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Cattle preferences differ when endophyte-infected tall fescue, birdsfoot trefoil, and alfalfa are grazed in different sequences

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Cattle preferences differ when endophyte-infected tall fescue, birdsfoot trefoil, and alfalfa are grazed in different sequences[1]

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ABSTRACT: We determined if sequence of ingestion affected use of endophyte-infected tall fescue (TF) when cattle also grazed birdsfoot trefoil (BFT) or alfalfa (ALF). Based on chemical characteristics of TF (alkaloids), BFT (tannins), and ALF (saponins), we hypothesized that cattle first allowed to graze ALF or BFT would subsequently spend more time grazing TF than cattle that first grazed TF followed by ALF or BFT. Sixteen bred heifers (478 ± 39 kg initial BW) were randomly assigned to 4 replicated pasture units. Each replicated unit consisted of 4 treatment sequences (TF→BFT, TF→ALF, BFT→TF, or ALF→TF), with 2 cows per sequence. Pastures were in the vegetative stage of growth at a height of 20 to 30 cm and provided ad libitum forage to cattle. We recorded foraging on TF, BFT, and ALF using scan sampling of individuals at 2-min intervals. The study was conducted in 4 phases run sequentially, for a total of 30 d. In phases 1 and 3, cattle in group 1 grazed TF pastures for 45 min and were then moved to BFT pastures for the next 45 min (TF→BFT); cattle in group 2 grazed in the reverse sequence (BFT→TF). In phases 2 and 4, cattle in group 1 grazed TF pastures for 45 min and then subsequently grazed ALF pastures for the remaining 45 min (TF→ALF); cattle in group 2 grazed in the reverse sequence (ALF→TF). Sequence of plant ingestion affected food selection. In phase 1, scans revealed grazing of TF by heifers was cyclic, and heifers tended to have more scans ($P = 0.52$) grazing TF when they grazed BFT→TF; scans for heifers grazing TF were consistently greater ($P < 0.05$) throughout phase 3 of the trial. In phase 2, heifers that grazed in the sequence ALF→TF spent considerably more scans ($P = 0.03$) foraging on TF from d 4 to 10 than heifers that grazed in the sequence TF→ALF, and they remained greater throughout phase 4 of the trial. Although the sequence ALF→TF appeared to be more effective than BFT→TF, consistent with the hypothesis of a complementary relationship between the steroidal alkaloids in TF and saponins in ALF, tannin concentrations in BFT were minimal (1.8%), which likely reduced the presumed inactivation of alkaloids by tannins. We also speculate that heifers needed to learn about the positive postingestive influence of sequence, a notion consistent with more similar scans spent foraging BFT and TF early in phases 1 (BFT→TF) and 2 (ALF→TF), and with the consistent and marked increase in scans spent foraging on TF for animals foraging in phases 3 (BFT→TF) and 4 (ALF→TF).

Key words: animal behavior, complementarity, diet selection, foraging, pasture diversity, plant secondary compound

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INTRODUCTION

Little is known about how the sequence of food ingestion influences forage intake and preference, although it appears to be important. Sheep eat more when foods with secondary compounds are offered in the morning followed by limited nutritious foods in the afternoon (Papachristou et al., 2007). Sheep also eat more food with terpenes when they first eat food with tannins (Mote et al., 2008); food with tannins eaten first remains in the gut up to 72 h (Silanikove et al., 1994), where tannins can interact with terpenes, whereas terpenes are highly soluble compounds absorbed quickly from the gastrointestinal tract and eliminated from the body (Foley and McArthur, 1994). Altering the sequence of forage ingestion also causes calves to spend more time eating grasses, such as endophyte-infected tall fescue (**TF**) and reed canarygrass, when they first

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eat legumes, such as alfalfa (**ALF**) or birdsfoot trefoil (**BFT**), and then eat endophyte-infected tall fescue or reed canarygrass (Lyman, 2008).

Our objective in the present study was to follow up on the study of Lyman (2008), which was more elaborate in the choices offered to fall-born calves, with a specific focus on foraging sequence and more limited choices offered to bred heifers. To do so, we determined if the sequence of grazing TF and legumes containing tannins (BFT) or saponins (ALF) increased use of TF by bred heifers. The alkaloids in TF are protein-like and steroidal in nature, whereas the tannins in BFT and the saponins in ALF are high molecular weight compounds, not absorbed through the rumen wall, with high affinity for binding to protein and lipid-like compounds such as the alkaloids in TF (Jones and Mangan, 1977; Malinow et al., 1979). We thus hypothesized cattle would eat more TF after eating forages with tannins (BFT) or saponins (ALF).

MATERIALS AND METHODS

Procedures followed the protocols for animal care and use (Institutional Animal Care and Use Committee protocol approval number 1372).

Chemical Characteristics of Plant Species

Alkaloids are small, fat-soluble molecules absorbed rapidly through the rumen epithelium. In large amounts, they can decrease food intake and animal performance (Cheeke and Shull, 1985). Tall fescue contains 2 groups of alkaloids, one associated with the plant and the other allied with the fungus *Neotyphodium coenophialum*. The intrinsic alkaloids perlolidine and perloline, which are steroidal, negatively affect rumen fermentation and food intake. The fungus-associated alkaloids *N*-acetylloline, *N*-formylloline, ergotamine, and ergovaline, which have lipid structures, reduce food intake and cause fescue toxicity.

Conversely, tannins and saponins are high-molecular-weight compounds (2,000 to 4,000 g/mol) that remain in the gut for many hours where they interact with many other compounds (Kumar and Singh, 1984; Min and Hart, 2003). Condensed tannins in plants like BFT bind to proteins in the rumen (Jones and Mangan, 1977); because alkaloids are N-based, we hypothesized including tannin-containing BFT in the diets of livestock would neutralize the alkaloids in TF, and stable complexes form between alkaloids and tannins (Okuda et al., 1982). Saponins in plants like alfalfa bind to lipids such as cholesterol in the gastrointestinal tract of animals causing their excretion in the feces (Malinow et al., 1979); because the endogenous alkaloids in TF are lipids, we hypothesized including ALF in the diet of animals grazing TF would also neutralize the alkaloids in TF. Given the rapid rate of absorption of alkaloids relative to tannins and saponins, we hypothesized that first grazing BFT or ALF would increase concentrations of tannins and saponins in the rumen and enable heifers to better use TF.

Pasture Design

Plant species with alkaloids, tannins, and saponins were seeded at the Utah Agricultural Experiment Station Pasture Research Facility in Lewiston (41°57′ N, 111°52′ W). In 2006, we planted monocultures of TF (*Festuca arundinacea*, Kentucky 31 endophyte-infected; Rottinghaus et al., 1991) with increased concentrations of alkaloids, BFT (*Lotus corniculatus* variety Goldie) with increased tannins (Terrill et al., 1992; Hedqvist et al., 2000), and ALF (*Medicago sativa* varieties Vernal and Lahontan) with increased saponins (Pedersen, 1975; Pedersen et al., 1976).

Pasture units, constructed with temporary electric fence, consisted of a 0.05-ha plot of TF, a 0.04-ha plot of ALF and a 0.04-ha plot of BFT planted in adjacent monoculture strips, with 2.25 ha of orchardgrass (**OG**) as a holding area (Figure 1). We had 4 replications of each unit with the following sequences of grazing: 1) TF followed by (→) ALF or BFT (sequence 1), and 2) ALF or BFT followed by (→) TF (sequence 2).

Grazing Trials

Sixteen bred 2-yr-old Black Angus heifers (478 ± 39 kg initial BW) were used in all phases of the trials. We randomly assigned 4 heifers to each of the 8 pasture units, with 2 heifers in each sequence within a unit. Each morning, cattle were moved from adjacent OG pastures to trial pastures for their morning meals. After each morning trial, cattle were moved back into OG pastures, where water and a low-moisture salt and mineral block supplement were provided. Pastures were in the vegetative stage of growth at a height of 20 to 30 cm, and all plots provided ad libitum forage to cattle. Cattle were weighed pre- and posttrial to estimate changes in BW during the 30-d trials. Cattle gained an average of 0.95 ± 0.54 kg of BW/(animal·d^{-1}).

Phase 1: TF and BFT. In the first phase, which lasted 10 d, cattle in group 1 grazed TF pastures for 45 min and were then moved to BFT pastures for the next 45 min (sequence 1, TF→BFT). Cattle in group 2 grazed in the reverse sequence (sequence 2, BFT→TF).

Phase 2: TF and ALF. In the second phase, which also lasted 10 d, cattle in group 1 grazed TF pastures for 45 min and then subsequently grazed ALF pastures for the remaining 45 min (sequence 1, TF→ALF). Cattle in group 2 grazed in the reverse sequence (sequence 2, ALF→TF).

Phase 3: TF and BFT (5 d). Given the experience animals gained in phase 1, we wanted to determine if their behavior was similar in phase 3 to that exhibited in phase 1. The third phase thus consisted of one 5-d period where cattle in group 1 first grazed TF pastures for 45 min and then grazed BFT for 45

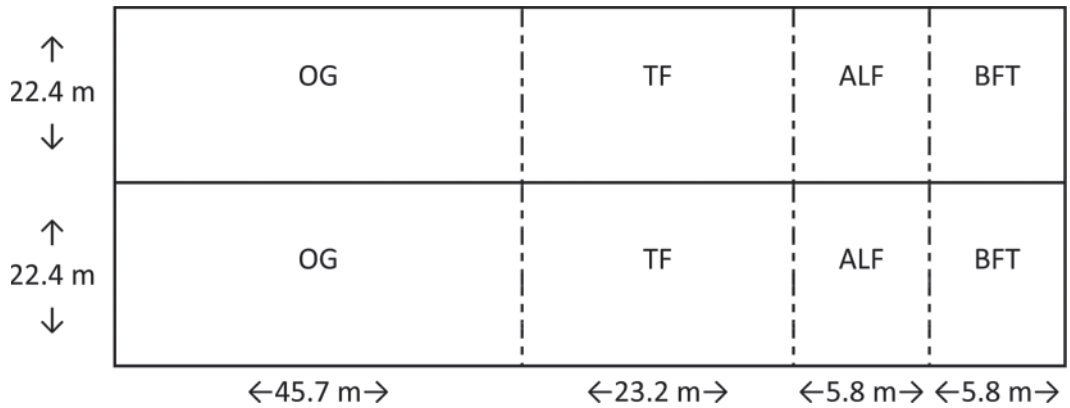

Figure 1. Depiction of one pasture unit containing adjacent monoculture pastures of tall fescue (TF), alfalfa (ALF), and birdsfoot trefoil (BFT) with orchardgrass (OG) holding areas. Within each pasture unit were 2 sequence treatments (sequence 1: TF → BFT or ALF and sequence 2: BFT or ALF → TF).

min for a 5-d period (sequence 1, TF→BFT). Cattle in group 2 grazed in the reverse sequence (sequence 2, BFT→TF).

Phase 4: TF and ALF (5 d). Given the experience animals gained in phase 2, we wanted to determine if their behavior was similar in phase 4 to that exhibited in phase 2. The fourth phase thus consisted of another 5-d period where cattle in group 1 first grazed TF pastures for 45 min and then grazed ALF for 45 min for a 5-d period (sequence 1, TF→ALF). Cattle in group 2 grazed in the reverse sequence (sequence 2, ALF→TF).

Scan Samples

In all phases of the study, one observer recorded behavioral data using scan samples of individually marked animals at 2-min intervals throughout daily trials from 0600 to 1030 h each day (Altman, 1974). Scans were taken from a 3-m-high platform centrally located to enable the observed to see all of the animals and whether or not they were grazing a particular forage. Animals were considered to be grazing only when they were actually biting and chewing forage; no grazing scan was recorded if an animal had its head in the sward, but was not biting or chewing forage. We then calculated the percentage of scans in which each animal spent grazing each forage each day.

Chemical Composition of the Forages

Representative forage samples, collected from plants along a paced transect across each pasture, were hand-harvested at the end of the study, placed in plastic bags covered with dry ice immediately after harvest, and transported to a freezer where they were kept at −20°C. The samples were subsequently freeze-dried, ground through a Wiley mill with a 1-mm screen, and analyzed for NDF (Goering and Van Soest, 1970) and N (method 990.03, AOAC, 2002), as well as condensed tannins (BFT; Terrill et al., 1992), saponins (ALF; Lee et al., 2001), and the alkaloid ergovaline (TF; Rottinghaus et al., 1991).

Statistical Design

The statistical design for the ANOVA was a repeated measures with 4 replications of 2 sequences (TF→ legume or legume → TF). Day was the repeated measure. Separate analyses were carried out for phases 1, 2, 3, and 4. The response variable was percentage of scans observed per forage.

RESULTS

Chemical Composition of the Forages

Tall fescue contained more fiber and less N than either ALF or BFT ($P < 0.05$; Table 1). Tall fescue had increased ($P < 0.0001$) in ergovaline, and ALF contained increased ($P < 0.05$) amounts of saponins, but BFT was low in tannins (Table 1).

Phase 1

The percentage of scans of cattle grazing TF did not differ between groups when cattle grazed TF→BFT or BFT→TF. Heifers spent 36% of the scans grazing TF in the sequence TF→BFT and 45% of the scans grazing TF in the sequence BFT→TF ($P = 0.52$, Figure 2A). Scans of heifers spent grazing BFT did not differ (TF→BFT = 98% vs. BFT→TF = 96%, $P = 0.27$), nor was there an effect of day ($P = 0.36$) or a day × sequence interaction ($P = 0.27$).

Phase 2

The percentage of scans of cattle grazing TF differed between groups when heifers grazed TF→ALF as opposed to ALF→TF. Heifers spent 28% of the scans grazing TF in the sequence TF→ALF and 51% of the scans grazing TF in the sequence ALF→TF ($P = 0.03$,

Table 1. Plant chemical analyses (means ± SE) for NDF, N, and for alkaloids (endophyte-infected tall fescue, *Festuca arundinacea*, Kentucky 31), tannins (birdsfoot trefoil, *Lotus corniculatus* variety Goldie), and saponins (alfalfa, *Medicago sativa* variety Vernal)

Plant species	NDF, %	N, %	Total condensed tannins, %	Saponins, %	Ergovaline, μg/kg
Birdsfoot trefoil	44.1 ± 2.5	3.4 ± 0.6	1.81 ± 0.5	—	—
Alfalfa	42.2 ± 5.2	3.8 ± 0.5	—	3.9 ± 0.6	—
Tall fescue	59.8 ± 3.0	1.9 ± 0.4	—	—	263 ± 52.7

Figure 2C). Groups did not differ in scans of heifers grazing ALF (TF→ALF = 98% vs. ALF→TF = 98%, $P = 0.52$). There was an effect of day ($P < 0.0001$) and day × sequence interaction ($P = 0.03$).

Phase 3

When heifers grazed TF and BFT a second time, the percentage of scans of heifers grazing TF tended to differ between groups for TF→BFT and BFT→TF. Heifers grazed 37% of the scans on TF in the sequence TF→BFT and 61% of the scans on TF in the sequence BFT→TF ($P = 0.18$, Figure 2B). Groups did not differ in scans of grazing BFT (BFT→TF = 98%, TF→BFT = 95%, $P = 0.27$). There was an effect of day ($P < 0.05$), but no day × sequence interaction was observed ($P = 0.27$).

Phase 4

When heifers grazed TF and ALF a second time, the percentage of scans of grazing TF differed between groups for TF→ALF and ALF→TF. Heifers grazed 55% of the scans on TF in the sequence TF→ALF and 85% of the scans on TF in the sequence ALF→TF ($P = 0.05$, Figure 2D). Groups did not differ in scans of grazing ALF (ALF→TF = 98%, TF→ALF = 98%, $P = 0.52$). There was an effect of day ($P < 0.01$), but there was no day × sequence interaction ($P = 0.67$).

DISCUSSION

We determined if foraging sequence and plant diversity enhanced use of TF when cattle foraged on legumes that contained tannins (BFT) or saponins (ALF), and we found that forage species and sequence of forage ingestion affected food selection. Scans of cattle spent grazing TF were cyclic, especially when heifers grazed TF→BFT in phase 1, and they tended to spend more of the scans grazing TF when they grazed BFT→TF, particularly on d 3, 5, 7, 9, and 10 of phase 1. Importantly, scans of heifers spent grazing BFT were consistently greater throughout phase 3 of the trial, which is consistent with previous findings when calves grazed significantly more in the sequence BFT→TF than TF→BFT (Lyman, 2008). In phase 2, heifers that grazed in the sequence ALF→TF spent considerably more of the scans foraging on TF from d 4 to 10 compared with heifers that grazed in the sequence TF→ALF, and they remained greater throughout phase 4 of the trial.

Scans spent grazing TF increased throughout the trials for animals grazing BFT→TF and ALF→TF, though heifers consistently grazed less on TF plots in phase 1 (BFT→TF) than in phase 2 (ALF→TF). Thus, although the sequence ALF→TF appeared to be more effective than BFT→TF, consistent with the hypothesis of a complementary relationship between the steroidal alkaloids in TF and saponins in ALF, tannin concentrations in BFT were minimal (1.8%), which may have reduced the presumed tannin-alkaloid interactions. Tannin concentrations of 6 to 10% appear to be most effective at influencing grazing behavior without causing any harmful effects on intake or performance (Jones and Mangan, 1977; Lyman et al., 2008). We also speculate that heifers needed to learn about the positive postingestive influence of sequence for BFT and ALF on TF, a notion consistent with more similar scans of heifers spent foraging early in phases 1 (BFT→TF) and 2 (ALF→TF), and with the consistent and marked increase in scans of heifers spent foraging on TF for animals in phases 3 (BFT→TF) and 4 (ALF→TF).

Our findings thus support hypotheses that complementarities and foraging sequences influence scans of animals spent feeding and forage intake, and are consistent with findings that intake of high-alkaloid varieties of TF and reed canarygrass both increase when sheep eat legumes with increased amounts of tannins (BFT) or saponins (ALF; Owens, 2008). Whereas these studies provide indirect evidence of secondary compound interactions for cattle or sheep, direct evidence comes from studies where lambs given intraruminal infusions of tannins or saponins increase use of endophyte-infected TF relative to lambs not infused with tannins or saponins when offered choices of BFT, ALF, TF, and OG (Lisonbee et al., 2009; Villalba et al., 2011). Direct evidence also comes from studies where sheep eat more when offered foods with increased tannins or saponins along with foods with increased alkaloids (Lyman et al., 2008). This could occur because stable complexes form between alkaloids and tannins (Okuda et al., 1982), and because alkaloids bind to saponins in the gastrointestinal tract, causing their excretion in the feces (Malinow et al., 1979).

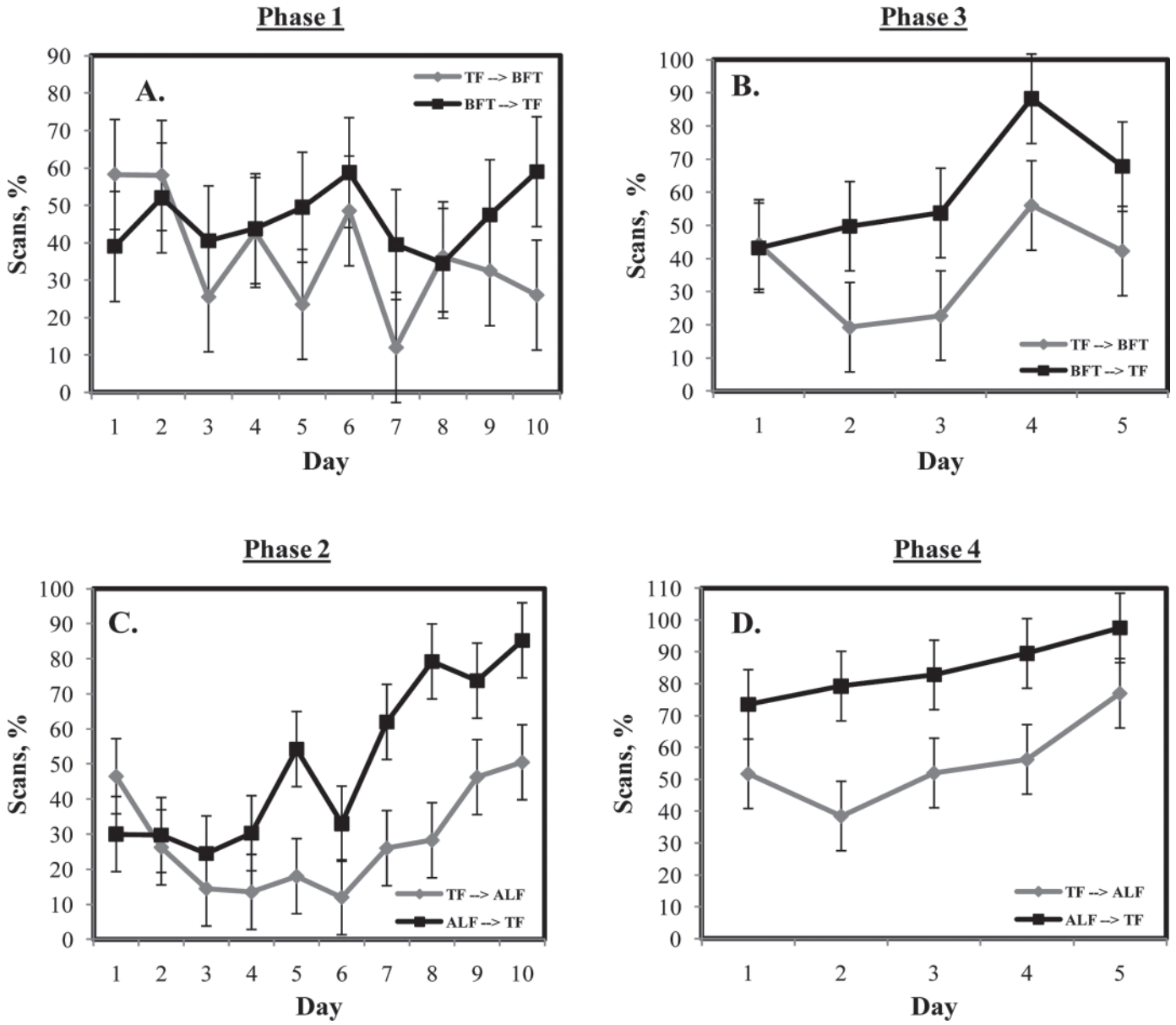

Figure 2. Percentage of scans in which heifers grazed tall fescue (TF) before or after a meal of birdsfoot trefoil (BFT; A and B), or alfalfa (ALF; C and D) in 4 phases. Scan samples were taken of individually marked animals from a 3-m-high, centrally located platform at 2-min intervals throughout daily trials to determine if cattle were grazing the allocated forage at each particular interval (Altman, 1974). Phases 1 and 3 lasted 10 d, whereas phases 2 and 4 lasted 5 d. Cattle first grazed TF for 45 min followed by a meal of BFT (A and B) or ALF (C and D) for 45 min or they first grazed BFT (A and B) or ALF (C and D) for 45 min followed by TF for 45 min. Bars are SE.

Obviously, more experimental analyses are necessary to assess the specific physiological and behavioral effects of interactions among secondary compounds, and to better understand higher-order interactions among primary and secondary compounds in various forages (Provenza et al., 2003). Both primary and secondary compounds in too great amounts can be toxic, whereas in appropriate amounts they interact to collectively benefit both nutrition and health (Provenza and Villalba, 2006, 2010). At the most simple levels, supplemental energy and protein enhance the abilities of animals to ingest forages with increased content of secondary compounds, particularly when animals must eat a diet with increased secondary compounds (e.g., Villalba et al., 2002a,b; see review by Provenza et al., 2003). In our study, additional protein in the legumes likely contributed to the differences in use of TF as additional protein helps facilitate detoxification processes (Foley and McArthur, 1994; Illius and Jessop, 1995; Foley et al., 1999), and protein supplementation increases intake of fiber (Van Soest, 1994). Meal size and length is larger in dairy cows fed a supplement before eating roughage than when the roughage is fed before the supplement (Morita et al., 1996). Whatever the higher-order interactions, findings from this and past studies show that forage complementarities and sequences facilitate intake of TF undoubtedly due to complex interactions among primary and secondary compounds.

Implications

Tall fescue is the primary grass growing on more than 14 million ha of pasture and hay land in the United States (Buckner et al., 1979). Most TF is endophyte-infected, and the negative impact of TF alkaloids on beef production was estimated at $600 million annually over 10 yr ago (Paterson et al., 1995). A conservative estimate places the total livestock-related losses at $500 million to $1 billion a year (University of Nebraska, Institute of Agriculture and Natural Resources; http://ianrnews.unl.edu/static/0106121.shtml). However, the alkaloids in TF, problematic for ruminants, make the plant highly resistant to drought and other stresses. Improved seedling performance and survival, as well as insect and nematode resistance, drought resistance, improved N assimilation, greater seed set, and overall increased survival are all benefits from using endophyte-infected TF in pasture systems (Pedersen et al., 1990).

Results from our study suggest ways for developing pastures and grazing systems that incorporate a variety of plant species with different secondary compounds to increase plant survivability as well as livestock productivity when dealing with forages such as TF. If, as our results suggest, tannin- or saponin-containing legumes can partially offset the negative effects of the alkaloids in TF, then new avenues for coping with fescue toxicosis would become available to producers willing to plant mixtures of forages. Our findings thus have the potential to create positive effects on the economic and environmental aspects of producing livestock on TF pastures.

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