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Influence of Supplemental Legumes that Contain Tannins and Saponins on Intake and Diet Digestibility in Sheep Fed Grasses that Contain Alkaloids

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INFLUENCE OF SUPPLEMENTAL LEGUMES THAT CONTAIN TANNINS AND SAPONINS ON INTAKE AND DIET DIGESTIBILITY IN SHEEP FED GRASSES THAT CONTAIN ALKALOIDS

by

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ABSTRACT

Influence of supplemental legumes that contain tannins

and saponins on intake and diet digestibility in sheep

fed grasses that contain alkaloids

by

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Utah State University, 2008

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My objectives were to determine if nutritional benefits occur when animals are offered foods with compounds -- alkaloids, saponins, and tannins – that are potentially complementary. I hypothesized that food intake and digestibility increase when lambs consume plants such as alfalfa ALF that contain saponins or birdsfoot trefoil (BFT) that contain tannins when the basal diet is endophyte-infected tall fescue (TF) or reed canarygrass (RCG) both of which contain alkaloids. I predicted that the nutritional status of lambs would be enhanced if basal diets of alkaloid-containing grasses were supplemented with ALF or BFT.

Lambs fed a basal diet of either endophyte-infected TF or RCG ate more food and consequently digested more dry matter, energy and nitrogen when supplemented with ALF or BFT. Lambs ingested more dry matter and digested more nutrients when fed a basal diet of RCG than one of TF, and supplementing with ALF and BFT was more beneficial for lambs fed TF than for lambs fed RCG. Increased intake of digestible nutrients was due to greater intake when lambs were offered more than one food, not due to an increase in digestibility.

In pen trials meant to complement the field trials, lambs were offered an alkaloid-containing (either gramine or 5-methoxy-N,N-dimethyltryptamine) total mixed ration and supplemented with a food that contained saponins or tannins. All rations were isocaloric (3.3Mcal/kg) and isonitrogenous (14% CP). Lambs fed a ration with either alkaloid and offered a food containing saponin digested approximately the same amount of dry matter, energy, nitrogen, and NDF as lambs not offered saponin. When lambs were fed a ration with either alkaloid and supplemented with food that contained tannins, tannin consumption adversely affected dry matter, energy, and NDF digestibility, but lambs offered food with tannins increased dry matter intake, and as a result, they digested the same amount of dry matter, energy, and NDF as lambs not offered the food with tannins. Lambs offered tannin digested and retained more nitrogen than lambs not offered tannin. These findings indicate a nutritional advantage for sheep eating mixtures as opposed to monocultures of foods with different profiles of secondary compounds and nutrients.

(63 pages)

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Jacob Owens

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CHAPTER 1

INTRODUCTION

All plants contain secondary compounds (PSC) that historically have been viewed as defenses against herbivory because they limit how much of any particular plant an herbivore can eat (Rosenthal and Janzen, 1979; Rosenthal and Berenbaum, 1992). When consumed in too large doses PSC can potentially damage the health of animals (Freeland and Janzen, 1974). Herbivores distinguish among plants that contain PSC, and limit their intake of PSC, through taste, odor, postingestive feedback, and past experience (Provenza, 1995; Provenza et al., 2000). These mechanisms both cause and enable herbivores to eat a variety of plants to meet nutritional requirements without over-ingesting any one PSC (Freeland and Janzen, 1974), and they underscore the importance of biochemical biodiversity in foraging specifically and ecological processes more generally (Provenza, 2003; Provenza, 2008).

Ironically, humans have selected for food crops and pasture plants low in PSCs. As food for humans, regardless of where they lived, our ancestors targeted a few species -- those that were abundant, palatable, easily cultivated and harvested -- for sampling and eventual use (Etkin, 1994). By focusing on a few species, people transformed the diverse world of plants into a manageable domain that generally met needs for nutrients, mainly energy, and through selection for low concentrations of PSC limited over-ingestion of PSC (Johns, 1994). As food for livestock, pasture plants often have been sown as monocultures. Eating only one plant species can lead to over-consumption of PSC, which can adversely affect food intake, nutritional status, and health of herbivores (Freeland and

Janzen, 1974; Provenza, 2003), so plant breeders have selectively reduced PSC concentrations in plants selected for livestock consumption.

Nowadays, people are just beginning to appreciate the diverse roles of PSC in herbivore health and production (Provenza, 2008). In the past, researchers focused on primary compounds such as nitrogen, phosphorus, and potassium and they viewed PSC as waste products of plant metabolism (Rosenthal and Janzen, 1979). Over the past 30 years, however, researchers have begun to recognize the importance of PSC in plant behavior, including reproduction, defense against herbivory, and recovery from injury (Rosenthal and Berenbaum, 1992).

With regard to foraging by herbivores, some PSC complement one another biochemically (Freeland and Janzen, 1974), which can increase intake of and preference for forages that consumed alone produce negative effects (Provenza, 2003). For example, mule deer eat more when offered sagebrush and juniper (12.3 g/kg BW) 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 consume more forage with amygdalin and LiCl or oxalate and nitrate than lambs with only one of these compounds in their diet (Burritt and Provenza, 2000). Sheep eat more when offered foods with terpenes, tannins, and oxalates than when offered foods with only one or two of these PSC (Villalba et al., 2004). Sheep also eat more of foods high in alkaloids when they can also eat foods high in tannins or saponins (Lyman, Provenza, and Villalba, 2008, unpublished data). While complementarities among secondary compounds are an important but little understood area of plant-herbivore interactions (Freeland and Janzen, 1974; Provenza, 2003), even less is known about how the sequences of eating plants with different compounds affects foraging. Sheep eat more food with terpenes when they first eat food with tannins (Mote et al., 2008). Cattle steadily decrease time eating endophyte-infected tall fescue when they grazed tall fescue first for 30 minutes and then birdsfoot trefoil and/or alfalfa alone for 60 minutes; however, when the sequence is reversed they foraged actively on both trefoil and/or alfalfa and fescue throughout the 90-minute meal (Lyman, Provenza, and Villalba, 2008, unpublished data). These patterns of foraging are analogous with birdsfoot trefoil/alfalfa and high-alkaloid reed canarygrass (Lyman, Provenza, and Villalba, 2008, unpublished data).

OBJECTIVES

While the aforementioned behavioral relationships are interesting and important, little is known about the influence of PSC on food intake and nutrient utilization by herbivores. I determined if a nutritional advantage exists when sheep were fed forage species with a mixture of alkaloids, saponins, and tannins as opposed to the forages that contained only alkaloids. My specific objectives were to determine dry matter, nitrogen, fiber, and energy digestibility when sheep fed a basal diet of the alkaloid-containing forages reed canarygrass *(Phalaris arundinacea L v.n.s.)* or endophyte-infected tall fescue *(Festuca arundinacea, Kentucky 31 endophyte)* were supplemented with a high-tannin variety of birdsfoot trefoil *(Lotus corniculatus variety Goldie)* and a high-saponin variety of alfalfa *(Medicago sativa variety Vernal)*.

I hypothesized that biochemical complementarities (among PSC and nutrients) exist in diverse PSC-containing foods which cannot be manifest in mono-specific diets. I predicted that forage intake and nutrient utilization increase when sheep eat a mixture of alkaloid-containing grass along with complementary saponin- or tannin-containing leguminous forage, as compared with eating only high-alkaloid forage. This hypothesis is based on the concept that a complementary relationship exists between 1) the alkaloids in tall fescue and saponins in alfalfa, and 2) the alkaloids in reed canarygrass and the tannins in birdsfoot trefoil. On this basis, I predicted that sheep fed mixtures of the above forages would maintain higher nutrient intake than sheep fed only the alkaloid-containing forages. Alkaloids in tall fescue are non-polar cholesterol-derived structures while alkaloids in reed canarygrass are proteinaceous in nature (tryptamine-like alkaloids). Saponins in alfalfa are non-polar steroidal compounds with an affinity for binding to cholesterol-derived compounds in the gastrointestinal tract causing them to be excreted in the feces (Malinow et al., 1979). Tannins in birdsfoot trefoil have an affinity for binding to proteins and protein like compounds in the rumen (Okuda et al., 1982).

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CHAPTER 2

LEWISTON STUDY: SUPPLEMENTING LAMBS FED TALL FESCUE OR REED CANARYGRASS WITH ALFALFA OR BIRDSFOOT TREFOIL

ABSTRACT: I hypothesized that legumes such as alfalfa (ALF) and birdsfoot trefoil (BFT) fed as supplements to grasses such as endophyte-infected tall fescue (TF) and older varieties of reed canarygrass (RCG) provide nutritional benefits. This hypothesis is based on the notion that complementary relationships exists between the alkaloids in tall fescue and reed canarygrass and the saponins in alfalfa and the tannins in birdsfoot trefoil that enable herbivores to eat more food when offered combination of plants as opposed to only one plant. On this basis, I predicted that sheep fed mixtures of the above forages maintain higher nutrient intake and hence digestibility than sheep fed only the alkaloid-containing forages. To determine the existence of a nutritional benefit when lambs were supplemented with leguminous forage, I conducted two trials. In each trial, 20 lambs were placed in individual metabolism stalls and offered freshly clipped forages each morning. In trial 1, 4 groups of 5 lambs were offered TF or RCG with ALF in a 2 x 2 factorial that included grass (TF or RCG) with or without ALF as follows: Group 1 TF with ALF, Group 2 RCG with ALF, Group 3 TF only, and Group 4 RCG only. Trial 2 was similar to trial 1 except lambs in Groups 1 and 2 were offered BFT as the supplemental legume instead of ALF. Forage, fecal, and urine samples were collected and analyzed to determine total dry matter intake (DMI) and apparent digestibility of dry matter (DM), energy (GE), nitrogen, and neutral detergent fiber (NDF). Supplementing lambs on basal diets of RCG or TF with ALF or BFT did not affect the digestibility of

nutrients. However, supplementing lambs with ALF or BFT increased intake and as a result increased the amount of nutrients digested. Lambs supplemented with ALF had higher total intake, and digested more dry matter, nitrogen, and energy. Lambs supplemented with BFT had higher total intake, and digested more dry matter and energy. These benefits were achieved when lambs ate less than 30% of their daily intake as ALF and less than 13% of their intake as BFT. The benefits of offering lambs BFT or ALF were of larger magnitude for lambs fed TF than for lambs fed RCG. Legumes thus enhanced intake, which increased the total amount of nutrients digested. These results are likely due to complementary relationships among secondary and primary compounds in the grasses and legumes.

INTRODUCTION

Because PSC limit forage intake, people have come to view them primarily as defenses against herbivory. As a result, we know little about how herbivores might use PSC for health and medicinal benefits (Provenza and Villalba, 2006). The outcomes of all biochemical interactions depend on the dose and the compounds involved in the interactions: both nutrients and PSC at excessive doses can be toxic, whereas at lower doses they can both be beneficial (Craig, 1999; Engel, 2002) and they can complement one another (Freeland and Janzen, 1974; Provenza, 2003). Lambs consume more forage with amygdalin and LiCl or oxalate and nitrate than lambs with only one of these compounds in their diet (Burritt and Provenza, 2000). Sheep eat more when offered foods with terpenes, tannins, and oxalates than when offered foods with only one or two of these PSC (Villalba et al., 2004). Sheep eat more terpene-containing food when they first eat food with tannins (Mote et al., 2007). Cattle steadily decrease time eating tall fescue when they first graze tall fescue alone for 30 minutes followed by birdsfoot trefoil and/or alfalfa alone for 60 minutes. However, when the sequence is reversed they forage actively on both trefoil/alfalfa and fescue throughout the 90-minute meal (Lyman, 2008, unpublished data). These patterns of foraging are analogous with trefoil/alfalfa and high-alkaloid reed canarygrass (Lyman, Provenza, and Villalba, 2008, unpublished data). Outside of these studies, we know little about complementarities among PSC that might lead to increases in forage intake and possibly nutrient digestion.

I used four forages with different PSC to examine if nutritional complementarities existed in foods: endophyte- infected tall fescue (*Festuca arundinacea Kentucky 31*) with high levels of alkaloids, reed canarygrass (*Phalaris arundinacea wild type*) with high levels of alkaloids, alfalfa (*Medicago sativa Vernal variety*) with high levels of saponins, and birdsfoot trefoil (*Lotus corniculatus Goldie variety*) with high levels of tannins.

The plant alkaloids in tall fescue are derived from cholesterol, while the saponins in alfalfa are non-polar steroidal compounds with an affinity for binding to cholesterol derived compounds in the gastro-intestinal tract of animals, causing their excretion in the feces (Malinow et al., 1979). The alkaloids in reed canarygrass, metabolized from the amino acid tryptophan, are proteinaceous in nature. Birdsfoot trefoil contains condensed tannins that bind to proteins in the rumen (Jones and Mangan, 1977). Based on these structural characteristics and binding affinities, I hypothesized that biochemical complementarities among PSC and nutrients exist which cannot be manifest in a monospecific diet. Thus, I predicted that forage intake and nutrient utilization would increase when sheep ate a mixture of alkaloid-containing grass along with complementary saponin- or tannin-containing leguminous forage, as compared with eating only high-alkaloid forage.

MATERIALS AND 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 et al., 1993) and reed canarygrass *(Phalaris arundinacea L v.n.s.)* with high alkaloids (Marten et al., 1973; Sheaffer et al., 1990), birdsfoot trefoil (*Lotus corniculatus variey Goldie*) with high tannins (Hedqvist et al., 2000; Terrill et al., 1991), and alfalfa (*Medicago* sativa *variety Vernal*) with high saponins (Pedersen et al., 1976; ARS, 1963). Our chemical analysis of each plant species confirmed appropriate levels of plant secondary compounds, which correlate with documented concentrations.

Twenty commercial Rambouillet-Columbia-Finn-Targhee and Suffolk lambs 4 months of age were placed in individual metabolism stalls at the Utah State University Pasture Project Facility. Lambs were offered daily freshly clipped forages to simulate pasture grazing conditions and to avoid inactivating PSM due to drying. I conducted two trials both approved by the Animal Care and Use Committee (Approval # 1317).

Trial 1

In Trial 1, lambs were offered plants high in alkaloids (TF and RCG) along with a plant high in saponins (ALF). Twenty lambs in 4 groups of 5 lambs/group were allocated to a 2 x 2 factorial with grass species (TF or RCG) and supplementation with ALF (yes, no) as the main factors as follows: Group 1 TF with ALF, Group 2 RCG with ALF, Group 3 TF only, and Group 4 RCG only. Lambs in Groups 1 and 2 were offered the leguminous forage (ALF) ad libitum for 30 min. After 30 min the leguminous forage was removed from the bunks and lambs were then offered TF or RCG ad libitum for 3½ h such that the lambs were pre-loaded with the supplemental forage prior to receiving the alkaloid-containing grass. Lambs in groups 3 and 4 were offered TF or RCG ad libitum, respectively, for the entire 4-h feeding period. The 4-h feeding period began at 0800 and ended at 1200. Forages were clipped at 0630 each morning.

Trial 2

Trial 2 was similar to Trial 1, except that a new set of 20 lambs were used and instead of supplementing with a legume high in saponins (ALF), animals were supplemented with a legume high in tannins (BFT). Thus, 20 lambs in 4 groups of 5 lambs/group were offered TF or RCG with BFT in a 2 x 2 factorial that included grass (TF or RCG) with or without BFT as follows: Group 1 TF with BFT, Group 2 RCG with BFT, Group 3 TF only, and Group 4 RCG only).

Collection Period

The above feeding protocol was a part of a 21-d adaptation period, which was followed by a 5-d collection period in which forages, feces, and urine were sampled daily to determine dry matter (DM) intake and nutrient digestibility. Forages were fed ad libitum with 30% refusal. During the 5-d collection period, forage and refusal samples were collected daily, weighed and then dried at 60 degrees centigrade for 72 h. After drying, samples were weighed again to determine dry matter content.Dried samples were ground in a Whiley mill with a 1mm filter, and 20 g of each forage sample were composited according to species to represent DM and nutrients fed during the 5-d collection period. A separate composite containing 20 g from each refusal per sheep was made according to species of plant. For instance, a sheep fed ALF and TF would have 2 composites, one per forage. Daily values for forage intake were averaged for the 5-d collection period and then matched for each animal with the composited 5-d samples of feces and urine to determine nutrient intake and digestibility.

Fecal and urine samples were weighed and collected daily. I weighed total fecal output and then made 1 composite for each sheep that consisted of 30g/d. Each fecal sample composite was dried at 60 degrees centigrade for 5 d to determine dry matter content of feces. Urine samples were collected in 10 ml of HCL to prevent NH_3 losses. Urine samples were collected and measured daily. I made 1 composite/lamb that consisted of 300ml of urine/d. From each composite, 100ml was freeze dried for use in nitrogen analyses. Forage and fecal samples were analyzed for 1) dry matter (DM) (AOAC, 1990); 2) nitrogen (Method 990.03 AOAC, 2002); 3) neutral detergent fiber

(NDF) (Goering and Van Soest, 1970); and 4) gross energy (AOAC, 1990). Dried composites were used to determine dry matter intake, nutrient intake, and energy intake. Nutrients consumed vs. excreted in feces were measured to assess apparent digestibility of DM, energy, and NDF (hemicelluloses, cellulose, lignin, and insoluble ash). Nitrogen consumed versus excreted in urine and feces were used to determine nitrogen utilization.

Statistical Analyses

The statistical design for each trial (1 and 2) was a 2 x 2 factorial with species of grasses (RCG or TF) and supplemental legume (yes or no) as the main effects. Animals (n = 5 per treatment) were nested within treatments. Day (n=5) was the repeated measure. ALF was the legume used in trial 1 and BTF was the legume used in trial 2. The response variables were dry matter intake and nutrient digestibility. Due to small sample sizes (n=5), I consider $P<0.10$ significant.

RESULTS

Grasses did not differ in NDF or gross energy content within a trial. Reed canarygrass had more nitrogen than tall fescue (Table 2-1). Legumes were similar to grasses except for NDF content, which was lower in the legumes. Grasses were lower in nitrogen in Trial 1 than 2, and trefoil had less nitrogen than alfalfa.

Nutrients digested were a function of forage intake, which was influenced by plant phenology during Trials 1 and 2. Generally speaking, lambs ate more dry matter during Trial 1, and as a result, with the exception of TF with ALF, they digested more nutrients during Trial 1 than during Trial 2 (Tables 2-1 to 2-4).

Table 2-1. Energy, neutral detergent fiber (NDF) and nitrogen (N) content of alfalfa (ALF), birdsfoot trefoil (BFT), reed canarygrass (RCG), and endophyte-infected tall fescue (TF) during Trials 1 and 2 (forage composites explained above were analyzed for nutrient content).

	Energy (Kcal/g)		NDF (%)		N (%)	
	Trail 1	Trial 2	Trail 1	Trial 2	Trail 1	Trial 2
ALF	4.3	-	43.7	-	3.6	-
BFT	-	4.3	-	45.8	-	2.7
RCG	4.2	4.1	63.1	60.5	2.9	3.7
TF	4.2	4.2	63.9	60.7	2.3	3.1

Trial 1: Alfalfa as a Supplement to Tall Fescue and Reed Canarygrass

Total Dry Matter Intake

Lambs ate more dry matter when offered RCG than when offered TF (Table 2-2). Lambs fed ALF before RCG or TF ate more dry matter than lambs fed only RCG or TF (Table 2-3). Grass and ALF did not interact (Table 2-3).

Grass Intake

Lambs ate less TF than RCG (Table 2-2). Lambs offered ALF ate less grass than lambs not offered ALF (Table 2-3). With TF, lambs fed ALF ingested 222g/d more than lambs not fed ALF (783g/d vs. 561g/d), yet their intake of TF was only 78g/d less than that for lambs fed only TF (561g/d vs. 639g/d). With RCG, lambs fed ALF ingested 206g/d more than lambs not fed ALF (1013g/d vs. 807g/d), and their intake of RCG was

95g/d less than that for lambs fed only TF (807g/d vs. 902g/d). Thus, there was a significant increase in intake due to ALF with both grasses. Grass species and ALF did not interact (Table 2-3).

Table 2-2. Intake and digestibility for lambs fed endophyte-infected tall fescue (TF) or reed canarygrass (RCG) in Trial 1.

	TF[1]	RCG[2]	Std. Error	Grass
Dry Matter Intake (g/d)	711	958	6	P<0.0001
Grass Intake (g/d)	600	855	6	P<0.0001
ALF[3] Intake (g/d)	215	207	4	P=0.78
DM[4] Digestibility (%)	70	72	2	P=0.34
DM Digested (g/d)	468	651	33	P<0.0001
Digested N[5] Retained (%)	47	46	5	P=0.84
N Retained/N Consumed (%)	40	45	5	P=0.38
N Retained (g/d)	7	14	2	P=0.0003
N Digested (g/d)	14	25	1	P<0.0001
N Digestibility (%)	76	82	1	P=0.0005
Energy Digestibility (%)	66	69	2	P=0.17
Energy Digested (Kcal/d)	1,895	2,726	139	P<0.0001
NDF[6] Digestibility (%)	63	67	2	P=0.14
NDF Digested (%)	250	357	21	P=0.0001

[1]Tall Fescue.
[2]Reedcanary Grass.
[3]Alfalfa.
[4]Dry matter.
[5]Nitrogen.
[6]Neutral detergent fiber.

Alfalfa Intake

There were no differences in alfalfa intake due to grass (Tables 2.2 and 2-3).

Table 2-3. Digestibilities for lambs fed endophyte-infected tall fescue (TF) or reed canarygrass (RCG) with or without alfalfa in Trial 1.

	TF[1] without ALF[2]	TF With ALF	RCG[3] without ALF	RCG with ALF	Std. Error	ALF	ALF* Grass
Dry Matter Intake (g/d)	639	783	902	1,013	8	P=0.009	P=0.70
Grass Intake (g/d)	639	561	902	807	8	P=0.055	P=0.83
DM[4] Digestibility (%)	73	66	71	73	3	P=0.28	P=0.05
DM Digested (g/d)	446	490	604	698	46	P=0.05	P=0.45
Digested N[5] Retained (%)	45	49	46	45	8	P=0.83	P=0.63
N Retained/N Consumed (%)	42	38	44	46	7	P=0.86	P=0.52
N Retained (g/d)	7	8	13	16	2	P=0.16	P=0.82
N Digested (g/d)	12	16	24	27	1	P=0.0009	P=0.75
N Digestibility (%)	76	75	81	83	2	P=0.86	P=0.33
Energy Digestibility (%)	68	64	67	72	3	P=0.93	P=0.07
Energy Digested (Kcal/d)	1,760	2,030	2,470	2,981	197	P=0.012	P=0.40
NDF[6] Digestibility (%)	67	60	65	70	4	P=0.67	P=0.04
NDF Digested (g/d)	249	250	332	382	30	P=0.24	P=0.26

[1]Tall Fescue.
[2]Alfalfa.
[3]Reedcanary Grass.
[4]Dry matter.
[5]Nitrogen.
[6]Neutral detergent fiber.

Dry Matter Digested

There was no difference in the digestibility of dry matter when lambs were fed TF or RCG (Table 2-2). However, lambs fed RCG ingested more dry matter than lambs fed TF, and as a result they digested more dry matter (Table 2-2). Supplementing lambs fed TF or RCG with ALF did not affect dry matter digestibility (Table 2-3), but their increased dry matter intake resulted in lambs digesting more dry matter when supplemented with alfalfa (Table 2-3). There was an interaction between grass and ALF (P=0.05). The digestibility of dry matter was lower for lambs fed TF supplemented with ALF (63%) than for lambs not supplemented (73%), and digestibility of dry matter was higher for lambs fed RCG supplemented with ALF (73%) than for lambs not supplemented (71%).

Nitrogen Digested

The digestibility of nitrogen was higher for lambs fed RCG than for lambs fed TF (Table 2-2). Lambs also digested more nitrogen on a basal diet of RCG than on a basal diet of TF (Table 2-2). The digestibility of nitrogen did not differ when lambs fed TF or RCG were supplemented with ALF, but they digested more nitrogen when the grasses were supplemented with alfalfa (Table 2-3). Grass and ALF did not interact to cause differences in nitrogen digestibity (P=0.63).

Nitrogen Retained

The percent of digested nitrogen retained by lambs did not differ for RCG or TF (Table 2-2). However, lambs fed RCG ate more dry matter than lambs fed TF and as a result they retained more nitrogen (Table 2-2). The percent of digested nitrogen retained did not differ when lambs fed grasses were supplemented with ALF. Lambs supplemented with alfalfa consumed more dry matter than lambs not supplemented which resulted in a trend for increased nitrogen retention when the grasses were fed with as opposed to without ALF (Table 2-3). Grass and ALF did not interact (P=0.52).

Kilocalories Digested

The digestibility of energy did not differ when lambs were fed RCG or TF. However, lambs fed RCG ingested more dry matter than lambs fed TF and as a result they digested more kilocalories (Table 2-2). There was no difference in the digestibility of energy, but due to increased intake lambs digested more kilocalories when the grasses were fed with as opposed to without ALF (Table 2-3). There was an interaction between grass and ALF (P=0.07). The digestibility of energy was lower for lambs fed TF supplemented with ALF (64%) than for lambs not supplemented (68%), and digestibility of energy was higher for lambs fed RCG supplemented with ALF (72%) than for lambs not supplemented (67%).

NDF Digested

Digestibility of NDF did not differ by species of grass (Table 2-2), but due to increased intake, lambs offered RCG digested more NDF than lambs offered TF. The digestibility of NDF did not differ when lambs were fed grasses with or without ALF (Table 2-3), but there was an interaction between grass and ALF (Table 2-3; P=0.04). The digestibility of NDF was lower for lambs fed TF supplemented with ALF (60%) than for lambs not supplemented (67%), and digestibility of NDF was higher for lambs fed RCG supplemented with ALF (70%) than for lambs not supplemented (75%).

Trial 2: Birdsfoot Trefoil as a Supplement to Tall Fescue and Reed Canarygrass

Total Dry Matter Intake

As in Trial 1, lambs ate more dry matter when fed RCG than when fed TF (Table 2-4). They also ate more dry matter when supplemented with BFT (Table 2-5). There was no interaction between BFT and grass (Table 2-5).

Table 2-4. Digestibility table for lambs fed endophyte-infected tall fescue (TF) or reed canarygrass (RCG) in Trial 2.

	TF[1]	RCG[2]	Std. Error	Grass
Dry Matter Intake (g/d)	583	843	38	P=0.0002
Grass Intake (g/d)	546	779	42	P=0.0013
BFT[3] Intake (g/d)	76	132	28	P=0.19
DM[4] Digestibility (%)	68	67	0.02	P=0.80
DM Digested (g/d)	364	539	39	P<0.0001
Digested N[5] Retained (%)	51	55	7	P=0.63
N Retained/N Consumed (%)	38	37	6	P=0.33
N Retained (g/d)	8	12	2	P=0.009
N Digested (g/d)	15	27	2	P<0.0001
N Digestibility (%)	79	82	1	P=0.03
Energy Digestibility (%)	68	68	3	P=0.96
Energy Digested (Kcal/d)	1,493	2,337	123	P<0.0001
NDF[6] Digestibility (%)	67	67	3	P=0.95
NDF Digested (%)	200	305	19	P<0.0001

[1]Tall Fescue.
[2]Reedcanary Grass.
[3]Birdsfoot Trefoil.
[4]Dry matter.
[5]Nitrogen.
[6]Neutral detergent fiber.

Grass Intake

Lambs fed RCG ate more grass than lambs fed TF (Table 2-4). There was an interaction between grass and BFT (Table 2-5). With TF, lambs fed BFT ingested 76g/d more than lambs not fed BFT (695g/d vs. 619g/d), and their intake of TF was 146g/d less than that for lambs fed only TF (619g/d vs. 473g/d). With RCG, lambs fed BFT ingested 129g/d more than lambs not fed BFT (869g/d vs. 740g/d), and their intake of RCG was 77g/d less than that for lambs fed only TF (740g/d vs. 817g/d). Thus, there was an increase in intake due to BFT with both grasses, but the effect was greater with RCG than with TF.

BFT Intake

There was a trend toward higher intake of BFT for lambs fed RCG as opposed to TF (Table 2-4).

Dry Matter Digested

Digestibility of dry matter did not differ when lambs were fed RCG or TF (Table 2-4). However, lambs ate more when fed RCG than when fed TF, which resulted in more dry matter digested by lambs fed RCG than by lambs fed TF (Table 2-4). The digestibility of dry matter did not differ due to BFT (Table 2-5). However, lambs digested more dry matter when grasses were fed with as opposed to without BFT due to increased intake (Table 2-5). Grass and BFT did not interact (P=0.53).

Table 2-5. Digestibility table for lambs fed endophyte-infected tll fescue (TF) or reed canarygrass (RCG) with and without birdsfoot trefoil (BFT) in Trial 2.

	TF[1] without BFTF[2]	TF with BFT	RCG[3] without BFT	RCG with BFT	Std. Error	BFT	BFT* Grass
Dry Matter Intake (g/d)	473	695	817	869	54	P=0.02	P=0.41
Grass Intake (g/d)	473	619	817	740	60	P=0.57	P=0.08
DM[4] Digestibility (%)	71%	65%	66%	68	3	P=0.31	P=0.13
DM Digested (g/d)	321	406	484	562	43	P=0.05	P=0.53
Digested N[5] Retained (%)	56	46	51	58	9	P=0.76	P=0.20
N Retained/N Consumed (%)	36	41	40	35	8	P=0.99	P=0.33
N Retained (g/d)	6	9	13	11	2	P=0.68	P=0.10
N Digested (g/d)	13	17	27	27	2	P=0.24	P=0.32
N Digestibility (%)	81	76	82	82	2	P=0.08	P=0.11
Energy Digestibility (%)	71	65	67	68	4	P=0.32	P=0.16
Energy Digested (Kcal/d)	1,322	1,664	2,234	2,439	173	P=0.04	P=0.58
NDF[6] Digestibility (%)	71	62	67	66	4	P=0.08	P=0.23
NDF Digested (g/d)	182	217	306	304	27	P=0.40	P=0.35

[1]Tall Fescue.
[2]Birdsfoot Trefoil.
[3]Reedcanary Grass.
[4]Dry matter.
[5]Nitrogen.
[6]Neutral detergent fiber.

Nitrogen Digested

The digestibility of nitrogen was higher for lambs fed RCG than for lambs fed TF (Table 2-4). The percent digestibility of nitrogen was higher for lambs fed grass plus BFT than for lambs fed only grass , but the total nitrogen digested did not differ with or without BFT (Table 2-5), nor did grass and BFT interact (Table 2-5).

Nitrogen Retained

The percent of digested nitrogen retained did not differ due to species of grass (Table 2-4), but lambs fed RCG retained more nitrogen than lambs fed TF (Table 2-4). The percent of digested nitrogen retained did not differ when lambs were supplemented with BFT (Table 2-5).

Kilocalories Digested

The digestibility of energy did not differ when lambs were fed RCG or TF (Table 2-4). However, due to increased intake, lambs fed RCG digested more energy than lambs fed TF (Table 2-4). The digestibility of energy was not affected when lambs were supplemented with BFT (Table 2-5), but due to higher intakes, lambs digested more kilocalories with as opposed to without BFT (Table 2-5). Grass and BFT did not interact (Table 2-5).

NDF Digested

The digestibility of NDF did not differ by species of grass (Table 2-4). However, due to increased intake lambs fed RCG digested more NDF than lambs fed TF (Table 2-4). The percent digestibility of NDF was lower for lambs supplemented with BFT than for lambs not supplemented (Table 2-5). The amount of NDF digested did not differ for lambs supplemented with BFT and lambs not supplemented with BFT (Table 2-5).Grass and BFT did not interact (Table 2-5).

DISCUSSION

I hypothesized nutritional benefits for lambs that consumed legumes such as alfalfa (saponins) and birdsfoot trefoil (tannins) in conjunction with grasses such as tall fescue and reed canarygrass that contain alkaloids based on the fact that secondary compounds limit how much of any particular food an animal can eat (Freeland and Janzen, 1974; Provenza, 2003). Based on this hypothesis, I predicted that food intake and digestibility of nutrients would be higher for lambs fed complimentary mixtures of plants as opposed to a monospecific diet.

Consistent with the predictions of my hypothesis, lambs offered ALF or BFT consumed more dry matter and digested more nutrients than lambs not offered these forages regardless of grass species (Table 2-2, 2-3, 2-4, and 2-5)**.** During both trials, lambs ate less TF than RCG, perhaps due to the differences in the kinds of alkaloids in TF and RCG and to the much higher alkaloid content in TF than in RCG (unpublished data). Lambs also ate more ALF than BFT indicating a preference for ALF.

Aflalfa

Lambs offered ALF digested more nitrogen and energy than lambs not offered ALF, even though they were fed ALF for only 30 min/d and less than 30% of their diet consisted of ALF. Lambs fed TF consumed about 27% of their daily intake as ALF (215g of 783g), and lambs fed RCG consumed about 20% of their daily intake as ALF (207g of 1,013g).

With TF, lambs fed ALF ingested 222g/d more than lambs not fed ALF (783g/d vs. 561g/d), yet their intake of TF was only 78g/d less than that for lambs fed only TF (561g/d vs. 639g/d). With RCG, lambs fed ALF ingested 206g/d more than lambs not fed ALF (1013g/d vs. 807g/d), and their intake of RCG was 95g/d less than that for lambs fed only TF (807g/d vs. 902g/d). Thus, there was a synergistic effect on intake provided by ALF with both grasses. Steers decrease intake of endophyte-infected tall fescue when offered high-quality forages (Goetsch et al., 1987), but the degree to which this occurs for both TF and RCG evidently is influenced by the sequence in which legumes such as alfalfa and trefoil are offered. Use of TF and RCG by sheep and cattle increases markedly when legumes are fed prior to eating the grasses (Lyman, Provenza, and Villalba, 2008, unpublished data; Lockard, Provenza, Villalba, and Cheney, 2008, unpublished data). Collectively, these findings show offering ALF for a short period each day increases intake which in turn increases animal performance on alkaloid containing grasses such as TF and RCG.

The nutritional benefits experienced by lambs offered ALF as a supplement to TF and RCG were in part due to the lambs' increased intake of dry matter, higher quality of the supplement, and possibly the predicted chemical interactions among PSC. Food quality increased with supplementation, as lambs fed TF ate 27% of their diet as a more nutritious plant (ALF) than lambs not supplemented (Table 2-1). ALF had less NDF and more nitrogen thus making it more easily digestible and more nutritious than the grasses (Table 2-5). Lambs also ate more food when offered a legume as opposed to eating only a grass, likely due to complementarities in PSC profiles. Thus, the increases in intake and digestibility we attribute to interactions among different primary and secondary chemicals derived from the combination of a legume and a grass. Lambs offered ALF had higher dry matter intake and digested more total grams of dry matter, but there was a trend toward lower dry matter digestibility when lambs fed TF were supplemented with ALF, and a trend toward higher dry matter digestibility when lambs fed RCG were supplemented with ALF. Increased intake can increase rate of passage of dry matter through the gastrointestinal tract (Van Soest, 1994). The lower digestibility of dry matter when lambs fed TF were supplemented with ALF could be attributed to increased rate of passage that lowered digestibility of dry matter. Lambs in both groups digested the same percent of total nitrogen consumed, but more grams of nitrogen were digested by lambs offered ALF (Table 2-2). Percent of consumed energy digested did not differ for lambs in either group, but lambs offered ALF digested more energy (Table 2-2). While lambs supplemented with ALF digested approximately the same percent of consumed nutrients as lambs not supplemented with ALF, lambs supplemented with ALF digested more nutrients than lambs not supplemented with ALF.

Birdsfoot Trefoil

Lambs did not consume as much BFT as ALF. Lambs in the TF group consumed only 13% of their daily intake from BFT (76g out of 587g), and lambs in the RCG group consumed only 15% of their daily intake from BFT (132g out of 869g). Nonetheless, a small amount of BFT in the lamb’s diet provided the nutritional benefits.

Lambs fed a basal diet of TF supplemented with BFT ate more TF than lambs fed only TF (Table 2-4). With TF, lambs fed BFT ingested 76g/d more than lambs not fed

BFT (695g/d vs. 619g/d), and their intake of TF was 146g/d less than that for lambs fed only TF (619g/d vs. 473g/d). With RCG, lambs fed BFT ingested 129g/d more than lambs not fed BFT (869g/d vs. 740g/d), and their intake of RCG was only 77g/d less than that for lambs fed only TF (740g/d vs. 817g/d). Thus, there was a synergistic effect on intake provided by BFT with both grasses, but the effect was greater with RCG than with TF. These results support findings that offering combinations of certain foods increases intake (Meuret et al., 1994).

Comparison of ALF and BFT

Several benefits are derived from adding legumes such as ALF and BFT to pasture mixtures. Until recently, nitrogen fertilizer was inexpensive and convenient to use, but that is not the case nowadays, and it is not likely to be in the future. Legumes have the ability to fix nitrogen and boost pasture productivity and quality for livestock consumption, as evidenced in this and other studies. Our findings show that intake of grasses such as TF and RCG both increased when lambs were supplemented with either ALF or BFT, and these increases in intake led to greater nutrient digestibility. In general, lambs ate more ALF than BFT, but a small amount of BFT made a big difference in intake and digestibility. My findings are consistent with field studies of the foraging behaviour of sheep on monocultures versus mixtures, carried out on the pastures where I collected the forages used in my trials (Lockard, Provenza, Villalba, and Cheney, 2008, unpublished data).

Other studies show benefits of animals consuming foods that complement one another. Alfalfa is good supplement for livestock eating range grasses (Holechek and

Herbel, 1986), and many forbs and shrubs provide cattle with nutritional benefits similar to those of offering alfalfa (Arthun et al., 1992a,b). When grazing adjacent pastures of sulla and Italian ryegrass, sheep spend more time eating than when grazing only Italian ryegrass (Molle et al., 2008). More generally, offering a variety of foods is beneficial. For instance, cattle in feedlots offered the ingredients in a total mixed ration free-choice consume more food than cattle offered only a total mixed ration (Atwood et al., 2001). Finally, plant diversity on rangelands can be used to increase intake and digestibility of nutrients, which improves animal performance. 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.

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CHAPTER 3

INFLUENCE OF SAPONIN OR TANNIN ON INTAKE AND NUTRIENT DIGESTION OF FOOD THAT CONTAINS DIFFERENT ALKALOID

ABSTRACT: I hypothesized lambs fed saponin or tannin as a supplement to foods containing alkaloids such as gramine (G) and 5-methoxy-N,N-dimethyltryptamine (T) have nutritional benefits over lambs fed alkaloids but not offered saponin or tannin. Saponins and tannins can bind to alkaloids, thus I predicted sheep fed saponins or tannins in foods would maintain higher nutrient intake and digestibility than sheep fed only alkaloid-containing foods. I conducted two trials to determine the existence of a nutritional benefit when lambs were supplemented with tannin or saponin. In trial 1, lambs were supplemented with saponin, and in trial 2, lambs were supplemented with tannin. All feeds were isocaloric (3.3Mcal.kg) and isonitrogenous (14% CP). During the collection period, forage, fecal, and urine samples were collected and analyzed to determine total dry matter intake and apparent digestibility of dry matter, energy, nitrogen, and NDF. Lambs offered saponin digested approximately the same amount of dry matter, energy, nitrogen, and NDF as lambs not offered saponin. Tannin consumption adversely affected dry matter, energy, and NDF digestibility. However, lambs offered tannin increased dry matter intake, and as a result, digested the same amount of dry matter, energy, and NDF as lambs not offered tannin. Lambs offered tannin also digested and retained more nitrogen than lambs not offered tannin.

INTRODUCTION

In the Lewiston study, the forages used varied in both primary and secondary chemistries. Hence, the degree to which PSC affected nutrient intake and digestibility could not definitively be determined. To better assess the possible roles of PSC in the Lewiston study, I conducted a study at Green Canyon in which I added PSC to food of uniform primary chemistry.

OBJECTIVES

My objectives were to determine if a simple subset of the secondary compounds (tannins, saponins, and alkaloids) of interest in the Lewiston study influenced the nutritional variations in lambs when the primary compounds were held constant.

MATERIALS AND METHODS

Twenty commercial Rambouillet-Columbia-Finn-Targhee and Suffolk lambs 4 months of age were placed in individual metabolism stalls at the Utah State University Green Canyon Ecology Center Facility as approved by the Animal Care and Use Committee (Approval # 1317).

Lambs were offered foods containing different PSC in two separate trials. For both trials, I created four total mixed rations with the same ingredients, such that the foods were isocaloric (3.3Mcal/kg) and isonitrogenous (14% CP). The only difference between the rations was the presence of different PSCs. I used two supplemental foods containing either saponin or tannin, and two alkaloids containing foods: methoxy-N,N-dimethyltryptamine (T) to simulate the ergot alkaloids produced by tall fescue and gramine (G) to simulate the alkaloids found in reed canarygrass. Tryptophan is the core structure of the ergot alkaloids (Tudzynski et al., 2001) produced by the endophyte-infected tall fescue. The alkaloids in reed canarygrass are derivatives of both gramine and tryptamine (Marten et al., 1973, 1981).

Diets

In Trial 1, lambs were offered food with the alkaloids 5-methoxy-N,N-dimethyltryptamine (T) or Gramine (G) in combination with a food high in saponins. Four groups of 5 lambs were offered G or T with saponin (S) in a 2 x 2 factorial that included alkaloid (G or T) with or without saponin as follows: Group G+S, Group T+S, Group G only, and Group T only. Trial 2 was similar to trial 1 except that I used a new group of 20 lambs and lambs in Groups 1 and 2 were offered the supplemental food with tannin instead of saponin.

I adjusted the amount of secondary chemicals in the lambs' diets according to the levels commonly found in plants. The tannin-containing food was 75.5% beet pulp, 14% soybean meal, 0.5% pomace, 2% soybean oil, and 8% tannin (quebracho tannin from Tannin Corp., Peabody, MA). The gramine-containing food was 74% beet pulp, 14% soybean meal, 9.8% pomace, 2% soybean oil, and 0.2% gramine (gramine from Sigma Chemical Co.). The saponin-containing food was 74.5% beet pulp, 14% soybean meal, 7.5% pomace, 2% oil, and 2% saponin (saponin from Sigma Chemical Co.). The dimethyltryptamine-containing food was 74% beet pulp, 14% soybean meal, 10% pomace, 2% oil, and 30ppm 5-methoxy-N,N-dimethyltryptamine (dimethyltryptamine from Sigma Chemical Co.). Thus, the primary chemistry of the foods was basically the same, which allowed us to better isolate the effects of the PSCs on food intake and nutrient digestion.

Collection Period

The above feeding protocol was a part of a 21-day adaptation period, which was followed by a 5-d collection period in which foods, feces, and urine were sampled daily to determine dry matter (DM) intake and nutrient digestibility. Foods were offered ad libitum. Food and refusal samples were weighed daily and dried at 60 degrees centigrade for 72 h. After drying, samples were weighed again to determine dry matter content. Dried samples were ground in a Whiley mill with a 1mm filter. Twenty grams of each food sample were composited according to food to represent DM and nutrients fed during the 5-day collection period. A separate composite containing 20 g from each refusal per sheep was made according to species of plant. For instance, a sheep fed G and saponin would have 2 composites, one per food. Daily values for forage intake were averaged for the 5-d collection period and then matched for each animal with the composited 5-d samples of feces and urine to determine nutrient intake and digestibility.

Fecal and urine samples were weighed and collected daily. I weighed total fecal output and then made 1 composite for each sheep that consisted of 30g/d. Each fecal composite was dried at 60 degrees centigrade for 5 d until completely dry to determine dry matter content of feces. Urine samples were collected in 10 ml of HCL to prevent NH_3 losses. Urine samples were collected and measured daily. I made one composite per lamb that consisted of 300ml of urine per day. One hundred ml from each composite was freeze dried for use in nitrogen analyses. Forage and fecal samples were analyzed for 1) dry matter (DM) (AOAC, 1990); 2) nitrogen (Method 990.03 AOAC, 2002); 3) neutral detergent fiber (NDF) (Goering and Van Soest, 1970); and 4) gross energy (AOAC, 1990). Dried composites were used to determine dry matter intake, nutrient intake, and energy intake. Nutrients consumed vs. excreted in feces were measured to assess apparent digestibility of DM, energy, and NDF (hemicelluloses, cellulose, lignin, and insoluble ash). Nitrogen consumed versus excreted in urine and feces were used to determine nitrogen utilization.

Statistical Analyses

The statistical design for each trial (1 and 2) was a 2 x 2 factorial with alkaloid (G or T) and supplemental food (yes or no) as the main effects. Animals (n = 5 per treatment) were nested within treatments. Day (n=5) was the repeated measure. Saponin containing food was the supplement used in trial 1 and tannin containing food was the supplement used in trial 2. The response variables were dry matter intake and nutrient digestibility. Due to the small sample sizes (n=5), I consider $P<0.10$ significant.

RESULTS

Trial 1: Alkaloids and Saponin

Total Dry Matter Intake

There were no differences in total dry matter intake due to alkaloid (see table 3-1) or saponin (see table 3-2). Nor were there interactions between alkaloid and saponin (see table 3-2), alkaloid and day (P=0.32).

Table 3-1. Digestibility table for lambs fed gramine vs. dimethyltryptamine in Trial 1.

	G[1]	T[2]	Std. Error	Alkaloid
Dry Matter Intake (g/d)	941	906	70	P=0.74
Alkaloid Intake (g/d)	770	790	54	P=0.80
Saponin Intake (g/d)	340	231	69	P=0.29
DM[3] Digestibility (%)	78	79	1	P=0.66
DM Digested (g/d)	3,600	3,455	292	P=0.73
Digested N[4] Retained (%)	41	37	3	P=0.47
N Retained/N Consumed (%)	40	44	3	P=0.24
N Retained (g/d)	51	54	7	P=0.77
N Digested (g/d)	83	85	7	P=0.89
N Digestibility (%)	67	70	2	P=0.24
Energy Digestibility (%)	77	77	1	P=0.84
Energy Digested (Kcal/d)	14,722	13,964	1205	P=0.66
NDF[5] Digestibility (%)	76	78	1	P=0.48
NDF Digested (%)	1,506	1,520	132	P=0.94

[1]Gramine.
[2]Tryptamine.
[3]Dry matter.
[4]Nitrogen.
[5]Neutral detergent fiber.

Intake of Foods with Alkaloids

Food intake did not differ due to alkaloid (Table 3-1). There were no interactions between alkaloid and saponin (Table 3-2). Saponin decreased the intake of food with gramine or tryptamine (Table 3-2).

Intake of Food with Saponin

There were no differences in intake of the food containing saponin due to alkaloids.

Table 3-2. Digestibility table for lambs fed foods with and without saponin in Trial 1 of the Green Canyon Study.

	G[1] without Sap[2]	G with Sap	T[3] without Sap	T with Sap	Std. Error	Sap	Sap*Grass
Dry Matter Intake (g/d)	940	941	950	863	100	P=0.68	P=0.67
Alkaloid Intake (g/d)	940	600	950	630	77	P=0.0005	P=0.90
DM[4] Digestibility (%)	78	79	78	80	2	P=0.56	P=0.92
DM Digested (g/d)	3,475	3,646	3,607	3303	412	P=0.80	P=0.64
Digested N[5] Retained (%)	40	42	40	35	5	P=0.49	P=0.86
N Retained/N Consumed (%)	39	40	44	43	4	P=0.15	p=0.93
N Retained (g/d)	48	54	58	51	10	P=0.94	P=0.52
N Digested (g/d)	79	88	87	83	10	P=0.85	P=0.53
N Digestibility (%)	65	69	68	71	2	P=0.15	P=0.93
Energy Digestibility (%)	76	78	77	78	2	P=0.55	P=0.83
Energy Digested (Kcal/d)	14415	15,030	14,681	13,248	1703	P=0.81	P=0.56
NDF[6] Digestibility (%)	76	77	78	78	2	P=0.59	P=0.84
NDF Digested (g/d)	1,455	1,557	1,584	1,455	187	P=0.95	P=0.55

[1]Gramine.
[2]Saponin.
[3]Tryptamine.
[4]Dry matter.
[5]Nitrogen.
[6]Neutral detergent fiber.

Dry Matter Digested

The digestibility of dry matter did not differ when lambs were fed gramine or tryptamine (Table 3-1). Nor did the digestibility of dry matter differ when lambs were supplemented with saponin (Table 3-2). Alkaloid and saponin did not interact (Table 3-2).

Nitrogen Digested

Nitrogen digestibility and grams of nitrogen digested did not differ for lambs fed gramine and tryptamine (Table 3-1). Nor did nitrogen digestibility and grams of nitrogen digested differ due to saponin (Table 3-2). Alkaloid and saponin did not interact (Table 3-2).

Nitrogen Retention

The percent of digested nitrogen retained did not differ due to alkaloid (Table 3-1) or saponin (see table 3-2). Nor did alkaloids and saponin interact (P=0.52).

Kilocalories Digested

There were no differences in energy digestibility or energy digested due to alkaloid (Table 3-1) or saponin (Table 3-2). Alkaloid and saponin did not interact (Table 3-2).

NDF Digested

There were no differences in NDF digestibility or NDF digested due to alkaloid (Table 3-1) or saponin (Table 3-2). Nor did alkaloid and saponin interact (Table 3-2).

Trial 2: Alkaloids and Tannins

Total Dry Matter Intake

Total dry matter intake did not differ due to alkaloid (Table 3-3). There was a trend toward increasing intake of G with tannin (Table 3-4). Alkaloid and tannin did not interact (Table 3-4).

Table 3-3. Digestibility table for lambs fed gramine vs. dimethyltryptamine in Trial 2 of the Green Canyon Study.

	G[1]	T[2]	Std. Error	Alkaloid
Dry Matter Intake (g/d)	965	914	50	P=0.48
Alkaloid Intake (g/d)	882	801	48	P=0.25
Tannin Intake (g/d)	166	226	30	P=0.20
DM[3] Digestibility (%)	76	77	1	P=0.53
DM Digested (g/d)	3582	3467	184	P=0.66
Digested N[4] Retained (%)	46	39	47	P=0.36
N Retained/N Consumed (%)	32	36	3	P=0.40
N Retained (g/d)	39	44	5	P=0.47
N Digested (g/d)	70	80	6	P=0.25
N Digestibility (%)	58	66	3	P=0.08
Energy Digestibility (%)	75	74	1	P=0.69
Energy Digested (Kcal/d)	1485	13356	737	P=0.17
NDF[5] Digestibility (%)	74	75	1	P=0.43
NDF Digested (%)	1602	1533	89	P=0.59

[1]Gramine.
[2]Tryptamine.
[3]Dry matter.
[4]Nitrogen.
[5]Neutral detergent fiber.

Table 3-4. Digestibility table for lambs fed foods with and without tannin in Trial 2 of the Green Canyon Study.

	G[1] without Tan[2]	G with Tan	T[3] without Tan	T with Tan	Std. Error	Tan	Tan* Grass
Dry Matter Intake (g/d)	887	1,042	904	923	70	P=0.23	P=0.35
Alkaloid Intake (g/d)	887	877	904	697	68	P=0.13	P=0.17
DM[4] Digestibility (%)	77	75	80	74	1	P=0.0026	P=0.20
DM Digested (g/d)	3,358	3,806	3,606	3,327	260	P=0.75	P=0.18
Digested N[5] Retained (%)	54	37	43	35	67	P=0.07	P=0.49
N Retained/N Consumed (%)	25	39	34	38	5	P=0.07	P=0.33
N Retained (g/d)	29	49	41	47	7	P=0.07	P=0.35
N Digested (g/d)	61	79	71	89	8	P=0.06	P=0.99
N Digestibility (%)	54	61	60	73	5	P=0.04	P=0.46
Energy Digestibility (%)	76	73	77	71	1	P=0.003	P=0.23
Energy Digested (Kcal/d)	14,012	15,682	13,924	12,883	1,043	P=0.74	P=0.23
NDF[6] Digestibility (%)	75	72	79	71	2	P=0.008	P=0.27
NDF Digested (g/d)	14,282	15,682	13,829	12,883	126	P=0.87	P=0.19

[1]Gramine.
[2]Saponin.
[3]Tryptamine.
[4]Dry matter.
[5]Nitrogen.
[6]Neutral detergent fiber.

Intake of Food with Alkaloids

There were no differences in intake due to alkaloid (Table 3-3) or tannin (Table 3-4). Nor were there interactions between alkaloids and tannin (Table 3-4).

Intake of Food with Tannin

There were no differences in tannin intake due to alkaloid.

Dry of Matter Digested

Dry matter digestibility and dry matter digested did not differ due to alkaloid (Table 3-3). Dry matter digestibility was lower for lambs supplemented with tannin (Table 3-4). However, due to increased intake when lambs were supplemented with tannin, there were no differences in dry matter digested due to tannin (Table 3-4). Alkaloid and tannin did not interact (Table 3-4)

Nitrogen Digested

Nitrogen digestibility was higher for lambs fed tryptamine than for lambs fed gramine, however, there were no differences in the amount of N digested (Table 3-3). Nitrogen digestibility was higher and lambs digested more nitrogen when supplemented with tannin than when not supplemented (Table 3-4). Alkaloid and tannin did not interact (Table 3-4).

Nitrogen Retention

There were no differences in the percent of digested nitrogen retained due to alkaloid (Table 3-3). Lambs retained a smaller percent of digested nitrogen when supplemented with tannin. However, due to increased intake, lambs offered G retained similar nitrogen as lambs offered T (Table 3-3). Alkaloid and tannin did not interact (Table 3-4).

Kilocalories Digested

There were no differences in energy digestibility or energy digested due to alkaloid (Table 3-3). Digestibility of energy was lower for lambs offered tannin than for lambs not offered tannin. However, due to increased intake, there was no difference in energy digested due to tannin (Table 3-4). There was a trend toward an interaction between alkaloid and tannin because tannin affected G differently from T. Lambs fed a basal diet of G and supplemented with tannin digested more energy than lambs fed only G, while lambs fed T and supplemented with tannin digested less energy than lambs fed T only (Table 3-4).

NDF Digested

NDF digestibility or the amount of NDF digested did not differ due to alkaloid (Table 3-3). NDF digestibility was lower for lambs supplemented with tannin than for lambs not supplemented. However, due to increased intake by lambs supplemented with tannin, there were no differences in NDF digested due to tannin (Table 3-4). Alkaloid and tannin did not interact (Table 3-4).

DISCUSSION

I hypothesized that saponins and tannins inactivate alkaloids due to their binding affinities, thus reducing the negative effects of alkaloids on nutrition and intake. Based on this hypothesis, I predicted that food intake and nutrient digestibility would be higher for lambs supplemented with saponin or tannin than for lambs not supplemented.

Saponin

Supplementing lambs with a saponin-containing food did not affect total dry matter intake or nutrient digestibility, but there were differences in alkaloid intake due to offering saponin to lambs. Lambs offered saponin during the first 30 min of feeding each day consumed less of the alkaloid-containing food than lambs not offered saponin (615g vs. 945g). This may be due in part to lambs eating the saponin-containing food and needing less of the alkaloid-containing food to meet their nutritional requirements. On the other hand, lambs not offered saponin had to eat more of the alkaloid-containing food to meet their nutritional requirements. Based on chemical structures and binding affinities, I predicted that saponin would complement the ergot alkaloids in endophyte-infected tall fescue. Due to difficulty locating a chemical supplier with ergovaline or ergotamine, dimethyltryptamine was used to simulate the ergot alkaloids of tall fescue. I would expect greater differences if this study was duplicated using one of the ergot alkaloids rather than dimethyltryptamine.

Tannin

Lambs supplemented with tannins digested a lower percent of consumed NDF, energy, and dry matter (Table 3-4). Nonetheless, there were no differences in the total amount of NDF, energy, and dry matter digested by lambs offered tannin and lambs not offered tannin because lambs fed tannin tended to consume more dry matter (Table 3-4). As a result, they digested approximately the same amount of dry matter, energy, and NDF as lambs not offered tannin.

Supplementing with tannin increased nitrogen utilization by lambs. Lambs supplemented with tannin digested more nitrogen than lambs not supplemented with tannin (Table 3-4). They also digested more consumed nitrogen (Table 3-4) than lambs not offered tannin. These results support the findings of Lyman (Lyman, Provenza, and Villalba, 2008, unpublished data) that suggest offering tannin to sheep reduces the adverse effects of alkaloids on intake. Other studies suggest similar advantages of offering lambs complementary PSC. When offered foods with terpenes, tannins, and oxalates, sheep eat more than when offered foods with only one or two of these PSC (Villalba et al., 2004).

The forage (Lewiston) study and the ration (Green Canyon) study show benefits of supplementing lambs with foods containing high concentrations of tannins. However, the benefits of supplementing with tannin were different for each study. Supplementing lambs fed alkaloid containing grasses with a high-tannin variety of birdsfoot trefoil (*Lotus corniculatus Goldie variety*) stimulated increased intake of dry matter and resulted in lambs digesting more energy and nitrogen, but did not affect the digestibility of these nutrients. In the ration study, however, supplementing with tannin increased digestibility of nitrogen, but did not affect dry matter intake. Several factors likely contributed to the differences in the two studies. The advantages experienced in the pasture study were undoubtedly due in part to interactions among primary and secondary compounds that affected forage intake. Conversely, the foods offered in the pen study were total mixed rations of the same ingredients that differed only in their contents of one PSC. Tannins increased nitrogen digestibility, but did not affect intake.

More generally, tannins are increasingly recognized as compounds important in health and nutrition, though historically they were thought by agriculturalists and ecologists alike to adversely affect herbivores. Eating plants high in tannins is a way for herbivores to reduce internal parasites (Min and Hart, 2003), and tannins alleviate bloat by binding to proteins in the rumen (Waghorn, 1990). By making the protein unavailable for digestion and absorption until it reaches the more acidic abomasum, tannins also enhance nutrition by providing high-quality protein to the small intestines (Barry et al., 2001). This high-quality-protein-bypass effect enhances immune responses and increases resistance to gastrointestinal nematodes (Niezen et al., 2002; Min et al., 2004). The resulting increase in essential and branched-chain amino acids improves reproduction efficiency in sheep (Min et al., 2001). Tannins in the diet are a natural way to reduce methane emission in ruminants (Woodward et al., 2004), which is an important issue regarding ongoing efforts to diminish the influence of livestock on global warming. Finally, tannins eaten in modest amounts by herbivores can improve the color and quality of meat for human consumption (Priolo et al., 2005). More generally, diverse assortments of SC in the diets of herbivores influence the flavor, color and quality of meat and milk for human consumption, often in ways that are positive (Vasta et al., 2008).

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CHAPTER 4

CONCLUSIONS AND RECOMMENDATIONS FOR MANAGEMENT AND RESEARCH

I determined if lambs fed combinations of foods containing alkaloids, tannins, and saponins ate more and had better nutrient utilization than lambs offered only alkaloid-containing foods. I hypothesized that food intake and digestibility increase when lambs eat plants such as alfalfa that contain saponins or birdsfoot trefoil that contain tannins along with plants such as endophyte-infected tall fescue or reed canarygrass that contain alkaloids. Based on this hypothesis, I predicted that the nutritional status of lambs would be enhanced if they were supplemented with alfalfa or birdsfoot trefoil.

There were differences in the results of the field (forage) trials and the pen (ration) trials. In the Forage Study, supplementing lambs fed TF and RCG (alkaloids) with ALF (saponin) or BFT (tannin) increased forage intake, which in turn increased nutrient digestibility. Nutrients from ALF and BFT were higher than nutrients in the grasses. Hence, nutrient intake was higher for lambs supplemented with ALF or BFT.

In the Ration Study, where all diets were isonitrogenous and isocaloric, there were no benefits to substituting one food with another except with regard to PSC. The only advantage of supplementing lambs fed feed containing alkaloids with feed containing tannin or saponin was increased intake and utilization of nitrogen by lambs supplemented with tannin.

Many factors likely contributed to the differences in the Forage and Ration studies. The advantages experienced in the Forage Study were undoubtedly due in part to interactions among primary and secondary compounds that affected forage intake. Conversely, the foods offered in the Ration Study were total mixed rations that consisted of the same ingredients and only differed in their content of one PSC. Thus, differences observed in intake and nutrient utilization could be attributed to differences in such PSC.

In the pen trials, I used only one compound (5-methoxy-N,N-dimethyltryptamine or gramine) in combination with a food high in saponins or tannins. Conversely, plants in the pasture trials were considerably more diverse in secondary compounds. Tall fescue has two major types of alkaloids, those associated with the plant and those due to infestation of the fungus *Neotyphodium coenophialum*. Alkaloids produced by the plant -- perlolidine and perloline -- can affect rumen fermentation. The fungus-associated alkaloids -- N-acetyl loline and N-formyl loline – are associated with fescue toxicity. Perlolidine and perloline are both steroidal in nature. N-acetyl loline and N-formyl loline both have lipid chemical structures (Cheeke and Schull, 1985; Cheeke, 1998). Reed canarygrass, in its wild form, contains eight alkaloids -- four derivatives of tryptamine, gramine, hordine, and two derivatives of β-carboline -- that when ingested in monocultures reduce intake and performance, and in extreme cases produce gross histopathology of the central nervous system (Phalaris staggers) (Marten et al., 1973, 1981). Alfalfa contains glycosides such as saponins (Lu and Jorgensen, 1987) and birdsfoot trefoil contains tannins (Ramirez-Restrepo and Berry, 2005), both of which reduce intake and performance when consumed as single plants in too large amounts.

In addition to differences in chemical characteristics of plants, differences in dry matter intake could in part be due to differences in the physical characteristic of the forages. Lambs in the Forage Study preferred to eat only the leaves of plants and refused the stems. Increased intake of certain combinations of plants could be due to the ability of lambs to harvest the leaves of the different plants. For instance, reed canarygrass had larger leaves and appeared easier for the lambs to harvest than the leaves of tall fescue. When lambs were offered either reed canarygrass or alfalfa, they appeared to spend more time eating and less time nosing through the food searching for desirable parts of plants than when lambs were offered tall fescue. In the Ration study, however, food intake and digestibility were not affected by differences in the primary chemistry or the physical structure of the foods.

RECOMMENDATIONS FOR MANAGEMENT

My findings provide a nutritional basis for the benefits of mixtures versus monocultures observed in grazing trials with sheep (Lockard, Provenza, Villalba, and Cheney, 2008, unpublished data) and cattle (Lyman, Provenza, and Villalba, 2008, unpublished data), and they suggest mixtures may enhance use of pastures for finishing livestock. Mixtures of plants increased dry matter intake and nutrient digestibility for lambs fed alkaloid-containing grasses. Planting alfalfa or birdsfoot trefoil along with tall fescue and reed canarygrass can enhance pasture productivity and provide animals on pastures with nutritional benefits that also enhance performance and health of pasture-finished lambs and calves as well.

While some argue the time to finish cattle on pastures is too great compared with feedlots, fall-born calves can gain 2.5 to 3.0 lbs/head/day on the nutritious plant mixtures we propose to use (Meek et al., 2004; Lyman, Provenza, and Villalba, 2008, unpublished data), and they can be slaughtered at as little as 14 months of age (Wiedmeier, Provenza, Villalba, 2005, unpublished data). Most importantly, the cost/unit of digestible energy on pastures is $0.029 while that on a typical high-cereal grain feedlot diet is $0.075 for a difference of 162% (Wiedmeier and Snyder 2005, unpublished data).

The discovery of endophyte-infected Kentucky-31 tall fescue, which now grows on 14 million hectares of pasture land in the U.S. (Buckner et al., 1979), was revolutionary for enabling livestock production in the so-called “transition zone” from Missouri and Arkansas to the east coast. Indeed, fescue made Missouri second in the nation in livestock production. Though endophyte-infected tall fescue is not typically classified as a toxic plant, the alkaloids it contains cause severe losses cattle, and a conservative estimate of the impact of fescue alkaloids on livestock exceeds $500 million annually (Paterson et al., 1995). At the same time, the alkaloids so problematic for livestock make the plant highly resistant to drought and other environmental stressors. Our research suggests consumption of alfalfa and trefoil can increase nutrient intake and digestion, and perhaps reduce fescue toxicity by tannins and saponins binding with alkaloids.

If saponin- or tannin-containing legumes can offset the negative effects of the alkaloids in endophyte-infected tall fescue and enhance livestock performance, the economic impact for beef producers coping with fescue toxicosis will be enormous. In that regard, it is indeed significant that cattle steadily decrease time eating endophyte-infected tall fescue when they grazed tall fescue first for 30 min and then birdsfoot trefoil and/or alfalfa alone for 60 min; yet, when the sequence is reversed they foraged actively on both trefoil and/or alfalfa followed fescue throughout a 90-minmeal (Lyman 2008, unpublished data). More generally, other toxic plant problems worldwide may benefit from similar research and applications on forage mixtures.

RECOMMENDATION FOR RESEARCH

The predicted chemical interactions that formed the basis of my hypothesis are that alkaloids in tall fescue and reed canarygrass bind with saponins (alfalfa) and tannins (birdsfoot trefoil) in the gastrointestinal tract and are excreted in their feces rather than absorbed. Based on these structural characteristics and binding affinities, I hypothesized that forage intake and nutrient utilization increase when sheep ate a mixture of alkaloid-containing grass along with complementary saponin- or tannin-containing leguminous forage, as compared with eating only high-alkaloid forage. Additional research is needed to determine if such binding occurs.

In my study, offering alfalfa to lambs fed tall fescue increased intake and as a result increased the amount of dry matter digested. However, offering alfalfa lowered the digestibility of dry matter. Further research could indicate if lower digestibility when offered alfalfa was due to the high levels of saponin in the alfalfa or the increase in intake and rate of passage. Further research should be done to determine if the advantages of supplementing lambs with tannin- and saponin-containing legumes can be attributed to the predicted chemical interactions that are the basis of our hypothesis. If such a chemical interaction exists, it would be expected that lambs supplemented with saponins or tannins digest fewer alkaloids and reduce the rate of alkaloid absorption, resulting in more alkaloids that are excreted in the feces. If so, such an interaction would reduce the occurrence of fescue toxicosis associated with the consumption and digestion of alkaloids and increase the health of animals grazing tall fescue.

More research could also indicate if supplementing lambs on high-alkaloid grasses with other legumes or forbs containing high concentrations of PSC result in similar benefits as supplementing with alfalfa and birdsfoot trefoil. Native forbs such as scarlet globemalow (*Sphaeralcea coccinea*) and leatherleaf croton (*Croton pottsii* Lam.) provide cattle on low quality grass diets with similar advantages as offering alfalfa (Arthun et al., 1992). Native forbs such as these and other pasture legumes such as the many different clovers may provide lambs with similar nutritional benefits as alfalfa or birdsfoot trefoil.

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