed tannins on gastro-intestinal nematode parasite infections in grazing wether goats. Small Ruminant Research 51,279-283

Mote, T., Villalba, J.J., Provenza, F.D., 2008. Sequence of food presentation influences intake of foods containing tannins and terpenes. Applied Animal Behavior in Science (In Press).

Niezen, J.H., Charleston, W.A.G., Robertson, H.A., Shelton, D., Waghorn, G.C., Green, R., 2002. The effect of feeding sulla (*Hedysarum coronarium*) or lucerne (*Medicago sativa*) on lamb parasite burdens and development of immunity to gastrointestinal nematodes. Veterinary Parasitology 105,229-245

Okuda, T., Mori, K., Shiota, M., 1982. Effects of interaction of tannins and coexisting substances. III Formation and solubilization of precipitates with alkaloids. Journal of the Pharmaceutical Society of Japan 102, 854-858.

Provenza, F.D., 2003. Twenty-five years of paradox in plant-herbivore interactions and “sustainable” grazing management. Rangelands 25, 4-15.

Provenza, F.D., 2008. What does it mean to be locally adapted to a landscape and who cares anyway? Journal of Animal Science 86, E271.

Tilman, D., 1982. Resource Competition and Community Structure. Princeton University Press, Princeton, NJ.

Villalba, J.J., Provenza, F.D., Han, G., 2004. Experience influences diet mixing by herbivores: implications for plant biochemical diversity. Oikos 107, 100-109.

Waghorn, G.C., 1990. Beneficial effects of low concentrations of condensed tannins in forages fed to ruminants. In: Akin, D.E., Ljungdahl, L.G., Wilson, J.R., Harris, P.J., (Eds.) Microbial and Plant Opportunities to Improve Lignocellulose Utilization by Ruminants. Elsevier Science Publications, New York, p. 137.

CHAPTER 2

TANNINS AND SELF-MEDICATION: IMPLICATIONS FOR SUSTAINABLE PARASITE CONTRL IN HERBAVORES

ABSTRACT

Internal parasitism is one of the greatest disease problems in grazing livestock worldwide, and it is a growing concern given increasing resistance to commonly used anthelmintics. As a result, considerable attention is being given to the antiparasitic properties of tannins, a class of highly reactive polymerized phenolic compounds. If herbivores learn to consume plants high in secondary compounds such as tannins to combat parasites (i.e., self-medicate), this could aid in developing management programs geared at seeding "medicinal" plants strategically in the environment, allowing herbivores to combat disease by themselves.

The goal of this study was to determine whether parasitized sheep increase intake of a low-quality food containing tannins (a natural antiparasitic agent) relative to non-parasitized sheep and if this behavior decreases parasite burdens as measured by fecal egg counts (FEC). Four groups of lambs (n=8 lambs/group) were assigned to a 2 x 2 factorial design with parasite burden (P = parasitized; NP = non-parasitized; treated with ivermectin) and supplemental tannins (yes, no) as the main factors. Lambs that received tannins were offered a low-quality supplement of 30% Quebracho tannins and 70% grape pomace. The remaining animals only received grape pomace (placebo). All lambs consumed more supplement without than with tannins ($P < 0.001$), but supplement intake was influenced by parasite burden. Parasitized lambs consumed more tannin-containing supplement than non-parasitized lambs for the first 12 d (P =

0.01; i.e., when parasitic burdens where the highest) and 21 d (P = 0.14) of the study. Fecal egg counts declined within days after the animals started to consume the supplement (P = 0.04 to P < 0.001), no differences in FEC were detected between groups of parasitized animals offered either tannins or placebo across sampling periods (parasitic burden x quebracho tannin x sampling period; P = .66). For parasitized lambs, ingestion of the tannin-containing supplement was inversely proportional to FEC ($P = 0.0007$; $R^2 = 0.43$). In contrast, no relationship was found between intake of the placebo and FEC (P = 0.24; $R^2 = 0.07$).

These findings lead me to suggest that domestic sheep increase consumption of tannins when experiencing parasite burdens, even when tannins are mixed with low-quality foods. Self-selection of tannins by parasitized livestock could reduce labor and costs associated with chemotherapy in programs of parasite control.

INTRODUCTION

Internal parasitism is one a major disease problem in grazing livestock worldwide (Min and Hart, 2003; Waller, 2006). Parasitism is a pervasive challenge to host survival and reproduction (Hutching 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 loads is not easy, particularly in recent time. 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 anthelmintics (Pomroy et al., 2002; Min et al., 2004).

A potential low-cost and sustainable alternative to parasite control involves managing the foraging behavior of herbivores. Just as foraging behavior is influenced by nutrients, 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; Housten et al., 2001), but recent evidence suggests animals can self-medicate (Huffman, 2003; Villalba et al., 2006).

Considerable attention has been given recently to the antiparasitic properties of tannins, a class of polymerized phenolic compounds. Livestock feeding on plants with tannin 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 tannin (Niezen et al., 2002; Coop and Kyriakis, 2001; Min and Hart, 2003; Min et al., 2004). The mode of action of tannin is primarily a direct anthelmintic effect (Athanasiadou et al., 2000), but tannin 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). Medicinal plants provide an important tool for rural communities where commercial anti-parasitic agents are inaccessible or too expensive. Moreover, in recent years there has been an increasing demand by consumers for products that are both “clean” and “green” (Waller , 2006). Use of PSM may be an effective way to treat animals for parasite loads in an “organic” production where no agro-chemical products are used.

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

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).

Experimental Design

Basal Diet. Lambs received a basal diet of beet pulp (95%) and alfalfa hay (5%) (3.5 Mcal DE/Kg; 9.8% 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).

Animals and Parasite Burdens. Lambs in the study were selected from a group of 32 commercial crossbred lambs (4 mo of age) that were 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^{o}C and analyzed the same day. Fecal egg counts (FEC) are used routinely as a reliable indicator of parasite infection in livestock (Niezen et al., 1995; Hutchings et al., 2003). The FEC were determined by a simple 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 4 lambs were randomly assigned to 4 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 x 2 factorial design that included parasite burden (P=parasitized; NP= non-parasitized) and offer of quebracho tannin (yes, no). Thus, I had 4 groups: Group 1 = NP + no tannin; Group 2 = NP + tannin; Group 3 = P + no tannin; and Group 4 = P + tannin.

The initial average body weight BW of the 32 lambs was 36 ± 1 Kg. Lambs were penned outdoors under a protective roof in individual, adjacent pens measuring 2.4 x 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 wk, during which they received 350 g of rolled barley grain/animal/d and free access to alfalfa pellets. Lambs were weighed at the end of the study and average daily gains (ADG) were estimated.

Feeding Protocol. From 0800 to 1200 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. The supplement contained (on an as-fed basis) a low-quality filler grape pomace (70%) and quebracho tannin (30%). Quebracho tannin is a powder and grape pomace was ground to a particle size < 1mm, which made the mix homogeneous. The supplement without tannin was just the filler (grape pomace; placebo). At 1200 refusals were collected and weighed, and I calculated supplement intake for each lamb. At 1300 lambs received 2 Kg of the basal diet of ground beet pulp (95%) mixed with ground alfalfa pellets (5%) and refusals were collected and weighed at 1700. Intake of the basal diet was estimated by comparing refusals weights with the weights of the feed that was offered. No other food was offered until the following day at 0800. We began feeding the supplements on August 16, 2006 and ended on October 4, 2006 for a total of 49 days.

Fecal egg counts were estimated initially 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.

Statistical Analyses

Data were analyzed as a 2x2 factorial design with two factors: 1) parasite burden (P, 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. For analyzing the 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 as 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 d after the animals started to consume the supplements (Aug 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.

RESULTS

Intake of Supplements

Averaged across all days, lambs consumed more supplement without tannins (12.0 g/Kg $BW^{0.75}$) than with tannins (2.3 g/Kg $BW^{0.75}$; SEM = 1.0; $P < 0.001$; Figure 2.1). Nevertheless, this pattern was influenced by the parasitic burdens of the lambs. When looking at individual supplement intakes, parasitized lambs consumed less grape pomace (placebo) than non-parasitized lambs throughout the study ($P < 0.05$; Figure 2.1). In contrast, parasitized lambs consumed more tannin-containing supplement than non-parasitized lambs for the first 12 d of the study ($P = 0.01$), a period when FEC showed the greatest decline relative to initial values (see below; Figure 2.2). 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 averaged for the 49 d of the study (P = 0. 58; Figure 2.1). These different patterns of supplement consumption by parasitized and non-parasitized lambs caused a parasitic burden (P, NP) x quebracho tannin (yes, no) interaction (P = 0.01; Figure 2.1).

Intake of the Basal Diet

Averaged across days, no differences in intake of the basal diet were observed between parasitized (64.7 g/Kg $BW^{0.75}$) and non-parasitized (64.4 g/Kg $BW^{0.75}$; P=0.87; SEM = 1.5) groups. Lambs offered the placebo (grape pomace) tended to consume more basal diet (66.0 g/Kg $BW^{0.75}$) than lambs offered the tannin-containing supplement (63.1 g/Kg $BW^{0.75}$; P = 0.19; SEM = 1.5). Non-parasitized animals consumed similar amounts of basal diet when offered the placebo (64.1 g/Kg $BW^{0.75}$) or the tannin supplement (64.7 g/Kg $BW^{0.75}$). In contrast, parasitized sheep tended to consume more basal diet when offered the placebo (67.9 g/Kg $BW^{0.75}$) than when offered the tannin supplement (61.5 g/Kg $BW^{0.75}$ (parasitic burden x quebracho tannin interaction (P = 0.12).

Fecal Egg Counts

Parasitized animals showed much higher FEC than non-parasitized animals (P < 0.0001; Figure 2.2). Fecal egg counts increased from the initial sampling period (July 20) to the day before the study started (Aug 15, P= 0.005). In contrast, FEC declined from the day before lambs started to receive their supplements (Aug 15) to the rest of the sampling periods (Aug 28, P = 0.001; Sep 6; P = 0.04; Oct 4, P < 0.001) (Figure 2.2). Nevertheless, no differences in FEC were detected between groups of parasitized animals offered either tannins or placebo across sampling periods (parasitic burden x quebracho tannin x sampling period; P = .66).

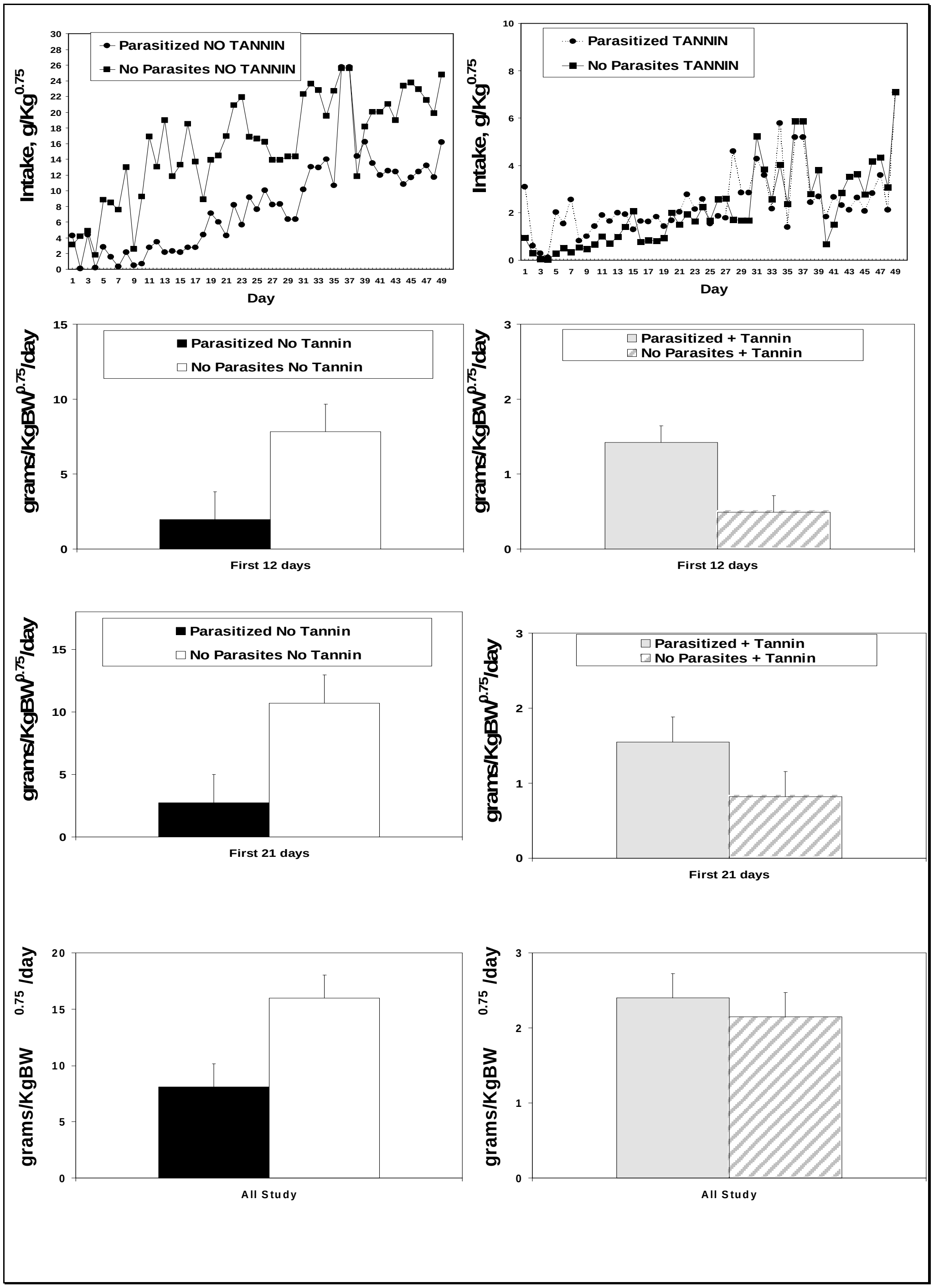

Figure 2.1. Intake of two supplements by parasitized and non-parasitized lambs receiving two kinds of supplements: grape pomace:quebracho tannin (70:30) (TANNIN) or grape pomace (NO TANNIN). Upper panels are daily intakes of the supplement without (NO TANNIN; SEM = 2.9) and with tannin (TANNIN; SEM = 0.8). Lower panels: Average intake of supplements for the first 12 and 21 days and overall intake for the 49 days of the study.

For parasitized lambs, ingestion of the tannin-containing supplement was inversely proportional to FEC, with slopes of the regression lines different from 0 (*P* = 0.0007; R^2 = 0.43; Figure 3). This suggests that FEC decreased with the ingestion of tannins in parasitized animals. In contrast, no relationships between intake of the tannin-containing food and FEC was observed in parasitized lambs offered a placebo (P = 0.24; R^2 = 0.07; Figure 3).

Average Daily Gains

Non-parasitized lambs tended to gain more weight than parasitized lambs (parasitic burden effect; P = 0.14), particularly when the parasitized lambs were offered a supplement with tannins (parasitic burden x quebracho tannin interaction; P = 0.19; Figure 2 final column of ADG).

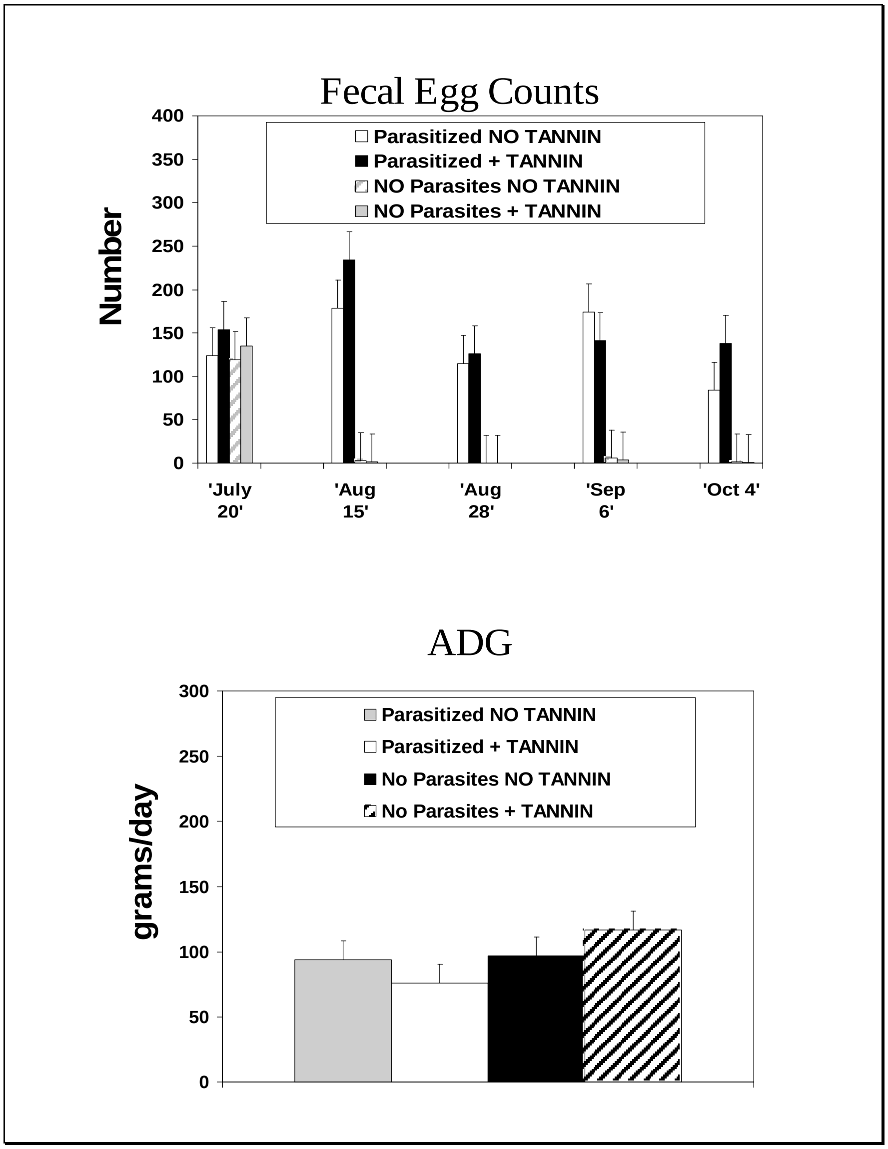

Figure 2.2. Upper panel. Fecal egg counts in parasitized and non-parasitized lambs receiving two kinds of supplements: one supplement was grape pomace:quebracho tannin (70:30) (TANNIN) and the other was just grape pomace (NO TANNIN). Non-parasitized lambs were treated with ivermectin before Aug 15 and on Aug 16 all animals started to receive their respective supplements. Lower panel: Average daily gains of the parasitized and non-parasitized lambs receiving the two kinds of supplements. Animals were weighed at the beginning and at the end of the study.

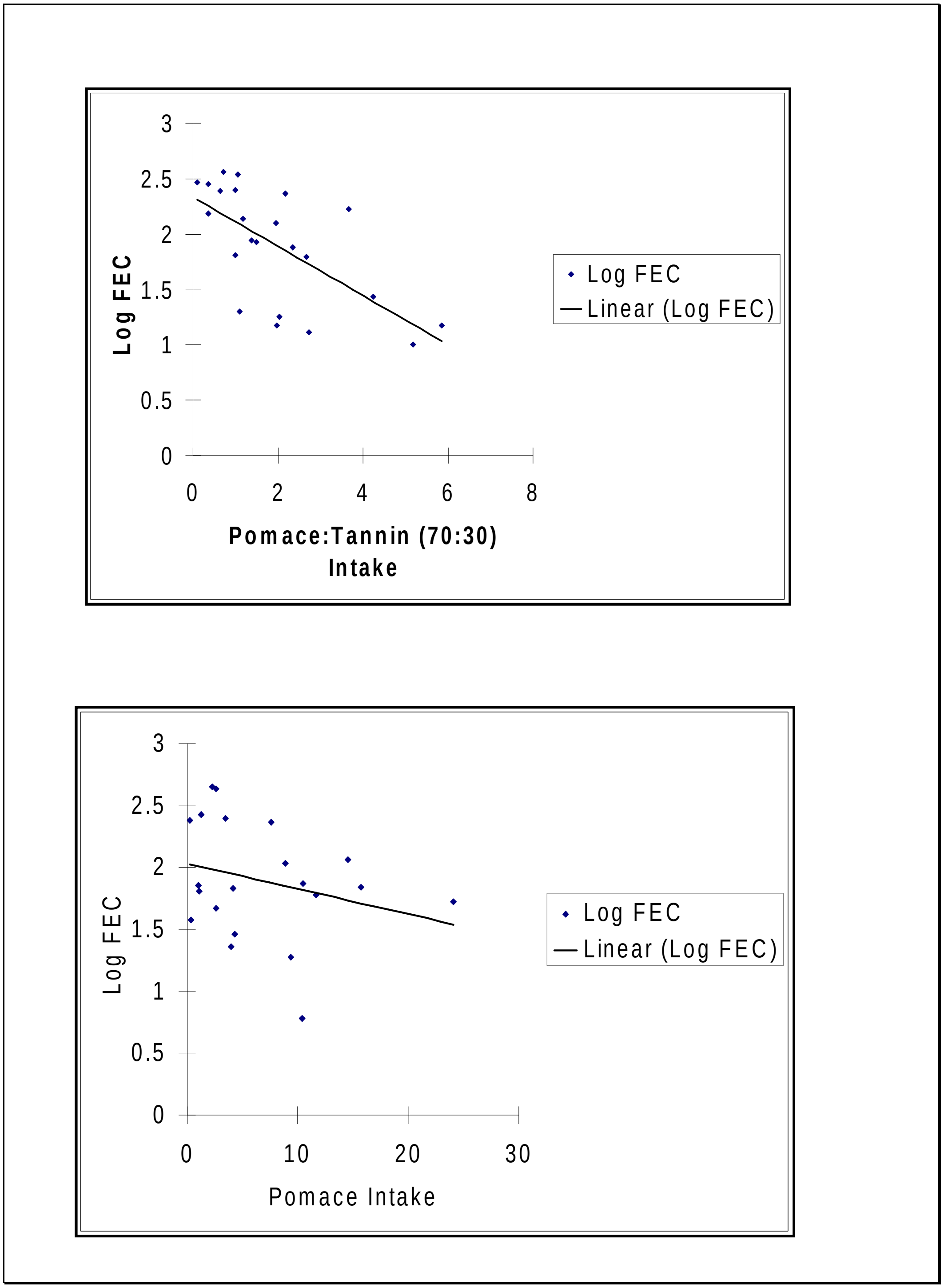

Figure 2.3. Linear regression analyses used to estimate the relationship between intake of supplements (grape pomace and grape pomace:tannin (70:30)) and FEC in parasitized lambs.

DISCUSSION

Ability of Sheep to Self-Medicate with Tannins When Challenged with Parasite Burdens

I began this study with the following question: Will lambs experiencing parasite burdens learn to eat foods containing tannins and will ingestion of tannins reduce the effects of a parasite burden, even when the tannin-containing food provides very low to nil nutritional value? To answer these questions I offered lambs a tannin supplement of very poor nutritional quality (grape pomace), but in doses considered therapeutic for combating parasitism (Coop and Kyriakis, 2001; Niezen et al., 2002; Min and Hart, 2003; Min et al., 2004).

Differential intake of the supplements with or without tannins by parasitized and non-parasitized groups of lambs suggests parasitic burdens influenced tannin consumption. Non-parasitized lambs consumed more placebo than parasitized lambs. In contrast, when presented with a tannin supplement, parasitized lambs ate more supplement than non-parasitized lambs and continued to do so for the first 3 wk of the study (Figure 2.1). Parasitized lambs evidently sensed the benefit (i.e., reduced parasitic burdens) of consuming a supplement with tannin, which encouraged further consumption. Consistent with this, higher tannin intakes were associated with lower FEC, whereas higher placebo intakes were not associated with lower FEC (Figure 2.3).

Consuming a diet of 50 g/Kg DM quebracho tannin reduces the worm populations in lambs (Butter et al., 2000). A drench of quebracho tannin extract at 8% by weight of food intake reduces worm (*Trichostrongylus colubriformis*) burdens and the number of eggs/g of feces (30%

compared with controls) such that 2 d after the first drench, FEC were approximately 50% of control sheep (Athanasiadou et al., 2000, 2001).

Principles of behavior maintain that positive consequences increase the likelihood of a behavior whereas aversive consequences do not (Chance, 1988). Tannin adversely affects parasite reproduction and in some cases even in their viability. Thus, it is clearly beneficial for the lambs with a parasitic burden to consume tannin. The non-tannin pomace supplement had little nutritional value and no medicinal effect and was therefore not a benefit to the lamb, reducing any motivation to consume the supplement.

Non-parasitized lambs ate very little tannin supplement initially, but they increased their intake through the end of the study, likely due to an increase in familiarity with the supplement. In contrast, parasitized lambs ate considerably more of the high-tannin diet during the first 12 and 21 d of the study, and their initial intake was more comparable with the average intake for the whole study than that displayed by non-parasitized lambs (Figure 2.1).

Increase in tannin intake over time, particularly in the non-parasitized animals, may also reflect additional benefits provided by daily consumption of small amounts of tannins including supplying by-pass protein (Reed, 1995) and reducing methane production (Min et al., 2006). Higher ADG in non-parasitized lambs consuming tannin may reflect these benefits (Figure 2.2).

Herbivores sample and consume a variety of plants containing secondary chemicals. Studies suggest primates use secondary compounds to control parasite loads (Wrangham and Goodall, 1989). Many of the plants being eaten have negligible nutritional value suggesting secondary chemicals are being sought. Our study also suggests lambs consumed tannin-containing foods, even though they did not provide elements that are classically considered to be nutrition.

Higher intakes of tannin, but not of placebo, were correlated with lower FEC, which suggests tannins reduced parasitic loads (Figure 2.3). Nevertheless, no significant differences in FEC were detected between groups of parasitized animals offered either tannins or placebo across sampling periods, except that the significant decline in FEC from Aug 15 to Sep 6 was due to tannin supplementation, as lambs receiving placebo displayed essentially the same FEC for both periods (178 and 174 eggs/g feces, respectively). Fecal egg counts were reduced to almost half in parasitized lambs receiving tannins: From 234 eggs/g (Aug 15) to 126 eggs/g (Aug 28) and 141 eggs/g (Sep 6), whereas this decline was much smaller or non-existent for parasitized animals receiving placebo: 178 eggs/g (Aug 15) to 115 eggs/g (Aug 28) and 174 eggs/g (Sep 6) (Figure 2.2). The lack of significant differences between lambs consuming tannins and placebo was likely due to the low FEC manifested by both groups of lambs by the end of the study (Oct 4) and to the high variability among individuals. Regarding the low FEC displayed by all lambs during Oct 4, it is likely that animals developed local or systemic immunity against the parasites in their gastrointestinal tract (Jackson and Miller, 2006). Fecal egg counts in lambs receiving placebo was essentially the same during Aug 15 (178 eggs/g) and Sep 6 (174 eggs d/g). Thus, it is likely lambs started to develop immunity against parasites during or after those periods (e.g., by Oct 4), which would explain the low FEC values in all parasitized lambs during the last sampling period (Figure 2.2).

CONCLUSION

Resistance to the three major broad-spectrum drug classes (eg., benzimidiazoles, imidothiazoles and macrocyclic lactones) used in the control of gastrointestinal parasites now occurs worldwide and is causing serious concern, particularly in the small ruminant industries (Waller, 2006). Drug resistance is testimony to the remarkable biological plasticity of nematode parasites in response to a sustained and almost exclusive reliance on specific chemicals for disease control (Waller, 2006). Thus it is likely that the more choices and the greater variety of controls – including anthelmintics – used in combination, the more effective and sustainable a program for combating parasites will be necessary (Waller, 2006). Tannins have anathematic and prophylactic qualities, and they are ingested in different forms for different purposes, likely including parasite regulation through self-medication (Villalba and Provenza , 2001).

Differences that developed over time indicate a learned aspect to self-medication through ingestion of tannin. This suggests that self medication is both physiological and behavioral in nature and can be learned providing that appropriate exposure to the medication is provided with discernable differences in dietary characteristics and body condition (flavor, texture, parasites, etc.).

REFERENCES

Athanasiadou, S., Kyriazakis, I., Jackson F., Coop, R.L., 2000. Effects of short-term exposure to condensed tannins on adult *Trichostrongylus colubriformis*. Veterinary Record 146,728-732.

Athanasiadou, S., Kyriazaki, I., Jackson, F., Coop, R.L., 2001. The Effects of condensed tannins supplementation of foods with different protein content on parasitism, food intake and performance of sheep infected with *Trichostrongylus colubriformis.* British Journal of Nutrition 86, 697-706.

Butter, N.L., Dawson, J.M., Wakelin, D., Buttery, P.J., 2000. Effect of dietary tannin and protein concentration on nematode infection (*Trichostrongylus colubriformis*) in lambs. Journal of Agriculture Science, 134, 89-99.

Chance, P., 1988. Learning and Behavior Wadsworth Publishing Company

Clayton, D.H., Wolfe, N.D., 1993. Trends in Ecology & Evolution, - csa.com The Adaptive Significance of Self-Medication. 8, 22, 60-63, 1993.

Coop, R.L., Kyriazakis, I., 2001. Influence of host nutrition on the development and consequences of nematode parasitism in ruminants. Trends in Parasitolology 17, 325-330.

Foley, W.J., Iason, G.R., McArthur, C., 1999. Role of plant secondary metabolites in the nutritional ecology of mammalian herbivores: how far have we come in 25 years Nutritional ecology of herbivores. Proceedings of the Fifth International Symposium on Nutritional Herbology American Society of Animal Science, Illinois pp. 130-209.

Hendrix, C.M., Robinson, E., 2006. Diagnostic Parasitology for Veterinary Technicians, pp. 236-237. Mosby Elsevier, Saint Louis, Missouri.

Houston, D.C., Gilardi, J.D., Hall, A.J., 2001. Soil consumption by elephants might help to minimize the toxic effects of plant secondary compounds in forest browse. Mammal Review, 31, 249-254.

Huffman, M.A., 2003. Animal self-medication and ethno-medicine: exploration and exploitation of the medicinal properties of plants. Proceedings of the Nutritional Society 62, 371-381.

Hutchings, M.R., Athanasiadou, S., Kyriazakis, I., Gordon, I.J., 2003. Can animals use foraging behavior to combat parasites? Proceedings of the Nutritional Society 62, 361-370.

Jackson, F., Miller, J., 2006. Alternative approaches to control – Quo vadit? Veterinary Parasitology 139, 371-384.

Janzen, J., 1978. Complications in interpreting the chemical defenses of trees against tropical arboreal plant-eating vertebrates. In: Montgomery, G., (Ed.), The Ecology of Arboreal Folivores. Smithsonian Institution Press, Washington, D.C., pp. 73-84.

Lozano, G.A., 1998. Parasitic stress and self-medication in wild animals. Advances in the Study of Behavior 27,291-317 Elsevier Science, London, U.K.

Makkar, H.P.S, Dawra, R.K, Singh, B., 1987. Protein precipitation assay for quantification of tannins – Determination of protein in tannin protein complex. Analytical Biochemistry 66, 435-439.

Min, B.R., Hart, S.P., 2003. Tannins for suppression of internal parasites. Journal of Animal Science 81, E102-E109.

Min, B.R., W.E. Pomroy, S.P. Hart, Sahlu, T., 2004. The effect of short-term consumption of a forage containing condensed tannins on gastro-intestinal nematode parasite infections in grazing wether goats. Small Ruminant Research 51, 279-283.

Min, R., Pinchak, W.E., Anderson, R.C., Fulford, J.D., Puchala, R., 2006. Effects of condensed tannins supplementation level on weight gain and in vitro and in vivo bloat precursors in steers grazing winter wheat. Journal of Animal Science 81, E102-E109. 84, 2546-2554.

Niezen, J.H., Waghorn, T.S., Charleston, W.A.G., Waghorn, G.C., 1995. Growth and gastrointestinal nematode parasitism in lambs grazing either lucerne (*Medicago sativa*) or sulla (*Hedysarum coronarium*) which contains condensed tannins. Journal of Agricultural Science Cambridge 125, 281-289.

Niezen, J.H., Charleston, W.A.G., Robertson, H.A., Shelton, D., Waghorn, G.C., Green, R., 2002. The effect of feeding sulla (*Hedysarum coronarium*) or lucerne (*Medicago sativa*) on lamb parasite burdens and development of immunity to gastrointestinal nematodes. Veterinary Parasitology 105,229-245

Plotkin, M.J., 2000. Medicine quest. In Search of Nature's Healing Secrets. Penguin Putnam Inc., New York NY.

Pomeroy, S.L., Tamayo, P., Gaasenbeek, M., Sturla, L.M., Angelo, M., McLaughlin, M.E., Kim, J.Y., Goumnerova, L.C., Black, P.M., Lau, C., Allen, J.C., Zagzag, D., Olson, J.M, Curran, T., Wetmore, C., Biegel, J.A., Poggio, T., Mukherjee, S., Rifkin, R., Califano, A., Stolovitzky, G., Louis, D.N., Mesirov, J.P., Lander, E.S., Golub, T.R., 2002. Prediction of central nervous system embryonal tumour outcome based on gene expression. Division of Neuroscience, Department of Neurology, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts.

Reed, J.D., 1995. Nutritional toxicology of tannins and related polyphenols in forage legumes. Journal of Animal Science 73, 1516-1528.

Villalba, J.J., Provenza, F.D., 2001. Preference for polyethylene glycol by sheep fed a quebracho tannin diet. Journal of Animal Science 79, 2066-2074.

Villalba, J. J., Provenza, F. D., Shaw, R. 2006. Sheep self-medicate when challenged with illness-inducing foods. Animal Behavior 71, 1131-1139.

Waller, P.J., 2006. Sustainable nematode parasite control strategies for ruminant livestock by grazing management and biological control. Animal Feed Science Technology 126,277-289.

Wrangham, R.W., Goodall, J., 1989. Chimpanzee use of medicinal leaves. In: Understanding Chimpanzees. Harvard University Press, Cambridge, Massachusetts, pp. 22-37

CHAPTER 3

EFFECTS OF TANNIN ON SELECTION BY SHEEP OF FORAGES CONTAINING ALKALOIDS, TANNINS, AND SAPONINS

ABSTRACT

Natural landscapes are diverse mixes of plants that occur in patches reflecting history of use in concert with particular soil, precipitation, and temperature regimes. For plants, diversity is the rule -- for species, phenologies, growth forms, and biochemistries. Regarding the latter, plants are nutrition centers and pharmacies with vast arrays of primary (nutrients) and secondary (pharmaceuticals) compounds useful in animal nutrition and health. Regrettably, we have neither understood nor valued diversity in agriculture, as evident in our persistent attempts to simplify ecological systems to maximize yields of crops and pastures in monocultures.

Interactions among plant secondary metabolites (PSM) may lead to complementarities wherein eating a variety of foods may be more beneficial than eating single foods. Tannins are a group of highly reactive chemical compounds with the potential to interact with other PSM such as alkaloids and saponins, neutralizing their negative effects. In this study I determined whether tannins modify the foraging behavior of sheep grazing on varieties of alfalfa, birdsfoot trefoil and tall fescue with high and low concentrations of saponins, tannins and alkaloids, respectively. Lambs that received intraruminal infusions of tannins increased their consumption of the high-saponin variety of alfalfa and the high-alkaloid variety of tall fescue relative to lambs not infused with tannin (Controls). Lambs offered choices among the three high-PSM varieties of the forages also manifested higher consumption of the high-alkaloid variety of tall fescue than Control

lambs. In contrast, lambs infused with tannin reduced their consumption of the high-tannin variety of birdsfoot trefoil. Thus, lambs modified their foraging behavior as a function of the presence/absence of tannins in their rumens. Preference increased for forage varieties with high concentrations of saponins and alkaloids, likely because tannins form insoluble complexes with these PSM. In contrast, lambs infused with tannins avoided tannin-containing birdsfoot trefoil. These results indicate ruminants are able to discriminate the specific postingestive effects of forage varieties with high concentrations of PSM, and further PSM complementarities are likely to increase the efficiency of use of diverse pastures with different biochemistries.

INTRODUCTION

Natural landscapes are diverse mixes of plants that occur in patches reflecting history of use in concert with particular soil, precipitation, and temperature regimes. For plants, diversity is the rule – for species, phenologies, growth forms, and biochemistries. Regarding the latter, plants are nutrition centers and pharmacies with vast arrays of primary (nutrients) and secondary (pharmaceuticals) compounds useful in animal nutrition and health (Engel, 2002). Regrettably, we have neither understood nor valued diversity in agriculture, as evident in our persistent attempts to simplify ecological systems to maximize yields of crops and pastures.

Simplifying agriculture to facilitate livestock production, coupled with a view of plant secondary metabolites (PSM) as toxins, has resulted in selecting for a biochemical balance in forages favoring primary compounds and nearly eliminating PSM. To increase intake of single-plant diets, one must reduce PSM as they limit how much of a food humans and livestock can consume (Provenza, 2003). The outcome is energy- and protein-dense monocultures low in the PSM. The alternative, which we have not pursued, is offering animals a variety of foods that differ in primary and secondary metabolites, thereby enabling them and us to obtain a much greater array of nutrition and health benefits from nature's bounty.

Interactions among PSM can lead to complementarities wherein eating a variety of foods is beneficial (Tilman, 1982; Provenza, 2003). Outside of a few studies, however, we know little about which PSM are biochemically complementary. When sheep choose between foods that contain either amygdalin or lithium chloride, they eat more than sheep offered a food with only one of these compounds; the same is true for nitrate and oxalate (Burritt and Provenza, 2000), and for tannin and the alkaloids; gramine and ergovaline (Lyman et al., 2007).

Tannins are a group of highly reactive chemical compounds increasingly recognized as important in health and nutrition, though historically they were thought by agriculturalists and ecologists alike to adversely affect herbivores. Eating plants high in tannin is a natural way for herbivores to reduce levels of internal parasites (Min and Hart, 2003), and tannin alleviates bloat by binding to proteins in the rumen (Waghorn, 1990). Tannin also can enhance nutrition by providing high-quality protein to the small intestines by binding to degradable protein in the rumen, making the protein unavailable for digestion and absorption until it reaches the more acidic abomasum (Barry et al. 2001). This high-quality-protein-bypass effect enhances immune responses and increases resistance to gastrointestinal nematodes (Niezen et al., 2002; Min et al., 2004). Tannins form complexes, mainly through hydrophobic/hydrogen interactions, with a wide variety of compounds including protein (Jones and Mangan, 1977), polysaccharides, nucleic acids, minerals (Min and Hart, 2003), alkaloids (Okuda et al., 1982) and saponins (Freeland et al., 1985).

We determined whether tannin influences forage selection by sheep when they were offered plants that varied in the concentration of saponins (alfalfa, Vernal [high] and Lahontan

[low]*, Medicago sativa)*, tannins (birdsfoot trefoil, Goldie [high]and Norcen [low], *Lotus corniculatus*), and alkaloids (tall fescue, Kentucky 31[with and without endophyte], *Lolium arundinaceum*). Tannins bind to and possibly deactivate the negative effects of alkaloids (Okuda et al., 1982) and saponins (Freeland et al., 1985). Thus, we predicted sheep receiving intraruminal infusions of tannins would 1) preferentially graze high-alkaloid varieties of tall fescue and high-saponin varieties of alfalfa, and 2) avoid high-tannin varieties of birdsfoot trefoil. In general, enhancing knowledge of the ability of animals to use PSM for nutrition and health will be useful for animal production systems – on organic land in particular – that seek to minimize the effects of secondary compounds in diverse pastures.

MATERIALS AND METHODS

Plant Species

Tall fescue is highly productive, well-adapted cool-season grass, ubiquitous in the U.S. Though breeding programs have reduced or eliminated many of the potentially toxic alkaloids in tall fescue, the lack of these compounds may have negative implications regarding plant persistence and adaptability. For example, Asay et al. (2001) stated “Differences in dry matter yield between ‘KY 31’ tall fescue infected with the *Neotyphodium endophyte* and its endophyte-free counterpart confirms earlier reports of the positive effect of this fungal organism on forage yield in tall fescue, particularly in water-limited environments.” Thus, there may be agronomic advantages if these chemical constituents are present in the grass, provided we can find ways to neutralize their adverse effects in animals. One way to accomplish this objective is to grow plants in mixtures whose secondary compounds are complementary.

In its wild form, tall fescue contains two groups of alkaloids that can adversely affect animals, especially as pastures in monocultures. One group is inherently associated with the plant and the other associated with the fungus *Neotyphodium coenophialum* (Burrows and Tyrl 2001). The inherent alkaloids are perlolidine and perloline, which negatively affect rumen fermentation. The fungus-associated alkaloids are N-acetyl loline and N-formyl loline, which are generally associated with fescue toxicity. Perlolidine and perloline are steroidal compounds. N-acetyl loline and N-formyl loline have lipid chemical structures.

Legumes such as alfalfa and birdsfoot trefoil also have application in irrigated pasture systems due to nitrogen fixing capabilities and complementary root profiles. Alfalfa contains glycosides such as saponins. Reduced feed intake and lowered performance occur when animals consume large amounts of high-saponin alfalfa. Saponins bind to lipids such as cholesterol in the gastro-intestinal tract of animals causing their excretion in the feces (Malinow et al. 1979). Birdsfoot trefoil contains condensed tannins that are known to firmly bind to soluble proteins in the rumen (Jones and Mangan 1977).

Pasture Design

Plant species with high and low concentrations of alkaloids, tannins, and saponins were seeded at the USU pasture research facility in Lewiston Utah [41 56’ N 111 52’W] in spring of 2006. To compare the effects of intraruminal infusions of tannins on preference for forages with different concentrations of PSM, sheep had access to strips of 0.18 ha each of: 1) birdsfoot trefoil, 2) alfalfa, and 3) tall fescue offered in a choice of two varieties with high and low concentration of PSM: Tall fescue (TF), *Festuca arundinaceum,* Kentucky 31 endophyte-infected and endophyte-free*;* birdsfoot trefoil (BFT), *Lotus corniculatus*, varieties Goldie and Norcen with high and low tannin, respectively; and alfalfa (AA), *Medicago sativa*, varieties Vernal and Lahontan with high and low saponins, respectively.

To assess the effects of intraruminal infusions of tannins on preference for forages with different combinations of PSM, sheep in a 4^{th} treatment had access to all three species with high levels of PSM. To determine the effects of intraruminal infusions of tannins on use of a plant species with low concentrations of PSM, animals in a 5^{th} treatment had access only to orchardgrass (OG; *Dactylis glomerata* L.). The five treatments constituted a pasture unit and there were 4 replications of pasture units in space (Figure 3.1). All plots provided ad libitum forage to all the animals. Pastures were constructed of temporary electric fencing, as illustrated by dashed lines in Figure 3.1.

Animals

During the study, 40 commercial crossbred lambs (4 mo of age, average weight 28 kg) were randomly allocated to the four pasture units (2 animals for each of the 5 treatments replicated 4 times). Pairs of lambs grazed their respective treatment plots throughout the study. Once formed, the same pairs of sheep, identified by specific numbers, always were tested together. When not in the pasture units, all animals were maintained on four 1-ha plots of OG, one per pasture unit. Every morning at 0900 pairs of animals were transported to their respective units to graze and at the end of the grazing period they were returned to the OG plots at 1100 where they grazed until 1600. Subsequently, animals were penned overnight with free access to shade, water and trace mineral blocks.

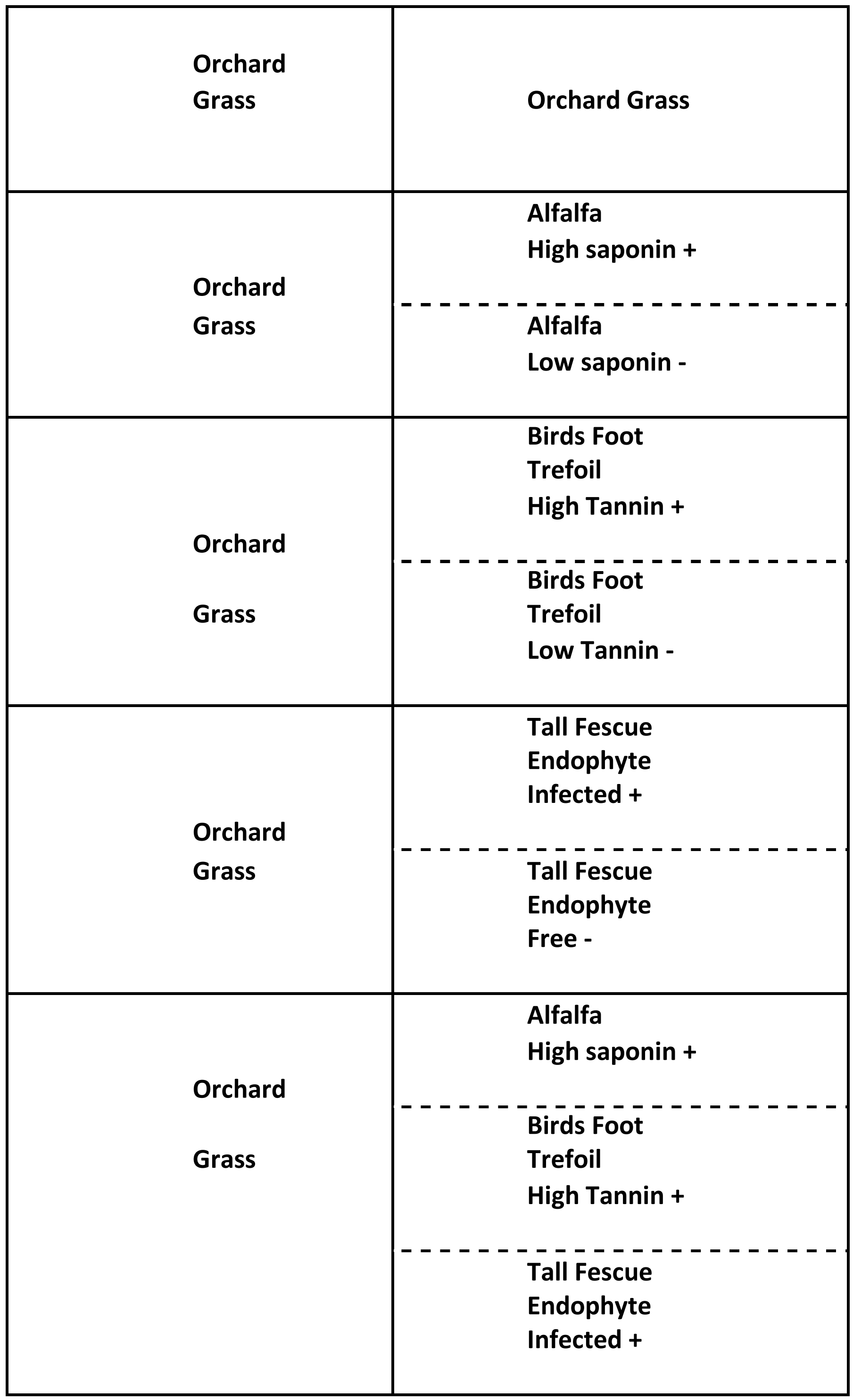

Figure 3.1. Experimental design for the grazing study (1 of 4 replications).

Adaptation Period

Pairs of lambs were allowed to graze for 2 h/d during a period of 18 days. An adaptation period of 2 days preceded the study in order to allow familiarization with the pastures and with experimental procedures described in the following section.

Baseline 1 – Without a Tannin Challenge. For 2 h/d, animals grazed on their assigned treatment plots. An observer recorded behavioral data, using the scan-sampling method (Altman, 1974), to determine the percent of time each animal spent grazing on each choice plot. Samples were taken at 5-min intervals from 0900 to 1100 for 6 d. Every 5 min, pairs were identified and the plant species they were consuming recorded. Pairs normally grazed together (>95% of the time) on the same plant species. If individuals were performing different behaviors we recorded each behavior for each individual.

Chemical analyses carried out in each pasture assessed the levels of alkaloids, tannins, and saponins in each plot. Representative samples of the biomass harvested by animals were taken from each of the four units at the end of the trial, placed in plastic bags covered with ice, transported to a freezer at -20 0C until they were freeze dried before chemical analyses.

Tannin Challenge. From 0900 to 0930 daily for 6 d animals in two of the four pasture units (20 lambs total; Tannin Treatment) received by oral intubation intraruminal infusions of quebracho tannin (Tannin Corporation, Peabody, MA), a condensed tannin extracted from the South-American quebracho tree (*Aspidosperma quebracho*) dissolved in 400 ml of water (d 1-3: 30 g; d 4-5: 40 g; d 6: 50 g). These levels of tannins have been used safely in sheep (Villalba et al., 2002), and represent less than the 40% of the levels that lambs can voluntarily consume in 4 h (Villalba et al., 2002). Animals in the other two pasture units (20 lambs; Control group) were not infused. Pasture units for Tannin Treatment and Control were selected at random.

Grazing and scan sampling occurred as described in the previous section.

Baseline 2- Without a Tannin Challenge. After testing for 6 d with a tannin challenge, all animals were tested for 6 d, as described for Baseline 1.

Statistical Analyses

The statistical design for the analysis of variance was a split-plot with spatial repetitions (experimental units) nested within treatment (Tannin, Water). Period (Baseline 1, Tannin Challenge, and Baseline 2) and day (1-6) were the repeated measures.

For Treatments 1-3, pasture choices (1-birdsfoot trefoil, 2- alfalfa, and 3-tall fescue offered in two varieties with high and low concentration of PSM [Table 1]) was the whole-plot factor and percentage of scans recorded on the variety with high concentration of PSM was the response variable.

For Treatment 4, species (1-birdsfoot trefoil, 2- alfalfa, and 3-tall fescue with high levels of PSM) was the whole-plot and percentage of scans recorded on each species was the response variable.

For all Treatments 1-5, treatment (1-birdsfoot trefoil, 2- alfalfa, and 3-tall fescue offered in two varieties with high and low concentration of PSM; 4-access to all 3 species with high levels of PSM, and 5-access only to orchard grass) was the whole-plot and percentage of scans recorded for grazing events was the response variable.

Plant variety	NDF, %	N, %	Total Condensed Tannins %	Saponins, %	Ergovaline,ppm	Yield Tons/ha
Alfalfa (High)[*]	44.1±0.4	3.4±0.2	—		—	117
Alfalfa (Low)[†]	40.9±1.7	3.9±0.1	—		—	161
Birdsfoot trefoil (High)	45.1±1.4	3.1±0.1	0.0747	—	—	188
Birdsfoot trefoil (Low)	43.8±0.8	3.9±0.4	0.0197	—	—	237
Tall Fescue (High)	55.9±2.5	1.9±0.2	—	—	181ppm	165
Tall Fescue (Low)	59.8±0.9	1.7±0.5	—	—	35ppm	161

[*]Variety with high concentrations of saponins (alfalfa), condensed tannins (birdsfoot trefoil) and alkaloids (ergovaline; tall fescue).

[†] Variety with low concentrations of saponins (alfalfa), condensed tannins (birdsfoot trefoil) and alkaloids (ergovaline; tall fescue).

Table 3.1. Fiber, nitrogen and plant secondary compound concentrations (dry matter basis; means ± SEM) and yields in the plant varieties of the study

RESULTS

Baseline 1 -Before Tannin Infusions

The objective of this period was to obtain a baseline for pasture use and preference before groups of lambs (Treatment, Control) received intraruminal infusions of tannins (Treatment) or vehicle (water, Control).

Total Number of Grazing Scans for all Groups. Lambs grazed almost all the time during the observation period (Figure3.2).

No differences in the total number of scans were observed between the Control and Treatment groups during this period ($P > 0.10$), except that Control lambs showed a lower number of scans on birdsfoot trefoil than Treatment lambs on d 3 ($P = 0.002$) and on tall fescue on d 1 and 3 ($P < 0.001$; Figure 3.3).

Averaged for all periods, lambs showed a higher number of scans on the variety of birdsfoot trefoil with high concentration of tannins than lambs on the variety of alfalfa with high concentration of saponins ($P = 0.008$) or lambs on the variety of tall fescue with high concentrations of alkaloids ($P=0.003$; Figure 3).

However, no differences in the number of scans recorded on the varieties with high concentrations of PSM were observed between Control and Treatment lambs (Pasture x Treatment interaction, $P = 0.48$; Figures 2 and 3).Only lambs in the Control group had fewer scans on the high-saponin variety of alfalfa on d 6 ($P = 0.09$) and more scans on the high-alkaloid variety of tall fescue on d 3 ($P = 0.06$) than lambs in the Treatment group (Pasture x Treatment x Day interaction; $P = 0.10$; Figure 2).

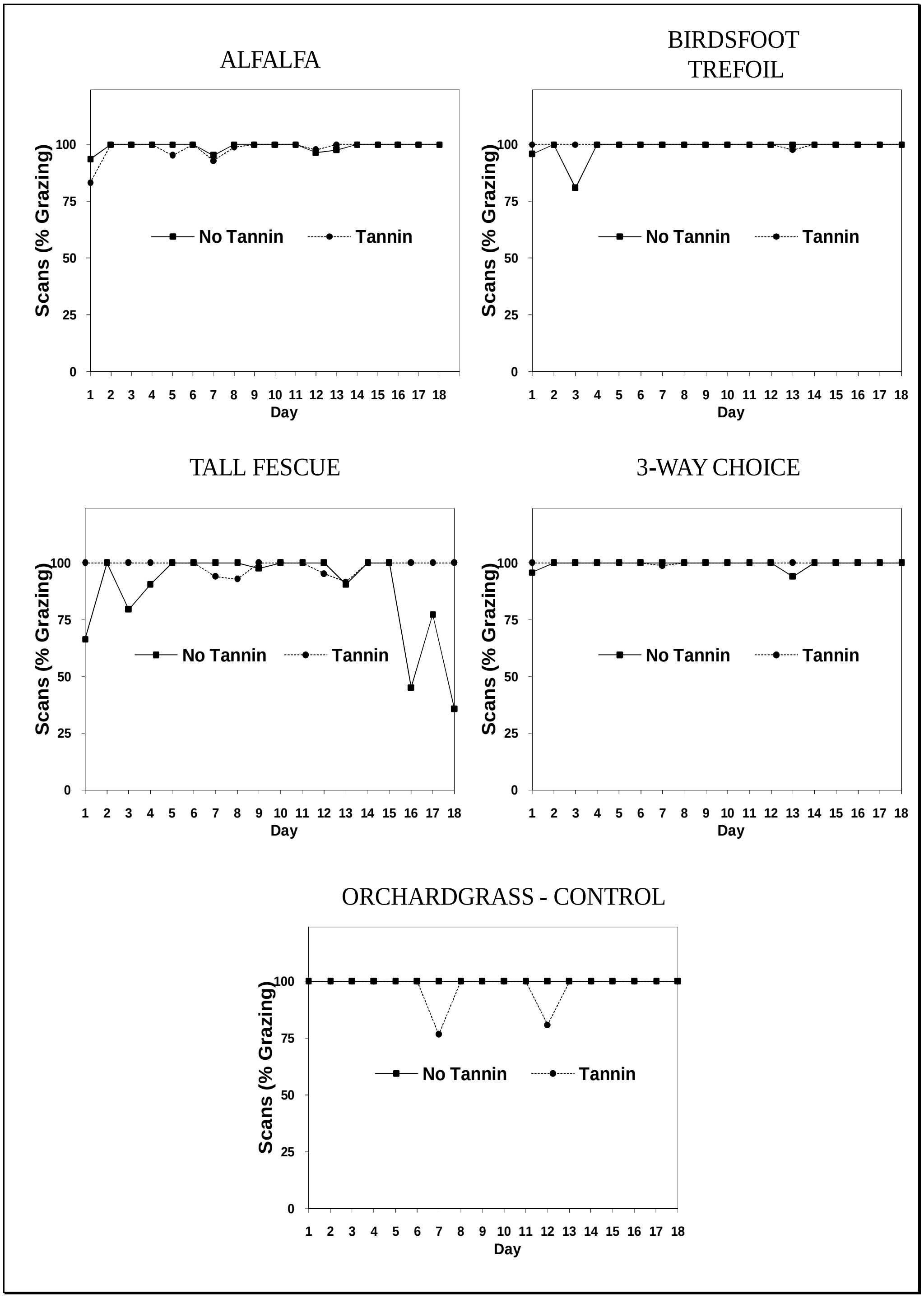

Figure 3.2: Total Grazing Scans for all treatments.

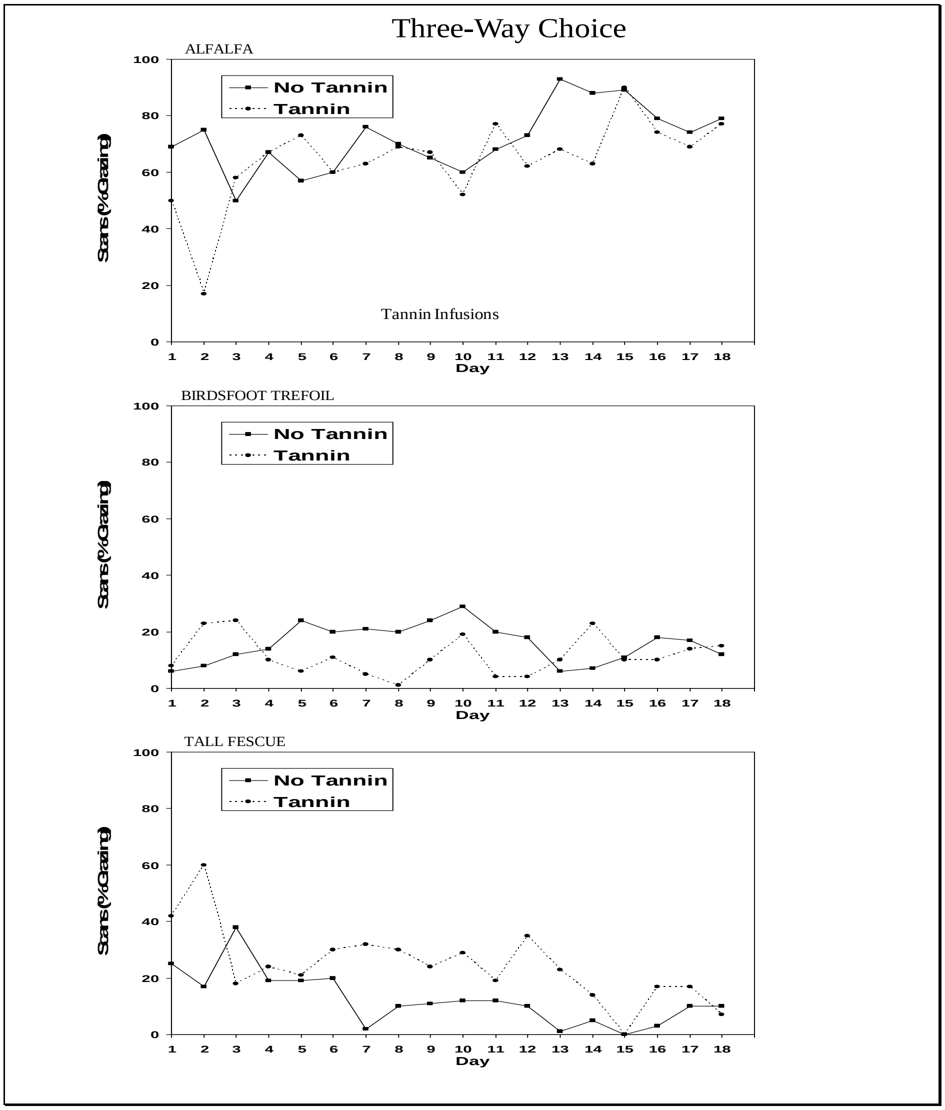

Figure 3.3. Grazing events on pastures with high concentrations of plant secondary metabolites (PSM) (Shown as a % of total grazing events). Pairs of lambs had simultaneous access to 3 species with high concentrations of PSM: 1) birdsfoot trefoil, 2) alfalfa, and 3) tall fescue. Lambs in the Treatment Group received intraruminal infusions of tannin during d 7-12, whereas lambs in the Control Group did not receive such infusions. Baseline 1 occurred between d 1-6 and Baseline 2 during d 13-18. During baseline periods no infusions of tannin were delivered.

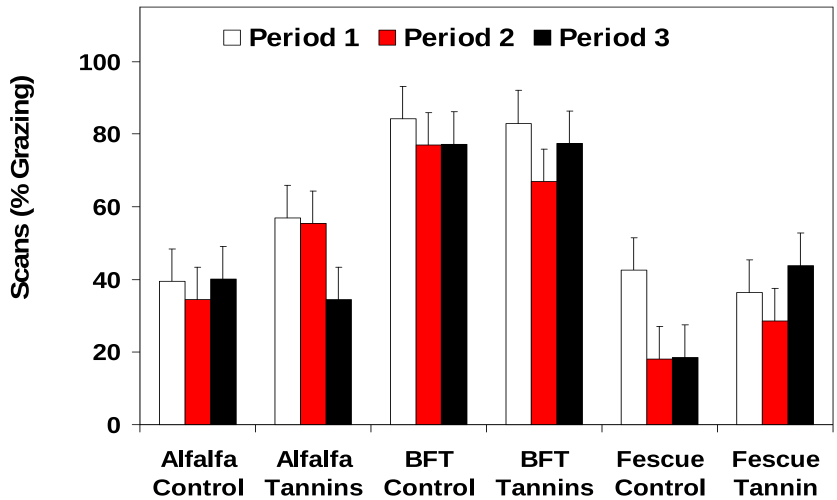

Figure 3.4. Grazing events on pastures with high concentrations of plant secondary metabolites (PSM) recorded during scan sampling (Shown as a % of total grazing events). Pairs of lambs had access to strips of: 1) birdsfoot trefoil, 2) alfalfa, and 3) tall fescue offered in two varieties with high and low concentration of PSM. Lambs in the Treatment Group received intraruminal infusions of tannins during d 7-12, whereas lambs in the Control Group did not receive such infusions. Baseline 1 occurred between d 1-6 and Baseline 2 during d 13-18. During baseline periods no infusions of tannins were delivered.

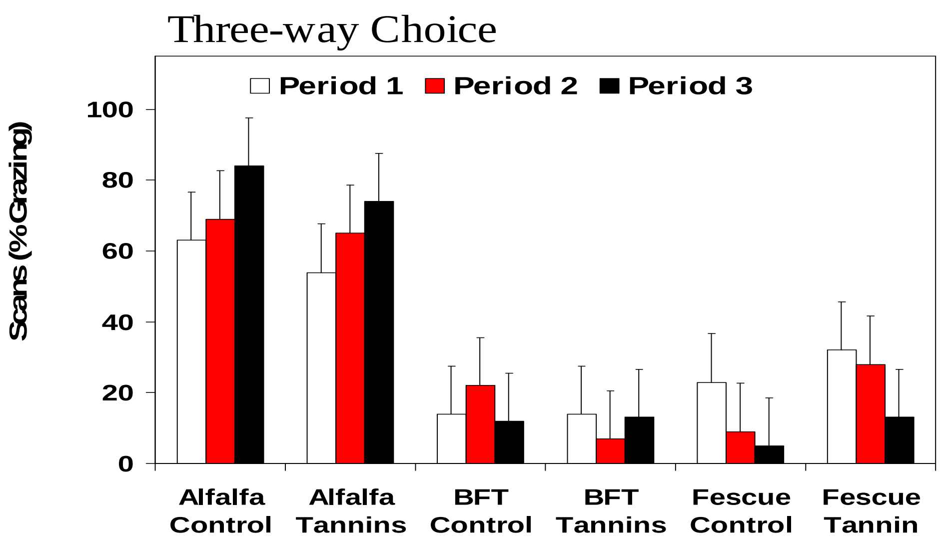

Figure 3.5. Average number of scans on pastures with high concentrations of plant secondary metabolites (PSM) (Shown as a % of total grazing events). Pairs of lambs had simultaneous access to 3 species with high concentrations of PSM: 1) birdsfoot trefoil, 2) alfalfa, and 3) tall fescue. Lambs were observed during 3 periods of 6 d each: Baseline 1 and 2 without tannin infusions, and Tannin Infusions, where lambs in the Treatment group received intraruminal infusions of tannin. Lambs in the Control group did not receive tannin infusions in any period.

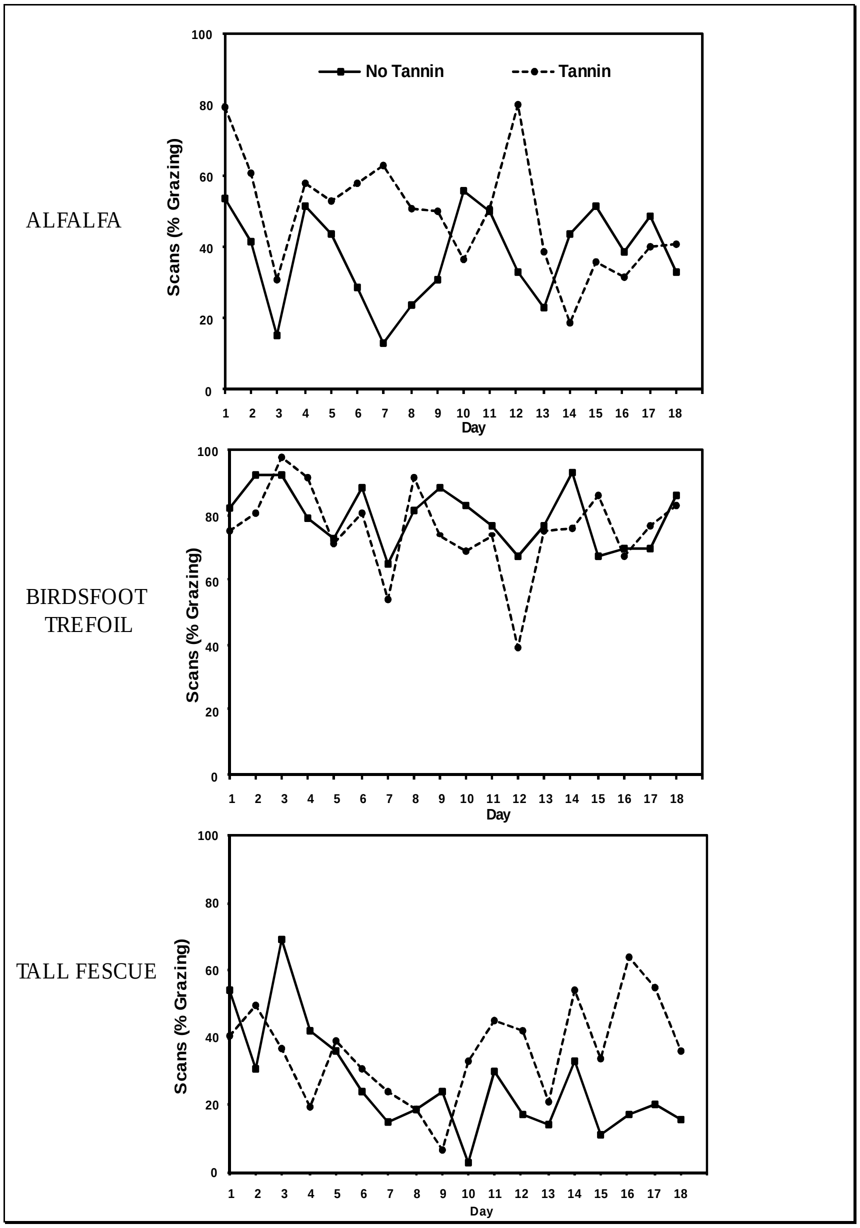

Figure 3.6. Average number of scans on pastures with high concentrations of plant secondary metabolites (PSM) (Shown as a % of total grazing events). Pairs of lambs had access to strips of: 1) birdsfoot trefoil, 2) alfalfa, and 3) tall fescue offered in two varieties with high and low concentration of PSM. Lambs were observed during 3 periods of 6 d each: Baseline 1 and 2 without tannin infusions, and with tannin Infusions, where lambs in the Treatment group received intraruminal infusions of tannins. Lambs in the Control group did not receive tannin infusions in any period.

Pasture Preference

Three-way Choices. Lambs differed in their preference for the three pastures offered during the choice (Pasture effect; P = 0.025). Averaged for all days of the period, lambs preferred alfalfa over birdsfoot trefoil (P = 0.01) and tall fescue (P = 0.02). No differences were observed in the number of scans recorded for birdsfoot trefoil and tall fescue (P = 0.73; Figures 5 and 6).

No differences between Control and Treatment lambs were observed in preferences for the three pastures offered during baseline (Pasture x Treatment interaction, P = 0.85; Figures 5 and 6). Only lambs in the Control group during d 2 manifested a higher number of scans on alfalfa (P = 0.008) and a lower number of scans on tall fescue (P = 0.05) than lambs in the Treatment group (Pasture x Treatment x Day interaction; P < 0.001; Figure 5).

During Tannin Infusions

The objective of this period was to determine pasture use and preference before lambs (Treatment, Control) received intraruminal infusions of tannins (Treatment) or vehicle (water, Control).

Total Number of Grazing Scans for all Groups. Lambs grazed almost all the time during the period of tannin infusions (Figure3. 5). No differences in the total number of scans were observed between the Control and Treatment groups for animals offered 2- and 3-way choices (P > 0.10). However, lambs treated with tannins (Treatment) had fewer scans on orchard grass on d 7 (P < 0.001) and 12 (P = 0.002) than Control lambs (Figure 3.5). Averaged across the period, lambs that grazed orchard grass after being infused with tannins had fewer grazing events than lambs offered 2-way choices of alfalfa (P= 0.12)and birdsfoot trefoil (P=0.05), or 3-way choices of alfalfa, birdsfoot trefoil, and tall fescue (P=0.05).

Pasture Preference – Two-Way Choices. Tannin infusions affected pasture preference in the 2-way choices (Pasture x Treatment interaction; P = 0.08). Lambs infused with tannins had more scans than Control lambs for the high-saponin variety of alfalfa during the first days (e.g., d 7 (P < 0.001), 8 (P = 0.12)) and last day (d12; P = 0.007) of infusions. During the last day (d 12) of infusions, lambs treated with tannin had fewer scans on the high-tannin variety of birdsfoot trefoil than lambs in the Control group (P = 0.11). In contrast, during the last days of infusions, lambs in the Treatment group had more scans grazing the high-alkaloid variety of tall fescue than Control lambs (d 10; P = 0.08; d 12; P = 0.14; Figure 3.2).

Pasture Preference – Three-way Choices. Tannin infusions did not affect the overall pattern of preference in the 3-way choice (Pasture x Treatment interaction; P = 0.56). However, lambs treated with tannin tended to show higher number of scans than Control lambs on the high-alkaloid variety of tall fescue during d 7 and 12 (P = 0.2).

Baseline 2 -After Tannin Infusions

The objective of this phase was to determine pasture use and preference by two groups of lambs (Control and Treatments) when tannin infusions were no longer administered to the Treatment group of animals.

Total Number of Grazing Scans for all Groups. Lambs grazed almost all the time during the observation period in Baseline 2 (Figure 3.6). No differences in the total number of scans were observed between the Control and Treatment groups during this period (P > 0.10), except that Control lambs had fewer scans on tall fescue than lambs infused with tannin during the previous period (d 16-18; P < 0.001; Figure3.5).

Pasture Preference – Two-Way Choices. After tannin infusions were removed, no differences in the number of scans recorded on the varieties with high concentrations of PSM were observed between Control and Treatment lambs (Pasture x Treatment interaction; P = 0.42; Figures 2 and 3). However, lambs that had received tannin infusions had more scans (d 14-15, 18; P = 0.2; d 16-17; P < 0.05) on the high-alkaloid variety of tall fescue than Control lambs (Pasture x Treatment x Day interaction; P = 0.003; Figure 3.5).

Pasture Preference – Three-way Choices. Tannin infusions did not affect the overall pattern of preference in the 3-way choice (Pasture x Treatment interaction; P = 0.63). However, lambs treated with tannin tended to have more scans than Control lambs on the high-alkaloid variety of tall fescue during d 7 and 12 (P = 0.2).

DISCUSSION

Effects of Tannin on Selection by Sheep of Forages Containing Alkaloids, Tannins and Saponnins

I determined if lambs receiving intraruminal infusions of tannin modify their diet selection when offered forages of high- and low- concentrations of saponins, tannins, and alkaloids. Can lambs learn to self-select foods that attenuate the adverse effects of tannins when they receive doses of tannin which they can voluntarily consume daily when exposed to high-tannin foods (Villalba et al., 2004)? Results from the present study suggest that they can.

Relative to Controls, lambs infused with tannins increased their preference for the high-saponin variety of alfalfa and the high-alkaloid variety of tall fescue (Figure 3). Lambs offered choices among the three high-PSM varieties of the forages tested also manifested higher preference for the high-alkaloid variety of tall fescue than Control lambs.

Plant secondary metabolites constrain intake. Thus, PSM satiate herbivores and the more quickly plants satiate foragers, the more likely these plants are to survive (Garcia, 1975).

From the animal perspective, reduced intake implies constraints on productivity. Moreover, at certain doses PSM negatively impact animal cells, tissues and metabolic processes (Cheeke and Shull, 1985; Cheeke, 1988; Osweiler et al., 1985). Nevertheless, there may be important advantages for plants and herbivores if PSM are present in grazing systems. From the plant perspective, PSM are vital for attracting pollinators and seed dispersers, helping plants recover from injury, protecting plants from ultraviolet radiation, and defending plants against pathogens and herbivores (Rosenthal and Janzen, 1979; Rosenthal and Berenbaum, 1992). Moreover, the lack of PSM may negatively impact plant persistence and adaptability (Asay et al., 2001). From the animal perspective, PSM at certain doses may provide beneficial effects to herbivores, such as combating pathogens (Athasianadou et al., 2001, Chapter 2), providing antioxidant and immunity enhancing activities (von Schonfeld et al., 1997; Mishima et al., 2004), and reducing nitrogen loses (Reed, 1995) and CH_4 emissions (Tedeschi et al., 2003).

A plant community with a diverse array of PSM may allow animals to harvest higher amounts of nutrients while maintaining PSM intake below toxic levels. This is because PSM that affect different organs and detoxification pathways are likely to be less toxic when ingested as a dilute mixture than when each PSM is ingested in a larger dose (Freeland and Janzen, 1974). When sheep choose between foods that contain either amygdalin or lithium chloride, they eat more than sheep offered a food with only one of these compounds; the same is true for nitrate and oxalate (Burritt and Provenza, 2000), and for tannin and the alkaloids; gramine and ergovaline (Lyman et al., 2007). A diverse intake of PSM may also lead to toxin neutralization or inactivation, which in turn could reduce susceptibility to PSM, as suggested by results of my study.

Tannins are highly reactive molecules that once in the gut are likely to interact with other PSM forming insoluble tannin-PSM complexes which reduce their negative postingestive effects. Tannins form complexes, mainly through hydrophobic/hydrogen interactions, with a wide variety of compounds including proteins, polysaccharides, nucleic acids, alkaloids, and minerals (Okuda et al., 1982; Min and Hart, 2003). Rats eat more of a combination of foods containing tannins and saponins because tannins and saponins chelate in the intestinal tract, reducing the negative effects of both components (Freeland et al., 1985). When sheep choose between foods that contain either tannin and alkaloids they eat more than sheep offered a food with only one of these compounds; the same is true for gramine and ergovaline (Lyman et al., 2007). Consistent with this, lambs in our study increased their preference for the high-saponin variety of alfalfa after tannin infusions (Figure 3.2). Thus, interactions among PSM-containing foods can lead to complementary relationships such that eating a combination of foods may exceed the benefit of consuming any one food in isolation (Tilman, 1982).

Cattle steadily increase intake of endophyte-infected tall fescue after grazing a high-tannin variety of birdsfoot trefoil (Lyman et al. 2007). Lambs in our study also increased their preference for endophyte-infected tall fescue during tannin infusions (Figure 3.2). This effect was also evident after tannin infusions in both two- and there-way choices (e.g., Baseline 2) which suggests the effects of tannin persisted across time. The retention of quebracho tannin in the gastrointestinal tract ranges from 48 h (free and soluble) to 72 h (bound to protein and fiber) (Silanikove et al., 1994, 1996), which could have accounted for the continued effect during baseline 2.

The chelating properties of tannin which may reduce the negative postingestive effects of insoluble PSM-tannin complexes and the increased preference for the high-PSM plant varieties during tannin infusions suggest lambs foraged in order to reduce the negative impacts of tannin forced into their gastrointestinal tract. The lower number of total grazing events recorded for lambs grazing only orchard grass (Control) than for lambs grazing a combination of PSM-containing forages during tannin infusions (Figure 23.) also suggests selection of PSM-containing forages benefited animals treated with a tannin load. Herbivores learn to consume foods that neutralize or ameliorate negative internal states. For instance, sheep ingest “medicines” such as polyethylene glycol (PEG), a substance that attenuates the aversive effects of tannins, when they eat foods high in tannin, and they titrate the dose of PEG in accord with the amount of tannin in their diet (Provenza et al., 2000). They discriminate the medicinal benefits of PEG from non-medicinal substances by selectively ingesting PEG after eating a meal high in tannins (Villalba and Provenza, 2001). They also choose to forage in locations where PEG is present when offered nutritious foods high in tannin in different locations (Villalba et al., 2002). Sheep fed acid-producing substrates such as grains subsequently ingest foods and solutions that contain sodium bicarbonate, which attenuates acidosis (Phy and Provenza, 1998). Sheep also learned to selectively ingest three medicines – sodium bentonite, polyethylene glycol, dicalcium phosphate – that lead to recovery from illness due to eating too high amounts of grain, tannins, and oxalic acid, respectively (Villalba et al., 2006). Collectively the information presented and observed in this study suggests lambs self-selected PSM-containing forages to increase their grazing time and counteract the negative postingestive effects of tannin.

Effects of Tannin on Selection by Sheep of Forages Containing Alkaloids, Tannins and Saponnins

During the last day of infusions, lambs infused with quebracho tannins had fewer scans on the high-tannin variety of birdsfoot trefoil than Control lambs (Figure 3.2). Likewise, oral gavage of a toxin (LiCl) causes dose-dependent decreases in intake of foods that contain the same toxin (Wang and Provenza, 1997). Herbivores regulate their intake of foods that contain single PSM to avoid poisoning. In contrast, a diverse feeding environment with multiple PSM may be beneficial. As mentioned, some PSM may contribute to ameliorate the negative effects of quebracho tannins (e.g., saponins, alkaloids) whereas other PSM may promote additive negative postingestive effects (e.g., tannins from birdsfoot trefoil) – as likely occurred in the present study – impact the same detoxification pathway or be antagonistic and as a consequence ingestion of PSM decreases (Burritt and Provenza, 2000). Reductions (tannins) and increases (alkaloids, saponins) in preference for diverse PSM-containing forages during tannin infusions suggests as in previous studies (Villalba et al., 2005) that herbivores can discriminate among the specific postingestive effects of different PSM.

IMPLICATIONS

Lambs modified their foraging behavior as a function of intraruminal infusions of tannin. Preference increased for forage varieties with high concentrations of saponins and alkaloids, likely because tannin forms insoluble complexes with these PSM. In contrast, a reduction in preference for tannin-containing birdsfoot trefoil was observed, which suggest animals avoided high-tannin foods after tannin infusions. Plant secondary metabolites have the potential to provide beneficial effects to both plants (e.g., persistence, pollination) and herbivores (e.g., nutrition and health). Thus, enhancing knowledge of the ability of animals to use PSM could be useful for animal production systems – on organic land in particular – that seek to minimize the negative effects of secondary compounds in diverse pastures. Differences among the various treatments developed over time indicating a learned aspect to self-medication while ingesting of foods with PSMs. This suggests that self medication is both physiological and behavioral in nature and can be learned when appropriate exposure to the medicinal forage is provided in concert with discernable differences in dietary characteristics (flavor, texture, etc.).

REFERENCES

Altman, J., 1974. Observational study of behavior: sampling methods. Behavior 49, 227.

Asay, K.H., Jensen, K.B., Waldron, B.L., 2001. Responses of tall fescue cultivars to an irrigation gradient. Crop Science 41, 350.

Athanasiadou, S., Kyriazakis I., Jackson F., Coop, R.L., 2001. The Effects of condensed tannins supplementation of foods with different protein content on parasitism, food intake and performance of sheep infected with *Trichostrongylus colubriformis.* British Journal of Nutrition 86, 697-706.

Barry, T.N., McNeill, D.M., McNabb, W.C., 2001. Plant secondary compounds: their impact on nutritive value and upon animal production. In: Procedures of the XIX International Grass Conference, Sao Paulo, Brazil, pp. 445-452.

Burritt, E.A., Provenza F.D., 2000. Role of toxins in intake of varied diets by sheep. Journal Chemical Ecology 26, 1991.

Burrows, G. E., Tyrl, R. J., 2001. Toxic Plants of North America, Iowa State Press, Ames, Iowa.

Cheeke, P.R., 1998. Natural Toxicants in Feeds, Forages, and Poisonous Plants. Interstate Publishing Incorporated, Danville, Illinois.

Cheeke, P., Shull, L.R., 1985. Natural Toxicants in Feeds and Poisonous Plants. Avi Publishing, Westport, Connecticut.

Engel, C., 2002. Wild Health. Houghton Mifflin Co., Boston, New York, NY.

Freeland, W.J., Calcott, P.H., Anderson, L.R., 1985. Tannins and saponin: interaction in herbivore diets. Biochemical System Ecology 13, 189-193.

Freeland, W.J., Janzen D.H., 1974. Strategies in herbivory by mammals: the role of plant secondary compounds. American Nature 108, 269-286.

Garcia, J., Hankins, W.G., 1975. The evolution of bitter and the acquisition oftoxiphobia. In: Denton, D.A., Coghlan, J.P. (Eds), Olfaction and Taste. V Proceedings of the 5th International Symposium in Melbourne, Australia. Academic Press, New York pp. 39-45.

Jones, W.T., Mangan, J.L., 1977. Complexes of condensed tannins of sainfoin (*Onobrychis viciifolia Scop.*) with fraction-1 leaf protein and with submaxillary mucoprotein, and their reversal by polyethylene-glycol and pH. Journal of Science Food and Agriculture 28, 126-136.

Lyman, T., Provenza, F.D., Villalba, J.J., 2007. Sheep foraging behavior in response to interactions among alkaloids, tannins and saponins: Implications for chemical and taxonomic diversity in pasture ecosystems. Journal of the Science of Food and Agriculture, (In Press).

Malinow, M.R., McLaughlin, P., Stafford, C., Livingston, A.L., Kohler, G.O., Cheeke, P.R., 1979. Comparative effects of alfalfa saponins and alfalfa fiber on cholesterol absorption in rats. American Journal of Chemical Nutrition 32, 1810-1812.

Min, B.R., Hart, S.P., 2003. Tannins for suppression of internal parasites. Journal of Animal Science 81E102-E109.

,

Min, B.R., Pomroy, W.E., Hart, S.P., Sahlu, T., 2004. The effect of short-term consumption of a forage containing condensed tannins on gastro-intestinal nematode parasite infections in grazing whether goats. Small Ruminant Research 51, 279-283.

Mishima, M., Pavicic, V., Grüneberg, U., Nigg, E.A., Glotzer, M., 2004. Cell cycle regulation of central spindle assembly. Research Institute of Molecular Pathology, Dr. Bohrgasse 7, A-1030 Vienna, Austria.

Niezen, J.H., Charleston, W.A.G., Robertson, H.A., Shelton, D., Waghorn, G.C., Green, R., 2002. The effect of feeding sulla (*Hedysarum coronarium*) or lucerne (*Medicago sativa*) on lamb parasite burdens and development of immunity to gastrointestinal nematodes. Veterinary Parasitology 105, 229-245.

Okuda, T., Mori, K., Shiota, M., 1982. Effects of interaction of tannins and coexisting substances. III Formation and solubilization of precipitates with alkaloids. Journal of the Pharmaceutical Society of Japan 102, 854-858.

Osweiler, G.D., Carson, T.L., Buck, W.B., Van Gelder, G.A., 1985. Clinical and Diagnostic Veterinary Toxicology. Third edition. Kendall/Hunt Publishing Company, Dubuque, Iowa.

Phy, T.S., Provenza, F.D., 1998. Sheep fed grain prefer foods and solutions that attenuate acidosis. Journal of Animal Science 76, 954-960.

Provenza, F.D., Burritt, E.A., Perevolotsky, A., Silanikove, N., 2000. Self-regulation of intake of polyethylene glycol by sheep fed diets varying in tannin concentrations. Journal of Animal Science 78, 1206-1212.

Provenza, F.D. 2003. Twenty-five years of paradox in plant-herbivore interactions and “sustainable” grazing management. Rangelands 25,4-15.

Reed, J.D. 1995. Nutritional toxicology of tannins and related polyphenols in forage legumes. Journal of Animal Science 73, 1516-1528.

Rosenthal, G.A., Janzen, D.H., (Eds.), 1979. Herbivores: Their Interaction with Secondary Plant Metabolites. Academic Press, New York, NY.

Rosenthal, G.A., Berenbaum, M.R., (Eds.), 1992. Herbivores: Their Interactions with Secondary Plant Metabolites. Second Ed. Academic Press, New York.

Silanikove, N., Nitsan, Z., Perevolotsky, A. 1994. Effect of a daily supplementation of polyethylene glycol on intake and digestion of tannin-containing leaves (*Ceratonia siliqua*) by sheep. Journal of Agricultural Food Chemistry 42, 2844-2847.

Silanikove, N., Gilboa, N., Nir, I., Perevolotsky, A., Nitsan, Z., 1996. Effect of a daily supplementation of polyethylene glycol on intake and digestion of tannin-containing leaves (*Quercus calliprinos, Pistacia lentiscus, Ceratonia siliqua*) by sheep. Journal of Agricultural Food Chemistry 44, 199-205.

Tedeschi, L.O., Fox, D.G., Tylutki, T.P., 2003.Potential Environmental Benefits of Ionophores in Ruminant Diets Animal Science Department, Cornell University, Ithaca, New York.

Tilman, D., 1982. Resource Competition and Community Structure. Princeton University Press, Princeton, New Jersey.

Villalba, J.J., Provenza, F.D., 2001. Preference for polyethylene glycol by sheep fed a quebracho tannin diet. Journal of Animal Science 79, 2066-2074.

Villalba, J.J., Provenza, F.D., Bryant, J.P., 2002. Consequences of the interaction between nutrients and plant secondary metabolites on herbivore selectivity: benefits or detriments for plants? Oikos 97:282-292.

Villalba, J.J., Provenza, F.D., Han, G., 2004. Experience influences diet mixing by herbivores: Implications for plant biochemical diversity. Oikos 107, 100-109.

Villalba, J.J., Provenza, F.D., Hall, J.O., Peterson, C., 2005. Phosphorus appetite in sheep: Dissociating taste from postingestive effects. Journal of Animal Science 84, 2213-2223.

Villalba, J.J., Provenza, F.D., Shaw, R., 2006. Sheep self-medicate when challenged with illness-inducing foods. Animal Behavior 71, 1131-1139.

Von Schonfeld, J.V., Hector, M.P., Evans, D.F., Wingate, D., 1997. Oesophageal acid and salivary secretion: is chewing gum a treatment option for gastro-oesophageal reflux? Digestion 58,111–114

Waghorn, G.C., 1990. Beneficial effects of low concentrations of condensed tannins in forages fed to ruminants. In: Akin, D.E., Ljungdahl, L.G., Wilson, J.R., Harris, P.J., (Eds.), Microbial and Plant Opportunities to Improve Lignocellulose Utilization by Ruminants. Elsevier Scientific Publishing, New York NY. p 137.

Wang, J., Provenza, F.D., 1997. Dynamics of preference by sheep offered foods varying in flavors, nutrients, and a toxin. Journal Chemical Ecology 23, 275.

CHAPTER 4

CONCLUSIONS

In the first study, I determined that sheep were able to identify and reduce parasite loads through self medication when condensed tannins were available. I found significant evidence of self medication in the test group where the behavior of the test lambs varied from that in the control groups. When presented with a tannin supplement the lambs with parasites consumed more supplement than the non-parasitized lambs. Conversely, the lambs with parasites consumed less of the non-tannin supplement than the parasite-free control lambs. Tannins adversely affect parasites suppressing and in some cases even killing the parasites, thus it is clearly beneficial for the lambs with a parasitic burden to consume tannin (Niezen et al., 2002; Coop and Kyriakis, 2001; Min and Hart, 2003; Min et al., 2004). As tannin intake increased, parasite burden (Chapter 2, Figures 2-4). Tannins also have value as a nutritional aid thereby supporting our finding that the parasite-free lambs increased tannin intake over time.

In the second study, I found that lambs modified their foraging behavior as a function of intraruminal infusions of tannin. Preference increased for forage varieties with high concentrations of saponins and alkaloids, likely because tannins form insoluble complexes with these PSM. In addition, a reduction in preference for tannin-containing birdsfoot trefoil was observed by the end of the period of infusions, which suggest animals avoided high-tannin foods after tannin infusions.

Plant secondary metabolites have the potential to provide beneficial effects to both plants (e.g., persistence, pollination) and herbivores (e.g., nutrition and health) (Engel 2002). Thus, enhancing knowledge of the ability of animals to use PSM could be useful for animal production systems – on organic land in particular – that seek to minimize the negative effects of secondary compounds in diverse pastures. There were differences in foraging behavior as the study periods progressed where utilization of beneficial forages increased as did the avoidance of detrimental ones (Figures 2.1-2.2, 2.3-2.4).

Differences among the various treatments developed through experience over time indicating a learned aspect to self-medication when ingesting of foods with PSMs. This suggests that self medication is both physiological and behavioral in nature and can be learned by the animal providing that appropriate exposure to the medication is provided in concert with discernable differences in dietary characteristics (flavor, texture, etc.).

MANAGEMENT IMPLICATIONS

Domestic animals are disadvantaged whenever they are confined or removed from natural environments. As stewards it is our responsibility to manage opportunities for diet selection and self-medication including parasite control. Studies like this help us understand how to manage animals more effectively at lower cost by allowing the animals to use naturally occurring secondary compounds to their advantage. It is clear that the necessary physiology exists within the domestic genotypes and that with adequate exposure to the right types of plants the learning process can be enhanced.

Using PSM such as tannins to control parasites and enhance intake of high-alkaloid plants such as endophyte-infected tall fescue and high-saponin alfalfa that can neutralize the negative effects of tannin could be very effective at maintaining animal health while controlling cost. There is also an advantage in planting forages with natural PSM levels because they can survive under stressful extremes including drought and heavy grazing which could help producers meet economic objectives.

Another important area that would be enhanced by taking these more natural management approaches would be in environmentally sensitive areas where secondary contamination from chemical anthelmintics are damaging natural systems. Many of the chemicals used to control internal nematodes also affect soil microbes in a negative manner, it is also noted that there is a correlation between anthelmintic usage and the decline of the dung beetle (Colwell et. al. 2002).

Studies like this aid in discovering which plants complement one another and provide producers with the added option of planting mixtures on pastures to benefit their operations. These findings can also be used by public land managers who must find alternative methods of eliminating or utilizing invasive plant species that more than likely contain high amounts of secondary compounds.

While these studies are not an answer to every management situation, and may not benefit all producers, they make important findings on a topic that needs to be studied more. Additional research is needed to discover other possible PSC combinations, and to detect the negative impacts that might come about from planting high PSC mixtures on pastures. It would also be helpful to develop better techniques of exposing animals to the different forages that will allow them to discriminate more clearly thereby making dietary associations more effective. These findings will hopefully stimulate further research on possible benefits of PSC and their influence on animal grazing behavior.

REFERENCES

Colwell, D.D., Fox, A.S., Floate, K.D., 2002. Reductions of non-pests insects in dung of cattle treated with endectocides: a comparison of four products. Bulletin of Entomology Research 92, 471-481.

Coop, R.L., Kyriazakis, I., 2001. Influence of host nutrition on the development and consequences of nematode parasitism in ruminants. Trends in Parasitology 17, 325-330.

Engel, C., 2002. Wild Health. Houghton Mifflin Co., Boston, New York, NY.

Min, B.R., Hart, S.P., 2003. Tannins for suppression of internal parasites. Journal of Animal Science 81, E102-E109.

Min, B.R., Pomroy, W.E., Hart, S.P., Sahlu, T., 2004. The effect of short-term consumption of a forage containing condensed tannins on gastro-intestinal nematode parasite infections in grazing wether goats. Small Ruminant Research 51, 279-283.

Niezen, J.H., Charleston, W.A.G., Robertson, H.A., Shelton, D., Waghorn, G.C., Green, R., 2002. The effect of feeding sulla (*Hedysarum coronarium*) or lucerne (*Medicago sativa*) on lamb parasite burdens and development of immunity to gastrointestinal nematodes. Veterinary Parasitology 105, 229-245.

APPENDICES

Appendix A. Bibliography

Altman, J., 1974. Observational study of behavior: sampling methods. Behavior 49, 227.

Asay, K.H., Jensen, K.B., Waldron, B.L., 2001. Responses of tall fescue cultivars to an irrigation gradient. Crop Science 41, 350.

Athanasiadou, S., Kyriazakis, I., Jackson, F., Coop, R.L., 2000. Effects of short-term exposure to condensed tannins on adult *Trichostrongylus colubriformis*. Veterinary Record, 146,728-732.

Athanasiadou S., Kyriazaki, I., Jackson, F, Coop, R.L., 2001. The Effects of condensed tannins supplementation of foods with different protein content on parasitism, food intake and performance of sheep infected with *Trichostrongylus colubriformis.* British Journal of Nutrition 86, 697-706.

Barry, T.N., McNeill, D.M., McNabb, W.C., 2001. Plant secondary compounds: their impact on nutritive value and upon animal production. In: Proc. XIX Int. Grass. Conference, Sao Paulo, Brazil, pp. 445-452.

Burritt, E.A., Provenza F.D., 2000. Role of toxins in intake of varied diets by sheep. Journal Chemical Ecology 26, 1991-2005.

Burrows, G.E., Tyrl R.J., 2001. Toxic Plants of North America, Iowa State Press, Ames Iowa.

Butter, N.L., Dawson, J.M., Wakelin, D., Buttery, P.J., 2000. Effect of dietary tannin and protein concentration on nematode infection (*Trichostrongylus colubriformis*) in lambs. Journal of Agriculture Science 134, 89-99.

Chance, P., 1988. Learning and Behavior, Wadsworth Publishing Company.

Cheeke, P.R., 1998. Natural Toxicants in Feeds, Forages, and Poisonous Plants. Interstate Publishing Incorporated, Danville, IL.

Cheeke, P., Shull, L.R., 1985. Natural Toxicants in Feeds and Poisonous Plants. Avi Publishing, Westport, Connecticut.

Clayton, D.H., Wolfe, N.D., 1993. Trends in Ecology & Evolution, - csa.com The Adaptive Significance of Self-Medication 8, 22, 60-63.

Colwell, D.D., Fox, A.S., Floate, K.D., 2002. Reductions of non-pests insects in dung of cattle treated with endectocides: a comparison of four products. Bulletin of Entomology Research 92, 471-481.

Coop, R.L., Kyriazakis, I., 2001. Influence of host nutrition on the development and consequences of nematode parasitism in ruminants. Trends in Parasitolology 17, 325-330.

Engel, C., 2002. Wild Health. Houghton Mifflin Company, Boston, New York NY.

Foley, W.J., Iason, G.R., McArthur, C., 1999. Role of plant secondary metabolites in the nutritional ecology of mammalian herbivores: how far have we come in 25 years? In: Jung, H.G. and G.C. Fahey, Jr. (eds.), Nutritional ecology of herbivores. Proceedings of the Fifth International Symposium on Nutritional Herbology American Society of Animal Science., IL, pp. 130-209.

Freeland, W.J., Calcott, P.H., Anderson, L.R., 1985. Tannins and saponin: interaction in herbivore diets. Biochemical System Ecology 13,189-193.

Freeland, W.J., Janzen D.H., 1974. Strategies in herbivory by mammals: the role of plant secondary compounds. American Nature 108, 269-286.

Green, R., 2002. The effect of feeding sulla (*Hedysarum coronarium*) or lucerne (*Medicago sativa*) on lamb parasite burdens and development of immunity to gastrointestinal nematodes. Veterinary Parasitology 105, 229-245.

Hendrix, C.M., Robinson, E., 2006. Diagnostic Parasitology for Veterinary Technicians, pp. 236-237. Mosby Elsevier Saint Louis, MO.

Houston, D.C., Gilardi, J.D., Hall, A.J., 2001. Soil consumption by elephants might help to minimize the toxic effects of plant secondary compounds in forest browse. Mammal Review, 31, 249-254.

Huffman, M.A., 2003. Animal self-medication and ethno-medicine: exploration and exploitation of the medicinal properties of plants. Proceedings of the Nutritional Society 62, 371-381.

Hutchings, M.R., Athanasiadou, S., Kyriazakis, I., Gordon, I.J., 2003. Can animals use foraging behaviour to combat parasites? Proceedings of the Nutritional Society 62, 361-370.

Jackson, F., Miller, J., 2006. Alternative approaches to control – Quo vadit? Veterinary Parasitolology 139, 371-384.

Janzen, J., 1978. Complications in interpreting the chemical defenses of trees against tropical arboreal plant-eating vertebrates. In: G. Montgomery (Ed.), The Ecology of Arboreal Folivores. Smithsonian Institution Press, Washington, D.C., pp. 73-84.

Jones, W.T., Mangan, J.L., 1977. Complexes of condensed tannins of sainfoin (*Onobrychis viciifolia Scop.*) with fraction-1 leaf protein and with submaxillary mucoprotein, and their reversal by polyethylene-glycol and pH. Journal of Science Food and Agriculture 28,126-136.

Lozano, G.A., 1998. Parasitic stress and self-medication in wild animals. Advances in the Study of Behavior 27:291-317 Elsevier Science, London, U.K.

Lyman, T., Provenza, F.D., Villalba, J.J., 2007. Sheep foraging behavior in response to interactions among alkaloids, tannins and saponins: Implications for chemical and taxonomic diversity in pasture ecosystems. Journal of the Science of Food and Agriculture, (In Press).

Makkar, H.P.S, Dawra, R.K, Singh, B., 1987. Protein precipitation assay for quantification of tannins – Determination of protein in tannin protein complex. Analytical Biochemistry 66, 435-439.

Malinow, M.R., McLaughlin, P., Stafford, C., Livingston, A.L., Kohler, G.O., Cheeke, P.R., 1979. Comparative effects of alfalfa saponins and alfalfa fiber on cholesterol absorption in rats. American Journal of Chemical Nutrition 32, 1810-1812.

Min, B.R., Hart, S.P., 2003. Tannins for suppression of internal parasites. Journal of Animal Science 81, E102-E109.

Min, B.R., Pomroy, W.E., Hart, S.P., Sahlu, T., 2004. The effect of short-term consumption of a forage containing condensed tannins on gastro-intestinal nematode parasite infections in grazing whether goats. Small Ruminant Research 51, 279-283.

Min, R., Pinchak, W.E., Anderson, R.C., Fulford, J.D., Puchala, R., 2006. Effects of condensed tannins supplementation level on weight gain and in vitro and in vivo bloat precursors in steers grazing winter wheat. Journal of Animal Science 84, 2546-2554.

Mishima, M., Pavicic, V., Grüneberg, U., Nigg, E.A., Glotzer, M., 2004. Cell cycle regulation of central spindle assembly. Research Institute of Molecular Pathology, Dr. Bohrgasse 7, A-1030 Vienna, Austria.

Mote, T., Villalba, J.J., Provenza, F.D., 2008. Sequence of food presentation influences intake of foods containing tannins and terpenes. Applied Animal Behavior in Science (In Press).

Niezen, J.H., Waghorn, T.S., Charleston, W.A.G., Waghorn, G.C., 1995. Growth and gastrointestinal nematode parasitism in lambs grazing either lucerne (*Medicago sativa*) or sulla (*Hedysarum coronarium*) which contains condensed tannins. Journal of Agricultural Science Cambridge 125, 281-289.

Niezen, J.H., Charleston, W.A.G., Robertson, H.A., Shelton, D., Waghorn, G.C., Green, R., 2002. The effect of feeding sulla (*Hedysarum coronarium*) or lucerne (*Medicago sativa*) on lamb parasite burdens and development of immunity to gastrointestinal nematodes. Veterinary Parasitology 105, 229-245.

Okuda, T., Mori, K., Shiota, M., 1982. Effects of interaction of tannins and coexisting substances. III Formation and solubilization of precipitates with alkaloids. Journal of the Pharmaceutical Society of Japan 102, 854-858.

Osweiler, G.D., Carson, T.L., Buck, W.B., Van Gelder, G.A., 1985. Clinical and Diagnostic Veterinary Toxicology. Third edition. Kendall/Hunt Publishing Company, Dubuque, Iowa, pp. 494.

Phy, T.S., Provenza, F.D., 1998. Sheep fed grain prefer foods and solutions that attenuate acidosis. Journal of Animal Science 76, 954-960.

Plotkin, M.J., 2000. Medicine Quest. In Search of Nature's Healing Secrets. Penguin Putnam Inc. New York, NY.

Pomeroy, S.L., Tamayo, P., Gaasenbeek, M., Sturla, L.M., Angelo, M., McLaughlin, M.E., Kim, J.Y., Goumnerova, L.C., Black, P.M., Lau, C., Allen, J.C., Zagzag, D., Olson, J.M, Curran, T., Wetmore, C., Biegel, J.A., Poggio, T., Mukherjee, S., Rifkin, R., Califano, A., Stolovitzky, G., Louis, D.N., Mesirov, J.P., Lander, E.S., Golub, T.R., 2002. Prediction of central nervous system embryonal tumour outcome based on gene expression. Division of Neuroscience, Department of Neurology, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts.

Provenza, F.D., Burritt, E.A., Perevolotsky, A., Silanikove, N., 2000. Self-regulation of intake of polyethylene glycol by sheep fed diets varying in tannin concentrations. Journal of Animal Science 78, 1206-1212.

Provenza, F.D., 2003. Twenty-five years of paradox in plant-herbivore interactions and "sustainable" grazing management. Rangelands 25, 4-15.

Provenza, F. D., 2008. What does it mean to be locally adapted and who cares anyway? Journal of Animal Science 86, E271–E284.

Reed, J.D. 1995. Nutritional toxicology of tannins and related polyphenols in forage legumes. Journal of Animal Science 73, 1516-1528.

Rosenthal, G.A., Janzen, D.H., (Eds.), 1979. Herbivores: Their Interaction with Secondary Plant Metabolites. Academic Press, New York, NY.

Rosenthal, G.A., Berenbaum, M.R., (Eds.), 1992. Herbivores: Their Interactions with Secondary Plant Metabolites. Second Ed. Academic Press, New York NY.

Silanikove, N., Nitsan, Z., Perevolotsky, A. 1994. Effect of a daily supplementation of polyethylene glycol on intake and digestion of tannin-containing leaves (*Ceratonia siliqua*) by sheep. Journal of Agricultural Food Chemistry 42, 2844-2847.

Silanikove, N., Gilboa, N., Nir, I., Perevolotsky, A., Nitsan, Z., 1996. Effect of a daily supplementation of polyethylene glycol on intake and digestion of tannin-containing leaves (*Quercus calliprinos, Pistacia lentiscus, Ceratonia siliqua*) by sheep. Journal of Agricultural Food Chemistry 44, 199-205.

Shaw, R. 2006. Sheep self-medicate when challenged with illness-inducing foods. Animal Behavior 71, 1131-1139.

Tedeschi, L.O., Fox, D.G., Tylutki ,T.P., 2003.Potential Environmental Benefits of Ionophores in Ruminant Diets Animal Science Department, Cornell University, Ithaca, New York NY.

Tilman, D., 1982. Resource Competition and Community Structure. Princeton University Press, Princeton, NJ.

Villalba, J. J., Provenza, F. D., 2001. Preference for polyethylene glycol by sheep fed a quebracho tannin diet. Journal of Animal Science 79, 2066-2074.

Villalba, J.J., Provenza, F.D., Bryant J.P., 2002. Consequences of the interaction between nutrients and plant secondary metabolites on herbivore selectivity: benefits or detriments for plants? Oikos 97, 282-292.

Villalba, J.J., Provenza, F.D., Han, G., 2004. Experience influences diet mixing by herbivores: implications for plant biochemical diversity. Oikos 107, 100-109.

Villalba, J.J., Provenza, F.D., Hall, J.O., Peterson, C.**,** 2005. Phosphorus appetite in sheep: Dissociating taste from postingestive effects. Journal of Animal Science 84, 2213-2223.

Villalba, J.J., Provenza, F.D., Shaw, R., 2006. Sheep self-medicate when challenged with illness-inducing foods. Animal Behavior 71, 1131-1139.

Von Schonfeld J.V., Hector M.P., Evans D.F., Wingate D., 1997. Oesophageal acid and salivary secretion: is chewing gum a treatment option for gastro-oesophageal reflux? Digestion 58, 111–114.

Waghorn, G.C., 1990. Beneficial effects of low concentrations of condensed tannins in forages fed to ruminants. in: Akin, D.E., Ljungdahl, L.G., Wilson, J.R., Harris, P.J., (Eds.) Microbial and Plant Opportunities to Improve Lignocellulose Utilization by Ruminants. Elsevier Science Publications, New York, p. 137.

Waller, P.J., 2006. Sustainable nematode parasite control strategies for ruminant livestock by grazing management and biological control. Animal Feed Science Technology 126, 277-289.

Wang, J., Provenza F.D., 1997. Dynamics of preference by sheep offered foods varying in flavors, nutrients, and a toxin. Journal Chemical Ecology 23, 275.

Wrangham, R.W., Goodall, J., 1989. Chimpanzee use of medicinal leaves. In: Understanding Chimpanzees .Harvard University Press, Cambridge, Massachusetts pp. 22-37.