LIVESTOCK FORAGING BEHAVIOR IN RESPONSE TO INTERACTIONS AMONG

ALKALOIDS, TANNINS, AND SAPONINS

by

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ABSTRACT

Livestock Foraging Behavior in Response to Interactions Among
Alkaloids, Tannins and Saponins

by

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Utah State University, 2007

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Plant secondary compounds abound in every plant mother nature has to offer. From common garden vegetables to poisonous plants, there are secondary compounds in every plant any animal, as well as we, chooses to eat. In the past, secondary compounds were mostly considered waste products of plant metabolism, but over the last several decades research has shown that these compounds play an active role in plant and animal behavior, health, and productivity. Though often seen only in terms of their negative impacts on intake and production, we are becoming increasingly aware of their beneficial roles in plant, animal, and human health. Providing herbivores with a diversity of plants to make up their diet allows them to regulate and mix foods so as to better utilize primary and secondary compounds, as well as enhancing economic and ecological performance.

The secondary compound gramine is an alkaloid found in reed canarygrass that is proteinaceous in nature. Endophyte-infected tall fescue contains the alkaloids perlolidine, perloline and ergotamine, which are all steroidal in nature. Tannins have a high affinity for binding proteins, and saponins are non-polar steroidal compounds with a high affinity for binding to lipids in the gastro-intestinal tract of animals. These findings suggest that animals ingesting foods with alkaloids may increase their preference for tannin or saponin-containing

foods to reduce the negative impacts of these secondary compounds. Moreover, tannins and saponins hasten alkaloid excretion from the body, which might also allow animals to eat more high-alkaloid forages when presented with tannins first.

I tested the hypothesis that cattle and sheep foraging behavior is influenced by eating different combinations and sequences of forages containing secondary compounds. In pen and pasture trials, I showed that 1) sheep fed basal diets high in alkaloids (gramine or ergotamine) ate more when supplemented with food containing either tannins or saponins ; 2) cattle that ate a 30-minute meal of tall fescue(alkaloid) subsequently preferred birdsfoot trefoil(tannin) to alfalfa (saponin), while cattle that first ate reed canarygrass(alkaloid) subsequently preferred alfalfa(saponin) to birdsfoot trefoil (tannin) ; and 3) cattle spent more time grazing tall fescue and reed canarygrass when they first ate birdsfoot trefoil (high in tannins) and alfalfa (high in saponins), respectively, than when they ate these forages in the reverse sequence.

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Tiffanny D. Lyman

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CHAPTER 1

INTRODUCTION

During the past several decades, people worldwide have expressed a growing interest in reconstructing ecosystems to enhance ecological, economic, and social values. Yet, to do so, we must find ways to enhance biodiversity, environmental quality and the sustainability of grazing lands. In Utah, most agricultural receipts are from livestock production on pastures and rangelands, and thus, sustaining grazing practices is particularly important for this region.

In all these instances, plants are the glue that binds soils, water, herbivores, and people. However, monocultures or simple grass-legume mixtures are not always ideal for intensively managed pastures, because of their seasonality, susceptibility to pests, and monotony of nutrient profiles. Diverse mixtures of plants may provide benefits monocultures cannot. Mixtures can affect nutrient and water use, and thus plant production. For instance, more diverse mixtures of plants are likely to capture and use nitrates that accumulate in the root zone during the growing season, so they are not leached by winter precipitation (MacAdam, 2002). A diverse mix of species may add biochemical structure, nutrient diversification, and cover during times of the year when such resources may be absent if producers rely on monocultures.

While plants and herbivores have interacted one with another for eons, we are just beginning to appreciate the complexities and subtleties of their interactions. During the past 30 years, our understanding has evolved from a focus on plant structure and nutrients to a growing appreciation for so-called secondary plant metabolites (Rosenthal and Janzen, 1979). Prior to that time, scientists had come to appreciate the roles of primary compounds such as nitrogen, phosphorus, and potassium, but during this era they discovered that a vast array of interactions in terrestrial and aquatic ecosystems are mediated by secondary compounds formerly thought to be waste products of plant metabolism (Rosenthal and Berenbaum, 1992). They also came to better understand how environmental factors - nutrients, water, and light - influence the evolution (Coley et al., 1985) and phenotypic expression (Bryant et al., 1983) of secondary compounds, and how secondary compounds influence herbivores.

The most common emphasis of plant secondary metabolites has always been the protective role they play in plant defense, and how they influence herbivore behavior. Plants that produce compounds that can quickly induce satiety stand a better chance of surviving, and most secondary compounds limit how much of a particular species an herbivore can eat, thereby spreading the load of herbivory across many species (Freeland and Janzen, 1974, Foley et al., 1999). Where once we thought only poisonous plants -- species that for one reason or another are a problem for herbivores (Provenza et al., 1992) -- contain compounds that were potentially toxic, there is increasing awareness that all plants, including garden vegetables, contain primary and secondary compounds that limit intake (Provenza et al., 2003).

Herbivores seldom consume enough primary or secondary compounds to result in poisoning because they regulate their intake of foods (Provenza, 1995, 1996; Foley et al., 1999). For instance, oral gavages of toxins cause dose-dependent decreases in intake of foods that contain the toxins (Wang and Provenza, 1997). Because secondary compounds in particular plants limit intake below needs for nutrients such as energy and protein, herbivores must eat a variety of plant species that contain different kinds of compounds. In principle, herbivores should be able to eat more foods with different kinds of secondary compounds because they affect the body in different ways and they are detoxified by different mechanisms (Freeland and Janzen, 1974). A diverse intake of secondary compounds may also lead to neutralization or inactivation, which in turn could reduce susceptibility to toxic doses.

Interactions among plants with secondary compounds 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). 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), though such complementarities were not observed with sheep (Burritt and Provenza, 2000). When sheep choose between foods that contain either amygdaline or lithium chloride, they eat more than lambs offered a food that contains only one of these toxins; the same is true with nitrate and oxalate (Burritt and Provenza, 2000). Sheep also eat more when offered three foods that contain terpenes, tannins, and oxalates than when offered foods with only one or two of these toxins (Villalba et al., 2004). Mule deer eat more when offered both sagebrush and juniper (12.3 g/kg BW), plants that contain different terpenes, than when they are offered only sagebrush (4.2 g/kg BW) or juniper (7.8 g/kg BW) (Smith, 1959). Brushtail possums that can select from two diets containing phenolics and terpenes eat more food than when they consume diets containing only one of these toxins (Dearing and Cork, 1999), and the same is true in principle with squirrels (Schmidt et al., 1998). Conversely, sheep offered foods containing either sparteine or saponin eat no more of both foods than lambs offered foods containing only one of these compounds because these toxins are not complementary.

It is clear that animals can meet their needs for nutrients and tolerate higher total intake of secondary compounds when they can choose from a variety of plants (Provenza and Villalba, 2005). Yet, outside of the aforementioned studies, very little has been learned about which plants high in secondary compounds are complementary and which are not, and up to this point we have had no information about SC complementarities in mixtures of plant species on pastures.

My objectives were to test the hypothesis that cattle and sheep foraging behavior is influenced by eating different combinations and sequences of forages containing secondary compounds. More specifically I wanted to determine 1) if sheep fed basal diets high in alkaloids (gramine or ergotamine) ate more when supplemented with food containing either tannins or saponins ; 2) if cattle that ate a 30-minute meal of tall fescue(alkaloid) subsequently preferred birdsfoot trefoil(tannin) to alfalfa (saponin), while cattle that first ate reed canarygrass (alkaloid) subsequently preferred alfalfa (saponin) to birdsfoot trefoil (tannin) ; and 3) if cattle spent more time grazing tall fescue and reed canarygrass when they first ate birdsfoot trefoil (high in tannins) and alfalfa (high in saponins), respectively, than when eating these forages in the reverse sequence.

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CHAPTER 2

SHEEP FORAGING BEHAVIOR IN RESPONSE TO INTERACTIONS AMONG ALKALOIDS, TANNINS, AND SAPONINS[1]

Abstract

BACKGROUND: A mixture of plant species adds biochemical diversity to pastures that may enhance productivity while decreasing reliance on herbicides and insecticides. All plants contain secondary metabolites (PSM) that interact in plant communities in a variety of ways. Our objective was to determine if PSM diversity enhanced food intake when sheep were allowed to select from foods that varied in concentrations of tannins, saponins, and alkaloids. We hypothesized that intake of foods containing alkaloids would increase when sheep were offered supplemental foods with tannins and saponins. The alkaloid gramine is proteinaceous in nature, tannins bind to proteins in the gut, which enhances excretion of proteins, so we hypothesized tannins would bind to gramine and enhance its excretion from the body. The alkaloid ergotamine is steroidal, saponins have a high affinity for binding to lipid and steroidal compounds in the gut and then being excreted, so we hypothesized saponins would bind to ergotamine and enhance its excretion from the body.

RESULTS: We found that sheep fed either gramine or ergotamine in combination with tannin- or saponin-containing foods had higher intakes of food than sheep offered only foods with gramine or ergotamine.

CONCLUSION: These findings are consistent with the hypothesis that animals can neutralize the negative effects of alkaloids by ingesting tannins and saponins. Our results show PSM can complement one another to increase herbivore's intake of unpalatable plant species, with the

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[1] Chapter 2 Coauthored by Tiffanny Lyman, Fredrick D. Provenza, and Juan J. Villalba. J. Sci. Food Agric. Vol 88: 824. 2008.

potential to maintain healthy animals and bio-diverse landscapes.

INTRODUCTION

Much work of the past 30 years emphasized the protective roles of plant secondary metabolites as plant defenses in interactions with herbivores. Most PSM limit how much of a particular species an herbivore can eat, in essence spreading the load of herbivory across many plant species .[1,2] Herbivores seldom consume enough PSM to result in poisoning because they regulate their intake of foods that contain PSM. [2-4] Because PSM in particular plants limit intake below needs for nutrients such as energy and protein, herbivores must eat a variety of plant species that contain different kinds of PSM to meet these needs.[1,2] In principle, herbivores should be able to eat more foods with diverse PSM because different PSM affect the body in different ways and they are detoxified by different mechanisms.[1,2] Ingesting a variety of phytochemicals also may lead to PSM-PSM neutralization or inactivation, which in turn could reduce susceptibility to PSM.[5]

In their efforts to describe the defensive roles of secondary compounds in plants, researchers have not considered the possible health benefits of these compounds in appropriate dosages.[6] Everything depends on the dose: Nutrients and toxins at too high doses can be toxic, while at lower doses they can both have health benefits.[7] Likewise, in our haste to increase the palatability of pasture species, researchers have selected for low concentrations of compounds such as alkaloids (e.g., reed canary grass and endophyte-infected tall fescue), tannins (e.g., birdsfoot trefoil), and saponins (e.g., alfalfa), not appreciating that these compounds in proper mixtures may actually benefit animals and plants by increasing animal intake of plants that assist in ecosystem sustainability, and increasing livestock production by offering a variety of foods.[6,8-10] Interactions among PSM in foods can lead to complementary relationships such that eating a mixture of foods may exceed the benefit of consuming any one food in isolation.[1, 11] 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,[5] though such complementarities were not observed in a study with sheep.[12] Studies with sheep (O*vis aries*),[12,13] mule deer (*Odocoileus hemionus*),[14] brushtail possums (*Trichosurus vulpecula*),[15] and squirrels (*Sciurus niger and S. carolinensis*) [16] also show that eating variety of PSM-containing foods enhances food intake relative to single PSM-containing foods.

While research suggests animals can meet their needs for nutrients and tolerate higher total intake of PSM when they can choose from a variety of plants,[7] outside of the aforementioned studies, we know very little about which PSM are complementary and which are not and we have no information about such complementarities in mixtures of plant species that can be planted on pastures. Thus, we sought to understand how plant diversity might enhance food intake when sheep were allowed to select from foods with tannins, saponins, and alkaloids. Our objectives were to determine if sheep offered foods containing alkaloids (gramine or ergotamine D tartrate) would eat more food with alkaloids when offered other foods containing saponins or tannins, through PSM-PSM inactivation or neutralization, compared with sheep offered only foods with alkaloids.

The alkaloid gramine, found in Reed Canarygrass (*Phalaris arundinacea*), is metabolized from the amino acid tryptophan, and is proteinaceous in nature. Tannins have a high affinity for binding proteins,[17] and they also have an affinity for binding alkaloids in vitro.[18] On this basis, we hypothesized animals ingesting food with gramine would increase their intake and preference for food with tannins.

Endophyte-infected tall fescue (*Lolium arundinaceum*) contains the alkaloids perlolidine, perloline and ergotamine, which are all steroidal in nature. Saponins in plants such as alfalfa (*Medicago sativa*) are non-polar steroidal compounds with detergent properties and a high affinity for binding to lipids and sterols in the gastro-intestinal tract, causing their excretion in the feces.[8] Because the alkaloids in tall fescue are steroidal, similar to some lipids in nature, we hypothesized animals eating food containing ergotamine would increase their intake and preference for food containing saponins.

MATERIALS AND METHODS

Sheep used in this study were 6 month old, commercial Rambouillet-Columbia-Finn-Targhee crossbred lambs, with no previous experience eating secondary compounds. Lambs averaged 40 Kg, and all were acquired from the same farm. Lambs were individually penned at the Green Canyon Ecology Center, Utah State University, Logan, with free access to trace mineralized salt blocks and fresh water. The lambs were kept outdoors, under a protective roof in individual, adjacent pens measuring 2.4 x 3.6 m. All procedures were approved by the Utah State University Institutional Animal Care and Use Committee (IACUC #1270).

Experimental Design. Three groups of lambs (n = 8/group) received a basal diet containing alkaloids in 2 Experiments. Each experiment involved 6 periods and each period lasted 5 days, except for Period 6 when animals were offered a choice of test foods on 2 consecutive days. The general protocol for both experiments involved offering each lamb 1000g of the basal diet for an hour, with or without the addition of alkaloids during different periods, followed by the test foods with or without the addition of potential complementary PSM (tannin, saponin or alkaloid) for 30 minutes.

In Experiment 1 (Table 1), we fed the basal diet and test foods without PSM during Periods 1, 3 and 5. During Period 2 we provided the basal diet with gramine (G) to all groups for 1 h, after which test foods containing tannins (T), saponins (S), or more gramine were presented to Groups 1 (tannin; G→T), 2 (saponins; G→S) and 3 (gramine; G→G) for 30 minutes. During

Period 4, sheep were first fed gramine and then fed either saponins in Group 1 (G→S) or tannins in Group 2 (G→T); Group 3 continued to receive gramine (G→G). During Period 6, all lambs were given a choice between tannin- and saponin-containing foods after eating a meal of food containing gramine.

In Experiment 2 (Table 1), three groups of lambs (n = 8/group), different from the animals used in Experiment 1, were provided food with the alkaloid ergotamine D tartrate, instead of gramine, using the same protocol as in Experiment 1.

Foods. Dried, ground alfalfa pellets (alfalfa was of a variety grown with little saponin content) and dried, ground beet pulp were the ingredients of the basal diet and test foods, respectively. During Periods 2, 4, and 6 the basal diet of ground alfalfa was mixed with the alkaloid 1) gramine (0.2% Experiment 1) or 2) ergotamine D tartrate (EDT) (30ppm, Experiment 2) and 2% onion powder to act as a flavor cue that signaled the addition of alkaloids to alfalfa. The test foods in periods 2, 4 and 6 were formed of beet pulp that contained tannins (10%), saponins (2%) or more gramine (30ppm). All beet pulp diets were mixed with 2% vegetable oil to reduce dust and inhalation of secondary compounds, particularly saponins, which tended to form a fine dust when mixed with food. Ergotamine D-tartrate (syn: Ergotamini tartras), Gramine [syn: 3-Dimethylaminomethyl)indole] and Saponin (purum) were obtained from Sigma-Aldrich or Spectrum chemical supply companies. The tannin-containing diet was formulated using quebracho tannin, a source of condensed tannin extracted from the South-American quebracho tree (*Aspidosperma quebracho*). Quebracho tannin is a complex of tannins, flavonoids, and other phenolic compounds. The quebracho we used contains approximately 85% tannin (Titus and Provenza, unpublished results), and was obtained from Tannin Corporation, Peabody, Massachusetts, USA.

The concentrations of PSM used were chosen to correspond with naturally occurring levels

Table 1. Sequence of feeding ground alfalfa (Basal Diet) and ground beet pulp (test foods) with or without plant secondary metabolites during two experiments.

Experiment 1: Gramine

Period	Group 1	Group 2	Group 3
1	←----------------*Basal Diet and test foods without PSM* --------------------→		
2	Gramine then Tannins	Gramine then Saponins	Gramine then Gramine
3	←----------------- --*Basal Diet and test foods without PSM* -------------------------→		
4	Gramine then Saponins	Gramine then Tannins	Gramine then gramine
5	←------------------*Basal Diet and test foods without PSM* -------------------→		
6	←----------- Gramine then Choice between Tannins and Saponins ------- ------→		

Experiment 2: Ergotamine D Tartrate (EDT)

Period	Group 1	Group 2	Group 3
1	←------------------*Basal Diet and test foods without PSM* ----------------→		
2	EDT* then Tannins	EDT then Saponins	EDT then EDT only
3	←------------------*Basal Diet and test foods without PSM*------------------------→		
4	EDT then Saponins	EDT then Tannins	EDT then EDT only
5	←-------------------*Basal Diet and test foods without PSM*----------------→		
6	←---------- -- EDT then Choice between Tannins and Saponins -----------------→		

*EDT: Ergotamine D Tartrate

in pasture species. Gramine at 0.2% in Reed Canarygrass is reported to reduce weight gains in sheep, [9] while ergotamine at 30 ppm causes physiological changes similar to those observed in animals suffering from fescue toxicosis.[10] Tannin concentrations of 10% were selected to be in the range of Lotus spp[19] and saponins at 2% in herbivorous diets cause decreases in food intake by sheep. [9]

Diets containing alkaloids, tannins, and saponins were fed to sheep ad libitum; food refusals were weighed to determine if lambs preferentially ate tannin or saponin foods when fed either gramine, in Experiment 1, or ergotamine, in Experiment 2. Baseline food intakes during Periods 1, 3, and 5 were recorded for 5 days, without onion flavor in the basal diet or secondary compounds in either the basal diet or test foods, to allow PSM to be eliminated from the body.

Statistical Analyses. The statistical design for the analysis of variance was a split-plot with lambs nested within group and repeated measures on each lamb. We ran three separate analyses for each Experiment: 1) For Choice data (P6) – Lambs in all groups had a choice between tannins and saponins. The response variable was the amount of tannin- and saponin-containing food consumed by lambs and day was the repeated measure; 2) for intake data (P1-P5) -- period and day within period were the repeated measures on each lamb in all groups. The response variables were the amount consumed of basal diet with alkaloids (P2 and P4) and the amount of basal diet consumed without alkaloids (P1, P3, and P5). 3) For intake in P2 and P4 -- period and day within period were the repeated measures on each lamb in all groups. The response variable was the amount of beet pulp with tannin, saponin (Groups 1 and 2), or alkaloid (Group 3) consumed.

RESULTS

Experiment 1: Gramine Alkaloid. For Experiment 1 there was a treatment x period interaction (intake of basal diet food, $P < 0.0001$; intake of beet pulp test food, $P=0.0015$; total food intakes, $p = .06$).

Period 1. During the first baseline period, sheep in Group 3 (G→G) ate more alfalfa (786 g vs. 697 g, $P < 0.0001$) and beet pulp (385 g vs. 337 g, $P = 0.0015$) than sheep in Group 2 (G→S).

Sheep in Group 1 (G→T) did not differ from sheep in either Group 2 or 3 in their intake of alfalfa (749g) or beet pulp (347g) (Figures 1 and 2). Sheep in Group 3 had significantly higher total intakes than sheep in Group 2 (1171g vs. 1034g, P=0.06) during this period, with neither being significantly different from sheep in Group 1 (1097g) (Figure 3).

Period 2. Sheep were first fed ground alfalfa with gramine, and then provided beet pulp with tannins (Group 1), saponins (Group 2), or gramine (Group 3). Intake of alfalfa with gramine was greater for sheep in Group 1 (G→T, 722g) than for sheep in Group 2 (G→S, 557g) or Group 3 (G→G, 589g) (P < 0.0001) (Figure 1). After eating alfalfa with gramine, sheep ate more beet pulp with gramine (Group 3) than with tannins (Group 1) (352g vs. 261g, P = 0.0015). Sheep in Group 2 ate an average of 311g of saponin-containing food, which was not different from Groups 1 or 3 (Figure 2). Sheep in Group 1 (G→ T) had higher total intakes than sheep in Group 2 (G→S) (980g vs. 868g, P=0.06), and neither group differed from Group 3 (G→G) (941g) (Figure 3).

Period 3. During the second baseline, sheep in Group 3 (G→G) ate more alfalfa (701 g vs. 620 g, P < 0.0001) and beet pulp (423g vs. 363 g, P = 0.0015) than did sheep in Group 2 (G→S) (Figures 1 and 2). Intake by sheep in Group 1 (G→T) did not differ from either group. Sheep in Group 2 ate less than sheep in Group 3 (1122g vs. 983g, P=0.0015), and they had lower total intakes than sheep in Group 1 (1070g, P=0.06) (Figure 3).

Period 4. Intake of gramine-containing food was higher for sheep in Group 1 (G→S) than in Group 3 (G→G) (680 g vs. 556 g, P < 0.0001). Group 2 (G→T) differed from Groups 1 and 3 with an average intake of 624g. When offered beet pulp with PSM, sheep ate more beet pulp with gramine (Group 3) than with either tannins (Group 1) or saponins (Group 2) (372g vs. 211g and 323g, P = 0.0015) (Figure 2). Sheep in Group 1 had higher total intakes of both foods than sheep in Group 2 (1002g vs. 834g, P=0.0015), and in Group 3 (928g, P=0.06) (Figure 3).

Period 5. During the final baseline period, there were no differences (P > 0.1) in intakes of alfalfa (Figure 1). Sheep in Group 3 ate more beet pulp than sheep in Groups 1 and 2 (468g vs. 422g and 383g. P = 0.0015) (Figure 2). Sheep in Group 3 had higher total intakes than sheep in Group 2 (1179g vs. 1055g, P=0.0015) and Group 1 (1091g, P=0.06) (Figure 3).

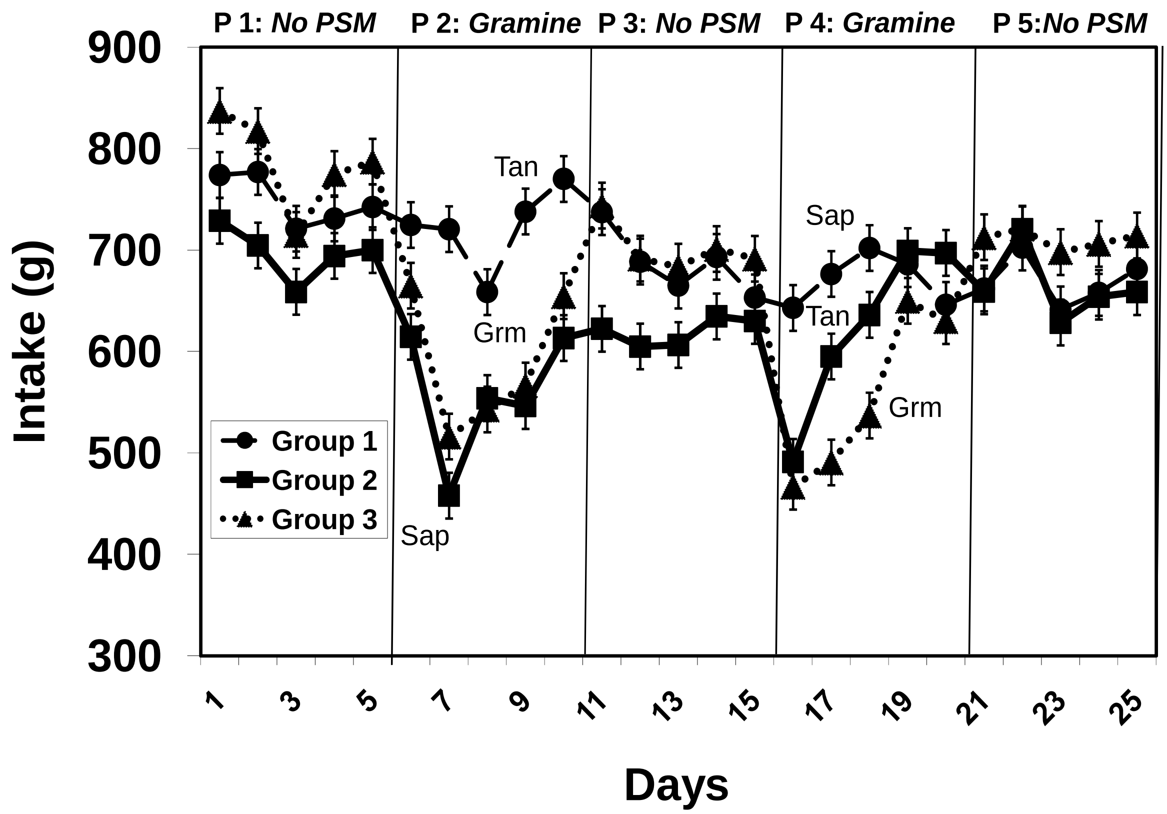

Figure 1: Intake by sheep, during Experiment 1, of alfalfa without gramine (Periods 1, 3, and 5) and alfalfa with gramine (Periods 2, and 4). During Period 2, Group 1 was supplemented with tannins, Group 2 was supplemented with saponins and the Group 3 was provided with more gramine. During Period 4, Group 1 was supplemented with saponins, Group 2 was supplemented with tannins, and Group 3 was given more gramine. During the Choice period all sheep were given a choice of tannins and saponins.

Period 6. Choice. Intake of gramine did not differ among groups for the choice period (P = 0.644). However, treatment and day interacted (P = 0.06) as sheep in Group 1 (G→T) had a higher preference for food with saponins on day 2 than on day 1 of the choice (Figure 4). Total intakes did not differ among groups during the choice period.

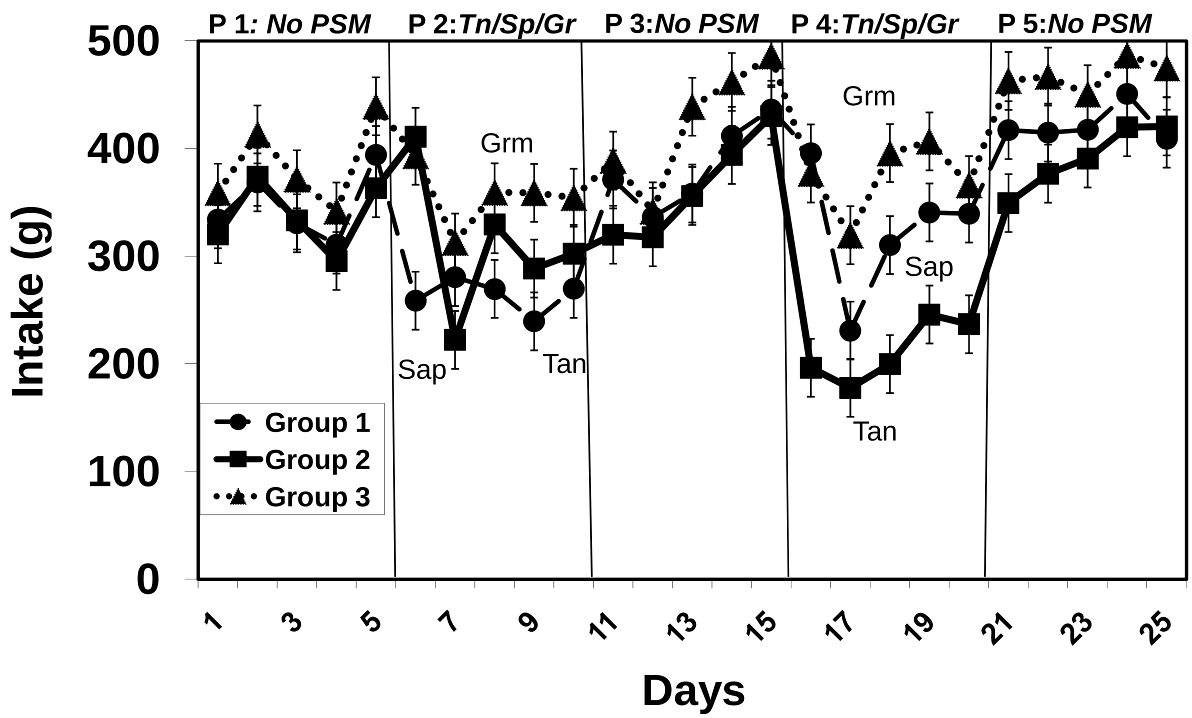

Figure 2: Intake by sheep during Experiment 1 of beet pulp without PSM (Periods 1, 3, and 5) and beet pulp with PSM (Periods 2 and 4). During Period 2, Group 1 was fed tannins, Group 2 was fed saponins, and Group 3 was fed more gramine in their beet pulp diets. During Period 4, Group 1 was then fed saponins, Group 2 was fed tannins, and Group 3 continued to be fed gramine in their beet pulp food.

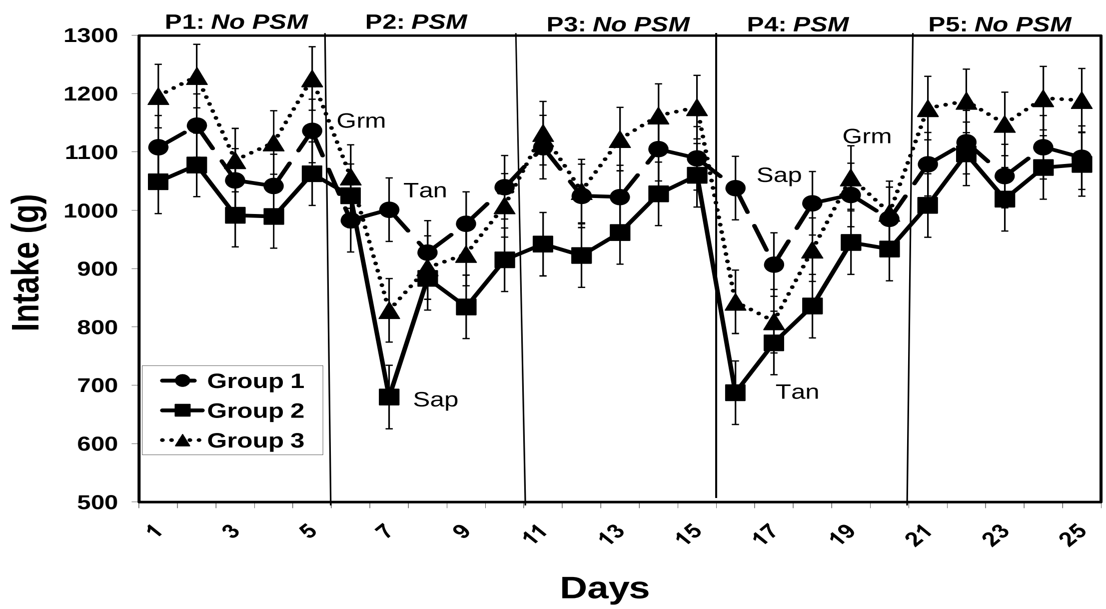

Figure 3: Total intake by sheep, during Experiment 1, of basal diet and test foods, with (Periods 2 and 4) and without PSM (Periods 1, 3 and 5).

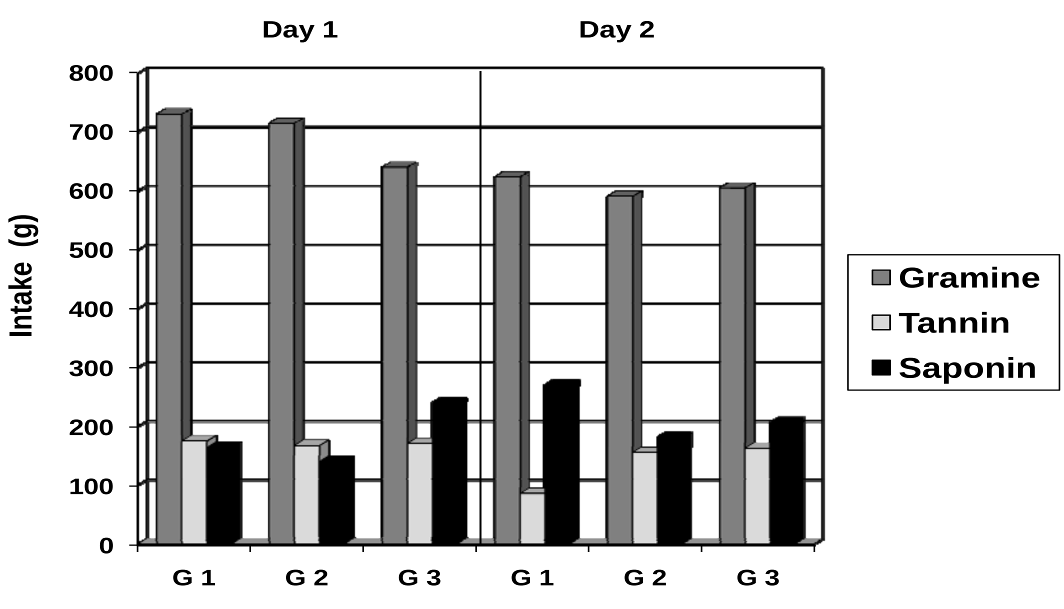

Figure 4: Intakes of alfalfa with gramine and beet pulp with tannin or saponin by sheep given a choice of tannin- or saponin-containing foods.

Experiment 2: Ergotamine Alkaloid. For Experiment 2 there was a treatment x period interaction for intake of the basal diets (P = 0.03; Figure 5) and the beet pulp test foods (P = 0.006; Figure 6). There was no treatment x period interaction for total food intakes (P = 0.33; Figure 7), though a trend in the data suggests sheep in Group 1 ate more than did sheep in Group 2, both of which generally ate more than did sheep eating only EDT (Group 3).

Period 1. Sheep in Groups 1 and 3 differed in intake of alfalfa (676g vs. 584g, P < 0.1) (Figure 5). Group 2 had an average intake of 667 g, which did not differ from Group 1, but was higher than for Group 2 (P=0.03) (Figure 7). Intake of beet pulp by all groups did not differ (P < 0.1) (Figure 6).

Period 2. Sheep in the Group 3 (G→G, 209g) ate less alfalfa with ergotamine than sheep in Groups 1 (G→T, 396g) and 2 (G→S, 393g) (P = 0.03) (Figure 5). Intake of beet pulp with PSM differed among groups 2 and 3 (344g vs. 393g, P < 0.1), but these groups did not differ from Group 1 (372 g) (Figure 6).

Period 3. During the second baseline, sheep in Group 1 (G→T, 800g) ate more alfalfa than sheep in Groups 2 (G→S, 693g) and 3 (G→G, 606g) (P = 0.03) (Figure 5). Sheep in Group 1 (G -> T) ate more beet pulp without PSM than Groups 2 and 3 (432 g vs. 344g and 346g, P < 0.1) during this period (Figure 6).

Period 4. Sheep in Group 3 (G→G, 209g) again ate less alfalfa with ergotamine than sheep in Group 1 (G→S, 383g) and Group 2 (G→T, 471g) (P = 0.03) (Figure 5). Sheep ate more beet pulp with PSM in Groups 3 (424g) and 2 (401g) than did sheep in Group 1 (314g) (P = 0.03) (Figure 6).

Period 5. During the final baseline, sheep in Group 3 (632g) ate less alfalfa than sheep in Groups 1 (753g) and 2 (772g) (P = 0.03). Sheep in Group 3 also ate less beet pulp than sheep in Group 1 (336g vs. 407g, P = 0.03) (Figures 5 and 6).

Period 6. Choice. Sheep in Groups 1, 2, and 3 all differed in their intakes of ergotamine-containing alfalfa (520g, 401g, 283g, P = 0.02) (Figure 8).

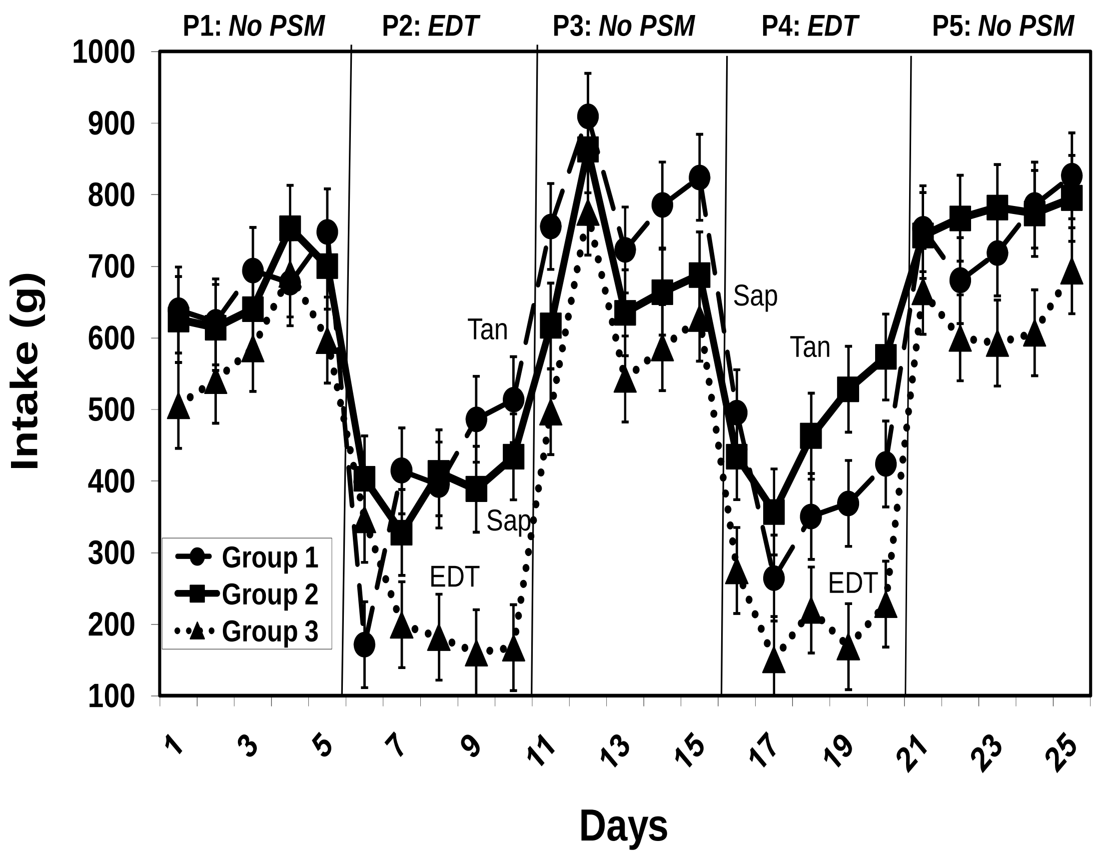

Figure 5: Intake by sheep during Experiment 2 of alfalfa without ergotamine d tartrate (Periods 1, 3, and 5) and alfalfa with ergotamine d tartrate (Periods 2, and 4). During Period 2, Group 1 was supplemented with tannins, Group 2 was supplemented with saponins, and Group 3 was supplemented with more E. During Period 4, Group 1 was supplemented with saponins, Group 2 was supplemented with tannins, and Group 3 was given more E. During the Choice period all sheep were offered a choice of tannins and saponins.

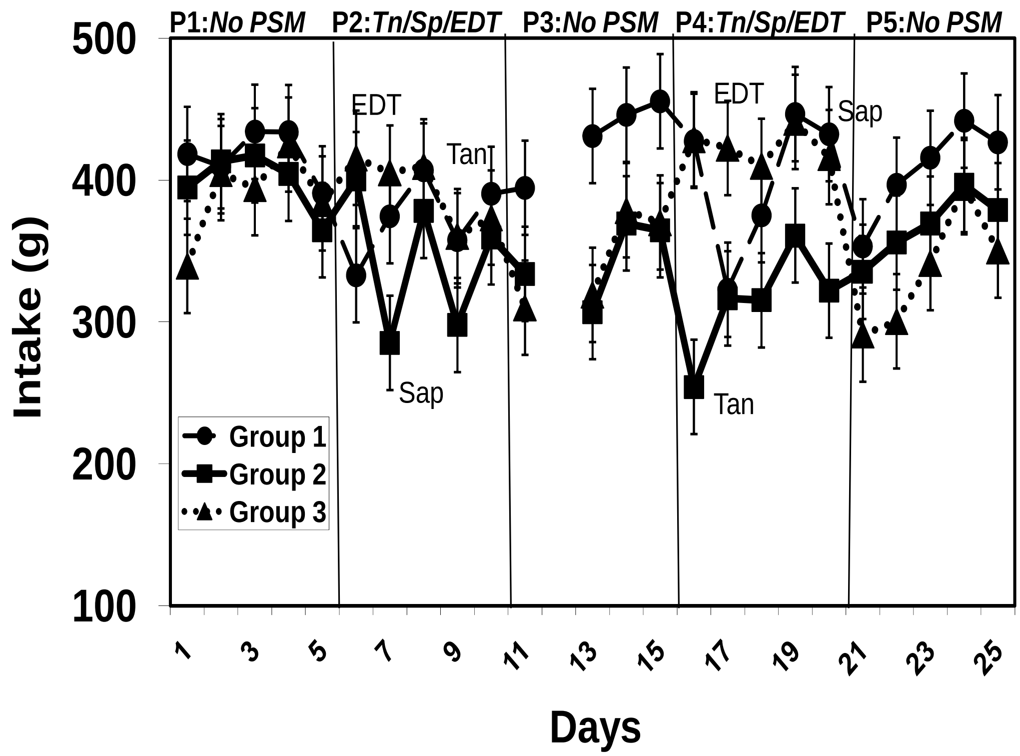

Figure 6: Intake by sheep during, Experiment 2, of beet pulp without PSM (Periods 1, 3, and 5) and beet pulp with PSM (Periods 2, and 4). During Period 2, Group 1 was fed tannins, Group 2 was fed saponins and Group 3 was fed more EDT in their beet pulp diets. During Period 4, Group 1 was then fed saponins, Group 2 was fed tannins and Group 3 continued to be fed EDT in their beet pulp food.

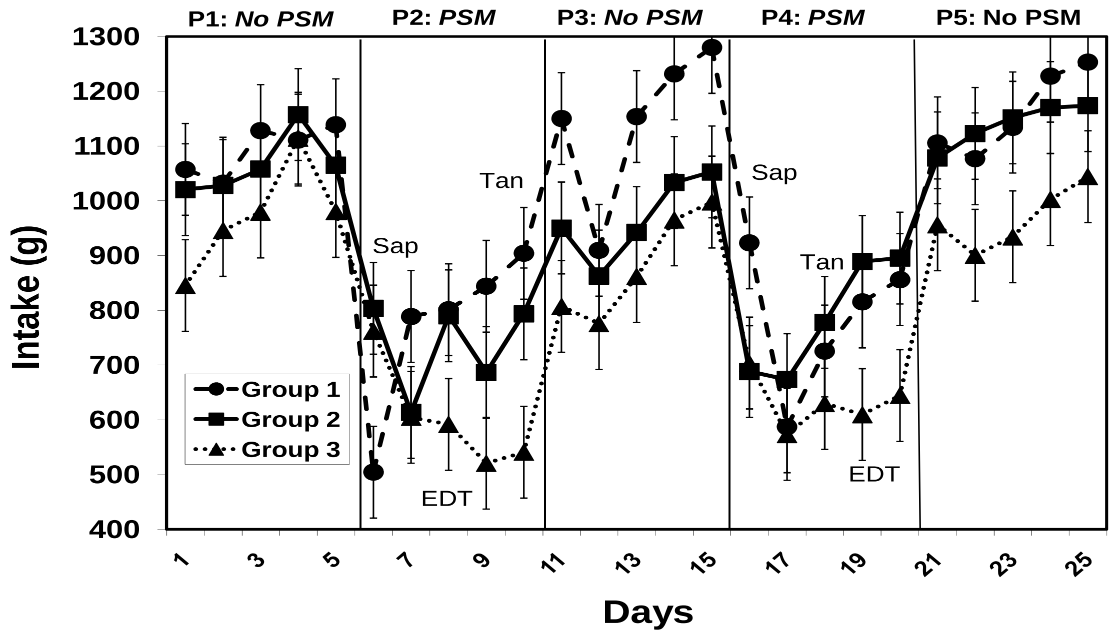

Figure 7: Total intake by sheep, during Experiment 2, of basal diet and test foods with, (Periods 2 and 4) and without PSM (Periods 1, 3, and 5).

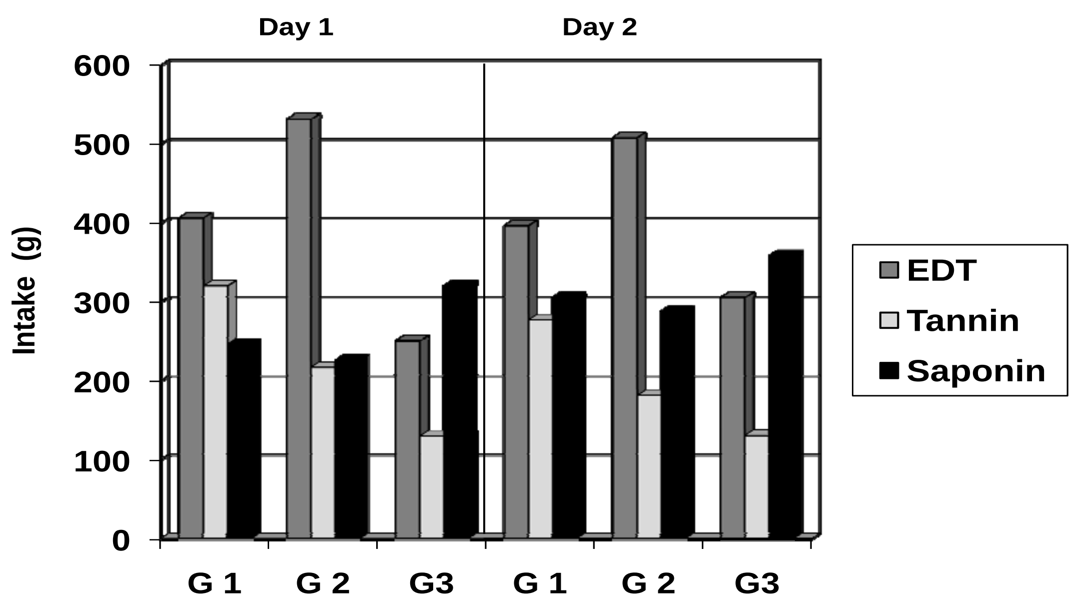

Figure 8: Intakes of alfalfa with Ergotamine D. Tartrate and beet pulp with tannin or saponin by sheep given a choice of tannin- or saponin-containing foods.

DISCUSSION

Sheep fed foods containing alkaloids (gramine or ergotamine D tartrate) increased intake of these alkaloid-containing foods when offered other foods containing saponins or tannins, relative to sheep fed only alkaloids. Based on the chemical characteristics of the two alkaloids, tannins, and saponins, we predicted sheep would increase intake of gramine-containing foods when supplemented with beet pulp-containing tannins. We further predicted sheep would increase intake of foods containing ergotamine D tartrate when supplemented with beet pulp-containing saponins. Overall our results support these hypotheses, and show that sheep ate more food with alkaloids when supplemented with tannin- and saponin-containing foods. Given a choice, we expected a stronger preference for tannins when sheep were fed gramine and a stronger preference for saponins when sheep were fed ergotamine D tartrate, but sheep showed no strong preference for tannin- or saponin-containing foods (Figures 4 and 8).

Experiment 1: Gramine Alkaloid. The data collected in Experiment 1, when sheep were fed a basal diet of alfalfa containing gramine (Periods 2, 4, and 6), suggest sheep supplemented with beet pulp containing tannin were able to eat more food with alfalfa-gramine than sheep supplemented with beet pulp containing either saponins or more gramine. This is consistent with the hypothesis that sheep can mitigate the negative effects of gramine by ingesting small amounts of tannins, because gramine is amino acid-like and tannins have a high affinity for binding to proteins in the gut of the animal and enhancing their excretion from the body.[18] During period 4, when the PSM test food fed to Groups 1 and 2 was switched (Group 1, saponin-beet pulp and Group 2 tannin-beet pulp), sheep in Group 1, initially fed tannin during period 2, had higher intakes of gramine than both other groups (Figure 1). We hypothesize that this group initially had higher intakes of food with gramine because their initial experience with Gramine was not as negative as Groups 2 and 3, because Group 1 was receiving the positive supplemental effects of the tannins. This idea is supported by sheep in Group 1as they gradually decreased their intake of gramine over the 5-day period, when they were no longer receiving the benefits of supplemental tannins; conversely, the new group receiving tannins (Group 2) gradually increased intake of food with gramine over the same period (Figure 1). Both observations are consistent with the hypothesis that tannins decrease the negative effects of the alkaloid gramine. Sheep in Group 3 (G→G) also increased their intake of food with Gramine during Period 4, which may imply they were able to eat more gramine over time, as their rumen adapted to this compound. Though Group 3 increased their intakes near the end of Period 4, both groups fed tannins and saponins in test foods had much higher intakes of gramine over most of the period.

When offered a choice during Period 6, we expected sheep to prefer the tannin-containing beet pulp over the saponin- or gramine-containing beet pulp, but that did not occur. Rather, all sheep fed alfalfa with gramine for 1 h and then given a choice of tannin-beet pulp or saponin-beet pulp showed a slight preference (P=0.06) for saponin-beet pulp on day 2 (Figure 4). Differences in the percentage of PSM in the food might have influenced intakes of beet pulp: the amount of tannin mixed with beet pulp 10%, while the amount of saponin was only 0.2%. Thus, the saponin-containing beet pulp contained less PSM in the mixture.

Another explanation for the preference of sheep for saponin-containing foods over tannin-containing foods is that while small amounts of tannins can increase intake of terpenes, [20, 21] high amounts of tannins can suppress intake.[22] No preference for the tannin-containing food occurred during this study, yet a small amount of tannins might be more effective at increasing intake of alkaloids than a higher dose, and thus sheep ate enough tannins to obtain the positive effect of binding to the alkaloid, but not enough to statistically show a preference for this compound or decrease their overall appetite.

Experiment 2: Ergotamine Alkaloid. During Experiment 2, a new group of sheep were fed a basal diet of alfalfa containing ergotamine D tartrate (EDT). Throughout this Experiment, sheep supplemented with either tannins or saponins had significantly higher intakes of EDT food than sheep fed EDT in both alfalfa and beet pulp (Group 3) (Figure 5). During Period 2, sheep in the group 3 had significantly lower intakes of alfalfa containing EDT than sheep supplemented with either tannin- or saponin-containing foods. In Period 4, the pattern was similar such that sheep fed only EDT ate significantly less EDT alfalfa than sheep fed tannins or saponins, while the group fed tannin had slightly higher intakes than the group fed saponin.

Sheep fed EDT ate more when supplemented with tannins or saponins, while sheep fed only EDT foods tended to decrease their intake of all foods over the entire trial. Intake was lower for these sheep not only during PSM periods but also during baseline periods when foods were presented without PSM. This has implications for herbivores grazing monocultures of one plant species, as ingesting only a single PSM may cause long-term food aversions that decrease intake and lower production.[4] Conversely, offering a variety of foods with different PSM may increase intake of plants on pastures as well as of problem species, such as weeds, unpalatable plants, thus providing other benefits to both the animal and the plant.

When offered a choice during Period 6, sheep in Group 2 ate the most alfalfa containing EDT, and the control group ate the least (Figure 8) Groups supplemented with tannins had the highest intakes of EDT-alfalfa throughout the trial, which suggests that tannins are a better supplement than saponins for sheep ingesting foods containing ergotamine. We hypothesized saponins would have a greater effect than tannins on increasing intake of EDT, but at the concentrations we fed these compounds, both PSM increased intake, tannins more than saponins.

Tannins may more effectively enhance intake of alkaloid-containing foods because tannins bind readily to a wide arrange of chemical groups and potentially inactivate PSMs.[17] For instance, tannins bind to alkaloids and there is evidence of synergism between tannins and terpenes.[18] Lambs eat more when allowed to ingest tannin- and terpene-containing foods than when offered either food individually.[13] Likewise, brushtail possums ingest more condensed tannins and terpenes when allowed to select from two diets containing different profiles of these PSM.[15] Finally, a small amount of tannin-containing food (100 g) enhances intake of terpene-containing foods in sheep.[20]

Throughout the trial, we fed alkaloid-containing foods first, followed by tannin- or saponin-containing foods. Reversing the sequence in which the foods were presented might have further enhanced intake as Mote et al. found when they fed lambs foods containing tannin-, terpene-, and alfalfa-barley in different sequences.[21] Food intake was higher when sheep first ate food with tannins and then ate food with terpenes than vice versa. Tannins remain in the gut up to 72 hours,[23, 24] where they can interact with terpenes, whereas terpenes are highly soluble compounds absorbed through the gastrointestinal tract and eliminated quickly from the body.[25] We conclude the increases in intake we observed might well have been greater had we reversed the order of food offerings, but the long retention of tannins (free and soluble) in the body evidently enabled tannins to interact with alkaloids from day to day, though likely to a lesser extent given their reactivity with other compounds in the gut. Saponins are high molecular weight triterpene glycosides, with a sugar group attached to either a sterol or another triterpene.[26] The chemical structures of saponins may also lead to a higher retention time in the gut thereby providing a residual effect of saponins on alkaloids.[27]

CONCLUSION

In the past, secondary compounds were often thought of only in terms of their negative effects on food intake and production in herbivores. An overabundance of any one secondary compound in the diet will decrease intake, cause health problems, and harm overall profitability and production. Yet, the positive effects of PSM at appropriate doses are only recently being discovered for plant and animal health.[28] Landscapes that allow animals to select among alternative forages enable individuals to meet needs for nutrients and to better cope with the negative effects of PSM.[29] When animals are presented with only one food in their diets, transient food aversions decrease intake even if an animal is suited to that particular nutrient or secondary compound profile.[4, 29] Herbivores can eat more than one PSM at a time as different compounds produce different effects and are detoxified by different mechanisms within the body.[1, 29] Our research shows that offering sheep a variety of foods allows them to ingest more foods that contain PSM, which can have important benefits ecologically for pastures and rangelands. Further study of forage plants is needed to validate this research for sheep and cattle foraging on pastures.

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CHAPTER 3

CATTLE PREFERENCES DIFFER WHEN FORAGES CONTAINING ALKALOIDS, TANNINS, AND SAPONINS ARE GRAZED IN DIFFERENT SEQUENCES

Abstract

Our objectives were to understand if plant diversity and sequence of plant ingestion affected food selection when cattle chose from forages that varied in concentrations of alkaloids, tannins and saponins. We hypothesized that animals can eat more of forages high in alkaloids when supplemented with legumes high in tannins (trefoil) or saponins (alfalfa), perhaps because tannins and saponins attenuate the negative effects of alkaloids. We determined whether cattle that ate high-alkaloid varieties of reed canarygrass or tall fescue as the first course of a meal subsequently ate high-tannin varieties of birdsfoot trefoil and high-saponin varieties of alfalfa more than when grazing low-alkaloid varieties of reed canarygrass or tall fescue. We also determined if intakes of high-alkaloid grasses varied when cattle foraged on high-tannin birdsfoot trefoil and high-saponin alfalfa before or after foraging on the grasses high in alkaloids.

We found cattle that ate tall fescue for 30 min subsequently preferred to eat birdsfoot trefoil, whereas cattle that ate reed canarygrass for 30 min showed a strong preference for alfalfa. Cattle grazing tall fescue and reed canarygrass also spent more time foraging on these grasses when they first ate legumes high in either tannins (trefoil) or saponins (alfalfa). Cattle decreased time eating tall fescue from 40% to 15% when they first grazed tall fescue for 30 min followed by birdsfoot trefoil for 60 min; remarkably, when the sequence was reversed they foraged actively on both trefoil and fescue throughout the 90-min meal. These patterns of foraging were analogous for reed canarygrass. We also found that cattle grazing high-alkaloid reed canarygrass or endophyte-free tall fescue ate significantly more alfalfa when given a

choice between birdsfoot trefoil (high and low) and alfalfa (high and low) than did cattle grazing endophyte-infected tall fescue or low-alkaloid reed canarygrass. These findings support the hypothesis that animals can eat more of forages high in alkaloids when supplemented with legumes high in tannins (trefoil) or saponins (alfalfa), perhaps because tannins and saponins attenuate the negative effects of alkaloids.

INTRODUCTION

While plants and herbivores have interacted one with another for eons, we are just beginning to appreciate the complexities and subtleties of their interactions. During the past 35 years, our understanding has evolved from a focus on plant structure and nutrients to a growing appreciation for so-called secondary compounds (SC) (Rosenthal and Janzen, 1979). Earlier work emphasized the protective roles of SC as plant defenses in interactions with herbivores. Plants that produce compounds that can quickly induce satiety in herbivores stand a better chance of surviving, and SC limit how much of a particular species an herbivore can eat, thereby spreading the load of herbivory across many species (Freeland and Janzen, 1974; Provenza, 1996, Foley et al., 1999). Thus, where once we thought that only poisonous plants, species that for one reason or another are a problem for herbivores (Provenza et al., 1992), contain compounds that were potentially toxic, there is increasing awareness that all plants contain SC that at too high concentrations limit intake and can be toxic (Provenza et al., 2003).

Herbivores seldom consume enough SC to result in poisoning because they regulate their intake of foods that contain SC (Provenza, 1995, 1996; Foley et al., 1999). For instance, oral gavages of toxins cause dose-dependent decreases in intake of foods that contain the SC (Wang and Provenza, 1997). Because the SC in any given plant limit intake below needs for nutrients such as energy and protein, herbivores must eat a variety of plant species that contain different kinds of SC. In principle, herbivores should be able to eat more foods with different kinds of SC because they affect the body in different ways and they are detoxified by different mechanisms (Freeland and Janzen, 1974). Eating a variety of phytochemicals also may lead to neutralization or inactivation of SC, which in turn could reduce susceptibility to toxicosis (Freeland et al., 1985).

Interactions among foods that contain high concentrations of SC 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). 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), though such complementarities were not observed with sheep (Burritt and Provenza, 2000). When sheep choose between foods that contain either amygdalin or lithium chloride, they eat more than lambs offered a food that contains only one of these compounds; the same is true with nitrate and oxalate (Burritt and Provenza, 2000). Sheep also eat more when offered three foods that contain terpenes, tannins, and oxalates than when offered foods with only one or two of these PSC (Villalba et al., 2004). The sequence in which complementary foods are ingested also influences preference. Sheep eat more terpene-containing foods after a meal of tannin-containing foods than when the sequence is reversed (Mote et al., 2008). While animals can meet needs for nutrients and tolerate higher total intake of SC when they can choose from a variety of plants (Provenza and Villalba, 2005), outside of the aforementioned studies, very little is known about which secondary compounds in plants are complementary and which are not when different forages are ingested in different sequences.

We sought to understand if plant diversity and foraging sequence might enhance food selection when cattle were allowed to eat forages that varied in concentrations of alkaloids, tannins and saponins. Intake of foods containing alkaloids increases when sheep are offered supplemental foods with tannins and saponins (Chapter 2). The alkaloid gramine is proteinaceous in nature, tannins bind to proteins in the gut, which enhances excretion of proteins, so we hypothesized tannins would bind to gramine and enhance its excretion from the body. The alkaloid ergotamine is steroidal, saponins have a high affinity for binding to lipid and steroidal compounds in the gut and then being excreted, so we hypothesized saponins would bind to ergotamine and enhance its excretion from the body. Sheep fed either gramine or ergotamine in combination with tannin- or saponin-containing foods have higher intakes of food than sheep offered only foods with gramine or ergotamine. These findings are consistent with the hypothesis that animals can neutralize the negative effects of alkaloids by ingesting tannins and saponins. These results show PSM can complement one another to increase herbivore's intake of "unpalatable" plant species, with the potential to maintain healthy animals and bio-diverse landscapes (Provenza, 2008).

We hypothesized that 1) cattle exposed to high-alkaloid varieties of reed canarygrass and tall fescue would then preferentially graze high- tannin birdsfoot trefoil or high- saponin alfalfa when given a four way choice between high- and low-tannin birdsfoot trefoil and high- and low-saponin alfalfa. We further hypothesized that 2) allowing cattle to ingest high-tannin and high-saponin legumes before grasses would assist cattle in eating high-alkaloid tall fescue and high-alkaloid reed canarygrass, and that the percentage of time cattle spent grazing these grasses would be greater for the sequence legumes → grass than for the sequence grass → legumes.

Wild varieties of reed canarygrass contain alkaloids, a class of compounds whose structures contain nitrogen. Tannins in birdsfoot trefoil bind to proteins in the gut, which enhances excretion of proteins (Okuda et al., 1982), so we hypothesized tannins would bind to the alkaloids and enhance their excretion from the body. The alkaloids in endophyte-infected tall fescue are mostly steroidal in nature, and saponins have a high affinity for binding to lipid and steroidal compounds in the gut and then being excreted, so we hypothesized that saponins would bind to the alkaloids and enhance their excretion from the body. Tannins and saponins are large molecules that interact with other compounds as they move slowly through the gastrointestinal tract. Thus, we further hypothesized that eating foods with tannins and saponins first in a meal increases the likelihood of interaction, and possible deactivation, of forages with alkaloids eaten subsequently in the meal.

A stronger preference for high-tannin and saponin varieties of forages when ingesting high-alkaloid varieties of plants would support the hypothesis that animals neutralize the negative effects of alkaloids by ingesting tannins and saponins. A greater incidence of eating grasses high in alkaloids after a meal of legumes high in tannins or saponins would also support the hypothesis that the sequence in which cattle are exposed to forage species with different SC affects their ability to cope with SC such as alkaloids, tannins and saponins.

OBJECTIVES

Sequence 1

Our objective was to determine if cattle eating high-alkaloid varieties of reed canarygrass or tall fescue used high-tannin varieties of birdsfoot trefoil or high-saponin varieties of alfalfa, respectively, more than when grazing on low-alkaloid grass varieties.

Sequence 2

Our objective was to determine if cattle that ingested high-tannin birdsfoot trefoil and high-saponin alfalfa before grazing high-alkaloid grasses would spend more time grazing these grasses than when presented with high-SC legumes after grazing high-alkaloid grasses.

MATERIALS AND METHODS

Plant Species. Reed canarygrass and tall fescue are highly productive, well-adapted coolseason grasses with value as forages in irrigated pastures. Tall fescue is ubiquitous in the U.S., and reed canarygrass is ubiquitous in wetter and poorly-drained sites. Reed canarygrass is drought-tolerant and efficient at removing applied nitrogen from irrigated soils (Carlson et al., 1996). Though breeding programs have reduced or eliminated many of the potentially toxic alkaloids in both of these grasses, the lack of these compounds may have negative implications regarding plant persistence and adaptability. For example, Asay et al. (2001) state, “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”(pp. 1). 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. Planting forage species in mixtures may provide animals with the opportunity to mix and match foods, so as to better choose plants that compliment one another.

In their wild forms, both species contain alkaloids that can adversely affect animals, especially as pastures in monocultures. Reed canarygrass contains eight alkaloids that reduce intake and performance, and in extreme cases produce gross histopathology of the central nervous system (*Phalaris* staggers) (Burrows and Tyrl, 2001). These include four derivatives of gramine, tryptamine, hordine, and two derivatives of beta-carboline. Thus, indole and carboline alkaloids are present. These alkaloids are all metabolized from the amino acid tryptophan and are thus proteinaceous. Tall fescue contains two groups of alkaloids, one 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 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). The toxic alkaloids in tall fescue are lipid in nature, thus we hypothesized that including saponin-containing alfalfa in the diet of cattle grazing endophyte-infected tall fescue would neutralize these alkaloids. Birdsfoot trefoil contains condensed tannins that bind to soluble proteins in the rumen (Jones and Morgan, 1977). The threshold tannin concentration at which a reduction in diet digestibility occurs is fairly high, about 8 to 9% of dry matter. Because the alkaloids associated with reed canarygrass are derived from tryptophane, and are thus proteinaceous in nature, we hypothesized that including tannin-containing birdsfoot trefoil in the diet of cattle grazing reed canarygrass would neutralize the alkaloids.

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 (41’57 N. 111’52 W.). In 2006, we planted monocultures of tall fescue (TF) (*Festuca arundinaceum, Kentucky 31 endophyte-infected and endophyte-free*)(Rottinghaus et al., 1991; Aldrich et al., 1993) and reed canarygrass (RCG) *(Phalaris arundinacea L. varieties v.n.s and palaton)* with high (+) and low (-) alkaloids (Marten, 1972; Sheaffer et al., 1990), birdsfoot trefoil (BFT) (*Lotus corniculatus varieties Goldie and Norcen*) with high (+) and low (-) tannins (Hedqvist et al., 2000; Terrill et al., 1992), and alfalfa (AA) (*Medicago* sativa *varieties Vernal and Lahontan*) with high (+) and low (-) saponins (Pedersen et al., 1976; ARS, 1963) (Figure 9). Our chemical analysis of each plant species confirmed appropriate levels of plant secondary compounds, which correlate with documented concentrations (Table 2). In this study, grass species averaged 60% fiber and 2% N, while legume species averaged 40% fiber and 3-5% N. There were four replications of each SC x grass treatment combination (Figure 9).

Pasture Trials. Thirty-two fall-born Angus calves (592 lbs±14 lbs initial BW) were randomly assigned to each of 16 pastures (2 cows/pasture) of tall fescue (8 pastures) or reed canarygrass (8 pastures) at high (n=4) or low (n=4) concentrations of alkaloids. Cattle spent most of their time in orchard grass areas, but were moved to trial pastures to eat their morning meals in two different sequences. Pastures were defined by temporary electric fence, as illustrated by dashed lines in Figure 1. They were in the vegetative stage at a height of 8 to 12 inches. Cattle were weighed pre and post trial to estimate changes in body weight during the 30-d trials.

Sequence 1: Grass → Legume Choice

In the first sequence, which lasted 12 d, cattle grazed on either high- or low- alkaloid grass (tall fescue or reed canarygrass) pastures for 30 min and were then offered a choice of high- and low-tannin and saponin legume pastures for 60 min.

Sequence 2: High PSM Legume → Grass

In the second sequence, which lasted 12 d, cattle grazed first on either high-tannin birdsfoot trefoil or high-saponin alfalfa, and then on either high-alkaloid tall fescue or high-alkaloid reed canarygrass.

During both sequences, observers recorded behavioral data through scan-samples of individually marked animals (Altman, 1974), and then calculated the percent of time each animal spent grazing on each choice plot. Scans were made at 2 min intervals from 0600 to 1030 each day. 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

		AA (high)	BFT (high)	AA (low)	BFT (low)
Orchard grass	Tall fescue (low)				
Orchard grass	Reed Canarygrass (high)				
Orchard grass	Tall fescue (high)				
Orchard grass	Reed Canarygrass (low)				

Figure 9. Experimental pastures grazed by cattle.

were taken from each of the 16 pastures at the end of the trial, placed in plastic bags, covered with ice, transported to a freezer at -20^{o} C and freeze- dried for chemical analyses.

The statistical design for the analysis of variance for Sequence 1 had four replications of two treatments: whole plot factors were secondary compound concentration (high-low) and grass (TF or RCG) in a 2x2 factorial randomly assigned to pastures within replicates. The sub-plot factor was legume, assigned in spatial sequence to strips within pastures. The repeated measure was day. The statistical design for the analysis of variance for Sequence 2 had four replications of two treatments: either high-tannin birdsfoot trefoil or high-saponin alfalfa assigned to pastures within replicates. The sub-plot factor was grass, either high-alkaloid tall fescue or high-alkaloid reed canarygrass, assigned in spatial sequence to strips within pastures. The repeated measure was day. The response variable for both analyses was percent of scans observed per forage.

RESULTS

Sequence 1: Grass → Legume Choice

During Sequence 1 cattle grazed TF (high and low) or RCG (high and low) for 30 min, and then grazed AA (high and low) and BFT (high and low) for 60 min.

Grass. Cattle spent 27 % of the time grazing both varieties of TF and 62% of the time grazing both varieties of RCG ($P = 0.0015$). They differed little in time spent grazing high- and low-alkaloid varieties of TF (24% vs. 31%, $P = 0.3448$), and the same was true for high- and low-alkaloid varieties of RCG (66% vs. 59%, $P = 0.3448$). However, in both cases cattle consistently decreased time spent grazing during the first sequence, though the effect was most pronounced for tall fescue ($P = 0.0029$, Figures 10 and 11).

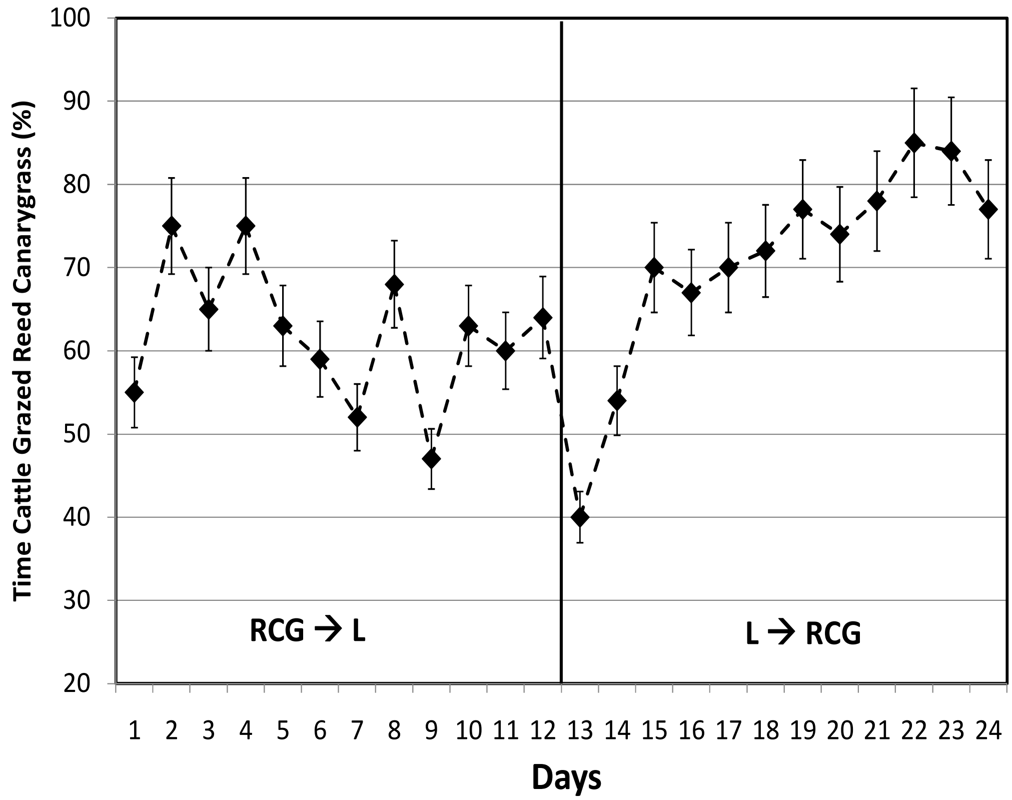

Figure 10. Percentage of time cattle spent grazing reed canarygrass during Sequence 1 (RCG →L) and Sequence 2 (L → RCG). During Sequence 1, cattle were first exposed to high- or low-alkaloid reed canarygrass(RCG) for 30 min and then given a choice of high- and low-saponin alfalfa and high- and low-tannin birdsfoot trefoil for 60 min (L). During Sequence 2, the order was reversed and cattle were given a choice of AA + or BFT + (L) before foraging on high- or low-alkaloid RCG pastures. Bars are standard errors.

Legume Choice. Reed Canarygrass: Cattle preloaded with high-alkaloid RCG preferred AA over BFT (33% vs. 16%, $P < 0.006$), whereas cattle preloaded with low-alkaloid RCG did not differ in choice of legumes (AA = 23% vs. BFT = 25%, $P < 0.05$) (Figure 12). Cattle preloaded with high-alkaloid RCG spent more time foraging on AA than cattle preloaded with low-alkaloid RCG (33% vs. 23%, $P = 0.006$) (Figure 12).

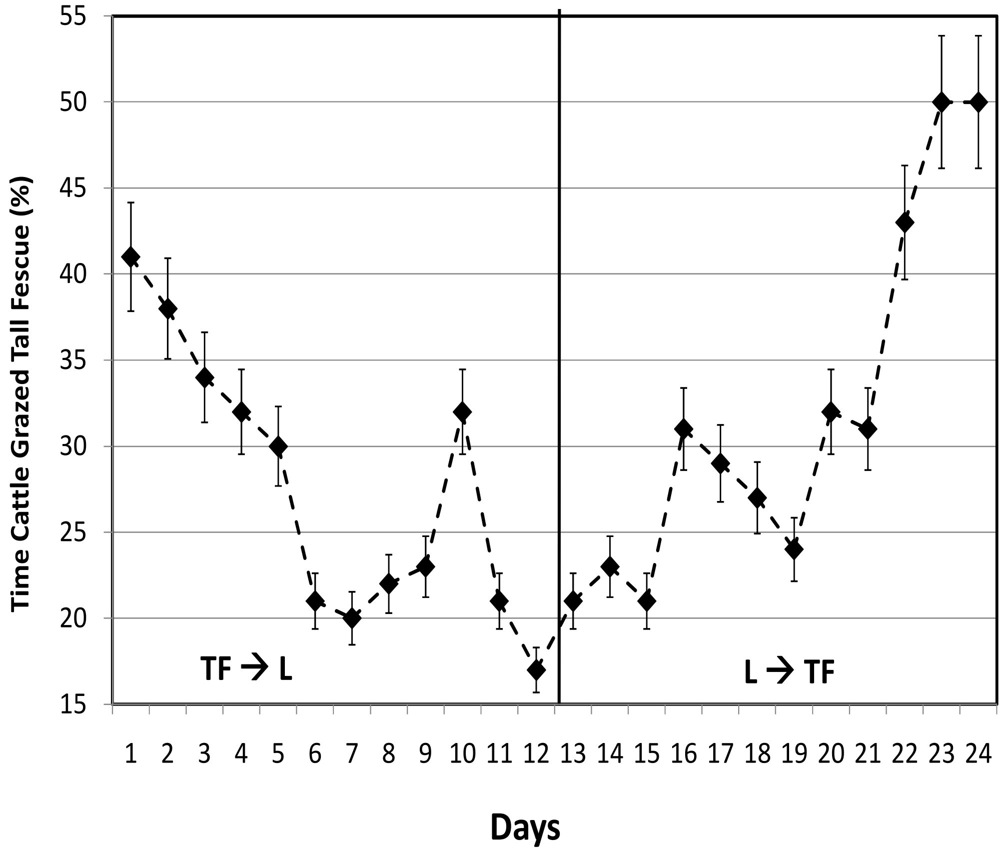

Figure 11. Percentage of time cattle spent grazing tall fescue during Sequence 1 (G → L) and Sequence 2 (L → G). In Sequence 1, cattle were first exposed to either high- or low-alkaloid tall fescue (TF) for 30 min and then given a choice of high- and low-saponin alfalfa and high- and low-tannin birdsfoot trefoil (L) for 60 min. In Sequence 2 (L→ TF), cattle were first exposed to either high-saponin alfalfa or high-tannin birdsfoot trefoil (L) before being offered a choice of high- or low-alkaloid tall fescue. Bars are standard errors.

Tall Fescue: Cattle preloaded with high-alkaloid TF did not prefer AA to BFT (25% vs. 23%, $p<0.05$), whereas cattle preloaded with endophyte-free TF preferred AA over BFT (30% vs. 19%, $P = 0.006$). Cattle preloaded with high-alkaloid TF spent more time foraging on BFT than cattle preloaded with the low-alkaloid variety of TF (23% vs. 19%, $P < 0.05$) (Figure 12).

Across all groups during Sequence 1, cattle offered BFT (high and low) and AA (high and low) preferred high-tannin BFT (36%) followed by low-saponin AA (29%) and high-saponin AA

(26%; p<0.0001). Cattle least preferred low-tannin BFT (6%; p<0.0001). With regard to sequence, cattle that first foraged on high-alkaloid RCG and low-alkaloid TF subsequently preferred AA over BFT, whereas cattle offered high-alkaloid TF and low-alkaloid RCG foraged on AA and BFT equally (Figure 12). Cattle spent significantly less time foraging on low-tannin BFT (6%), which reduced overall preference for BFT in Figure 12.

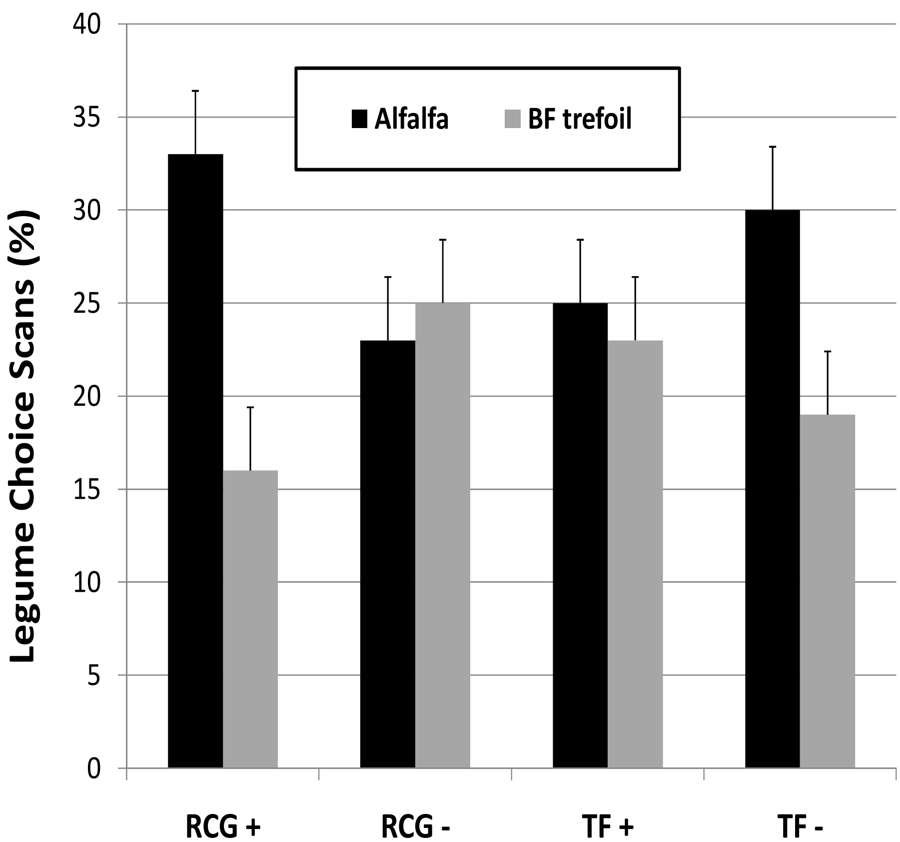

Figure 12. Percentage of time cattle spent grazing alfalfa and birdsfoot trefoil after eating high- and low-alkaloid reed canarygrass or tall fescue in Sequence 1. Bars are standard errors.

Sequence 2: High PSM Legume → Grass

During Sequence 2 cattle were first given a choice between high-tannin BFT and high-saponin AA for 30 min, after which they were offered high- and low-alkaloid varieties of TF and RCG for 60 min.

Grass. Cattle grazing TF spent less time foraging than cattle grazing RCG (32% vs. 71%, P = 0.0005). Again, no differences were observed between high- and low- alkaloid varieties (TF + 29%, TF- 35%, RCG+ 75%, and RCG - 66%, P = 0.324). All groups grazed more at the end of the sequence than at the beginning (63% vs. 31%, P < 0.0001). Cattle grazing RCG spent more time foraging than cattle grazing TF over the entire sequence, yet both groups consistently increased the percentage of time grazing throughout the sequence (P = 0.0159; Figures 10 and 11).

Legume Choice. Cattle grazing TF pastures slightly preferred BFT over AA (56% vs. 44%, P = 0.08), and cattle grazing RCG slightly preferred AA over BFT (50% vs. 44%, P = 0.08).

Weight gains. All cattle gained 1.45 kg/head/day (3.2 lbs/head/day). Groups did not differ in weight gains.

DISCUSSION

We sought to understand if plant diversity and foraging sequence might enhance food selection when cattle were allowed to eat forages that varied in concentrations of alkaloids, tannins and saponins. Intake of foods containing alkaloids increases when sheep are offered supplemental foods with tannins and saponins (Chapter 2). We hypothesized that both foraging choice and foraging sequence would affect time cattle spent grazing and we tested this hypothesis with forages that differed in concentrations of alkaloids (TF and RCG), tannins (BFT) and saponins (AA).

Sequence 1. For Sequence 1, we hypothesized cattle exposed to high-alkaloid varieties of

RCG and TF would then preferentially graze high-tannin BFT or high-saponin AA when given a four way choice of high- and low-saponin AA and high- and low-tannin BFT. We also hypothesized that time spent grazing would decrease over a 12-day trial if cattle received no benefit from ingesting forages with tannin and saponin after eating forages with alkaloids.

While we hypothesized that cattle eating RCG would prefer BFT and cattle eating TF would prefer AA, we observed the opposite (Figure 4). Cattle preloaded with high-alkaloid RCG spent more time eating AA than cattle preloaded with low-alkaloid RCG (33% vs. 23%, Figure 12), which suggests saponins in AA may complement alkaloids in RCG. Cattle preloaded with high-alkaloid TF spent more time foraging on BFT than cattle preloaded with low-alkaloid TF (23% vs. 19%, Figure 12), which suggests the tannins in BFT may have benefitted cattle more when they were ingesting alkaloids in endophyte-infected TF.

By far, the most striking observation of Sequence 1 was that the percentage of time cattle spent grazing TF and RCG steadily decreased when they first grazed these grasses and then were offered a choice of the legumes. This was particularly evident with TF pastures, where cattle grazed only 16% of the time by the end of the 12-day trial (Figure 11). A similar, though less dramatic, pattern occurred with cattle grazing RCG pastures (Figure 10).

Sequence 2. For Sequence 2, we hypothesized that first eating high-tannin and high-saponin legumes would enable cattle to eat more high-alkaloid TF and RCG such that the percentage of time spent grazing TF and RCG would be greater for Sequence 2 (legumes → grass) than for Sequence 1 (grass → legumes). The findings from this trial support these hypotheses. Cattle grazing RCG pastures preferred AA (high) while cattle grazing TF pastures preferred BFT (high). Overall, cattle grazing RCG continued to graze more on grass pastures during this sequence than cattle grazing TF (71% vs. 32%, P = 0.0005, Figures 10 and 11).

Contrary to Sequence 1, the most important finding was that the percent of time grazing RCG and TF steadily increased when cattle were given a choice of high-tannin BFT or high-saponin AA first and then placed on the high-alkaloid grasses. This behavior is consistent with the hypothesis that cattle ate more high-alkaloid grasses when legumes containing tannins or saponins were provided first. Obviously, more experimental analyses are necessary to assess the specific effects of secondary compounds, as these findings could also be attributed to interactions among secondary compounds and high amounts of protein found in legumes, which enhance the use of fiber from grasses (Van Soest, 1994).

Individual differences play an important role in grazing preferences, yet they are often overlooked when relying on group averages (Provenza et al., 2003). During Sequence 1, cattle drastically decreased time grazing TF; when the sequence was reversed, they markedly increased consumption, but that varied by individual. Some individuals, both within and among groups, began sampling and grazing TF several days before others, while still others continued to avoid the plant all together. The raw data show cyclical patterns of grazing for each individual, with grazing behavior varying from day to day. When ingesting foods high in SC, intakes are typically cyclical with individuals eating high amounts of a particular forage one day and low amounts of the forage the next day (Provenza, 1996; Pfister et al., 1997).

Complementarities among plant species are an important but little understood area of plant-herbivore interactions (Freeland and Janzen, 1974, Provenza et al., 2003), although recent studies are beginning to discover these interactions and their influence on herbivore behavior. Sheep decrease intake of tall fescue in a meal, unless they receive an oral gavage of tannins prior to the meal, in which case they eat tall fescue throughout the meal; conversely, they eat trefoil readily unless they receive an oral gavage of tannins prior to the meal in which case they eat less trefoil (Lisonbee, 2008). Sheep eat more when offered foods high in tannins or saponins along with foods high in alkaloids (Chapter 2), perhaps because tannins and saponins bind with alkaloids reducing their adverse effects on animal health and nutrition. Collectively, these findings suggest herbivores regulate intake of plants as a function of interactions among primary and secondary compounds.

Studies with wildlife also hint at the importance of complementary plants in animal diets. Mule deer (*Odocoileus hemionus*) eat more when offered both sagebrush and juniper (12.3 g/kg BW), plants that contain different terpenes, than when they are offered only sagebrush (4.2 g/kg BW) or juniper (7.8 g/kg BW) (Smith, 1959). Brushtail possums (*Trichosurus vulpecula*) that can select from two diets containing phenolics and terpenes eat more food than when they consume diets containing only one of these toxins (Dearing and Cork, 1999), and the same is true in principle with squirrels (*Sciurus niger and S. carolinensis*) (Schmidt et al., 1998). Conversely, sheep offered foods containing either sparteine or saponin eat no more of both foods than lambs offered foods containing only one of these compounds, possibly because these compounds are not complementary (Burritt and Provenza, 2000).

Little is known about how the sequences of eating plants with different SC affects foraging, though they appear to be critical. Sheep eat more of foods with SC when offered them in the morning followed by limited nutritious foods in the afternoon (Papachristou et al., 2007). The sequence in which foods containing tannins-, terpenes-, and alfalfa-barley are eaten by lambs also influences intake (Mote et al., 2007b). Intake is higher when sheep first eat food with tannins and then eat food with terpenes than vice versa. Tannins remain in the gut up to 72 hours (Silanikove et al., 1994, 1996), where they can then interact with terpenes, whereas terpenes are highly soluble compounds absorbed through the gastrointestinal tract and eliminated quickly from the body (Foley and McArthur, 1994). Thus, when eating plants containing SC, the sequence in which they are ingested may play a role in how well the animal is able to detoxify and utilize these forage species on landscapes (Seefeldt, 2005).

Plant secondary compounds interact with herbivores in various ways, many of which may have implications to our management of natural resources. These interactions should be studied further in an effort to determine how to best utilize plants with high amounts of SC, so as to sustain plants, animals and people.

IMPLICATIONS

Tall fescue is a major grass forage grown on more than 14 million ha of pasture and hay land in the United States (Buckner et al., 1979). Most tall fescue is endophyte-infected, which causes various animal performance problems. The highly negative impact of tall fescue alkaloids on beef production in the U.S. was estimated at $600 million annually more than 10 years ago (Paterson et al., 1995). A conservative estimate places the total livestock-related losses nationwide related to tall fescue at $500 million to $1 billion a year (Univ. Nebraska, Institute of Agriculture and Natural Resources). However, the alkaloids in tall fescue, so problematic for ruminants, make the plant highly resistant to drought and other stress.

Our findings are particularly important for those dealing with endophyte-infected tall fescue, a productive grass dominant in much of the U.S. Improved seedling performance and survival, as well as insect and nematode resistance, drought resistance, improved nitrogen assimilation, higher seed set, and overall increased survival are all benefits from using endophyte-infected tall fescue in pasture systems (Pedersen et al., 1990). However, problems arise in animal production as alkaloids decrease intake and performance, which in turn decreases economic viability and efficiency. Results from this study suggest ways for developing pastures and grazing systems that incorporate a variety of plant species with different secondary compounds as a way to increase plant survivability as well as livestock productivity when dealing with forages such as tall fescue. If, as our results suggest, tannin or saponin-containing legumes can offset the negative effects of the alkaloids in tall fescue, the economic impact for beef producers coping with fescue toxicosis would be enormous. The discovery of Kentucky-31 tall fescue was revolutionary in the so-called "transition zone" from Missouri and Arkansas to the east coast, allowing this region of the U.S. to engage in beef production. Our findings have the potential to create a similar positive impact on the beef industry, particularly in this region. We also hope these findings spark interest in discovering other complementary plant species that will enhance livestock production, landscape vigor, and economic prosperity.

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CHAPTER 4

CONCLUSIONS

In the beginning of this research we set out to determine if cattle and sheep foraging behavior would vary when subjected to different plant secondary compounds (SC) in their diets. More specifically we asked three questions: 1) Will sheep fed basal diets high in alkaloids (gramine or ergotamine d tartrate) eat more food with these alkaloids if supplemented with foods containing tannins or saponins? 2) Do cattle preloaded with tall fescue and reed canary grasses show a preference for alfalfa or birdsfoot trefoil? 3) Are cattle grazing tall fescue and reed canarygrass able to eat more of these grasses when they are allowed to ingest legumes high in tannins (trefoil) and saponins (alfalfa) first?

This research was based on hypotheses regarding how secondary compounds might interact to influence food selection. The SC gramine in reed canarygrass is proteinaceous in nature. Tannins have a high affinity for binding proteins (Jones and Mangan, 1977), which suggests that animals ingesting foods with Gramine may increase their preference for tannin-containing foods to reduce the negative impacts of this SC. Moreover, tannins hasten alkaloid excretion from the body (Okuda et al., 1982), which might also allow animals to eat more high-alkaloid forages and foods when presented with tannins first. Endophyte-infected tall fescue contains the alkaloids perlolidine, perloline and ergotamine, which are all steroidal in nature. Saponins are non-polar steroidal compounds that have a high affinity for binding to lipids in the gastro-intestinal tract of animals, causing their excretion in the feces (Malinow et al., 1979). Because the secondary compounds in tall fescue are steroidal, similar to lipids in nature, animals eating foods containing ergotamine should increase their intake of other foods containing saponins, thereby eliminating ergotamine from the body. Furthermore, animals ingesting saponins first should be able to eat more of high-alkaloid forages and foods.

With regard to the first question, we found that sheep fed basal diets high in alkaloids (gramine or ergotamine) ate more of their alkaloid diet when supplemented with food containing either tannins or saponins. We hypothesize these response were due to SC neutralization, i.e. tannins and saponins binding to alkaloids in the gut and enhancing excretion, that enabled sheep to more effectively cope with toxins. These results suggest that animals offered a variety of foods with complementary SC would benefit with regard to weight gains, health and overall production.

With regard to questions 2 and 3, we found that cattle preloaded with tall fescue (alkaloid) preferred birdsfoot trefoil (tannin), while cattle preloaded with reed canarygrass (alkaloid) preferred alfalfa (saponin). More importantly we observed that cattle grazing tall fescue and reed canarygrass spent more time grazing these grasses when the sequence was such that they were first given legumes high in tannins (trefoil) and saponins (alfalfa), respectively. The hypothesis that SC interact with one another, often in a positive way, and that herbivores are able to distinguish these relationships and use them to their benefit is an idea not often considered in livestock production systems. Plants that are good at surviving in extreme climates or landscapes often contain high amounts of SC, which can limit intake by animals. Yet, the results of these studies suggest that it is possible to utilize these compounds to benefit livestock, ecosystems, and producers.

MANAGEMENT IMPLICATIONS

Worldwide, landscape managers are constantly looking for ways to improve production as well as ecological integrity. Unearthing ways to sustainably feed and clothe humanity is an important factor for producers, who will increasingly be challenged to meet the world's needs for food and enhance and maintain the integrity of natural systems. Utilizing plants, such as endophyte-infected tall fescue, that can survive under stressful extremes including drought and heavy grazing is one way producers can meet both environmental and economic objectives. Yet, these plants often contain high amounts of secondary compounds, which limit intake by herbivores.

The results of this research suggest ways to encourage intake of SC by herbivores, without expensive or exhaustive approaches. Discovering which plants compliment one another provides producers with the added option of planting mixtures on pastures to benefit their operation. These findings could also be used by public land managers who must find alternative methods of eliminating invasive plant species, that more than likely contain high amounts of secondary compounds.

While these studies are certainly not a cure-all for every land issue, and may not benefit all producers, they are important findings on a topic that has not been studied near enough. More research is needed to discover other possible SC combinations, and to detect any negative impacts that might come about from planting SC mixtures on pastures. These findings will hopefully stimulate further research on possible benefits of SC and their influence on animal grazing behavior.

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APPENDICES

Appendix A. Permission Letter

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Appendix B. Bibliography

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