Behavioral, Nutritional, and Toxicological Responses of Cattle to Ensiled Leafy Spurge

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ABSTRACT: Yearling cattle (n = 25; 416.1 ± 25.9 kg) were stratified by weight and gender across five groups. Group 1 (OAT) was offered oat/rape haylage (ORH) for ad libitum consumption during two daily feeding periods. Group 2 (SPURGE) was offered leafy spurge/grass haylage (LSGH) for ad libitum consumption during the same feeding periods. Group 3 was offered ORH in an amount equal to the average amount of LSGH consumed by SPURGE at the previous feeding. Group 4 (MIX) was offered LSGH mixed with ORH for ad libitum consumption during the two feeding periods. Group 5 (PAIR) received the equivalent amount of ORH consumed by MIX at the previous feeding. The DMI for OAT, SPURGE, and MIX were similar at the first feeding (P = .52). The SPURGE group consumed very little LSGH thereafter and was removed from the trial. The OAT and MIX groups consumed similar amounts of DM daily on d 1 to 4 when the ration offered to MIX was only 7% LSGH (P = .33). When LSGH made up ≥ 21% of the mixture (d 7 to 32), the OAT group consumed more daily DM than did MIX (P < .05). The spurge/oatlage ration offered to MIX was less digestible than the oatlage-only ration offered to PAIR (P ≤ .01). Even though blood chemistry did not indicate that LSGH consumption caused organ damage, its intake caused minor alterations (P ≤ .05) in serum albumin, calcium, gamma glutamyltransferase, P, K, and urea nitrogen. No gross or microscopic lesions, infectious agents, or significant numbers of parasites were detected in any of the carcasses or tissues examined. The MIX group had diarrhea for much of the trial. In Trial 2, five yearling cattle were adapted to a mixture of 21% LSGH and 79% ORH. Then they were simultaneously offered three mixtures of spurge and oat haylages: 1) spurge ensiled with a microbial inoculant (LSGH); 2) spurge ensiled with the same inoculant and a cellulolytic/hemicellulolytic enzyme (ENZ); and 3) spurge ensiled with the same inoculant and molasses (MOL). The mixture with ENZ was preferred over those with MOL or LSGH (P < .001), but the amounts consumed were low and similar to those for LSGH-ORH in Trial 1 when amounts of ENZ and LSGH in the mixtures were similar. The ENZ mixture may have been more palatable than LSGH and MOL because it had less (P < .05) lactic acid, but intake of ENZ indicates that it had aversive characteristics, like LSGH. Ensiling leafy spurge did little, if anything, to improve its palatability to cattle.

Key Words: *Euphorbia esula*, Cattle, Haylage, Feeding Behavior, Toxicology

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Introduction

Leafy spurge (*Euphorbia esula*) is an aggressive noxious weed that costs farmers, ranchers, and associated communities in the Northern Great Plains approximately $130 million annually (Leistritz et al., 1995). Sheep and goats graze it (Landgraf et al., 1984; Walker et al., 1994), but cattle graze little, if any, leafy spurge (Lym and Kirby, 1987; Hein and Miller, 1992), and they are also reluctant to eat it as hay (Muller et al., 1990). This disparity between species seems to be linked at least partially to negative postingestive feedback experienced by cattle after they consume small amounts of leafy spurge. This negative feedback leads to a learned aversion to the plant (Kronberg et al., 1993).

The aversive chemicals in spurge may be terpenes (Kronberg et al., 1995). Terpenes can degrade under acidic conditions (Narasimhan et al., 1993) and during anaerobic fermentation (Rama Devi and Bhattcharya, 1977). It is plausible that anaerobic

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fermentation occurring during the ensiling process could degrade the substance(s) in leafy spurge that cause the aversive response in cattle or change other characteristics that affect palatability. Therefore, we ensiled leafy spurge and used it in two trials to determine the potential of this noxious weed as haylage for cattle. We are not aware of any literature concerning the nutrient availability or toxicity of leafy spurge to cattle, so no hypotheses were made in respect to nutritional or toxicological aspects of leafy spurge haylage for cattle.

Materials and Methods

Cutting and Ensiling Leafy Spurge/Grass Haylage

Leafy spurge and associated vegetation were harvested at approximately 40% DM from a site near Veblen in northeastern South Dakota. The vegetation was harvested in late June 1995, when the leafy spurge was in late bloom. Nine 1/3-m^2 plots were clipped in areas considered to be representative of the harvested area. These samples were sorted by species, dried, and weighed to determine botanical composition. The forage was chopped to reduce it to about 19 mm in length. The fresh chopped material was mixed with a microbial inoculant (1174, Pioneer Hi-Bred International, Des Moines, IA) at the rate of 9×10^{10} cfu per 907 kg of fresh forage. The lactic acid-forming bacteria present in the inoculant were *Lactobacillus acidophilus* and *L. plantarum*. The chopped vegetation was placed in 200-L barrels that were lined with plastic bags and packed by trampling. After each barrel was filled, air was evacuated from the bag with a portable vacuum and the bag was sealed. Each barrel contained about 114 kg of haylage. Four of the barrels received a liquid molasses treatment (**MOL**) sprayed onto the chopped material as it was placed in the barrels. Molasses was included at 3% of the fresh weight of the forage (approximately 3.4 kg/barrel) along with the same rate of microbial inoculant at 9×10^{10} cfu/907 kg of forage. Four additional barrels of chopped spurge/grass were treated with a cellulolytic/hemicellulolytic "Grasszyme" mixture (**ENZ**; Finn Feeds International, Wiltshire, U.K.) sprayed on at a rate of .2 L/1,000 kg fresh forage. The enzymes were produced by fermentation of *Trichoderma* spp. and *Aspergillus* spp. The same microbial inoculant was also applied to this haylage. Oat/rape haylage (**ORH**), used as the control feed, was harvested when the oats were in the dough stage and sealed in a large silage bag in late July 1995. All haylages were left undisturbed until late August when they were sampled for chemical analysis.

Chemical Analysis

Core samples of each type of haylage were obtained in late August 1995. Four samples were taken from the ORH at different locations in the bag. Six barrels of leafy spurge/grass haylage (**LSGH**) were randomly selected for sampling; one sample was collected from each barrel. Two barrels each of the MOL and the ENZ spurge/grass haylages were also sampled. Bags were resealed after the cores were removed. Each sample was thoroughly mixed, and a small portion was removed and sealed in a plastic bag. Samples were combined with deionized water and allowed to equilibrate for 10 to 15 min; then, pH was determined. The remaining portion of each sample was dried at 40°C for 5 d, ground in a Wiley mill through a 1-mm screen, and stored at –20°C. The haylage samples were analyzed for CP, DM, NH_3 N, ash, ADF, and ADL (AOAC, 1990). They were also analyzed for NDF (Goering and Van Soest, 1970, as modified by Mertens, 1991).

Lactic acid concentration was measured on ORH and LSGH samples taken on d 1 of the stair-step feeding trial and on samples of each of the three types of spurge taken on d 1 of the cafeteria trial. Samples were sealed in airtight bags and stored at –20°C. Later, they were thawed, weighed, and mixed with a known quantity of double-distilled water. Each sample was water-extracted (Wiseman and Irvin, 1957). One portion of the filtrate was analyzed with HPLC as described by Parekh and Cheryan (1990) to determine the lactic acid and ethanol content. Another portion of the filtrate (5 mL) was mixed with 1 mL of 25% weight/volume metaphosphoric acid and analyzed by gas chromatography (**GC**) for VFA content. Chemical compositions of the haylages were compared by analysis of variance and protected LSD means separation (SAS, 1991).

Stair-Step Feeding Trial

Fifteen castrated male and 10 female *Bos taurus* yearlings (416.1 ± 25.9 kg) that had no previous exposure to leafy spurge were adapted for 2 wk to ensiled feed (alfalfa haylage) and to trial procedures. The cattle were stratified by weight and sex across five treatment groups so that each group contained two heifers and three steers.

On d 1 of the trial, all cattle were offered treatment diets. Group 1 (**OAT**) was offered ad libitum access to ORH twice daily for 1 h each time. Group 2 (**SPURGE**) was offered ad libitum access to LSGH twice daily for 1 h each time. Group 3, a paired feeding control for SPURGE, was offered an amount of ORH equal to the amount of LSGH that SPURGE consumed at the previous feeding. Group 4 (**MIX**) was offered ad libitum access to a mixture of LSGH and ORH for 1 h twice daily. This mixture contained 7% LSGH on d 1 to 4, 14% LSGH on d 5 and 6, 21% LSGH on d 7 and 8, 28% LSGH on d 9 to 11, 35% LSGH on d 12 to 14, 42% LSGH on d 15 and 16, 25% LSGH on d 17 and 18, and 21% LSGH on d 19 to 32. Group 5 (**PAIR**), another paired-feeding control, was offered as much ORH as

the MIX group consumed of the mixture at the previous feeding.

Cattle were held in individual pens and given access to feed for 1 h at 0700 and again at 1700; their DMI was determined for each feeding. When not in individual pens, cattle were released into a drylot together, where they had free choice access to water and trace mineralized salt. Body weights were measured after a 12-h shrink on d −2 and 29. Daily DMI for each animal was analyzed by the GLM procedures of SAS (1991) as a repeated measures design with day as the repeated measure. The protected LSD procedure was used to separate means when effects were significant ($P \leq .05$).

Blood and Serum Sampling and Analysis

One week before the stair-step trial began (d −7), two jugular blood samples were collected from each animal in OAT, MIX, and PAIR groups. One sample was allowed to clot and serum was harvested. The following serum variables were quantified using Sigma Diagnostics test kits (St. Louis, MO) and an Abbott VP automated analyzer (Chicago, IL): albumin, aspartate aminotransferase (**AST**), bilirubin, chloride (Cl), creatinine kinase (**CK**), gamma glutamyltransferase (**GGTP**), globulin, glucose, magnesium (Mg), phosphorus (P), total protein, and urea nitrogen. Serum calcium (Ca) was determined with a test kit from Abbott Diagnostics (Chicago, IL) with an Abbott VP automated analyzer. Serum sodium (Na) and potassium (K) were determined using a Nova I sodium potassium electrode analyzer. Serum albumin and globulin were determined by cellulose acetate electrophoresis (Helena Laboratories test kit, Beaumont, TX). The second blood sample was collected into heparinized tubes and analyzed for hemoglobin, hematocrit, and white blood cell counts with an Abbott Cell DYN Cell Counter (Abbott Diagnostics). Jugular serum samples were also collected on d 7, 21, and 29 and analyzed as previously described. On d 29, a second sample was collected into an EDTA-treated tube. This sample was analyzed for hemoglobin, hematocrit, and white blood cell counts with a Coulter Model 2F cell counter (Plymouth, MN). All blood samples were analyzed by Wolff Laboratories (Minneapolis, MN). Levels of the blood variables were evaluated as indicators for a variety of potentially toxic responses to leafy spurge ingestion. These blood variables can indicate the following: albumin for liver, kidney and gastrointestinal disease, AST for soft tissue damage, bilirubin for hepatic function, Ca for Ca metabolism and renal function, Cl for electrolyte balance, CK for skeletal and cardiac muscle deterioration, GGTP for hepatic disorders, globulin for immune response and acute inflammation, hematocrit for percentage of blood composed of erythrocytes, hemoglobin for O_2 transport capacity, glucose for energy balance and endocrine function, Mg for Mg metabolism, Na for electrolyte balance, P for P metabolism and renal function, K for electrolyte balance, total protein for protein status and acid-base balance, urea nitrogen for renal function and nitrogen status, and white blood cell count for immune response (Duncan and Prasse, 1986; Kaneko, 1989).

Blood variable data were analyzed with the GLM procedures of SAS (1991) as a repeated measures design with day of blood collection as the repeated measure. Analyses of covariance using the pretrial value (d −7) for each variable as a covariate were also conducted. Blood variables near the end of the trial (d 29) were considered the most important because these represented the animals' health after 14 consecutive days of consistent treatment.

Tissue Sampling and Histopathological Examinations

At the end of the 32-d stair-step feeding trial, MIX and PAIR groups were euthanatized. The tongue, nasal passages, mouth, and esophagus were visually examined for any lesions or evidence of tissue irritation. The rest of the carcass and organs were also visually examined for abscesses or other tissue damage. Sections of the heart, lungs, kidneys, colon, rumen, abomasum, esophagus, jejunum, ileum, diaphragm, and skeletal muscle were placed in 10% neutral-buffered formalin. They were processed by standard paraffin techniques (Armed Forces Institute of Pathology, 1992), sectioned to 5 μm, stained with hematoxylin and eosin (H&E), and examined by light microscopy.

Two liver and two brain samples from animals in each group were selected randomly for bacterial culturing. The tissue was seared over a Bunsen burner and cut with a scissors dipped in alcohol and flame-sterilized. The aerobic culture was done on a heart infusion base agar to which blood had been added. It was placed in an incubator supplemented with CO_2 and stored at 35°C overnight. It was evaluated, incubated a 2nd d, evaluated again, and reported. The anaerobic culture was performed on the same agar in a Coy anaerobic hood at 35 to 37°C overnight and observed for 2 d.

Ileal sections were cut open with scissors. The inner portion was swabbed with cotton and cultured on Brilliant Green agar and Heltoen enteric agar to screen for *Salmonella*.

Viral isolations of the spleen for bovine viral diarrhea were made according to the procedures of Bolin (1990). Fecal samples from the colon of each animal were examined for internal parasites with the Wisconsin double-centrifugation fecal flotation technique (Cox and Todd, 1962).

Digestion Trial

Chromic oxide (Cr_2O_3) was used as an external marker to determine the apparent digestibility of the

Table 1. Chemical analysis of the oat/rape and leafy spurge haylages

Item	Oat/rape haylage	Leafy spurge haylages[a]		
		LSGH	MOL	ENZ
DM, %	35.46 ± 1.60[bc]	34.87 ± .86[bc]	39.87 ± .88[b]	30.99 ± 1.28[c]
CP, % DM	12.38 ± .06[b]	11.41 ± .16[d]	10.81 ± .06[c]	11.45 ± .05[d]
NH_3 N, mg/g DM	1.58 ± .0004[b]	1.51 ± .03[b]	1.26 ± .07[c]	1.55 ± .04[b]
NH_3 N, % N	8.66 ± .002[b]	8.25 ± .16[b]	7.26 ± .42[c]	8.48 ± .22[b]
NDF, % DM	52.72 ± .03[b]	54.11 ± .50[b]	51.57 ± 1.22[c]	53.85 ± .30[b]
ADF, % DM	34.98 ± .22[b]	41.04 ± .27[c]	39.31 ± .30[d]	41.72 ± .27[c]
ADL, % DM	4.13 ± .08[b]	7.19 ± .16[c]	7.02 ± .32[c]	6.95 ± .02[c]
pH	4.21[fb]	4.00 ± .02[c]	4.15 ± .09[b]	4.08 ± .04[bc]
Lactic acid, % DM	9.06 ± .18[b]	10.89 ± .06[c]	9.88 ± .02[d]	7.31 ± .05[e]
Acetic acid, % DM	1.02 ± .12[b]	1.23 ± .19[b]	.94 ± .10[b]	1.22 ± .04[b]
Butyric acid, % DM	.09 ± .02[b]	.02 ± .002[c]	.01 ± .004[c]	.05 ± .002[bc]
Ethanol, % DM	.14 ± .01[b]	.48 ± .02[c]	.97 ± .02[d]	.23 ± .01[e]
GE, Mcal/kg DM	4.35 ± .01[b]	4.66 ± .01[c]	4.66 ± .0003[c]	4.67 ± .003[c]
Ash, % DM	9.18 ± .04[b]	6.47 ± .12[c]	6.63 ± .11[c]	6.38 ± .07[c]

[a]LSGH, MOL, and ENZ = types of leafy spurge haylages. LSGH = leafy spurge ensiled with a microbial inoculant, MOL = leafy spurge ensiled with the same microbial inoculant and molasses, and ENZ = leafy spurge ensiled with the same microbial inoculant and a cellulolytic/hemicellulolytic enzyme.
[b,c,d,e]Means within a row with different superscripts differ ($P \leq .05$).
[f]Samples were pooled before analysis.

DM, CP, ADF, NDF, and GE components of both the ORH and 21% LSGH/79% ORH mixtures. Animals in MIX and PAIR were given 70 g of Cr_2O_3 mixed with a small amount of their silage that was fed to each animal prior to each feeding period. This resulted in 2.9% Cr_2O_3 in their diets on a DM basis. The Cr_2O_3 was offered for a 7-d equilibration period, followed by a 2-d sample collection. Fecal grab samples were taken after each feeding during the 2-d collection period. Fecal samples were weighed, dried at 60°C for 4 d, allowed to equilibrate for 24 h, and reweighed. They were then ground through a 1-mm screen, composited by animal, and analyzed for chromium concentration according to the procedures of Costigan and Ellis (1987). Dry matter, CP, ADF, NDF, and ash content were determined as described previously. Gross energy was determined using a Parr 1261 oxygen bomb calorimeter (Parr, Moline, IL). Digestion coefficients were calculated as described by Kartchner and Campbell (1979). Digestibility data from each animal were analyzed by analysis of variance as a completely randomized design (SAS, 1991).

Cafeteria Trial

A group (**CT**) of five *Bos taurus* crossbred yearlings (417.3 ± 27.9 kg) was used for a cafeteria trial. Before this trial began, they were offered 7% LSGH mixed with ORH for 4 d, 14% LSGH mixed with ORH for 2 d, and then 21% LSGH mixed with ORH for 6 d. At each of the six feedings of this trial, the cattle were simultaneously offered 21% mixtures of 1) LSGH (with the microbial inoculant only), 2) leafy spurge ensiled with the microbial inoculant and a cellulolytic/hemicellulolytic enzyme mixture (ENZ), and 3) spurge haylage ensiled with the microbial inoculant and molasses (MOL). Oat/rape haylage composed the other 79% of the feed offered with all three types of spurge haylage. The animals were fed in individual pens and allowed access to the feeds for 1 h in the morning and 1 h in the evening for 3 d. They had ad libitum access to the three mixtures during these six 1-h periods. All uneaten haylage was weighed. The position of each type of spurge haylage was rotated at every feeding. Data for the cafeteria trial were analyzed with the GLM procedures of SAS (1991) using a repeated measures design. Daily intake of the haylages was the repeated measure. When $P \leq .05$, the protected LSD procedure was used to compare treatment means.

Results

Botanical Composition

The leafy spurge/grass harvested for ensiling consisted of 54 ± 18.2% leafy spurge and 46 ± 18.2% other vegetation. Most of the other vegetation was Kentucky bluegrass (*Poa pratensis*), smooth brome (*Bromus inermus*), and wheatgrasses (*Agropyron* spp.).

Chemical Analysis

Results of the chemical analyses of the haylages are presented in Table 1. The LSGH and ORH haylages did not differ in DM, NH_3 N (as a percentage of DM and as a percentage of total nitrogen), acetic acid, or NDF ($P \leq .05$). The LSGH feed was lower in CP, butyric acid, ash, and pH than ORH ($P \leq .05$) but had more ADF, ADL, lactic acid, ethanol, and GE than ORH ($P \leq .05$).

Stair-Step Feeding Trial

All animals consumed very little of the new haylages during the first feeding. The OAT group consumed only .48 ± .18 kg DM of ORH, SPURGE consumed .48 ± .24 kg DM of LSGH, and MIX consumed .88 ± .37 kg DM of ORH/LSGH. The DMI by all groups were similar (P = .52).

The MIX group received 7% LSGH mixed with ORH on d 1 to 4 and consumed an average of 4.18 kg DM/d (Figure 1). The OAT group consumed an average of 4.71 kg DM/d over those same days. The two groups had similar DMI (P = .33). In contrast, SPURGE consumed much less DM than either OAT or MIX on d 1 to 3 ($P \leq .001$). They consumed essentially no LSGH after the first feeding and were removed from the trial on d 4 together with their paired feeding group (Group 3). The MIX group received a mixture of 14% LSGH and 86% ORH on d 5 and 6 and consumed an average of 5.94 kg DM/d, and the OAT group consumed an average of 6.64 kg DM/d. Daily DMI of OAT and MIX were similar during this period (P = .07). The MIX animals began to have diarrhea on d 5, and this continued for the remainder of the trial. On d 7 and 8, MIX was offered a mixture with 21% LSGH. The MIX animals consumed 5.29 kg DM/d, and OAT

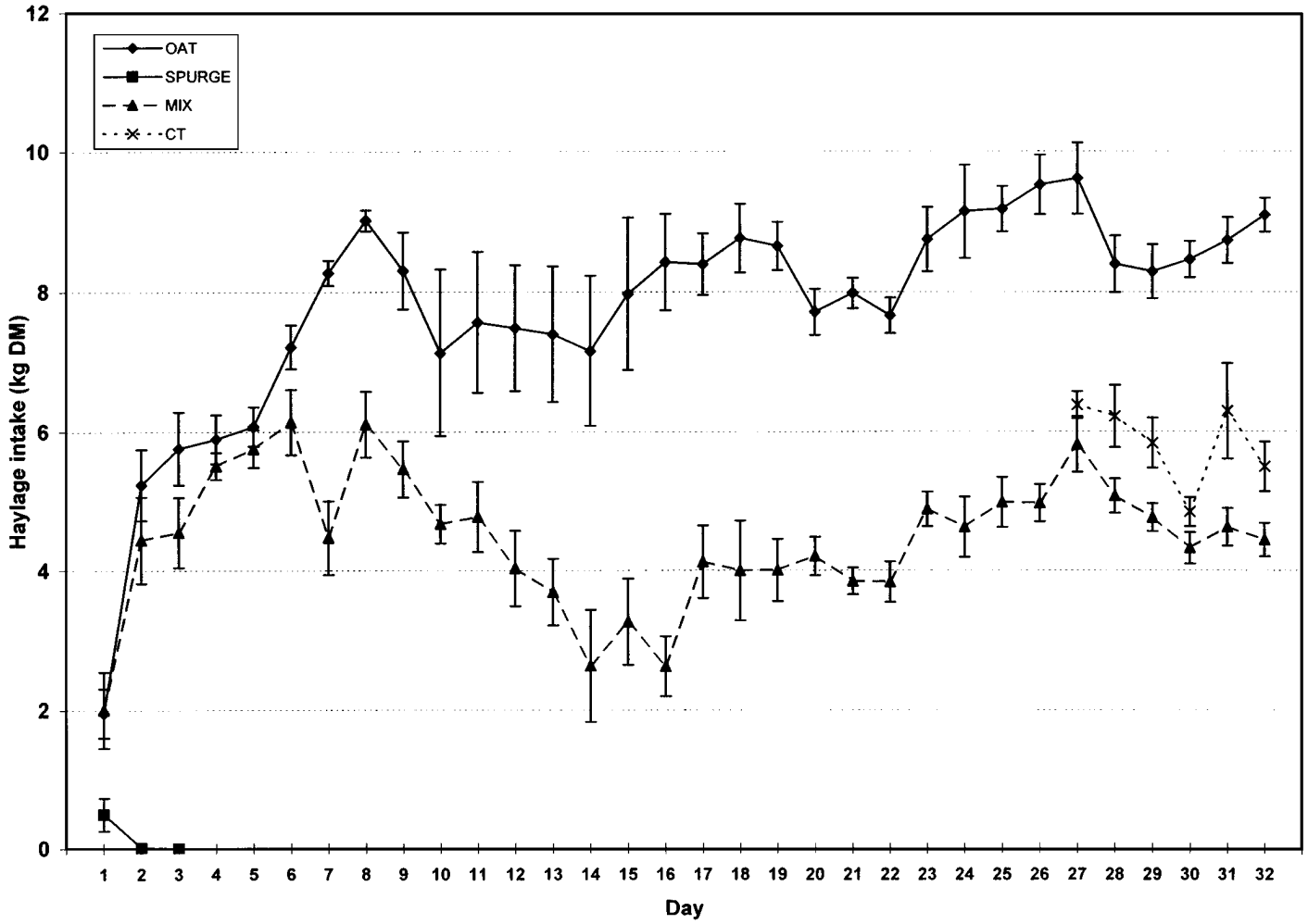

Figure 1. Dry matter intake of haylage by four groups of yearling cattle during a stair-step feeding trial. One group (OAT) consumed only oat/rape haylage (ORH) during the trial. Another group (SPURGE) consumed only leafy spurge/grass haylage (LSGH) during the trial. A third group (MIX) consumed various amounts of LSGH mixed with ORH during the trial, and a fourth group (CT) consumed 21% LSGH mixed with 79% ORH on d 27 to 32 during their adaptation period for the cafeteria trial. The mixture consumed by MIX was composed of 7% LSGH on d 1 to 4, 14% LSGH on d 5 to 6, 21% LSGH on d 7 to 8, 28% LSGH on d 9 to 11, 35% LSGH on d 12 to 14, 42% LSGH on d 15 to 16, 25% LSGH on d 17 to 18, and 21% LSGH on d 19 to 32. The balance of all of these mixtures was ORH. The SPURGE group consumed less DM on d 1 to 3 than did MIX or OAT ($P < .01$). Intakes by OAT and MIX were similar on d 1 to 4 (P = .33) and d 5 to 6 (P = .07). On d 7 to 32, MIX consumed less DM than did OAT ($P < .02$). On d 27 to 32, CT consumed more DM than MIX (P = .02), and CT and MIX both consumed less DM than did OAT ($P < .01$). Bars equal SEM.

consumed more DM (8.64 kg/d) on these days ($P < .001$; Figure 1), with MIX being 39% lower. On d 9 to 11, when MIX was offered a mixture with 28% LSGH, MIX animals consumed 4.96 kg DM/d, and OAT consumed 7.66 kg DM/d during this time ($P = .02$), with MIX being 35% lower. On d 12 to 14, when MIX was fed 35% LSGH, MIX animals consumed only 3.71 kg DM/d, which was 49% less than OAT ($P = .01$). The OAT group consumed 7.34 kg DM/d during these days. On d 15 to 16, MIX received a mixture containing 42% LSGH and their DMI was only 2.94 kg/d. OAT continued to consume more DM (8.19 kg DM/d) than MIX during these days ($P \leq .01$; Figure 1). It was concluded that MIX was not consuming enough protein and energy to maintain body weight (NRC, 1984) when the higher amounts of LSGH were included in the mixture. Therefore, the amount of LSGH in their haylage diet was reduced to 25% for d 17 and 18. During these days, MIX consumed only 4.06 kg DM/d, which was still 53% less DM ($P \leq .01$) than consumed by OAT (8.57 kg DM/d). Finally, the LSGH in the MIX diet was reduced to 21% and held at that level until the trial ended. During d 19 to 25, MIX consumed 4.34 kg DM/d, and OAT consumed 8.43 kg DM/d. Intake differences between the two groups remained large during this part of the trial ($P \leq .001$). On d 26 to 32, MIX consumed 4.86 kg DM/d, and OAT consumed 8.92 kg DM/d; intake was 46% lower ($P \leq .001$) for MIX. After d 7, intake of DM from LSGH in MIX averaged only 1.14 kg daily (range of .91 to 1.39), indicating that some factor inhibited intake to this maximum.

Serum Chemistries and Hematological Examinations

The covariables (d −7 values) for AST, bilirubin, Ca, Mg, globulin, hemoglobin, hematocrit, and white blood cell counts were not significant ($P \geq .07$). Therefore, the trial levels of these variables were not adjusted. The covariables for albumin, Cl, CK, GGTP, glucose, K, Na, P, total protein, and urea nitrogen were significant ($P \leq .05$). Therefore, the adjusted means for these variables are presented.

By the end of the trial (d 29), treatment altered levels of albumin, Ca, GGTP, P, K, and urea nitrogen ($P \leq .05$). Treatment values for all serum and blood variables are presented in Tables 2 and 3. The time × treatment interaction was significant ($P \leq .05$) only for P, K, and urea nitrogen.

The groups OAT and MIX had similar levels of albumin on d 29 ($P = .47$), but PAIR had higher levels than both other groups ($P < .01$). Calcium levels were 6% lower in MIX than in OAT and PAIR on d 29 ($P < .05$). The PAIR group had 16 and 30% lower levels of GGTP than OAT and MIX, respectively, on d 29 ($P < .05$). On d 29, serum levels of P were approximately 27% higher for MIX than for OAT and PAIR ($P \leq .01$). Serum K levels were similar for OAT and PAIR ($P = .82$) on d 29, but MIX was 6% higher than both ($P \leq .03$). On d 29, OAT and PAIR had similar ($P = .77$) levels of urea nitrogen, but MIX had approximately 27% higher levels than OAT and MIX ($P = .02$). Hematocrit, hemoglobin, and white blood cell number were similar for all groups on d 29 ($P > .05$).

Carcass and Histopathological Examinations

None of the carcasses had any gross lesions of the nasal passages, oral cavity, tongue, esophagus, or viscera. Microscopically, no significant lesions were present in any of the tissues sampled.

Fecal flotation examinations for internal parasites were negative for three animals in MIX and for two animals in PAIR. Two animals from MIX harbored low to moderate numbers of strongyle ova and *Eimeria bovia* (coccidia) ova. Examination of three animals in PAIR showed low numbers of strongyle ova.

Aerobic and anaerobic liver and brain cultures yielded no significant isolates. Ileal cultures were negative for *Salmonella* and other significant bacteria. Viral isolations from the spleen were negative for BVD.

Digestion Trial

There were no differences between MIX and PAIR for intake of DM, GE, CP, NDF, or ADF ($P \geq .64$; Table 4). The diet consumed by MIX had lower apparent digestibility for DM, CP, NDF, and ADF than the diet ingested by PAIR ($P \leq .01$), but the amount of DE consumed by the two groups did not differ ($P = .71$).

Body Weights

On d −2 of the stair-step trial, body weights of OAT, MIX, and PAIR were 416 ± 10, 416 ± 13, and 417 ± 15 kg, respectively, and similar ($P = 1.0$). On d 29, body weights were 454 ± 14, 409 ± 15, and 416 ± 12 kg for OAT, MIX, and PAIR, respectively. The OAT group weighed more than MIX ($P \leq .05$), and PAIR was similar to OAT and MIX ($P \geq .05$).

Cafeteria Trial

During d 27 to 32 of the stair-step feeding trial, the CT group was offered a 21% LSGH/79% ORH diet, as was MIX. The CT group consumed 5.84 kg DM/d and MIX consumed 4.84 kg DM/d. Intake by CT was higher than that by MIX ($P = .02$). During this same period, OAT had greater intake of DM (8.72 kg of ORH/d) than either CT or MIX ($P \leq .001$; Figure 1).

During the 3-d cafeteria trial (Figure 2), CT consumed an average of .37 kg of DM per feeding of the mixture containing LSGH, .27 kg DM of the mixture containing MOL, and 4.56 kg DM of the mixture containing ENZ ($P \leq .001$).

Table 2. Serum variables for the three treatment groups on d 29 of the trial

Variable	Day 29 OAT	MIX	PAIR	SE	*P*
Albumin, g/dL	2.76[b]	2.70[b]	3.06[c]	.06	.002
Aspartate aminotransferase, U/I	70.0	64.8	66.6	3.0	.48
Bilirubin, mg/dL	.14	.18	.20	.03	.28
Calcium, mg/dL	9.52[b]	8.98[c]	9.48[b]	.14	.03
Chloride, meq/dL	100.9	100.9	101.4	1.4	.96
Creatinine kinase, U/I	234	264	214	32	.56
Gamma glutamyltransferase, U/I	17.8[b]	15.4[c]	20.0[d]	.75	.005
Globulin, g/dL	3.76	3.58	3.62	.14	.64
Glucose, mg/dL	82.0	77.9	81.3	1.2	.10
Magnesium, mg/dL	2.44	2.58	2.50	.13	.23
Phosphorus, mg/dL	9.10[b]	11.79[c]	9.41[b]	.57	.01
Potassium, meq/dL	4.43[b]	4.70[c]	4.45[b]	.07	.04
Sodium, meq/dL	148	146	146	.80	.13
Total protein, g/dL	6.49	6.28	6.72	.11	.06
Urea nitrogen, mg/dL	15.93[b]	20.63[c]	16.42[b]	1.1	.03

[a]OAT, MIX, and PAIR = groups of cattle that were offered haylage diets during a 32-d trial. OAT = cattle offered ad libitum amounts of oat/rape haylage during two daily feeding periods, MIX = cattle offered ad libitum amounts of leafy spurge/grass haylage mixed with oat/rape haylage during two daily feeding periods, and PAIR = a paired-feeding group to MIX that received the equivalent amount of oat/rape haylage consumed by MIX at the previous feeding.
[b,c,d]Means within a row with different superscripts differ ($P \leq .05$).

Discussion

Chemical Analysis

All haylage types were similar in nutrient content, and their chemical composition was consistent with high intake-potential silages (Buchanan-Smith, 1990; McDonald et al., 1991). The ORH and leafy spurge haylages had high concentrations of lactic acid, low levels of acetic and butyric acids, and a desirable pH. They also contained low levels of NH_3 N (as a percentage of total nitrogen) and ethanol. Thus, the low intakes of LSGH evidently were due to one or more aversive compounds, not to poor-quality silage.

Stair-Step Feeding Trial

The consumption of haylage by OAT, SPURGE, and MIX groups at the first feeding was similar. This reveals that the LSGH was as palatable initially as ORH. The SPURGE group (offered 100% LSGH) refused their rations by d 3. Decreases in intake of LSGH by SPURGE and MIX reveals that they developed a learned aversion to the LSGH.

Muller et al. (1990) reported that total intake by cows decreased as the percentage of leafy spurge hay in a spurge-straw mixture was increased. The DMI of MIX decreased as the percentage of LSGH in the mixture increased. Ruminants seem to regulate their intake of aversive compounds as a function of the concentration of the compound in the feed (duToit et al., 1991; Provenza, 1995; Pfister et al., 1997; Wang and Provenza, 1997). The MIX cattle seemed to regulate their intake of LSGH by lowering total intake of the LSGH/ORH mixture as the amount of LSGH in the mixture increased. Pfister et al. (1997) observed that cattle seem to regulate their consumption of the nutritious but toxic tall larkspur (*Delphinium barbeyi*) to a level just below that which would produce

Table 3. Plasma variables for the three treatment groups on d 29 of the trial

Variable	Treatment[a] OAT	MIX	PAIR	*P*	SE
Hematocrit, %	37.2	33.2	36.7	.27	1.89
Hemoglobin, g/dL	12.0	10.8	11.5	.32	.60
White blood cells, per mm^3	7,580	5,580	6,480	.07	540

[a]OAT, MIX, and PAIR = groups of cattle that were offered haylage diets during a 32-d trial. OAT = cattle offered ad libitum amounts of oat/rape haylage during two daily feeding periods, MIX = cattle offered ad libitum amounts of leafy spurge/grass haylage mixed with oat/rape haylage during two daily feeding periods, and PAIR = a paired-feeding group to MIX that received the equivalent amount of oat/rape haylage consumed by MIX at the previous feeding.

Table 4. Daily intakes and apparent digestion of nutritional variables in diets by MIX and PAIR groups during a digestion-balance trial

	Treatment[a]		
Item	MIX	PAIR[b]	*P*
Intake			
DM, kg/d	4.87 ± .33	4.88 ± 0	.98
CP, kg/d	.593 ± 2.43	.604 ± 0	.79
GE, Mcal/d	21.51 ± 1.46	21.24 ± 0	.86
DE, Mcal/d	16.68 ± 1.27	17.16 ± .03	.71
NDF, kg/d	2.58 ± .18	2.57 ± 0	.96
ADF, kg/d	1.77 ± .12	1.71 ± 0	.64
Apparent digestion, %			
DM	51.9 ± 2.4	59.6 ± .37	.01
CP	50.9 ± 3.1	61.6 ± .44	.01
NDF	48.4 ± 2.4	58.2 ± .44	.01
ADF	45.7 ± 2.8	56.8 ± .54	.01

[a]MIX and PAIR are groups of cattle that were offered haylage diets during a digestion-balance trial. MIX = cattle offered ad libitum amounts of a mixture of leafy spurge/grass haylage (21%) and oat/rape haylage (79%) during two daily feeding periods; PAIR = a paired feeding group to MIX that received the equivalent amount (mean) of oat/rape haylage consumed by MIX at the previous feeding.
[b]All animals in PAIR consumed the same amount of haylage.

signs of overt toxicity. We suspect that MIX cattle were regulating their intake of LSGH in a similar manner. The SPURGE group (offered 100% LSGH) refused their rations by d 3.

The MIX and CT groups received 21% LSGH in the mixture from d 27 to 32. The MIX group (exposed earlier to 42% LSGH in their diet) consumed less than CT (exposed to a maximum of only 21% LSGH in their diet) during d 27 to 32. duToit et al. (1991) reported that sheep that received higher doses of the aversive compound lithium chloride after exposure to a novel feed consumed much less of the novel feed on the following day than control or low-dosage groups. Sheep that received higher intraruminal doses of leafy spurge after exposure to a novel feed consumed much less of that novel feed on the following days than the control or low-dosage groups (S. L. Kronberg, unpublished data).

Neither MIX nor CT exhibited evidence of adaptation to LSGH. Their diarrhea continued as long as they received LSGH, and they did not seem to increase their intake of the 21% LSGH/79% ORH mixture as the trial progressed. In fact, their intake of LSGH remained nearly stable throughout the trial.

The diarrhea exhibited by MIX was not caused by infectious agents or by changes in gastrointestinal morphology. Other causes of diarrhea in ruminants that are applicable to this situation are changes in gastrointestinal motility, forestomach disorders, and osmotic overload (Smith, 1990). Perhaps LSGH caused diarrhea in MIX through one or more of these mechanisms.

Digestion Trial

The DM, CP, ADF, and NDF fed to MIX was less digested than that fed to PAIR, even though intake was the same between these two groups. The diarrhea suffered by MIX may have contributed to this. Motility of the gastrointestinal tract is often increased when ruminants ingest toxic compounds (Smith, 1990). If gastrointestinal motility was increased in MIX, then the increased rate of passage through the tract could have decreased the digestibility of various components of the diet (Merchen, 1988). Leafy spurge/grass haylage effects on the forestomach also may have altered digestibility. If ruminal microbes were affected, the haylage may not have been fully digested. However, in vitro studies by Clark et al. (1993), Thomas et al. (1994), and Roberts and Olson (1996) and an in situ study by Thomas et al. (1994) indicate that microbial function of the ovine rumen was not negatively affected by leafy spurge. Ensiled leafy spurge may possess properties different from those of the dried plant. It is also possible that ruminal microbes may respond differently to leafy spurge in the bovine and the ovine.

Serum Chemistries and Hematological and Histopathological Examinations

Limited intake of LSGH by MIX did not affect AST, bilirubin, Cl, CK, globulin, glucose, Mg, sodium, total protein, hematocrit, hemoglobin, or white blood cell counts at the end of the trial (14 consecutive days consuming haylage with 21% LSGH). Intake of LSGH by MIX altered end-of-trial levels of Ca, GGTP, P, K, and urea nitrogen relative to both groups receiving only ORH. Increased serum urea nitrogen is associated with renal dysfunction or nutritional status (Kaneko, 1989). All groups remained within normal clinical range. However, had the cattle not refused to consume more of the LSGH, their levels of urea

nitrogen and other blood variables may have been very abnormal. The CP intake by MIX and PAIR was marginal for medium-framed steers and heifers of their body weight and gaining only .2 kg/d (NRC, 1984). The digestion coefficient for the CP fed to MIX was lower than that for PAIR. If MIX was at or near a negative nitrogen balance, catabolism of body proteins (Kaneko, 1989) and increased recycling of urea to the rumen via the bloodstream (Duncan and Prasse, 1986) could have caused higher urea nitrogen levels in those cattle.

Serum P of MIX was above published norms. Serum P can be elevated by increased dietary intake of P, decreased renal excretion of P, and hormonal imbalances associated with Ca metabolism (Duncan and Prasse, 1986). The P content of the LSGH was lower than that of the ORH (data not presented); therefore, dietary P levels are unlikely to be the cause of the higher serum P in MIX. It is possible that LSGH intake interfered with Ca metabolism or that renal function was altered. Altered renal function may cause elevated K, P, and urea nitrogen.

Serum K of MIX was elevated in comparison to OAT and PAIR, although their K levels were still within clinical norms. Serum K may be elevated when renal excretion decreases (Kaneko, 1989).

In respect to serum Ca differences in treatment groups, there are a large number of things that can cause depressed Ca. It was not apparent in this study which mechanisms were involved.

Serum GGTP was also within clinical norms for MIX. Increases in GGTP are normally associated with liver damage (Duncan and Prasse, 1986; Kaneko, 1989). The lower relative GGTP in MIX is unlikely to be a sign of any serious problems. We cannot explain why the PAIR group had increased GGTP, but it appeared leafy spurge may have protected the MIX group from the same fate and even caused a decrease of GGPT in their serum.

The parasites found were judged to be insignificant in relation to the health of the cattle. The lack of any significant lesions in the tissues of MIX supports the conclusion that no apparent organ damage occurred to cattle consuming LSGH.

Cafeteria Trial

Higher levels of voluntary DMI associated with forages fermented with cellulolytic and hemicellulolytic enzymes have been reported (McDonald et al., 1991). This has been attributed to greater fiber

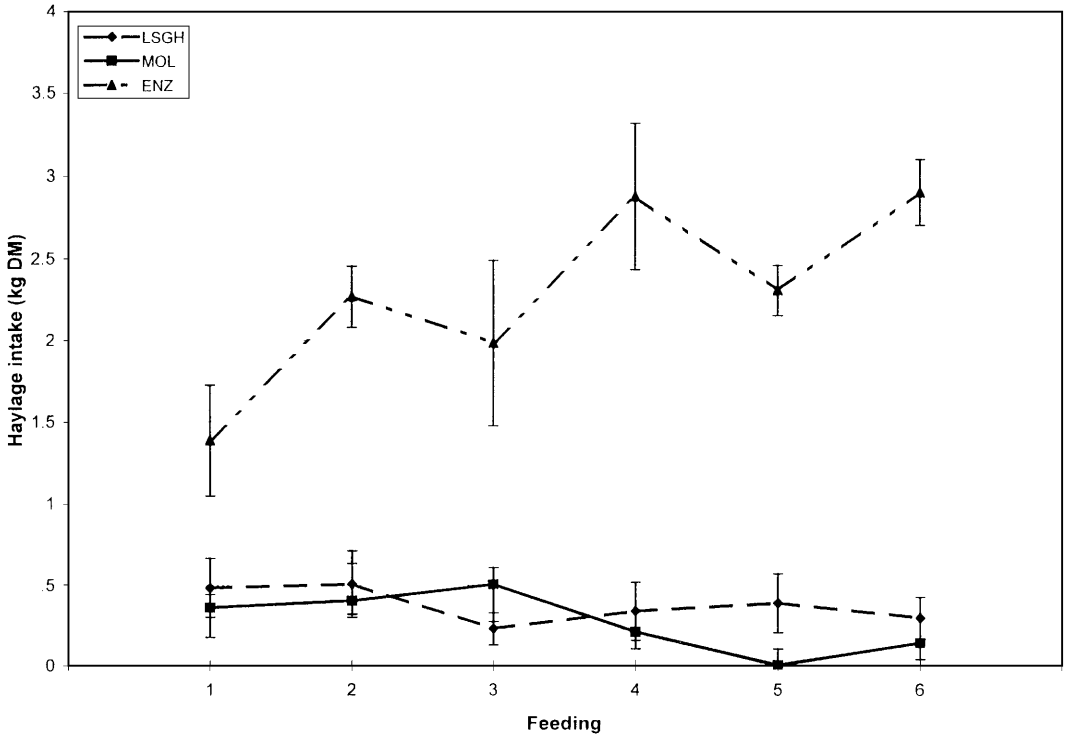

Figure 2. Dry matter intake of three mixtures of leafy spurge and oat/rape haylages by a group of yearling cattle (CT) in a cafeteria trial. Each mixture contained 21% of one of the three types of leafy spurge haylage and 79% of oat/rape haylage. The three types of leafy spurge haylage were LSGH, MOL, and ENZ. The LSGH notation equals leafy spurge ensiled with a microbial inoculant, MOL equals leafy spurge ensiled with the microbial inoculant and molasses, and ENZ equals leafy spurge ensiled with the microbial inoculant and a cellulolytic/hemicellulolytic enzyme. The mixture containing ENZ was preferred over the mixtures containing LSGH or MOL ($P < .01$). Bars equal SEM.

breakdown during ensilation and therefore higher digestibility of enzyme-treated silages. Because the fiber levels in ENZ haylage were similar to those in the other spurge haylages, higher intake of ENZ does not seem to be a result of differences in fiber levels among LSGH, MOL, or ENZ. Digestibility was not measured for the MOL or ENZ spurge haylage types. The CT group consumed more of the mixture containing the ENZ haylage type at the first feeding of the cafeteria trial. This reveals that the mixture containing ENZ haylage initially was preferred over the mixtures containing the MOL or LSGH haylage types. The lower lactic acid levels in the ENZ type may account for this. The continued preference for the haylage mixture containing ENZ during the next five feedings could have resulted from less negative and(or) more positive postingestive feedback compared to mixtures containing the LSGH or MOL haylage types (Provenza, 1995). Less negative postingestive feedback could have occurred if the enzyme treatment had reduced the level of aversive chemicals in the ENZ haylage. Because the identity of the aversive compounds in leafy spurge is unknown, it is difficult to determine whether ensiling leafy spurge with these enzymes reduced the level of the aversive compounds. We suspect that the aversive nature of leafy spurge was changed little by the addition of the enzyme during ensilation. More positive postingestive feedback could have occurred if the enzyme treatment had increased the nutrient availability from the haylage. However, ENZ had levels of CP and fiber similar to those of LSGH and(or) MOL, so it is unlikely that CT cattle received any more positive postingestive feedback from ENZ haylage. Also, one must remember that only 21% of each haylage mixture offered during the cafeteria trial was ENZ, LSGH, or MOL. The other 79% was ORH.

Intake of ORH by OAT was much higher than intake of the ENZ/ORH mixture offered during the cafeteria trial, so we suspect there was more negative (and less positive) postingestive feedback experienced by CT than by OAT. The ENZ haylage had lower levels of CP and higher levels of ADF and ADL than did ORH, so it is likely that cattle in CT received less positive postingestive feedback than cattle in OAT that consumed only ORH.

Implications

Leafy spurge/grass haylage was initially as palatable to cattle as oat haylage, but cattle quickly learned to avoid it. Cattle consumed leafy spurge/grass haylage repeatedly only when it was mixed in small amounts with oat haylage. Cattle seemed to regulate leafy spurge/grass haylage intake by decreasing total intake as the amount of leafy spurge in the mixture increased. Cattle offered greater amounts of leafy spurge mixed with oat haylage seemed to develop stronger aversions to leafy spurge/grass haylage than those offered lesser amounts. When leafy spurge was ensiled with a hemicellulolytic and cellulolytic enzyme mixture and microbial inoculant, cattle preferred it to leafy spurge ensiled with either a microbial inoculant or with molasses plus the inoculant. However, cattle did not consume greater amounts of this type of leafy spurge haylage. Leafy spurge haylage may be used as feed for cattle, but only when it is included as a small amount (< 1 kg/d) of the ration.

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