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Effects of Foraging Sequence on the Ability of Lambs to Consume Endophyte-Infected Tall Fescue (Alkaloids), Birdsfoot Trefoil (Tannins), and Alfalfa (Saponins)

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EFFECTS OF FORAGING SEQUENCE ON THE ABILITY OF LAMBS TO CONSUME ENDOPHYTE-INFECTED TALL FESCUE (ALKALOIDS), BIRDSFOOT TREFOIL (TANNINS), AND ALFALFA (SAPONINS)

by

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ABSTRACT

Effects of Foraging Sequence on the Ability of Lambs to Consume the Forages Endophyte-Infected Tall Fescue (Alkaloids), Birdsfoot Trefoil (Tannins), and Alfalfa (Saponins)

by

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All plants contain primary and secondary compounds. Primary compounds are needed by plants and herbivores for maintenance, growth, and reproduction, while secondary compounds play roles as diverse as protecting plants from ultraviolet radiation, defenses against herbivores, pollination attraction, and stress resistance. Secondary compounds have nutritional and medicinal benefits for herbivores as well, especially when eaten in diverse combinations that complement one another. While complementarities among secondary compounds are an important but little understood area of plant-herbivore interactions, even less is known about how the sequences of eating plants with different compounds affects foraging behavior, though they may be critical. In three trials, I determined if the sequence in which lambs ate endophyte-

infected tall fescue (alkaloids), birdsfoot trefoil (tannins), and alfalfa (saponins) affected their foraging behavior.

When lambs grazed on monocultures they spent similar amounts of time grazing regardless of which forage they grazed. Lambs that grazed in a sequence of different forages tended to subsequently eat less alfalfa pellets in pens than lambs that grazed a monoculture, which suggests they better met their nutritional needs on mixtures than on monocultures. Likewise, lambs that grazed a monoculture of alfalfa or fescue spent more time grazing during the first 45 min than in the subsequent 45 min, while lambs that grazed alfalfa during the first 45 min and then fescue spent more time grazing in the subsequent 45 min, suggesting lambs satiate faster when they have fewer choices.

While the foraging sequences I examined generally allowed animals to consume more than they would if they grazed in monocultures, there is still a need to further explore how different plants and foraging sequences influence the level of consumption by livestock of forages on pastures that contain various secondary compounds.

(49 pages)

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Emily C. Lockard

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CHAPTER I

INTRODUCTION

Herbivores prefer nutritious foods and they generally limit intake of foods high in secondary compounds (Freeland and Janzen, 1974). They balance their intake of primary and secondary compounds through a combination of past experience, especially learning from mother and peers, and learning through feedback-mediated trial and error learning linked to previous experiences with mother and peers (Provenza et al., 2003a). To assess the positive (nutritional) and negative (toxicological) effects of eating various forages, animals sample small amounts of novel foods and they rely on the ensuing postingestive feedback to assess the benefits and costs associated with ingesting a particular food or combination of foods (Provenza, 1995, 1996).

As complex as primary and secondary compounds are individually, they also interact with each other to influence foraging behavior. These interactions cause herbivores to eat a variety of plant species that contain different types of primary and secondary compounds to meet their nutritional and health needs. Too high of concentrations or imbalances of primary and secondary compounds limit how much of any one food an herbivore can eat (Provenza, 1995, 1996). Secondary compounds limit intake, and in principle, herbivores should be able to eat more foods with different types of compounds such as tannins and alkaloids because they produce different effects in the body and they are detoxified by different mechanisms (Freeland and Janzen, 1974).

To date, little is known about how different secondary compounds interact to influence food intake, but it is clear they do. When lambs 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). Mule deer also 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 consume more total food than when they consume diets containing only one of these secondary compounds (Dearing and Cork, 1999), and the same is true in principle with squirrels (Schmidt et al., 1998). Lambs also eat more forage and digest more nutrients when they eat foods containing alkaloids (endophyte-infected tall fescue or reed canarygrass) in combination with foods containing either saponins (alfalfa) or tannins (birdsfoot trefoil) (Owens, 2008). Conversely, lambs 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 compounds are not complementary (Burritt and Provenza, 2000).

Foods are defined as being complementary when the benefit of consuming those foods together exceeds the average benefit of consuming those foods alone (Tilman, 1982). While complementarities among secondary compounds are an important but little understood area of plant-herbivore interactions (Freeland and Janzen, 1974; Provenza et al., 2003a), even less is known about how the sequences of eating plants with different compounds affects foraging, though they appear to be critical. Sheep eat more food with terpenes when they first eat food with tannins (Mote et al., 2008). Cattle steadily decrease time eating tall fescue when they first graze tall fescue alone for 30-min followed by

trefoil, alfalfa, or alfalfa-trefoil combination alone for 60-min; when the sequence is reversed they forage actively on trefoil, alfalfa, trefoil-alfalfa combination and fescue throughout the 90-min meal (Lyman, 2008). These patterns of foraging are analogous with trefoil, alfalfa and high-alkaloid reed canarygrass (Lyman, 2008). Thus, both combination and sequence greatly influence intake of tall fescue and reed canarygrass by cattle. Sheep similarly decrease intake of tall fescue in a meal, unless they receive intraruminal infusions of tannins prior to the meal, in which case they eat tall fescue throughout the meal (Lisonbee, 2008). Conversely, they eat trefoil readily unless they receive intraruminal infusions of tannins prior to the meal in which case they eat less trefoil (Lisonbee, 2008). When sheep eat foods high in tannins or saponins along with foods high in alkaloids, the tannins and saponins bind with alkaloids reducing their adverse effects on intake (Lyman et al., 2008).

Collectively, these findings suggest that cattle and sheep regulate intake of plants as a function of interactions between tannins, saponins, and alkaloids and that the sequence in which they eat forages is crucial for increasing their intake of plants that differ in secondary compounds. We do not know if they learn to forage in sequences that optimize intake of secondary compounds or if they simply "eat the best and leave the rest" (Provenza, 2003a, b).

Objectives

My objective was to determine if different sequences of use of pasture plants that differ in secondary compounds affected diet selection and time spent foraging by lambs. I determined whether or not foraging time on various plant species, and intake of alfalfa

pellets as a complement to the forages on pasture, increased when lambs were rotated in sequences on pastures of two different forages compared with grazing monocultures.

I used three forages that differed in secondary compounds to examine the existence of complementary relationships and the resulting effects on foraging. The forages were endophyte-infected tall fescue (*Lolium arundinacea*) with high levels of alkaloids, alfalfa (*Medicago sativa*) with high levels of saponins, and birdsfoot trefoil (*Lotus corniculatus*) with high levels of tannins. The plant-derived alkaloids in tall fescue are steroidal, while the saponins in alfalfa are non-polar steroidal compounds with an affinity for binding to cholesterol and cholesterol-based compounds in the gastro-intestinal tract of animals, causing their excretion in the feces (Malinow et al., 1979). Birdsfoot trefoil contains condensed tannins, compounds of high molecular weight that remain in the rumen where they interact with endogenous enzymes, microbial protein, and dietary proteins (Jones and Mangan, 1977). Saponins are also high molecular weight triterpene glycosides, containing a sugar group attached to either a sterol or other triterpene, which leads to a higher retention time in the gut (Wallace, 2004). Tannins bind to amino acids and other N-containing compounds and thus they can also bind to alkaloids because alkaloids are N-containing compounds.

Hypothesis

I hypothesized that secondary compounds that bind in the gastrointestinal tract due to their structural characteristics and affinities would reduce their negative postingestive action relative to when animals ingest a single compound in monocultures. Thus, I predicted that forage intake would be higher when lambs ate 1) a combination of

high-tannin and high-alkaloid containing forages as compared with eating only a high-alkaloid or a high-tannin forage, 2) a combination of high-tannin and high-saponin containing forages as compared with eating only a high-tannin or a high-saponin forage, and 3) a combination of high-saponin and high-alkaloid containing forages as compared with eating only high-saponin or high-alkaloid forages. I also hypothesized that some sequences of forage ingestion would be more beneficial than others.

I predicted an increase in the consumption of alfalfa pellets when the lambs were previously offered non-complementary forages, as alfalfa pellets were used to supplement lambs as they foraged and indirectly indicated the amount forage ingested by lambs. I did not measure intake directly while lambs foraged on pastures. Rather, I used scan samples to estimate the percentage of time each lamb spent foraging while on pasture, and the ensuing intake of alfalfa pellets by each lamb as indicators of the degree to which different forages and foraging sequences were complementary in meeting nutritional needs. As lambs would be able to consume more when they grazed complementary forages, alfalfa pellets were used as an indicator of a beneficial sequence of a combination of forages. Thus, lambs that had a higher intake on pastures were predicted to have a lower intake of alfalfa pellets.

CHAPTER II

FORAGING SEQUENCE WITH TALL FESCUE, BIRDSFOOT TREFOIL, AND ALFALFA

I hypothesized that diverse foods can be complementary such that the benefit of consuming more than one food is greater than the average benefit of consuming the foods in isolation. I further hypothesized that some sequences of forage ingestion would be more beneficial than others. Based on these hypotheses I predicted that mixtures of foods and the sequence in which lambs consume foods with different secondary compounds can affect intake of those foods. In three trials, I determined whether the percent of time lambs spent foraging was affected when they were offered 1) monocultures versus mixtures of trefoil and fescue, alfalfa and trefoil, and alfalfa and fescue in different sequences. I also determined how intake of alfalfa pellets was affected when lambs grazed under each of these conditions. I assumed lambs would use pellets to compensate for the nutrients that they were unable to consume while grazing. For all trials, lambs grazed for a period of 45 min in one forage (sequence 1) and then for a second period of 45 min (sequence 2) in a different forage (Mixture, Treatment) or again on the same forage (Monoculture, Control). After grazing, all lambs received alfalfa pellets in pens. Lambs grazed on alternate days and during the days when they did not graze they received alfalfa pellets in pens.

For Trial 1, I examined the relationship between forages with tannins (trefoil) and alkaloids (fescue). Based on structural characteristics and binding affinities, I predicted that lambs offered trefoil and fescue would spend more time foraging on fescue and

trefoil and eat less alfalfa pellets. Conversely, I predicted lambs on monocultures would eat more alfalfa pellets, thus reducing the food-limiting effects of a meal of plants with only alkaloids or tannins, as they would not spend as much time foraging as the lambs that ate forages with complementary secondary compounds. I also predicted lambs would graze more, and thus eat fewer pellets, when they ate the high-tannin trefoil before they ate high-alkaloid tall fescue. Forage type and sequence influenced time spent foraging and amount of alfalfa pellets eaten, but the effects involved complex, higher-order interactions that were subtle and different from what I predicted for sequences.

For Trial 2, I examined the relationship between forages with tannins (trefoil) and saponins (alfalfa). Based on structural characteristics and binding affinities, I predicted that forage intake would be higher when lambs ate a combination of high-tannin and high-saponin forages as compared with eating only high-tannin or high-saponin forage. Lambs spent similar amounts of time grazing on monocultures and mixtures, but they often ate less alfalfa pellets if they foraged on a sequence than on a monoculture. This is consistent with findings that tannins (trefoil) and saponins (alfalfa) interact in the gastrointestinal tract in ways that enable higher intake of a combination of foods than of a food that contains either compound alone.

For Trial 3, I examined the relationship between forages with saponins (alfalfa) and alkaloids (fescue). I predicted that lambs given a choice would spend more time foraging on alfalfa and tall fescue and eat less alfalfa pellets, while lambs on monocultures would eat more alfalfa pellets, as they were unable to eat as much as lambs given a choice of forages with a complementary secondary compounds. When lambs

were offered *only* alfalfa or fescue (Monoculture, Control) they grazed more in the first sequence (97%, sequence 1) than in the second sequence of 45 min (94%, sequence 2). They tended to eat more alfalfa pellets after foraging on monoculture (499g) compared with a mixture (418g). This suggests lambs may satiate more on a monoculture of fescue or alfalfa than when grazing on a mixture of the two forages. When lambs grazed *both* alfalfa and fescue they grazed more in the second sequence (98%, sequence 2) than in the first sequence (94%, sequence 1). This could be due to secondary compound complementarities as the lambs grazed two forages. Lambs could also have satiated on the first forage and the increase in foraging in the second sequence could be a response to a new forage with different nutrient and secondary compound profiles.

Some of the foraging sequences I examined encouraged lambs to spend more time grazing on mixtures versus monocultures, and there is need to further explore how different plants and foraging sequences influence the level of consumption by livestock of forages on pastures containing various secondary compounds. While secondary compounds may often be a driving force in the grazing patterns of livestock, nutrients and physical characteristics may have also played a role in my results. As livestock graze they are influenced by the whole plant including nutrients and secondary compounds and we must look at their behavior as a result of their interactions with the plant as a whole.

Introduction

Little is known about how the combinations of secondary compounds found in trefoil (tannins), fescue (alkaloids), and alfalfa (saponins) might influence intake of these forages in mixtures. Ingesting various combinations of these plants in the correct

sequence may decrease the negative effects associated with their secondary compounds. When animals are able to combine plants with complementary secondary compounds they should be able to consume more (Freeland and Janzen, 1974; Provenza et al., 2003). PSC may attenuate the negative effects of each other by binding to each other and decreasing absorption. For example tannins attach to proteins and N-containing compounds and saponins bind to tannins and to cholesterol and cholesterol derivatives (Malinow et al., 1979; Kumar and Singh, 1984). If a ruminant consumes compounds such as tannins of high molecular weight that are not fat soluble the likelihood of those compounds remaining in the rumen for a long period increases. In contrast, fat-soluble compounds such as alkaloids may be absorbed very fast and the likelihood of binding to other chemicals in the rumen will diminish. Moreover, eating plants in appropriate sequences may further mitigate the negative effects of secondary compounds (Mote et al., 2008; Lyman et al., 2008). If these findings apply more broadly to pasture forages, they suggest specific mixtures and sequences will help livestock operations better utilize plants thought to be unusable or less desirable due to the negative effects of secondary compounds.

Trial 1

Objectives and hypothesis

I determined if the percent of time lambs spent foraging was affected when they grazed 1) monocultures versus mixtures of trefoil and fescue, and 2) trefoil and fescue in different sequences. I also determined how intake of alfalfa pellets was affected when

lambs grazed under each of these conditions. I assumed lambs would use pellets to compensate for the nutrients that they were unable to consume while grazing.

Lambs were assigned to 1 of 4 groups:

Group 1: birdsfoot trefoil (tannins) → tall fescue (alkaloids) → alfalfa pellets

Group 2: tall fescue (alkaloids) → birdsfoot trefoil (tannins) → alfalfa pellets

Group 3: tall fescue (alkaloids) → tall fescue (alkaloids) → alfalfa pellets

Group 4: birdsfoot trefoil (tannins) → birdsfoot trefoil (tannins) → alfalfa pellets

Based on my hypotheses about complementary secondary compounds, I predicted lambs in groups 1 and 2 offered forages in different sequences would spend more time foraging on fescue and trefoil and eat less alfalfa pellets, while lambs in groups 3 and 4 would eat more alfalfa pellets, as they would not eat as much on pasture as lambs in groups 1 and 2, who ate forages with complementary secondary compounds, thus reducing the presumed intake-limiting effects of a meal of only one food that contained either alkaloids or tannins. I also predicted that lambs would graze more, and thus eat fewer pellets, when they ate the high-tannin trefoil before they ate high-alkaloid tall fescue because tannins already in the gut would bind to alkaloids as lambs ate fescue.

Methods

Plant species with high 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 (*Festuca arundinaceum, Kentucky 31 endophyte-infected*) (Rottinghaus et al., 1991; Aldrich, 1993) birdsfoot trefoil (*Lotus corniculatus variey Goldie*) with high tannins (Terrill et al., 1992; Hedqvist et al., 2000),

and alfalfa (*Medicago* sativa *variety Vernal*) with high saponins (ARS, 1963; Pedersen et al., 1976). Our chemical analysis of each plant species confirmed appropriate levels of plant secondary compounds, which correlate with documented concentrations (Unpublished data). During my trials, the average dry matter weight in tall fescue pastures was 1.52 tons/ha, in alfalfa pastures was 0.97 tons/ha, and in birdsfoot trefoil pastures was 1.25 tons/ha. The tall fescue pastures averaged 2.5% nitrogen (N) and 58% neutral detergent fiber (NDF), the alfalfa pastures averaged 3.3% N and 50% NDF, and the birdsfoot pastures averaged 3.1% N and 41% NDF.

Twenty-four lambs of similar age were randomly divided into eight groups of three lambs (2 groups/treatment). Because lambs are reluctant to graze in isolation, we formed groups of 3 lambs at random and this was considered the experimental unit. Once formed, the same groups of 3 lambs, identified by specific numbers spray painted on their wool, were always tested together. Lambs were 4 mo old and weighed an average of 51 kg. Lambs were born in March and reared on native-grass pastures. I moved them from native pastures to pastures in Lewiston in June. Lambs (6/pen) were kept in pens (6m x 6m) next to the pastures when they were not grazing. They had shade and ad libitum access to water and salt in the pens. All procedures were approved by the Animal Care and Use Committee (Approval # 1320).

For each 14-day trial, there were 2 spatial replications of each of the 4 aforementioned treatments. Each pasture within a replication was 0.23 ha in size (Figure 1). Lambs were randomly assigned to 1 of 8 groups, each of which was further split into 2 groups of 3 lambs. It was not possible to have both groups within a treatment graze

each day so groups grazed on alternate days. The first group grazed on even days and the second group grazed on odd days.

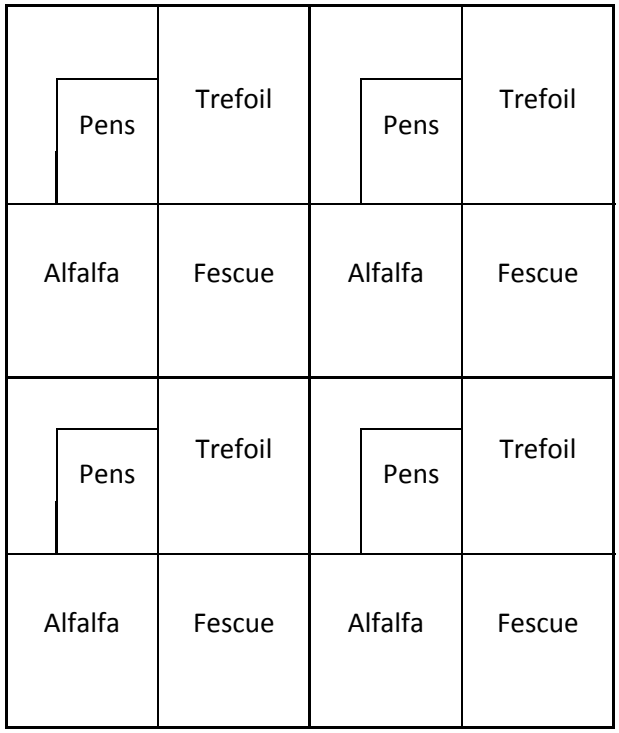

Figure 1. Physical layout of experimental pastures and holding pens.
The total area was 9 acres, and each holding pen was 18m by 20m.

Each morning at 0700, the lambs that grazed that day were moved from group pens to the first pasture where they grazed for 45 min. For my study, sequences are referred to as sequence 1, which is the forage lambs were offered for the first 45 min, and sequence 2, which is the forage lambs were offered in the second 45 min. Lambs offered two different forages were moved to the next pasture after 45 min, while lambs that grazed only one type of forage remained on the same pasture for another 45 min. Lambs

were then put back into the group pens until 1700 when the procedure was repeated. I conducted the trials in the morning and evening because there is evidence plants have higher sugar concentrations and are more nutritious in the evening than in the morning (Fisher et al., 1999; Burritt et al., 2005).

After grazing, lambs were offered alfalfa pellets ad libitum (1000g to 1500g) for 30 min, at which time the remaining alfalfa pellets were removed and weighed. Lambs that did not graze remained in individual pens and were offered alfalfa pellets ad libitum for 2 h, which was the same amount of time the other lambs spent grazing and eating alfalfa pellets. Each group of lambs remained together in a group pen overnight.

Each lamb was marked with spray paint for visual recognition. I used scan samples to estimate the percent of time lambs spent foraging (Altman, 1974). I scanned all lambs at 3-min intervals and recorded whether individuals were grazing and if they were eating something other than trefoil or fescue during the 90 min they grazed each morning and each evening. From the scan samples, I was able to calculate the percentage of time each lamb foraged in each of the pastures in each sequence.

I assumed the amount of time spent grazing reflected the intake of the lambs, which is the case assuming similar bite sizes and bite rates on a particular pasture. By measuring intake of alfalfa pellets, I was able to indirectly assess intake on pasture by measuring how much less they ate after foraging on the experimental pastures.

For the scan samples, the statistical design for the analysis of variance was a split-split plot with 2 spatial replications of 4 treatments with 3 lambs in each replication. The whole-plot was a 2 x 2 factorial with food (fescue or trefoil), choice (yes or no) and their

interactions. The sub-plot was sequence (fescue→trefoil or trefoil→fescue) and its interactions with food and choice. The sub-sub-plot was time (morning or afternoon) and its interactions with food, choice and sequence. The repeated measure was day (n = 14) and its interactions with food, choice, sequence and time. The dependent variable was percent scans foraging for each lamb. The variables are defined as food (fescue, trefoil, or alfalfa), choice (one forage or two forages), sequence (first 45 min or second 45 min), time (morning or afternoon), and day (day of trial from 1 to 7 or 8).

For the intake of alfalfa pellets, the statistical design for the analysis of variance was a split-split plot with 2 spatial replications of 4 treatments with 3 lambs in each replication. The whole-plot was a 2 x 2 factorial with food (fescue or trefoil), choice (yes or no) and their interactions. The sub-plot was time (morning or afternoon) and its interactions with food and choice. The sub-sub-plot was graze (yes or no) and its interactions with food, choice and time. The repeated measure was day (n = 14) and its interactions with food, choice, sequence and time. The dependent variable was intake of alfalfa pellets by each lamb. The variables are defined as food (fescue, trefoil, or alfalfa), choice (one forage or two forages), time (morning or afternoon), graze (each day lambs either grazed on pasture or did not) and day (day of trial from 1 to 7 or 8).

Means were compared using the LSD test. Due to small sample sizes, I consider differences between means significant at $P<0.10$.

Results

Scan samples on pastures. For all four treatment groups, lambs foraged significantly more in the first 45 min than in the second 45 min (97% vs. 87%;

P=0.0786), but the degree to which this pattern occurred was influenced by food, choice, sequence, and time (P=0.0917; Figure 2). Lambs offered *only* trefoil and *only* fescue tended to spend more time grazing in the first sequence (AM 1) than in the second sequence (AM 2) in the morning (P<0.20) (Figure 2).

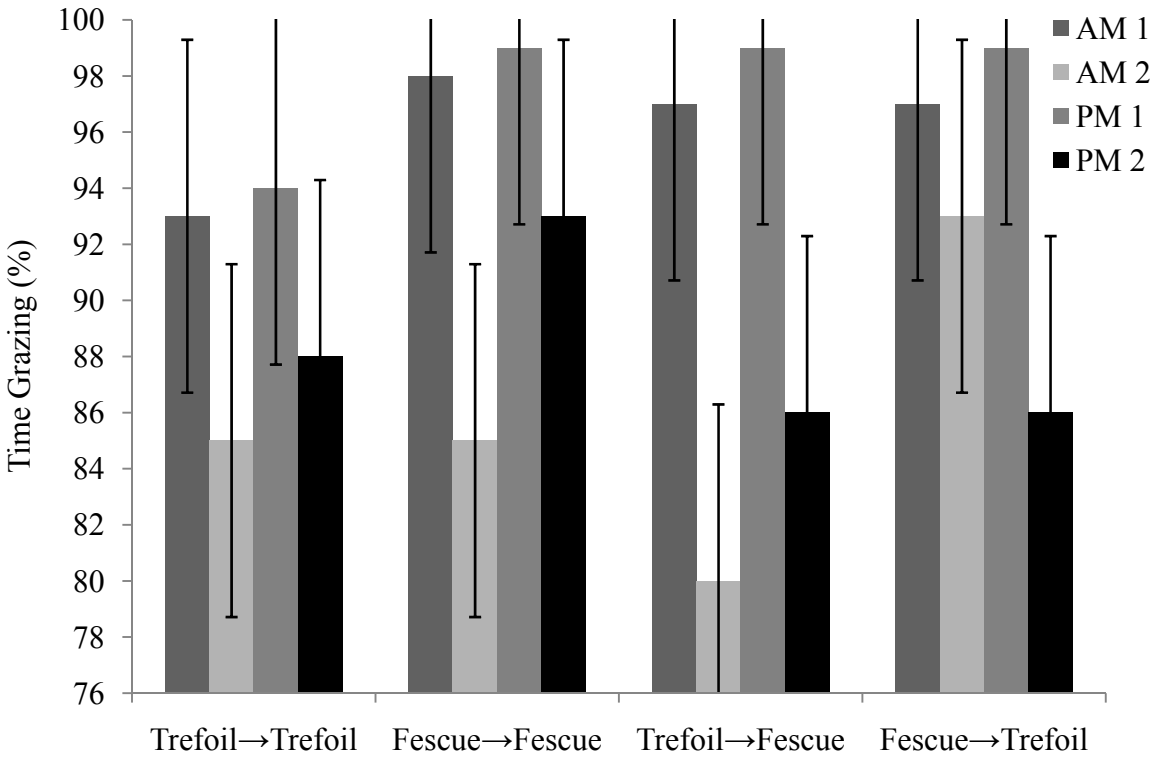

Figure 2. Interaction between food, choice, sequence, and time for lambs grazing on monocultures or mixtures in different sequences in Trial 1. Bars are standard errors.

When lambs were grazing a mixture and the sequence was trefoil→fescue, they spent significantly more time grazing trefoil than fescue in the morning (P<0.05) and in the afternoon (P<0.10) (Figure 2). However, when the sequence was fescue→trefoil,

there was no significant difference in the time lambs spent grazing fescue and trefoil in the morning (P>0.20), but they tended to spend more time grazing fescue than trefoil in the afternoon (P<0.20) (Figure 2).

Intake of pellets in pens. Lambs ate less alfalfa pellets when they grazed on pasture than when they did not graze (720 vs. 1,067g; P<0.0001). Time, graze, and day interacted (P=0.0019; Figure 3), and cyclic patterns of intake were evident throughout the trial (Figure 3). Neither forage (P=0.2216) nor its presentation (as a mixture or a monocultures) affected intake of alfalfa pellets (P=0.5474; Figure 4).

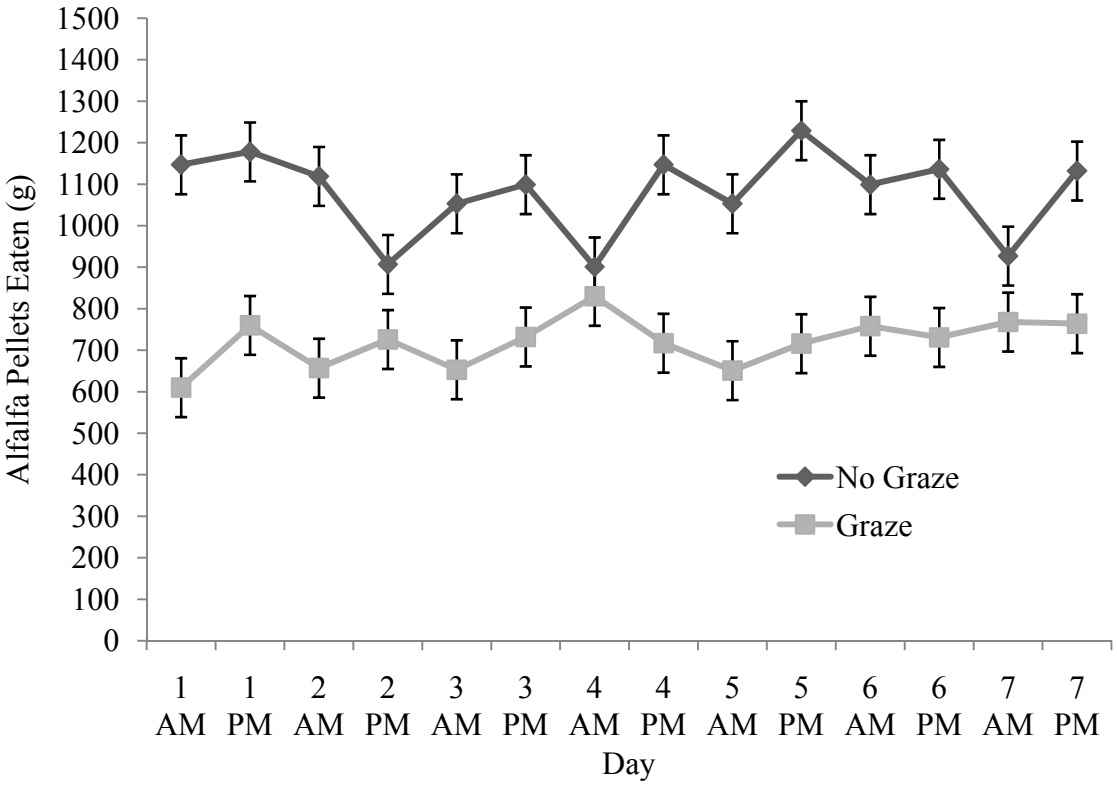

Figure 3. Interaction between time, graze, and day when lambs grazed or did not graze on monocultures or mixtures in different sequences in Trial 1. Bars are standard errors.

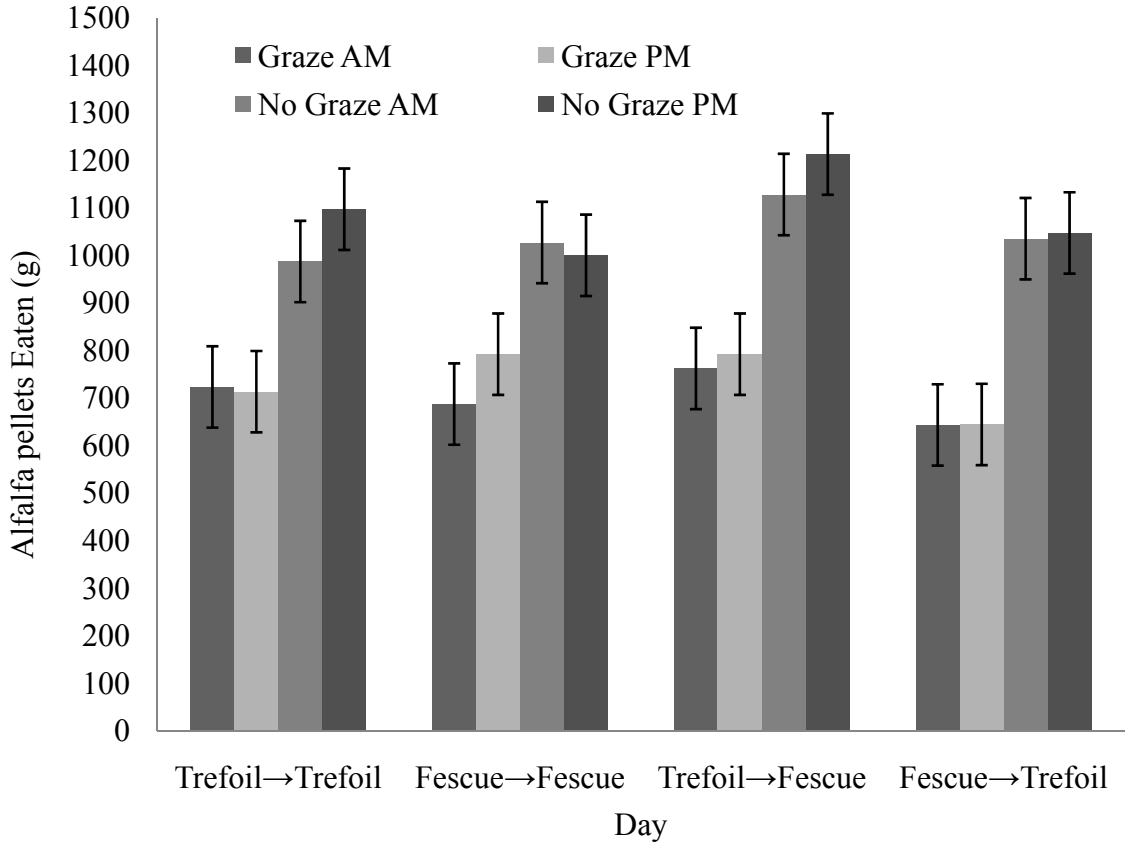

Figure 4. Grams of alfalfa pellets consumed by lambs foraging in monocultures or mixtures and in different sequences in Trial 1. Bars are standard errors.

Discussion

I hypothesized that lambs offered forages in the sequence of trefoil→fescue or fescue→trefoil would spend more time foraging on fescue and trefoil and eat less alfalfa pellets, while lambs in the sequence fescue→fescue and trefoil→trefoil would eat more alfalfa pellets as they would not spend as much time foraging as the lambs that ate forages with complementary secondary compounds, thus reducing the food-limiting effects of a meal of plants with only alkaloids or tannins. I also predicted lambs would

graze more, and thus eat fewer pellets, when they ate the high-tannin trefoil before they ate high-alkaloid tall fescue. Forage type and sequence influenced time spent foraging or amount of alfalfa pellets eaten, but the effects involved complex, higher-order interactions that were subtle and different from what I predicted for sequences. Lambs supplemented their intake on pastures with alfalfa pellets. They ate less pellets after they grazed on pasture and increased their intake of pellets on the days they did not graze no matter what sequence they had grazed the previous day (Figures 3 and 4).

Linking time foraging and pellet intake in monocultures. Lambs on a monoculture spent similar time grazing trefoil and fescue (Figure 2), and they ate similar amounts of alfalfa pellets (Figure 4). These findings suggest lambs ingested similar amounts of trefoil and fescue when foraging on monocultures.

Linking time foraging and pellet intake in mixtures. Lambs that grazed in the sequence trefoil→fescue spent more time grazing trefoil than fescue in the morning and in the afternoon (Figure 2). Conversely, lambs that grazed in the sequence fescue→trefoil spent similar amounts of time grazing fescue and trefoil in the morning and they tended to spend more time grazing fescue than trefoil in the afternoon (Figure 2). Lambs in the fescue→trefoil sequence also tended to consume less pellets when they returned from grazing and less pellets than the trefoil→fescue group when they did not graze (Figure 4). This suggests that lambs in the fescue→trefoil sequence were better able to meet their nutritional needs on pasture than lambs foraging in the trefoil→fescue sequence. It may be as well that the sequence trefoil→fescue→alfalfa pellets influenced lambs to eat more alfalfa pellets than the sequence fescue→trefoil→alfalfa pellets. Fescue has a higher

sugar concentration in the afternoon relative to its sugar concentration in the morning. This may have contributed to lambs preferring fescue first in the afternoon. Trefoil has a better nutritional value than fescue and lambs may have preferred trefoil to fescue in the morning. Lambs also may have satiated on a legume (trefoil) and had less motivation to compliment with a legume compared to a lamb coming from a grass (fescue) to eat alfalfa pellets (legume).

In digestion balance trials carried out at the same time on the same pastures, lambs offered trefoil and fescue in the sequence trefoil→fescue consumed only 13% of their daily intake from trefoil (76g of 587g) (Owens, 2008). Nonetheless, that a small amount of trefoil in their diet increased intake compared with lambs fed fescue only, and the trefoil provided nutritional benefits as lambs digested more energy and nitrogen when offered trefoil and fescue as opposed to fescue alone. Thus, trefoil helped lambs meet their nutritional needs when combined with fescue.

Comparing time foraging and pellet intake in monocultures and mixtures. Based on the complementary forage hypothesis, I predicted lambs would graze more on pasture and consume less alfalfa pellets when they grazed two different forages than when they grazed only on a monoculture. However, the patterns did not differ for mixture versus monoculture for time spent grazing (Figure 2) or intake of alfalfa pellets (Figure 4).

I also predicted lambs would graze more and eat fewer pellets when they ate high-tannin trefoil before they ate high-alkaloid fescue, but I found the opposite. In the first sequence in the morning, lambs in the fescue→trefoil sequence spent as much time

grazing fescue as lambs in the trefoil→fescue sequence spent grazing trefoil. In the second sequence in the morning, however, lambs in the fescue→trefoil group grazed significantly more on trefoil than lambs in the trefoil→fescue group grazed on fescue (Figure 2). This behavior may have been due to interactions among nutrients and secondary compounds. For instance, trefoil is more nutritious than fescue and it also contains tannins. In the morning, the nutrients and the tannins could have caused lambs to eat more trefoil following a meal of fescue both for a nutrient boost and to alleviate the effects of the alkaloids in the fescue they just ingested (Provenza et al., 2003). Those effects may not be so pronounced in the afternoon compared to the morning as fescue is more nutritious in the afternoon than in the morning (Fisher et al., 1999).

This is in contrast to studies where cattle spent markedly more time foraging when the sequence is trefoil→fescue than when the sequence is fescue→trefoil (Lyman, 2008). In those studies, cattle steadily decreased time eating tall fescue from 40% to 15% when they first grazed tall fescue alone for 30-min followed by birdsfoot trefoil alone for 60-min. When the sequence was reversed, they foraged actively on both trefoil and fescue throughout the 90-min meal (Lyman, 2008).

It is not clear why sheep and cattle differ with regard to the sequences of ingesting trefoil and fescue. In studies with cattle, the basal ration was an orchard grass pasture, while in the studies with sheep the basal ration was alfalfa pellets. Both plants are nutritious and should complement fescue and trefoil. Thus, it is not likely they would affect the pattern of food ingestion in the sequences. Fescue and trefoil are both high in secondary compounds that limit intake, though their effects evidently differ for sheep and

cattle in ways that may have affected food preferences during the various sequences. Cattle grazed trefoil and fescue every day, while lambs grazed trefoil and fescue every other day, which may have allowed lambs enough time to detoxify reducing the effects of tannins and alkaloids compared to cattle that grazed trefoil and fescue every day.

Trial 2

Objectives and hypothesis

In Trial 2, I examined the relationship between forages with tannins (trefoil) and saponins (alfalfa). 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). Based on these findings and the aforementioned structural characteristics and binding affinities, I hypothesized forage intake would be higher when lambs ate a combination of high-tannin and high-saponin forages as compared with eating only a high-tannin or a high-saponin forage.

Lambs were randomly assigned to the following groups:

Group 5: alfalfa (saponins) → birdsfoot trefoil (tannins) → alfalfa pellets

Group 6: birdsfoot trefoil (tannins) → alfalfa (saponins) → alfalfa pellets

Group 7: birdsfoot trefoil (tannins) → birdsfoot trefoil (tannins) → alfalfa pellets

Group 8: alfalfa (saponins) → alfalfa (saponins) → alfalfa pellets

Based on my hypothesis, I predicted that lambs in groups 5 and 6 would spend more time foraging on birdsfoot trefoil and alfalfa and consume less alfalfa pellets, while lambs in groups 7 and 8 would consume more alfalfa pellets, as they would not graze as

much as lambs in groups 5 and 6, who would eat forages with complementary secondary compounds to reduce the negative effects of a meal of only saponins or tannins.

Methods

I used the same methods and procedures in Trial 2 as in Trial 1, but with 24 new lambs (average weight 51 kg). Trial 2 lasted 14 d.

Results

Scan samples on pastures. Percent time foraging was influenced by forage type, choice, and sequence (P=0.0105). Lambs that grazed *only* trefoil foraged less in the first than in the second 45 min foraging bout (84% vs. 99% P<0.05), whereas lambs that grazed *only* alfalfa foraged more in the first than in the second 45 min bout (100% vs. 89% P<0.05). Lambs that foraged in the sequence alfalfa→trefoil spent more time grazing in alfalfa compared to trefoil (97% vs. 91%; P<0.05) and lambs foraging in the sequence trefoil→alfalfa tended to spend more time grazing trefoil compared to alfalfa (98% and 94%; P<0.20).

Food, choice and time interacted (P=0.0650; Figure 5). Lambs that grazed *only* alfalfa spent more time grazing alfalfa in the morning than in the afternoon (P<0.10), whereas lambs that grazed *only* trefoil spent less time grazing trefoil in the morning than in the afternoon (P<0.05). Lambs that grazed in the sequence trefoil→alfalfa did not spend a greater amount of time in either trefoil or alfalfa, but lambs in the sequence alfalfa→trefoil spent less time grazing in the morning than in the evening (P<0.10).

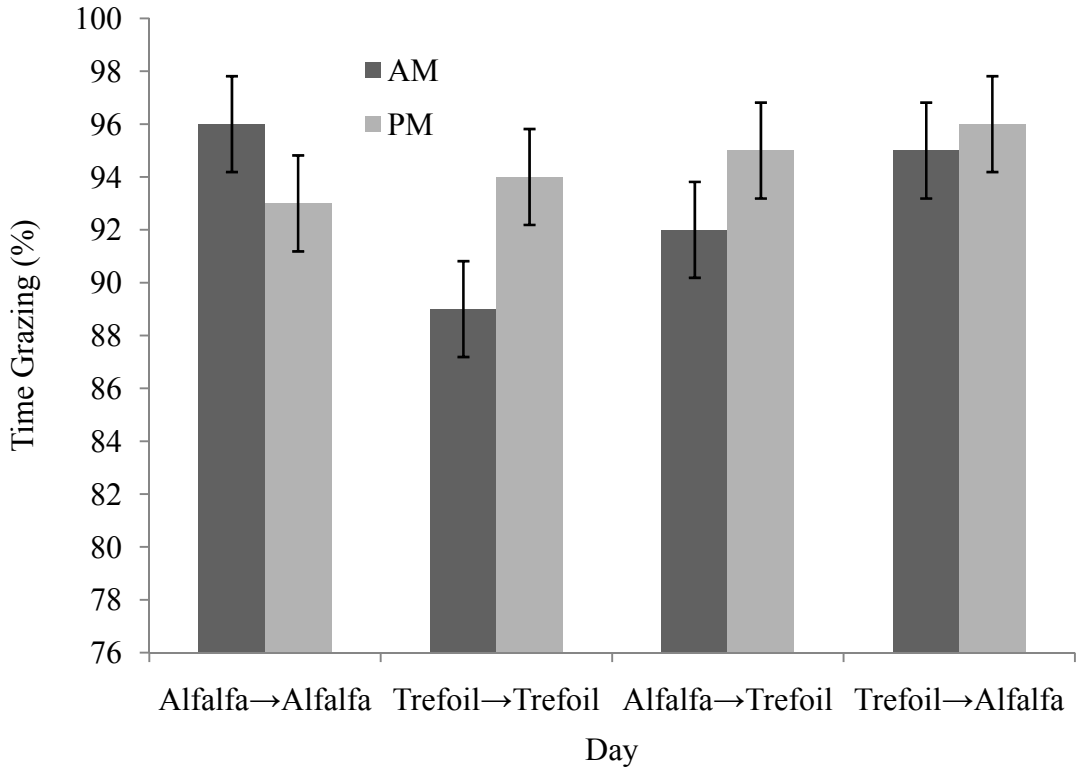

Figure 5. Interaction between food, choice, and time by lambs foraging in monocultures or mixtures and in different sequences in Trial 2. Bars are standard errors.

Sequence, time and day interacted (P=0.0299). Lamb foraging behavior was cyclic throughout the trial (Figure 6). In the morning lambs spent more time grazing in sequence 1 on day 7 ($P<0.05$) and more time grazing in sequence 2 on day 8. In the evening lambs spent significantly more time grazing in sequence 2 on days 4, 5, and 8 ($P<0.05$) and lambs spent more time grazing in sequence 2 on day 6 ($P<0.10$).

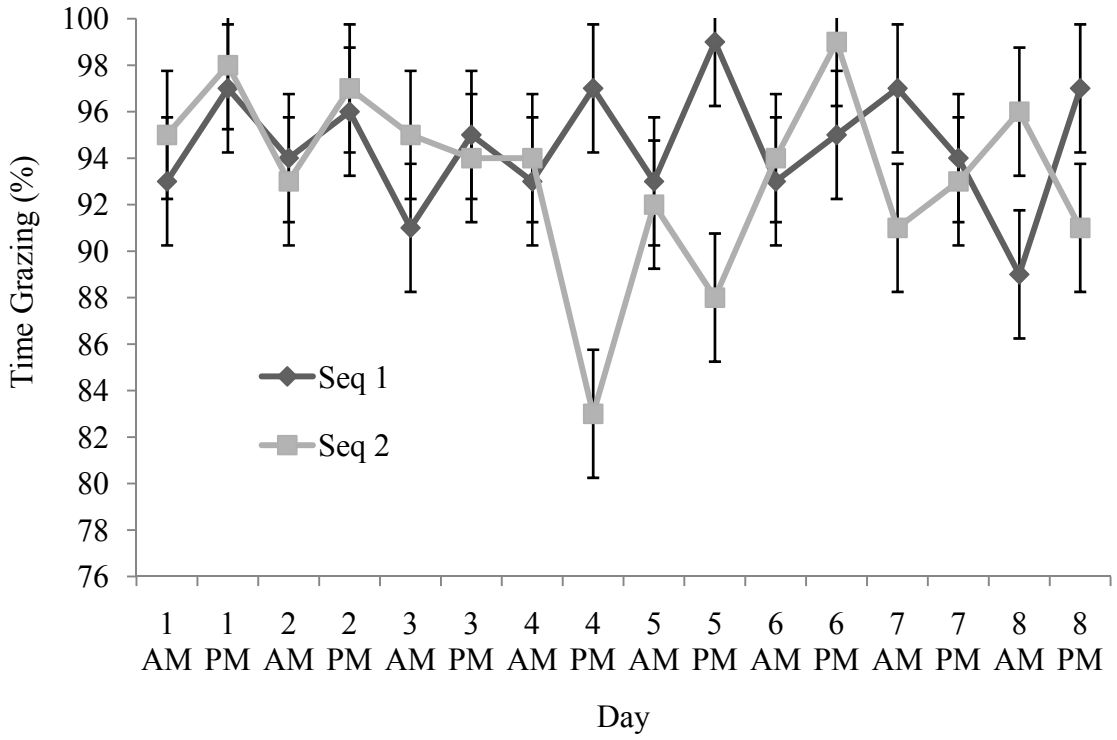

Figure 6. Interaction between sequence, time, and day by lambs foraging in monocultures or mixtures and in different sequences in Trial 2. Bars are standard errors.

Intake of pellets in pens. Intake of alfalfa pellets was influenced by forage type, choice, and whether or not lambs grazed (P=0.0044). Lambs that grazed *only* trefoil ate more alfalfa pellets than lambs that grazed *only* alfalfa on days when they grazed (799g vs. 434g; P<0.05), and the same occurred when they did not graze (1,270g vs. 1,192g; P<0.5). Intake of alfalfa pellets did not differ for lambs that grazed in the sequence trefoil→alfalfa or alfalfa→trefoil (573g vs. 535g, respectively; P>0.2) on days that they both grazed. When they did not graze, lambs in the trefoil→alfalfa group ate more alfalfa pellets compared to the alfalfa→trefoil group (1,207g vs. 1,112g; P<0.05). When lambs

grazed mixtures or monocultures they all ate more alfalfa pellets on days they did not graze compared with days that they did graze ($P<0.05$).

Choice, graze, and day interacted ($P=0.0270$; Figure 7). During the days when all animals had only alfalfa pellets, lambs in the mixed-forage treatment ate less alfalfa pellets on days 1 and 2 ($P<0.05$) and 3 and 4 ($P<0.10$) compared with lambs that grazed monocultures. On days when they grazed, lambs on monocultures ate more alfalfa pellets than lambs offered a mixture of forages on days 6 to 8 ($P<0.05$).

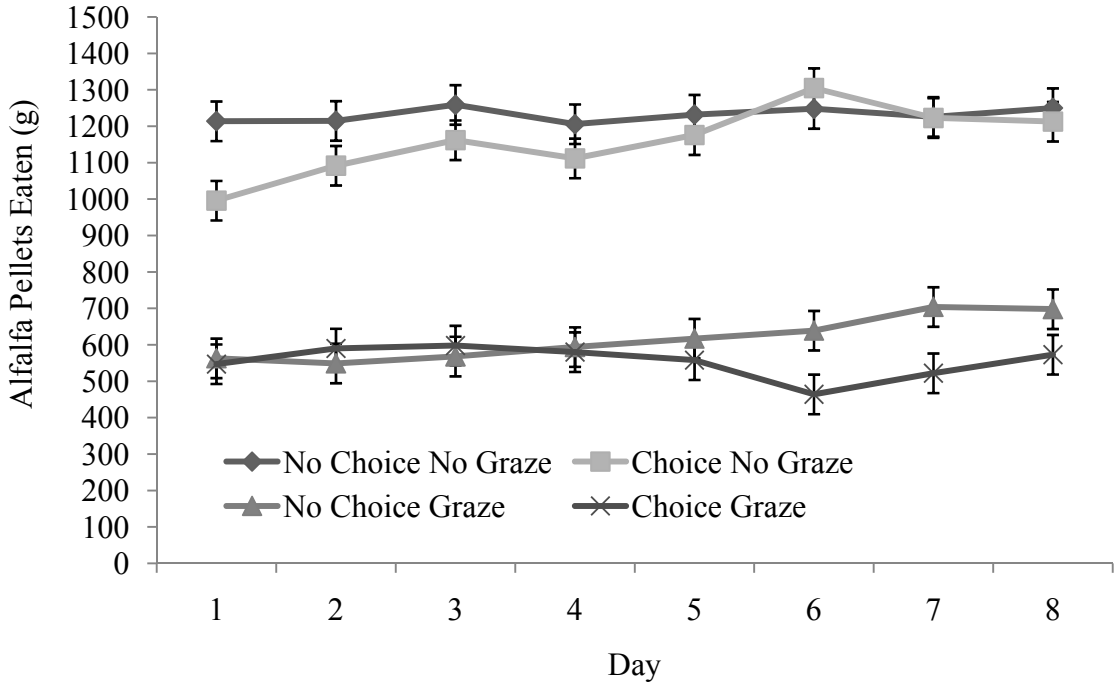

Figure 7. Interaction between choice, graze, and day by lambs foraging in monocultures (no choice) or mixtures (choice) and in different sequences in Trial 2. Bars are standard errors.

Time, graze, and day also interacted (P=0.0754; Figure 8). Lambs ate less alfalfa pellets if they grazed than if they did not graze (P<0.05), and they generally ate more pellets in the evening than in the morning. When lambs did not graze, they ate more pellets in the evening of days 1 to 4 (P<0.05), there was a trend of eating more alfalfa pellets in the evening on day 5, and on days 6 to 8 there was no difference in the amount of alfalfa pellets consumed (P>0.20). When lambs grazed, they ate more pellets in the evening on all days (P<0.05), except day 7 when there was a trend of higher consumption in the afternoon (P<0.10). Cyclic patterns of intake were most evident for lambs on days when they did not graze for both morning and evening throughout the trial.

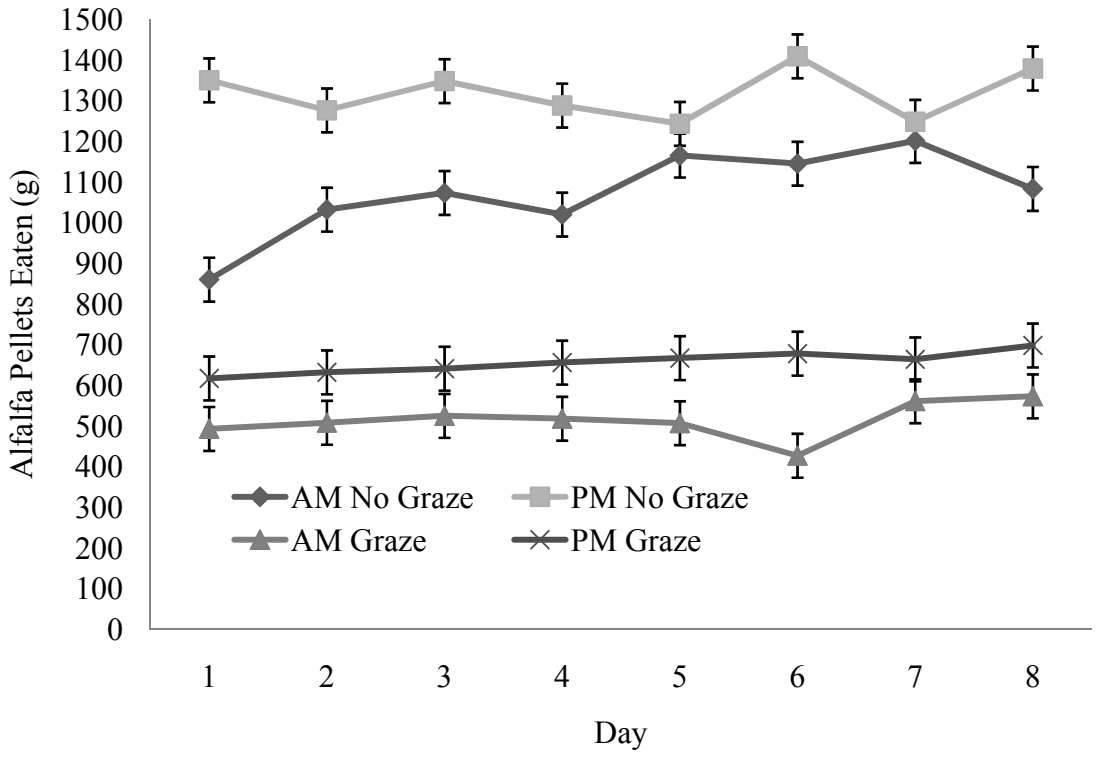

Figure 8. Interaction between time, graze and day by lambs foraging in monocultures or mixtures and in different sequences in Trial 2. Bars are standard errors.

Discussion

I hypothesized that lambs would spend more time grazing when they ate a combination of high-tannin and high-saponin forages as compared with eating only high-tannin or high-saponin forage. I also predicted lambs that ate a combination of forages would eat less alfalfa pellets than lambs that ate only one forage.

Linking time foraging and pellet intake in monocultures. Lambs on a monoculture of either trefoil or alfalfa spent similar times grazing, but lambs that grazed *only* trefoil ate significantly more alfalfa pellets than lambs that grazed *only* alfalfa on days when they grazed (799g vs. 434g), and the same occurred when they did not graze (1,270g vs. 1,192g). These results may be due both to lower preference for trefoil and satiation on alfalfa. Lambs evidently preferred alfalfa to trefoil, which is consistent with the findings in digestion balance trials carried out at the same time on the same pastures (Owens, 2008). Those trials showed lambs offered fresh-cut trefoil and fescue consumed only 13% of their daily intake from trefoil (76g of 587g), whereas lambs fed fresh-cut alfalfa and fescue consumed about 27% of their daily intake as alfalfa (215g out of 783g). Lambs on a monoculture of trefoil also may be responding to the variety of grazing trefoil and then eating alfalfa pellets, whereas the lambs grazing on alfalfa may satiate on alfalfa in pasture and consequently not eat as many alfalfa pellets (Provenza, 1996). Lambs that previously grazed alfalfa could have satiated on alfalfa and therefore consumed less alfalfa pellets when they returned from grazing.

Lambs offered only alfalfa tended to spend more time grazing in the morning than in the afternoon, whereas lambs offered only trefoil tended to spend more time grazing in

the evening than in the morning. Sheep prefer clover to grass in the morning and then switch to grass in the afternoon (Parsons et al., 1994), likely due to interactions involving primary and secondary compounds (Provenza et al., 2003). Lambs in my study likely responded to primary and secondary compounds in alfalfa and trefoil.

I speculate that alfalfa was relatively more nutritious than trefoil in the morning, hence the greater time foraging on alfalfa in the morning, whereas trefoil was relatively more nutritious in the afternoon. Forages are generally higher in energy in the afternoon than in the morning so lambs may have been better able to meet their needs for energy in the afternoon (Fisher et al., 1999; Burritt et al., 2005), with less time spent grazing alfalfa and more time grazing trefoil. The high-tannin content of trefoil may have caused lambs to avoid it more in the mornings and to use it more in the afternoon, when nutritive value relative to tannins was likely greater (Bryant et al., 1983).

Linking time foraging and pellet intake in mixtures. Lambs did not spend more time grazing in the sequence alfalfa→trefoil than trefoil→alfalfa, but lambs that grazed in the sequence trefoil→alfalfa ate more pellets on days when they did not graze (1,207g vs. 1,112g). This suggests consuming trefoil (tannins) before alfalfa (saponins) was not beneficial under the conditions of my study, and lambs may have compensated for the lower intake of trefoil by consuming alfalfa pellets to meet their nutritional needs. Lambs that ate alfalfa (saponins) before trefoil (tannins) appeared to benefit from this sequence and evidently required less alfalfa pellets to meet their nutritional needs.

Comparing time foraging and pellet intake in monocultures and mixtures. Lambs spent similar time grazing on monocultures and mixtures (Figure 5). They ate

more alfalfa pellets when they returned from grazing only one forage in either sequence compared with lambs that grazed two difference forages (days 6 to 8 choice, graze, and day; $P<0.05$). When lambs did not graze they ate less alfalfa pellets on days 1 to 2. This is consistent with findings that tannins (trefoil) and saponins (alfalfa) interact in the gastrointestinal tract in ways that enable higher intake of a combination of foods that contain tannins and saponins than of a food that contains either compound alone. When mice consume tannins and saponins at the same time they do not experience the adverse effects of tannins or saponins alone (Freeland et al., 1985).

Regardless of context (monoculture or mixture), sequence and whether or not lambs grazed, they ate more pellets in the evening than in the morning throughout this trial (Figure 8). This behavior evidently was not because the lambs ate less while on pasture in the evenings, as the same behavior occurred for lambs that did not graze. Rather, it suggests a greater appetite in the evening than in the morning.

Trial 3

Objectives and hypothesis

In trial 3, I examined the relationship between forages with saponins (alfalfa) and alkaloids (fescue). Based on the aforementioned structural characteristics and binding affinities, I hypothesized that forage intake would be higher when lambs ate a combination of saponin- and alkaloid-containing forages as compared with eating only a high-alkaloid or a high-saponin forage.

Lambs were randomly assigned to the following groups:

Group 9: alfalfa (saponins) → tall fescue (alkaloids) → alfalfa pellets

Group 10: tall fescue (alkaloids) → alfalfa (saponins) → alfalfa pellets

Group 11: tall fescue (alkaloids) → tall fescue (alkaloids) → alfalfa pellets

Group 12: alfalfa (saponins) → alfalfa (saponins) → alfalfa pellets

Based on my hypothesis, I predicted that lambs in groups 9 and 10 would spend more time foraging on alfalfa and tall fescue and eat less alfalfa pellets, while lambs in groups 11 and 12 would eat more alfalfa pellets, as they were unable to eat as much as lambs in groups 9 and 10, who ate a forage with a complementary secondary compounds to reduce the negative effects of a meal of only saponins or alkaloids.

Methods

I used the same methods and procedures in Trial 3 as in Trials 1 and 2, but with 24 new lambs (average weight 36 kg). Trial 3 lasted 14 d.

Results

Scan samples on pastures. Forage choice and sequence interacted ($P=0.0624$). When lambs grazed *only* alfalfa or fescue they tended to graze more in the first sequence than in the second sequence (97% and 94%, respectively; $P<0.20$). When lambs grazed *both* alfalfa and fescue they tended to graze more in the second sequence than in the first sequence (98% and 94%; $P<0.20$).

Food and sequence interacted ($P=0.0265$). When grazing only alfalfa or fescue, lambs tended to spend more time grazing alfalfa in the first sequence than in the second sequence (97% and 93%, respectively; $P<0.10$), and they tended to spend less time

grazing fescue in the first sequence (92%) than in the second sequence (92% and 99%, respectively; P<0.10).

Finally, sequence, time and day interacted (P=0.0703; Figure 9 and 10). Lambs tended to graze more during sequence 2 in the morning on days 3, 4, and 6 (P<0.20), whereas they tended to graze more during sequence 1 in the evening on days 3, 5, and 7 (P<0.20).

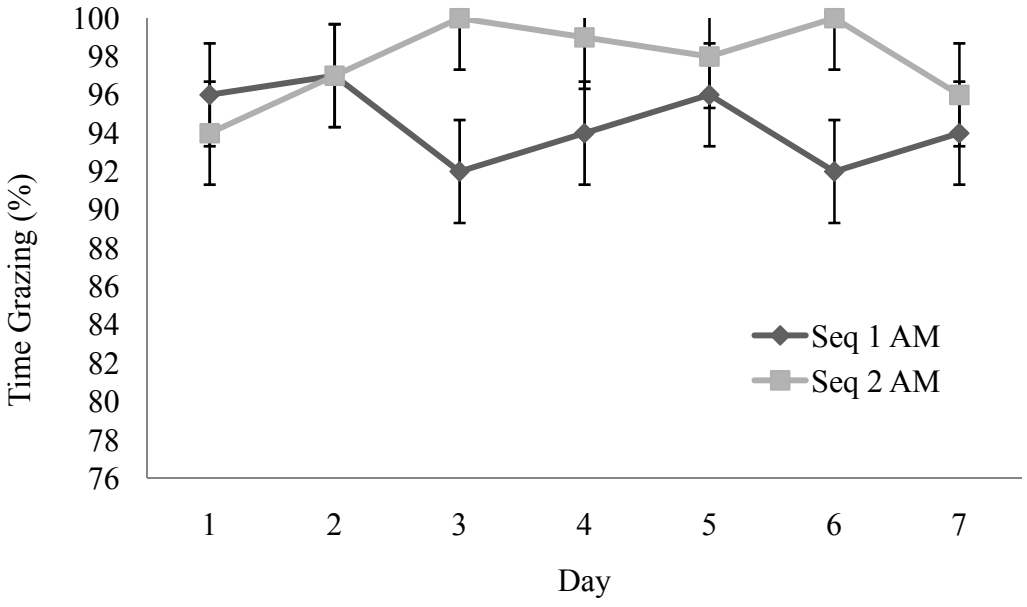

Figure 9. Interaction between sequence, time, and day for lambs grazing in two sequences in the morning in Trial 3. Bars are standard errors.

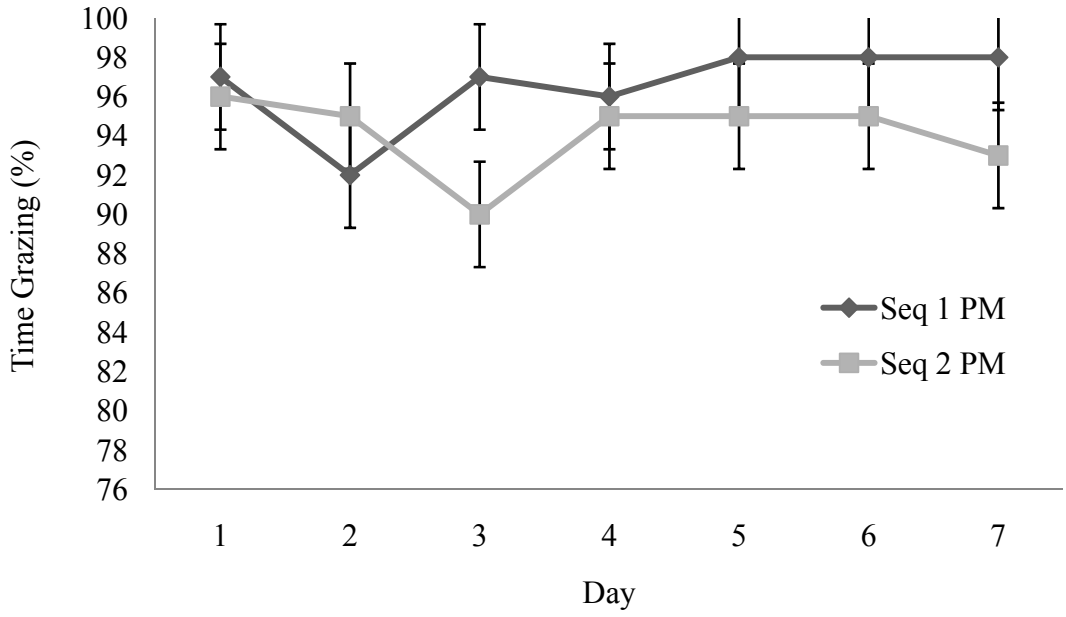

Figure 10. Interaction between sequence, time, and day for lambs grazing in two sequences in the evening in Trial 3. Bars are standard errors.

Intake of pellets in pens. Lambs ate less alfalfa pellets when they grazed than when they did not graze (459g vs. 830g; P=<0.001). Graze interacted with availability of forages (mixture vs. monoculture) (P=0.0818). When lambs grazed, they consumed more alfalfa pellets when they had previously grazed only one forage compared to two different forages (418g vs. 499g; P<0.05). When lambs did not graze, they tended to eat more alfalfa pellets if they had grazed two different forages the previous day (853g vs. 807g; P<0.2).

Time, graze, and day also interacted (P=0.0014; Figure 11). When lambs did not graze they ate more alfalfa pellets in the afternoon on days 3, 4, 6, and 7 (P<0.05). Cyclic patterns of intake were evident for all lambs in both mornings and evenings throughout the trial (Figure 11).

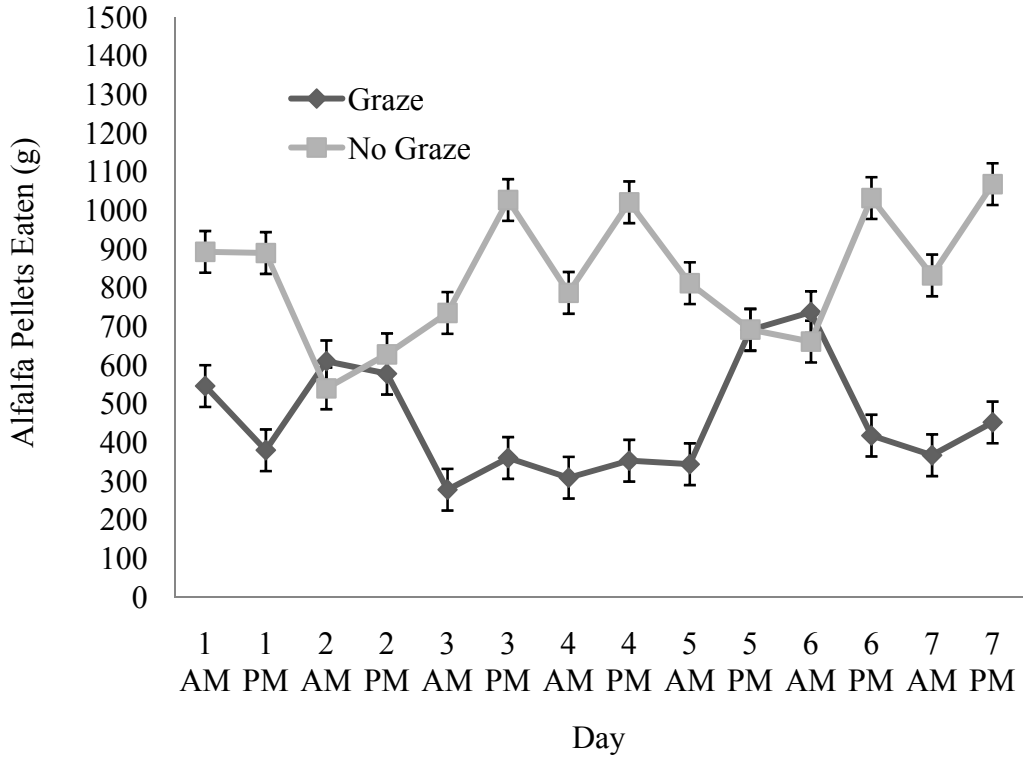

Figure 11. Interaction between time, graze, and day by lambs foraging in monocultures or mixtures and in different sequences in Trial 3. Bars are standard errors.

Discussion

Linking time foraging and pellet intake in monocultures. In Trial 3, I examined the relationship between forages with saponins (alfalfa) and alkaloids (fescue). Based on my hypothesis, I predicted that lambs given a choice would spend more time foraging on alfalfa and tall fescue and eat less alfalfa pellets, while lambs on monocultures would eat more alfalfa pellets, as they were unable to eat as much as lambs given a choice of forages with a complementary secondary compounds.

When lambs were offered *only* alfalfa or fescue they tended to graze more in the first sequence than in the second sequence (97% vs. 94%; $P<0.20$). They also ate more

alfalfa pellets after foraging on monocultures compared with mixtures on days when they grazed (499g vs. 418g; $P<0.05$). This suggests lambs satiate more on a monoculture of fescue or alfalfa than when grazing on a mixture of the two forages.

Linking time foraging and pellet intake in mixtures. When lambs grazed *both* alfalfa and fescue they grazed more in the second sequence than in the first sequence (98% vs. 94%; $P<0.20$). This behavior could be due to secondary compound complementarities as the lambs grazed two forages. Lambs could also have satiated on the first forage and the increase in foraging in the second sequence could be a response to new forage. Cattle steadily decrease time eating tall fescue when they first graze tall fescue alone for 30-min followed by alfalfa/trefoil for 60-min; when the sequence is reversed they forage actively on alfalfa/trefoil and fescue throughout the 90-min meal (Lyman, 2008). The same patterns of foraging occur when cattle are offered only alfalfa or trefoil in combination with fescue (Lyman and Provenza, unpublished data). Thus, both combination and sequence greatly influence intake of tall fescue by cattle.

Comparing time foraging and pellet intake in monocultures and mixtures. Lambs ate more alfalfa pellets after foraging on monoculture compared with mixtures on days when they grazed (499g vs. 418g; $P<0.05$). Conversely, they tended to eat more alfalfa pellets when they previously grazed on mixtures as opposed to monoculture on days when they did not graze (853g vs. 807g; $P<0.20$). This suggests lambs better met their needs for nutrients on mixtures than on monocultures.

In a related study, lambs fed a basal diet of tall fescue supplemented with alfalfa ingested 222g/d more than lambs not fed alfalfa (783g/d vs. 561g/d), yet their intake of

fescue was only 78g/d less than that for lambs fed only fescue (561g/d vs. 639g/d) (Owens, 2008). Thus, there was a synergistic effect on intake provided by alfalfa.

Having a choice on days when they grazed likely increased the amount of nutrients they were able to consume and digest. Alfalfa in combination with fescue increases the dry matter intake and digestion, nitrogen intake and retention, and Kcal digested opposed to when fescue is fed without alfalfa (Owens, 2008).

CHAPTER III

CONCLUSIONS AND RECOMMENDATIONS

I determined whether or not foraging time on various plant species and intake of alfalfa pellets as a complement to the forages on pasture increased when lambs were rotated in sequences on pastures containing a high-alkaloid variety of tall fescue (endophyte infected), a high-tannin variety of birdsfoot trefoil (Goldie), and a high-saponin variety of alfalfa (Vernal). Based on structural characteristics and binding affinities, I predicted forage intake would be higher when lambs ate 1) a combination of high-tannin and high-alkaloid containing forages as compared with eating only a high-alkaloid or a high-tannin forage, 2) a combination of high-tannin and high-saponin containing forages as compared with eating only a high-tannin or a high-saponin forage, and 3) a combination of high-saponin and high-alkaloid containing forages as compared with eating only high-saponin or high-alkaloid forages. I also predicted an increase in consumption of alfalfa pellets when lambs grazed monocultures as opposed to mixtures.

For Trial 1, I hypothesized lambs offered trefoil and fescue would spend more time foraging on fescue and trefoil and eat less alfalfa pellets, while lambs on monocultures would eat more alfalfa pellets as they would not spend as much time foraging as the lambs that ate forages with complementary secondary compounds, thus reducing the food-limiting effects of a meal of plants with only alkaloids or tannins. I also predicted lambs would graze more, and thus eat fewer pellets, when they ate the high-tannin trefoil before they ate high-alkaloid tall fescue. Forage type and sequence influenced time spent foraging and amount of alfalfa pellets eaten, but the effects

involved complex, higher-order interactions that were subtle. For Trial 2, I examined the relationship between forages with tannins (trefoil) and saponins (alfalfa). Based on structural characteristics and binding affinities, I hypothesized that forage intake would be higher when lambs ate a combination of high-tannin and high-saponin forages as compared with eating only a high-tannin or a high-saponin forage. Lambs spent similar time grazing on monocultures and mixtures, but they often ate less alfalfa pellets if they foraged on a sequence than on a monoculture when they grazed and when they did not graze. This is consistent with findings that tannins (trefoil) and saponins (alfalfa) interact in gastrointestinal tract in ways that enable higher intake of a combination of foods than of a food that contains either compound alone.

For Trial 3, I examined the relationship between forages with saponins (alfalfa) and alkaloids (fescue). Based on my hypothesis, I predicted that lambs given a choice would spend more time foraging on alfalfa and tall fescue and eat less alfalfa pellets, while lambs on monocultures would eat more alfalfa pellets, as they were unable to eat as much as lambs given a choice of forages with complementary secondary compounds. When lambs were offered *only* alfalfa or fescue they grazed more in the first sequence (97%) than in the second sequence (94%). They also tended to eat more alfalfa pellets after foraging on monoculture (499g) compared with choice (418g) on the days when they grazed. This suggests lambs may satiate more on a monoculture of fescue or alfalfa than when grazing on a mixture of the two forages. When lambs grazed *both* alfalfa and fescue they grazed more in the second sequence (98%) than in the first sequence (94%). This could be due to secondary compound complementarities as the lambs grazed two

forages. Lambs could also have satiated on the first forage and the increase in foraging in the second sequence could be a response to new forage. Alfalfa in combination with fescue increases the dry matter digestion, nitrogen intake, and Kcal digested opposed to when fescue is fed without alfalfa (Owens, 2008).

This research indicates the importance of the interactions between forages with different secondary compounds, but we are only beginning to understand the complexities involved in diet sequencing based on a limited number of forages and compounds. Herders in France use empirical understanding of forage and landscape diversity to stimulate food intake and more fully use the range of plants available by herding in grazing circuits (Hubert, 1993; Meuret et al., 1994; Meuret, 2008). The circuit includes a moderation phase, which provides sheep access to plants that are abundant but not highly preferred to calm a hungry flock; the next phase is a main course for the bulk of the meal with plants of moderate abundance and preference; then comes a booster phase of highly preferred plants for added diversity; and finally a dessert phase of palatable plants that complement previously eaten forages. Daily grazing circuits are designed to stimulate and satisfy an animal's appetite for different nutrients, and they enable animals to maximize intake of nutrients and regulate intake of different toxins. Moving animals to fresh pastures, or moving them to new areas on rangelands, has the same effect (Provenza, 1996; Bailey and Provenza, 2008). The new areas offer nutritious forages and a change of scenery. The idea of variety of foods and scenery increasing "foraging motivation" may seem counter intuitive, but to the French herders this idea is

the essence of the way they stimulate a flock's appetite throughout a grazing circuit (Meuret, 2008).

As we explore how primary and secondary compounds influence herbivores choices as they graze, we are better able to understand the reasons for their decisions. We still know little about the foraging sequences livestock should follow to enhance complementarities, but mixing appears to benefit livestock. We can use French herders as examples of how to guide sheep in foraging circuits that require empirical knowledge of the landscape and of the plants that individual sheep find palatable within and among meals across seasons.

When we rely on livestock to guide themselves, they often "eat the best and leave the rest." When we teach and provide some guidance, we can assist animals to learn beneficial sequences and encourage them to use areas previously underutilized but high in nutritious plants or high in plants with secondary compounds. For example, observations of lambs allowed free access to the forages in the pastures I used showed alfalfa in preference to trefoil and they virtually ignore tall fescue. Designing grazing sequences such as the ones I used to mitigate negative effects of secondary compounds may be effective and lower in costs compared with conventional methods of weed removal such as chemicals and machines that are fossil-fuel intensive. The role of sequences in the ability of livestock to better utilize secondary compounds requires further investigation with animals given options of different potentially complementary forages.

While the number of possible interactions of primary and secondary compounds may be great, more research is warranted to bring us closer to understanding the reasons for preferences for specific sequences and to help managers anticipate which sequences will benefit animals. Research to better understand relative rates of intake of different meals in a sequence would also be beneficial. A better understanding of forage sequences will help land managers as they consider altering the landscape by seeding pastures to assist their livestock. Also, the ability to isolate satiety from toxicological effects would be beneficial in understanding the reason for a cessation of grazing due to meeting nutrient needs or negative postingestive feedback.

As we seek to understand the role of forage sequencing in diet selection, we can look to the French herders as examples of how to use landscapes. The science behind complex interactions of sequences is yet to be fully understood, but we have strong reasons to believe that forage sequences are a vital part of how animals select and interact with their diet selections.

REFERENCES

Agricultural Research Station, 1963. Saponin Content of Alfalfa. ARS, U.S Dept. of Agriculture., pp. 34-44.

Aldrich, C.G., Rhodes, M.T., Miner, J.L., Kerley, M.S., Paterson, J.A., 1993. The effects of endophyte-infected tall fescue consumption and use of a dopamine antagonist on intake, digestibility, body temperature, and blood constituents in sheep. J. Anim. Sci. 71,155-163.

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

Bailey, D.W., Provenza, F.D., 2008. Mechanisms determining large-herbivore distribution. In: Prins, H.T.T., and van Langevelde, F. (Eds.), Resource Ecology: Spatial and Temporal Dynamics of Foraging. Springer. Dordrecht, Netherlands., pp. 7-28.

Bryant, J.P., Chapin III, F.S., Kline, D.R., 1983. Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. Oikos 40,357-368.

Burritt, E.A., Mayland, H.F., Provenza, F.D., Miller, R.L., Burns, J.C., 2005. Effect of added sugar on preference and intake by sheep of hay cut in the morning versus the afternoon. Appl. Anim. Behav. Sci. 94, 245-254.

Burritt, E.A., Provenza, F.D., 2000. Role of toxins in intake of varied diets by sheep. J. Chem. Ecol. 26, 1991-2005.

Dearing, M.D., Cork, S., 1999. Role of detoxification of plant secondary compounds on diet breadth in a mammalian herbivore, Trichosurus vulpecula. J. Chem. Eco. 25, 1205-1219.

Fisher, D.S., Mayland, H.F., Burns, J.C., 1999. Variation in ruminants' preference for tall fescue hays cut either at sundown or at sunup. J. Anim. Sci. 77, 762-768.

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

Freeland, W.J., Calcott, P.H., Anderson, L.R., 1985. Tannins and saponins: interaction in herbivore diets. Biochem. Syst. Ecol. 13, 189-193.

Hedqvist, Helena, Mueller-Harvey, Irene, Reed, Jess D., Krueger, Christian G., Murphy, Michael., 2000. Characterization of tannins and in vitro protein digestibility of several Lotus corniculatus varieties. Anim. Feed Sci. and Tech. 878, 41-56.

Hubert, B., 1993. Modeling pastoral land-use practices. In: Brossier, J., de Bonneval, L. (Eds), Systems Studies in Agriculture and Rural Development. INRA Press, Paris, France, pp. 235-258.

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. J. Sci. Food Agric. 28, 126-136.

Kumar, R., Singh, M., 1984. Tannins: their adverse role in ruminant nutrition. J. Agric. Food Chem. 32, 447-453.

Lisonbee, L., 2008. Plant secondary metabolites and self medication: implications for sustainable parasite control in herbivores. M.S. thesis, Utah State Univ., Logan.

Lyman, T.D., 2008. Foraging behavior of cattle grazing tall fescue, reed canarygrass, birdsfoot trefoil, and alfalfa in different sequences. M.S. thesis, Utah State Univ., Logan.

Lyman, T.D., Provenza, F.D., Villalba, J.J., 2008. Sheep foraging behavior in response to interactions among alkaloids, tannins and saponins. J. Sci. Food Agric. 88, 824-831.

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. Am. J. Clin. Nutr. 32, 1810-1812.

Meuret, M., 2008. MENU Model: The Herder as a Restaurant Chef. Institut National de la Recherche Agronomique, In press.

Meuret, M., Viauz, C., Chadoeuf, J., 1994. Land heterogeneity stimulates intake during grazing trips. Ann. Zootech. 43, 296.

Mote, T.E., Villalba, J.J., Provenza, F.D., 2008. Sequence of food presentation influences intake of foods containing tannins and terpenes. Appl. Anim. Behav. Sci. in press.

Owens, J., 2008. Nutritional affects of sheep consuming legumes that contain tannins and saponins in conjunction with grasses that contain alkaloids. M.S. thesis, Utah State Univ., Logan.

Parsons, A.J., Newman, J.A., Penning, P.D., Harvey, A., Orr, R.J., 1994. Diet preference of sheep: effect of recent diet, physiological state and species abundance. J. Anim. Ecol. 63, 465-478.

Pedersen, M.W., Barnes, D.K., Sorensen, E.L., Griffin, G.D., Nielson, M.W., Hill, R.R. Jr., Frosheiser, F.I., Sonoda, R.M., Hanson, C.H., Hunt, O.J., Peaden, R.N., Elgin Jr., J.H., Devine, T.E., Anderson, M.J., Goplen, B.P., Elling, L.J., Howarth, R.E., 1976. Effects of low and high saponin selection in alfalfa on agronomic and pest resistance traits and the interrelationship of these traits. Crop Husbandry 16, 193-199.

Provenza, F.D., 1995. Postingestive feedback as an elementary determinant of food preference and intake in ruminants. J. Range Manage. 48, 2-17.

Provenza, F.D., 1996. Acquired aversions as the basis for varied diets of ruminants foraging on rangelands. J. Anim. Sci. 74, 2010-2020.

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

Provenza, F.D., 2003b. Foraging Behavior: Managing to Survive in a World of Change. Utah State Univ., Logan.

Provenza, F.D., Villalba, J.J., Dziba, L.E., Atwood, S.B., Banner, R.E., 2003. Linking herbivore experience, varied diets, and plant biochemical diversity. Small Rum. Res. 49, 257-274.

Rottinghaus, G.E., Garner, George B., Cornell, Creighton N., Ellis, James L., 1991. HPLC method for quantitating ergovaline in endophyte-infested tall fescue: seasonal variation of ergovaline levels in stems with leaf sheaths, leaf blades, and seed heads. J. Agric. Food Chem. 39, 112-115.

Schmidt, K.S., Brown, J.S., Morgan, R.A., 1998. Plant defense as complementary resources: a test with squirrels. Oikos 81, 130-142.

Smith, A.D., 1959. Adequacy of some important browse species in overwintering mule deer. J. Range Manage. 12, 9-13.

Terrill, T. H., Rowan, A. M., Douglas, G. B., Barry, T. N., 1992. Determination of extractable and bound condensed tannin concentrations in forage plants, protein concentrate meals and cereal grains. J. Sci. Food Agric. 58, 321.

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

Wallace, J., 2004. Antimicrobial properties of plant secondary metabolites. Proc. Nutr. Soc. 63, 621-629.