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Evaluation of perennial ryegrass straw as a forage source for ruminants[1]

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ABSTRACT: Two experiments were conducted to evaluate perennial ryegrass straw as a forage source for ruminants. Experiment 1 evaluated digestion and physiological variables in steers offered perennial ryegrass straw containing increasing levels of ergot alkaloid, lolitrem B. Sixteen ruminally cannulated Angus × Hereford steers (231 ± 2 kg BW) were blocked by weight and assigned randomly to one of four treatments. Steers were provided perennial ryegrass straw at 120% of the previous 5-d average intake. Before straw feeding, soybean meal was provided (0.1% BW; CP basis) to meet the estimated requirement for degradable intake protein. Low (L) and high (H) lolitrem B straws (<100 and 1,550 ppb, respectively; DM basis) were used to formulate treatment diets: 100% L; 67% L:33% H; 33% L:67% H; 100% H (DM basis). Intake and digestibility of DM and OM, and ruminal pH, total VFA, and NH_3-N were not affected by increasing lolitrem B concentration. Ruminal indigestible ADF (IADF) fill increased linearly ($P = 0.01$) and IADF passage rate decreased linearly ($P = 0.04$) as lolitrem B increased. Experiment 2 evaluated performance and production by 72 Angus × Hereford cows (539 ± 5 kg BW) consuming perennial ryegrass straw containing increasing lolitrem B during the last third of gestation. Cows were blocked by body condition score and randomly assigned to one of three treatments. Cows were provided perennial ryegrass straw ad libitum and supplemented with soybean meal (0.1% BW; CP basis) to meet the estimated requirement for degradable intake protein. Mixtures of a L and H lolitrem B straw (467 and 2,017 ppb, respectively) were used to formulate treatment diets: 100% L, 50% L:50% H, 100% H (DM basis). Thirteen of 24 cows on the 100% H treatment exhibited signs of ryegrass staggers and were removed from the study; nevertheless, lolitrem B concentration did not influence pre- or postcalving weight or body condition score change. These data suggest that feeding perennial ryegrass straw containing up to 1,550 ppb lolitrem B (DM basis) did not adversely affect nutrient digestion or physiological response variables in steers. However, providing straw with a lolitrem B concentration of approximately 2,000 ppb (DM basis) resulted in 54% of cows exhibiting signs of ryegrass staggers. These data suggest that blending straws with a high (>2,000 ppb) and low (<500 ppb) concentration of lolitrem B can be a successful management practice.

Key Words: Alkaloid, Beef Cattle, Endophyte, Lolitrem B, Perennial Ryegrass, Straw

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Introduction

In the Pacific Northwest, grass seed is a major agricultural product. One of the most common grasses grown is perennial ryegrass (*Lolium perenne*). The traditional manner of straw disposal following seed harvest has been burning; however, the large amount of smoke produced adversely affects the environment and can create situations that may be dangerous and/

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or fatal to humans (Hovermale and Craig, 2001). An alternative way of disposing of grass seed straw is use by ruminant livestock. Straw can be a low-cost winter forage resource for livestock operations in the Pacific Northwest; however, a majority of the straw is exported to countries within the Pacific Rim, primarily Japan, Korea, and Taiwan. These countries imported 286,414 t of Oregon's perennial ryegrass straw during the 2000 to 2001 market year (Young, 2001).

In recent years, much of the grass seed industry's focus has been on producing "turf-type" grasses (Evers et al., 1996; Hannaway et al., 1999). Many of the "turf-type" perennial ryegrass varieties contain the endophytic fungus, *Neotyphodium lolii*. This can be a problem because *N. lolii* produces the ergot alkaloid, lolitrem B, which can have toxic effects when consumed by livestock (Tor-Agbidye et al., 2001). Recently, researchers and producers from Japan have expressed concerns related to impaired health and performance of cattle consuming imported perennial ryegrass straw (Miyaszaki et al., 2001). Therefore, the objectives of our study were to evaluate the effect of increasing lolitrem B concentration in perennial ryegrass straw on physiological response variables, ruminal fermentation characteristics, straw intake and digestibility, performance, and milk production of beef cattle.

Materials and Methods

Experiment 1: Steer Digestion/Physiology Study

Sixteen Angus × Hereford ruminally cannulated steers (231 ± 2 kg BW) were used in a randomized complete-block design experiment (Cochran and Cox, 1957). Steers were blocked by weight and assigned randomly to one of four treatments (**TRT**). Animals were housed in individual pens (2 × 4 m) within an enclosed barn with continuous lighting and unrestricted access to fresh water and a trace mineralized salt block (≥96.00% NaCl, ≥0.20% Mn, ≥0.10% Fe, ≥0.10% Mg, ≥0.05% S, ≥0.025% Cu, ≥0.01% Co, ≥0.008% Zn, and ≥0.007% I). In addition, all steers received an i.m. injection of vitamins A, D, and E (500,000; 50,000; and 1,500 IU, respectively; Vitamin E-AD 300, Agrilabs, St. Joseph, MO) at the start of the trial to safeguard against deficiency. Perennial ryegrass straw was provided at 120% of the previous 5-d average intake at 0730, with orts from the previous day determined before feeding. Before straw feeding (0700), soybean meal (**SBM**) was provided (0.1% BW; CP basis) to meet the estimated requirement for degradable intake protein assuming an 11% microbial efficiency (NRC, 1996; level 1). Mixtures of low (**L**) and high (**H**) lolitrem B straw (<100 and 1,550 ppb, respectively; Table 1) were used to formulate TRT diets. The TRT were **LOW** (100% L), **LOW-MIX** (67% L:33% H), **HIGH-MIX** (33% L:67% H), and **HIGH** (100% H; all rations on a DM basis). The nutrient content of the straws and SBM is provided in Table 1. The Institutional Animal Care and Use Committee at Oregon State University approved the experimental procedures used in this study.

The experimental period was 25 d, with the first 13 d used as an adaptation period. At 0700 on d 14, each steer was intraruminally pulse-dosed with 4 g of Co-EDTA in a 150-mL aqueous solution (Uden et al., 1980) for determination of ruminal fluid fill and dilution rate. The Co marker was administered throughout the rumen using a stainless-steel probe with a perforated tip. Ruminal fluid (approximately 100 mL) was collected by suction strainer (Raun and Burroughs, 1962; 19-mm diameter, 1.6-mm mesh) 0 (before SBM supplementation), 3, 6, 9, 12, and 24 h after SBM supplementation. Samples were immediately analyzed for pH and subsampled by placing 5 mL of ruminal fluid in 1 mL of 25% (wt/vol) meta-phosphoric acid and stored (–20°C) for later analysis of NH_3-N and VFA. Also, 20 mL of ruminal fluid was stored (–20°C) for later analysis of Co concentration. Frozen NH_3-N and VFA samples were prepared for analysis by thawing, centrifuging (15,000 × *g*; 10 min), and collecting the supernatant. Volatile fatty acids were analyzed as described by Harmon et al. (1985), and NH_3-N was analyzed by a modification (sodium salicylate was substituted for phenol) of the procedure described by Broderick and Kang (1980), using a UV/visible light spectrophotometer (Spectronic 710 spectrophotometer, Bausch & Lomb, Inc., Rochester, NY). Frozen ruminal fluid samples were prepared for Co analysis by thawing, centrifuging (2,000 × *g*; 20 min), and collecting the supernatant. Cobalt concentration in ruminal fluid was analyzed by atomic absorption using an air/acetylene flame (model 351 AA/AE spectrophotometer, Instrumentation Laboratory, Inc., Lexington, MA.) Ruminal fluid fill and dilution rate were determined by regressing the natural logarithm of Co concentration against sampling time, as described by Warner and Stacey (1968).

Intake and orts were monitored throughout the experiment; however, official measurements were taken on d 14 to 19 and d 15 to 20 for intake and orts, respectively. Samples (approximately 200 g) of L straw, H straw, and SBM were collected on d 14 to 19. Orts were collected and a subsample was obtained (10% wet weight) on d 15 to 20. Samples were dried in a forced-air oven (55°C; 48 h) and reweighed for calculation of DM. Orts were composited by steer, whereas samples of L straw, H straw, and SBM were independently composited. Straw, SBM, and orts samples were ground in a Wiley mill (1-mm screen).

On d 15, reticuloruminal contents were manually removed (Lesperance et. al., 1960) 4 h after straw feeding to determine TRT effects on ruminal indigestible ADF (**IADF**) fill and passage rate. Reticuloruminal contents were weighed, thoroughly hand mixed, and subsampled in triplicate (approximately 400 g). Remaining contents were then replaced. Samples were weighed, dried in a forced-air oven (55°C; 96 h), re-

Table 1. Feedstuff nutrient content (DM basis)

	Experiment 1 (Steer study)			Experiment 2 (Cow study)			
Item	Low perennial ryegrass straw[a]	High perennial ryegrass straw[a]	Soybean meal	Low perennial ryegrass straw[a]	High perennial ryegrass straw[a]	Meadow grass hay	Soybean meal
CP, %	4.6	5.5	45.6	5.4	6.2	6	51.8
OM, %	95	95	90	95	95	89	92
NDF, %	63	64	20	67	64	60	16
ADF, %	33	34	6	36	33	31	4
Lolitrem B, ppb	<100	1,550	N/A[b]	467	2,017	N/A	N/A
Ergovaline, ppb	<10	160	N/A	40	200	N/A	N/A

[a]Low and High are indicative of lolitrem B concentration.
[b]N/A = not applicable.

weighed for DM, composited by steer, and ground as described previously.

Steers were fitted with fecal bags at 0630 on d 16, with bags changed once every 24 h for a total fecal collection period of 6 d. Daily fecal samples were weighed, hand mixed, and a 2.5% subsample (wet-weight) collected. Subsamples were weighed, dried in a forced-air oven (55°C; 96 h), reweighed for DM, composited by steer, and ground as described previously.

Ground samples were analyzed for DM, OM (AOAC, 1990), N (Leco CN-2000, Leco Corp., St. Joseph, MI), and NDF (Robertson and Van Soest, 1981) and ADF (Goering and Van Soest, 1970) using procedures modified for use in an Ankom 200 fiber analyzer (Ankom Co., Fairport, NY). Also, samples were analyzed for IADF as described by Bohnert et al. (2002). Average fecal IADF recovery was 96 ± 1%. Digesta kinetics techniques described by Van Soest (1994) were used to determine IADF passage by dividing IADF intake by the quantity of IADF in the rumen 4 h after feeding. Straw samples were analyzed for lolitrem B and ergovaline with HPLC as described by Hovermale and Craig (2001).

Heart rate (**HR**; audibly monitored with a stethoscope in the area behind the left front elbow), respiration rate (appraised by flank movement), and rectal temperature were measured at 1300 on d 16 to 21. In addition, 10 mL of blood was collected from the jugular vein by venipuncture 4 h after straw feeding on d 22 to 25. Blood was immediately transferred to a Vacutainer tube and allowed to clot overnight at 4°C. Samples were then centrifuged (1,500 × *g*; 15 min; 4°C) and the serum harvested and stored (–20°C) for prolactin analysis as described by Hockett et al. (2000; intraassay CV = 5.6).

Data were analyzed as a randomized complete block using the GLM procedure of SAS (SAS Inst., Inc., Cary, NC). Steer, TRT, and block were included in the model. Contrast statements were 1) linear effect of increasing lolitrem B concentration and 2) quadratic effect of increasing lolitrem B concentration. Data for ruminal pH, NH_3-N, and VFA, collected at fixed time points after SBM supplementation, were analyzed using the REPEATED statement with the MIXED procedure of SAS. The model included steer, TRT, block, hour, and TRT × hour. Also, physiological variables and serum prolactin, collected 4 h after straw feeding on fixed days, were analyzed using the REPEATED statement with the MIXED procedure of SAS. The model included steer, TRT, block, day, and TRT × day. The same contrasts described above were used to partition TRT effects for ruminal pH, NH_3-N, VFA, physiological variables, and serum prolactin.

Experiment 2: Cow Performance/Production Study

The Institutional Animal Care and Use Committee at Oregon State University approved experimental procedures used in this experiment. Seventy-two pregnant (approximately 200 d gestation) Angus × Hereford cows (539 ± 5 kg BW) were stratified by body condition score (**BCS**; 1 = emaciated; 9 = obese; Herd and Sprott, 1986) and assigned randomly to one of 18 pens (four cows/pen; six pens/TRT) and one of three TRT in a randomized complete block design (Cochran and Cox, 1957). All cows had ad libitum access to fresh water, a loose mineral mix (≥21.00% NaCl, 2,600 ppm Mn ≥12.00% P, ≥11.00% Ca, ≥2.50% Mg, ≥2.50% K, 3,000 ppm Zn, 2,000 ppm Cu, 140 ppm Se, 60 ppm Co, 60 ppm I, ≥136,078 IU/kg vitamin A, and ≥27 I.U./lb vitamin E; DM basis), and trace mineralized salt (≥95.00% NaCl, ≥3,500 ppm Mn, ≥3,500 ppm Zn, ≥2,300 ppm Fe, ≥120 ppm I, ≥90 ppm Se, and ≥60 ppm Co; DM basis). Low and H lolitrem B straws (467 and 2,017 ppb, respectively; Table 1) were used to formulate TRT diets. The TRT (DM basis) were ad libitum access to LOW (100% L), **MIX** (50% L:50% H), or HIGH (100% H) lolitrem B straw. Also, SBM was provided (0.1% BW; CP basis) at 0700 to meet the estimated requirement for degradable intake protein assuming an 11% microbial efficiency (NRC, 1996; Level 1). Straw samples (approximately 100 g) for alkaloid analysis were obtained twice weekly until all cows calved. Low and H straw samples were analyzed for lolitrem B and ergovaline as described by Hovermale and Craig (2001). Also, additional samples (approximately 200

Table 2. Perennial ryegrass clinical sign evaluation scale[a]

Score	Clinical signs
0	No clinical signs
1	No resting tremors or incoordination; low-intensity tremor and incoordination with handling
2	No resting tremors or incoordination; moderate-intensity tremors and incoordination with handling
3[b]	Spontaneous low-intensity tremors and incoordination at rest; moderate to severe tremors and incoordination with handling
4	Pronounced resting tremors and incoordination; convulsive tremors and severe incoordination with handling
5	Severe spontaneous tremors and incoordination at rest, usually accompanied by convulsive episodes

[a]Adapted from Galey et al. (1991).
[b]Removal from the study occurred with a scale reading of 3.

g) of L and H straws and SBM were collected weekly, dried in a forced-air oven (55°C; 48 h), reweighed for calculation of DM, ground in a Wiley mill (1-mm screen), and composited by source for analysis of NDF, ADF, IADF, N, and OM as described in Exp. 1. Nutrient content of L and H straws and SBM is provided in Table 1.

A visual appraisal of all cows was conducted daily at 0630, with cows resting and walking within their pen. Daily "clinical sign" scores were assigned to cows based on an evaluation scale adapted from Galey et al. (1991; Table 2). Cows receiving a score of 3 or higher were removed from the experiment by relocating them, with minimal excitement, to an isolated, quiet pen with ad libitum access to meadow hay and fresh water. Once clinical signs receded, cows were turned out to pasture with meadow hay provided at approximately 11.3 kg(cow^{-1}·d^{-1} (DM basis) and supplemented with SBM (2.7 kg·cow^{-1}·supplementation event^{-1}) on Monday, Wednesday, and Friday.

Cow BW and BCS were measured at study initiation, d 28, and every 14 d thereafter until calving. All weights were obtained following an overnight shrink (16 h). Three trained technicians independently evaluated BCS of cows. The same technicians were utilized throughout the trial. Also, cow weight, BCS, and calf weight were obtained within 24 h following parturition.

Eighteen cows (one cow/pen; six cows/TRT) were randomly selected and dosed with an intraruminal Cr-releasing device (**IRCRD**; Captec, Nufarm, Auckland, New Zealand) on d 28. Fecal grab samples (approximately 400 g) were collected on d 35 to 39 at 0730. Fecal samples were dried in a forced-air oven (55°C; 96 h), composited by cow, and ground as described in Exp. 1. Samples were later analyzed for IADF as described in Exp. 1. Fecal samples were prepared as described by Williams et al. (1962) and analyzed for Cr by atomic absorption spectroscopy (air/acetylene flame; model 351 AA/AE spectrophotometer, Instrumentation Laboratory, Inc.). Chromium payout rate (951 ± 13 mg/d) of the IRCRD was validated using four steers in Exp. 1 and was 103 ± 1% of the IRCRD distributor's estimated payout. Fecal output was estimated by dividing IRCRD Cr payout by fecal Cr concentration. Also, DM digestibility was estimated with IADF as an internal marker (Cochran and Galyean, 1994). Consequently, DMI was estimated as fecal output divided by diet indigestibility.

Following parturition, cows and calves remaining on experimental TRT were placed in a common pasture (7.3 ha) that had been harvested for hay earlier in the year, and managed as a single group. Cows were provided approximately 11.3 kg·cow^{-1}·d^{-1} (DM basis) of meadow hay and supplemented with SBM (2.7 kg·cow^{-1}·supplementation event^{-1}) on Monday, Wednesday, and Friday for approximately 8 wk. Samples (approximately 200 g) of meadow hay and SBM were collected weekly. Samples were dried in a forced-air oven (55°C; 48 h) and reweighed for calculation of DM. Meadow hay and SBM samples were ground in a Wiley mill (1-mm screen) and composited by source for analysis of NDF, ADF, N, and OM as described in Exp. 1. Nutrient content of meadow hay and SBM is provided in Table 1.

Approximately 53 ± 1 d after calving (May 7, 2003), milk production was estimated by weigh-suckle-weigh (**WSW**) after an 8-h separation (Williams et al., 1979). Calf excretory (fecal and urinary) losses during suckling were considered minimal and were not collected as suggested by Lampkin and Lampkin (1960). In addition, 10 mL of blood was collected from the jugular vein of cows by venipuncture. Blood was immediately transferred to a Vacutainer tube and allowed to clot overnight. Samples were then centrifuged (1,500 × *g*; 15 min) and serum harvested and stored (–20°C) for prolactin analysis as described by Hockett et al. (2000; intraassay CV = 5.0).

Data were analyzed as a randomized complete block using the GLM procedure of SAS. Pen, TRT, and block were included in the model. Contrast statements were 1) linear effect of increasing lolitrem B concentration and 2) quadratic effect of increasing lolitrem B concentration. All data from cows removed from the experi-

ment (13 cows) for exhibiting clinical signs of ryegrass staggers were removed from dataset and not used in calculating pen means (all pens contained at least one cow).

Results and Discussion

We are unaware of previous research evaluating the effect of feeding perennial ryegrass straw containing increasing lolitrem B on physiological response variables, ruminal fermentation characteristics, straw intake and digestibility, performance, and milk production in ruminants. Alkaloid concerns with perennial ryegrass are normally associated with lolitrem B, whereas concerns with tall fescue are associated with ergovaline. Stamm (1992) conducted a survey of several grass straws harvested in the Willamette Valley of Oregon and reported the concentration of the alkaloid ergovaline contained within the various species and varieties. Results indicated that 42% of the perennial ryegrass fields sampled had straw containing greater than 200 ppb ergovaline, whereas only 14% of the tested tall fescue fields had an ergovaline concentration greater than 200 ppb. This suggests that perennial ryegrass has the potential to cause symptoms of both lolitrem B and ergovaline toxicosis. However, ergovaline levels within the current study (Table 1) were below the critical threshold (400 to 750 ppb) suggested to cause fescue toxicosis in cattle (Hovermale and Craig, 2001; Tor-Agbidye et al., 2001).

Experiment 1: Steer Digestion/Physiology Study

Neither straw nor total DMI was affected by increasing lolitrem B concentration ($P > 0.61$); straw and total DMI averaged 19.4 g/kg BW and 21.8 g/kg BW, respectively (Table 3). Similarly, straw and total OM intake was not affected by increasing lolitrem B concentration ($P > 0.60$), with straw and total OM intake averaging 18.4 g/kg BW and 20.6 g/kg BW, respectively. These results are inconsistent with those of Bluett et al. (2001). These researchers allowed lambs to graze one of two cultivars of perennial ryegrass (Aries HD: 3,420 ppb lolitrem B and 160 ppb ergovaline; Yatsyn 1: 2,420 ppb lolitrem B and 450 ppb ergovaline). Lambs consuming the grass with a higher concentration of lolitrem B had 12% greater herbage intake than did lambs consuming the variety with a lower lolitrem B level. However, their results may have been influenced by the ergovaline concentration of the two varieties (Yatsyn 1 contained less lolitrem B than Aries HD; however, Yatsyn 1 contained more than twice the level of ergovaline as Aries HD).

Previous work has indicated that consumption of ergovaline can depress feed intake when livestock are under environmental stress. Work by Hemken et al. (1981) noted that DMI of Holstein calves consuming endophyte-free or -infected tall fescue and maintained in an environment of 23°C or less did not differ. However, they reported that when temperatures exceeded 34°C, a marked decrease in DMI was observed in calves consuming endophyte-infected vs. endophyte-free tall fescue. The relatively low environmental temperature (9°C) may have masked any potential negative effect of increased alkaloid concentrations on forage and total DMI in the current study.

Intake of N and NDF was not affected ($P > 0.19$) by increasing lolitrem B concentration. Also, apparent total-tract DM, OM, and NDF digestibility did not differ ($P > 0.13$) between TRT. Similarly, Bluett et al. (2001) reported no difference in OM digestibility when lambs grazing a perennial ryegrass variety with a high concentration of lolitrem B were compared with lambs grazing a variety with a lower lolitrem B concentration. Additionally, our results agree with those of Stamm et al. (1994) in reporting no difference ($P > 0.10$) in straw and total DMI or apparent digestibility of DM and NDF by steers consuming straw with an increasing alkaloid (ergovaline) concentration.

No TRT effects were observed for IADF intake ($P > 0.18$); however, ruminal IADF fill increased linearly ($P = 0.01$) and IADF passage rate (%/h) decreased linearly ($P = 0.04$) as lolitrem B level increased (Table 3). It is possible that reticuloruminal smooth muscle activity may have been reduced as lolitrem B concentration increased, subsequently reducing ruminal IADF passage. Smith et al. (1997) inhibited gastrointestinal tract smooth muscle activity in sheep by dosing lolitrem B into the jugular vein. Furthermore, McLeay et al. (1999) noted that lolitrem B inhibited the frequency of reticular and ruminal contractions in sheep compared with those not receiving lolitrem B. However, these effects on gut motility are contradicted by the lack of a difference in DM and OM intake and ruminal fluid dilution rate (discussed below; Table 4) as lolitrem B level increased in the current study.

No TRT × hour interactions ($P > 0.08$) were noted for ruminal NH_3-N, pH, total VFA, or molar proportions of acetate, propionate, isobutyrate, butyrate, isovalerate, or acetate:propionate ratio. Therefore, only overall TRT means are discussed. A TRT × hour interaction ($P = 0.01$) was observed for the molar proportion of valerate; however, after reviewing the data, we concluded that the interaction did not seem to be biologically relevant. Consequently, overall TRT means for valerate are reported.

Increasing lolitrem B concentration did not affect ($P > 0.15$) ruminal NH_3-N, pH, or total VFA (Table 4). Molar proportions of propionate, isobutyrate, butyrate, valerate, and acetate:propionate ratio were not affected by increasing lolitrem B level ($P > 0.09$). However, a quadratic influence was observed for acetate and isovalerate ($P < 0.05$), with the greatest molar proportion of acetate occurring with LOW-MIX and HIGH-MIX and the greatest proportion of isovalerate occurring with LOW and HIGH TRT.

Whereas other ruminal fermentation data evaluating increasing lolitrem B levels within perennial rye-

Table 3. Effect of increasing lolitrem B concentration on nutrient intake and diet digestibility by steers consuming perennial ryegrass straw

	Treatment[a]					*P*-value[c]	
Item	LOW	LOW-MIX	HIGH-MIX	HIGH	SEM[b]	Linear	Quadratic
Daily DMI, g/kg BW							
Straw	19.7	19.7	19.4	18.9	1.1	0.62	0.81
Supplement	2.4	2.4	2.4	2.4			
Total	22.1	22.1	21.8	21.3	1.1	0.62	0.81
Daily OM intake, g/kg BW							
Straw	18.7	18.7	18.4	17.9	1.1	0.61	0.81
Supplement	2.2	2.2	2.2	2.2			
Total	20.9	20.9	20.6	20.1	1.1	0.61	0.81
Daily N intake, g/kg BW	0.326	0.335	0.341	0.345	0.010	0.20	0.82
Daily NDF intake, g/kg BW	12.8	13.0	12.9	12.7	0.7	0.88	0.81
Total-tract apparent digestibility, %							
DM	59.9	61.9	59.2	58.9	0.9	0.19	0.23
OM	60.6	63.1	60.8	60.3	0.9	0.44	0.14
NDF	51.6	55.4	52.4	51.9	1.4	0.72	0.16
IADF intake, g/kg BW[d]	3.1	3.1	2.9	2.8	0.2	0.19	0.78
IADF fill, g/kg BW[d]	6.7	7.0	6.6	7.7	0.2	0.01	0.06
IADF passage, %/h[d]	1.93	1.82	1.86	1.51	0.12	0.04	0.31

[a]LOW = 100% low straw (<100 ppb lolitrem B); LOW-MIX = 67% low straw:33% high straw (1,550 ppb lolitrem B); HIGH-MIX = 33% low straw:67% high straw; HIGH = 100% high straw.
[b]n = 4.
[c]Probability of linear and quadratic effects of increasing lolitrem B concentration.
[d]Indigestible ADF.

grass are unavailable, Stamm et al. (1994) did report ruminal fermentation parameters of cattle consuming tall fescue straw with increasing ergovaline concentration. Similar to the current study, the authors did not report a TRT difference in ruminal NH_3-N. However, they did observe a linear decrease in ruminal pH as ergovaline content of the diet increased. Additionally, they reported that total VFA increased linearly as ergovaline concentration increased. The current study used a high-alkaloid straw that contained one-third the ergovaline used by Stamm et al. (1994; 160 ppb vs. 475 ppb). Consequently, it is possible that ergovaline intake by steers in Exp. 1 was not sufficient to cause an effect on ruminal pH or total VFA.

There was no difference ($P > 0.13$) in ruminal fluid volume or dilution rate as lolitrem B concentration increased (Table 4). This is similar to results reported by Forcherio et al. (1995). They evaluated energy and protein supplementation effects on ruminal fermentation by cows consuming endophyte-infected tall fescue hay and did not see a difference in ruminal fluid passage rate as alkaloid concentration increased. How-

Table 4. Effect of increasing lolitrem B concentration on ruminal fermentation, and liquid volume and dilution rate in steers consuming perennial ryegrass straw

	Treatment[a]					*P*-value[c]	
Item	LOW	LOW-MIX	HIGH-MIX	HIGH	SEM[b]	Linear	Quadratic
NH_3-N, m*M*	2.9	3.0	3.2	3.7	0.6	0.35	0.73
pH	6.6	6.2	6.6	6.5	0.1	0.16	0.36
Total VFA, m*M*	81.7	81.0	80.2	86.7	3.5	0.39	0.33
Proportion, mol/100mol							
Acetate	69.0	70.7	69.2	68.4	0.4	0.10	0.01
Propionate	17.2	16.8	17.7	17.9	0.4	0.14	0.56
Isobutyrate	0.66	0.65	0.75	0.72	0.56	0.26	0.87
Butyrate	11.4	10.2	10.6	11.1	0.5	0.86	0.11
Isovalerate	1.06	0.83	0.93	1.22	0.11	0.24	0.04
Valerate	0.72	0.70	0.76	0.70	0.45	0.98	0.70
Acetate:propionate ratio	4.0	4.2	3.9	3.9	0.1	0.10	0.24
Liquid volume, mL/kg BW	152.7	161.4	152.5	148.5	12.6	0.71	0.63
Dilution rate, %	14.5	14.2	13.7	13.0	0.7	0.14	0.78

[a]LOW = 100% low straw (<100 ppb lolitrem B); LOW-MIX = 67% low straw:33% high straw (1,550 ppb lolitrem B); HIGH-MIX = 33% low straw:67% high straw; HIGH = 100% high straw.
[b]n = 4.
[c]Probability of linear and quadratic effects of increasing lolitrem B concentration.

Table 5. Effect of increasing lolitrem B concentration on serum prolactin and physiological variables in steers consuming perennial ryegrass straw

	Treatment[a]					*P*-value[c]	
Item	LOW	LOW-MIX	HIGH-MIX	HIGH	SEM[b]	Linear	Quadratic
Serum prolactin, ng/mL	10.0	5.7	11.2	4.4	4.6	0.60	0.79
Heart rate, beats/min	82	77	78	77	5	0.55	0.70
Respirations, breaths/min	29	34	32	29	2	0.94	0.03
Temperature, °C	38.7	38.9	39.2	38.9	0.1	0.05	0.03

[a]LOW = 100% low straw (<100 ppb lolitrem B); LOW-MIX = 67% low straw:33% high straw (1,550 ppb lolitrem B); HIGH-MIX = 33% low straw:67% high straw; HIGH = 100% high straw.
[b]n = 4.
[c]Probability of linear and quadratic effects of increasing lolitrem B concentration.

ever, Hannah et al. (1990) reported a linear decrease in ruminal fluid volume and a linear increase in ruminal fluid dilution rate in lambs consuming diets with increasing ergovaline concentration. The increased ruminal fluid dilution rate was probably because of a shorter ruminal retention time, which also decreased ruminal digestibility of OM, NDF, and cellulose.

No TRT × day interactions ($P > 0.32$) were noted for serum prolactin, HR, respiration rate, or rectal temperature. Therefore, only overall TRT means are discussed. Alkaloid (lolitrem B and ergovaline) concentration did not influence serum prolactin or HR ($P > 0.41$); however, a quadratic effect ($P = 0.03$) was noted for respiration rate, with the greatest values occurring with LOW-MIX and HIGH-MIX TRT (Table 5). Rectal temperature increased quadratically ($P = 0.03$) as lolitrem B increased, with the highest temperature observed with the HIGH-MIX TRT. These results may be because of increased ergovaline in the diet as lolitrem B level increased. Paterson et al. (1995), in their review of the effects of fescue toxicosis on beef cattle productivity, stated that animal temperature and respiration rate are normally increased, and serum prolactin decreased, with increasing ergovaline intake. However, the magnitude of change observed in temperature and respiration rate as alkaloid level increased in the current study is small (0.5°C and 5 breaths/min, respectively). Also, the quadratic effects do not correspond with what is normally expected following a linear increase in ergovaline intake (linear increase in temperature and respiration rate; Paterson et al., 1995). Therefore, the response observed for temperature and respiration rate in this study may not be related to ergovaline intake. This is supported by the lack of a TRT effect on serum prolactin. Additionally, Hemkin et al. (1981) reported that Holstein calves consuming endophyte-free or endophyte-infected tall fescue did not have different respiration rates or rectal temperatures at environmental temperatures of 23°C or less. In the current study, the average environmental temperature was 9.3 ± 0.3°C, suggesting it was conducted in an environment that would not be expected to cause the same physiological response trends reported by Paterson et al. (1995). Also, Stamm et al. (1994) reported no difference in HR, respiration rate, or rectal temperature of steers consuming tall fescue straw with increasing ergovaline concentration. However, these authors reported a weekly decrease in serum prolactin as ergovaline concentration increased. This contradicts serum prolactin results in the current study, probably because of the lower ergovaline concentration used in this study compared with that of Stamm et al. (1994; 160 ppb vs. 475 ppb).

Experiment 2: Cow Performance/Production Study

During the course of the experiment, 13 of 24 (54%) cows consuming HIGH had a "clinical sign" score of three or higher, indicating that they suffered from ryegrass staggers. These cows were removed from the study. The first removal occurred on d 8 and the last was on d 43. It should be noted that none of the 13 cows was observed to have difficulty calving; all had healthy calves, all weaned a calf, and all rebred within a 45-d breeding period (determined by pregnancy at weaning). One cow on the HIGH TRT expired during the trial. On multiple days, a "clinical sign" score of 2 had been assigned to her; however, she had received a score of 0 for the 2 d previous to her death. Cause of death was inconclusive following necropsy and histopathological analysis.

Pre- and postcalving BW and BCS change were not affected ($P > 0.10$) by increasing lolitrem B concentration (Table 6). These data concur with those of Eerens et al. (1997b), who noted no difference among TRT in prelambing BW change by ewes grazing endophyte-free or endophyte-infected perennial ryegrass/white clover mixed pasture.

Similar to Exp. 1, DMI by cows was not affected by increasing lolitrem B concentration (Table 6). Also, our data agree with those of Stamm et al. (1994), who reported no difference in DMI among steers consuming tall fescue straw with increasing concentration of the alkaloid ergovaline. In contrast, Bluett et al. (2001) allowed lambs to graze one of two varieties of perennial ryegrass (Aries HD: 3,420 ppb lolitrem B and 160 ppb ergovaline; Yatsyn 1: 2,420 ppb lolitrem B and 450 ppb ergovaline) and noted a 12% greater herbage intake by

Table 6. Effect of increasing lolitrem B concentration on dry matter intake, digestibility, and performance by cows consuming perennial ryegrass straw

	Treatment[a]				*P*-value[b]	
Item	LOW	MIX	HIGH	SEM[c]	Linear	Quadratic
Initial						
Body weight, kg	527	547	558			
Body condition score	5.4	5.4	5.4			
Precalving change[d]						
Body weight, kg	55.8	53.9	51.4	5.3	0.57	0.96
Body condition score	–0.14	–0.05	0.06	0.08	0.11	0.94
Postcalving change[e]						
Body weight, kg	7.8	15.2	19.4	6.1	0.22	0.85
Body condition score	–0.03	–0.11	–0.08	0.08	0.64	0.62
DMI, g·kg BW^{-1}·d^{-1}	11.7	11.3	13.6	1.3	0.13	0.81
DM digestibility, %	52	50	49	0.5	<0.01	0.57
Days to calving	68	65	63	2	0.13	0.81
Calf birth weight, kg	39.8	38.6	39.7	1.2	0.96	0.45
Calf gain, kg/d of age[f]	1.10	1.06	1.04	0.07	0.58	0.93
Milk production, kg[f]	11.7	11.3	13.6	0.6	0.03	0.08
Serum prolactin, ng/mL[f]	86.7	88.2	97.9	17.3	0.66	0.85

[a]LOW = 100% low straw (467 ppb lolitrem B); MIX = 50% low straw:50% high straw (2,017 ppb lolitrem B); HIGH = 100% high straw.
[b]Probability of linear and quadratic effects of increasing lolitrem B concentration.
[c]n =6.
[d]Within 14 d of calving.
[e]Within 24 h after parturition.
[f]Obtained 53 ± 1 d after calving.

lambs consuming the variety with a higher concentration of lolitrem B than lambs consuming the variety with a lower lolitrem B level.

Contrary to the results of Stamm et al. (1994), Bluett et al. (2001), and Exp. 1, a linear decrease ($P < 0.01$) in digestibility of perennial ryegrass straw was observed as alkaloid level increased (Table 6). It is not readily apparent why this difference occurred; however, it may have been because of variety differences and/or harvest conditions of the two straws used in Exp. 2.

Eerens et al. (1997a) reported delayed parturition in ewes grazing endophyte-infected perennial ryegrass pasture compared to ewes grazing endophyte-free perennial ryegrass/white clover pasture. This is contrary to the results of this study, which noted no difference ($P > 0.12$) in days to calving among the three TRT groups (Table 6). It is possible that an alkaloid × species interaction exists that accounts for the difference between the sheep research conducted by Eerens et al. (1997a) and the current study with cows. Also, calf birth weight did not differ ($P > 0.44$) across TRT. This is similar to results reported by Eerens et al. (1997b), in which lamb birth weight was not affected by ewes grazing endophyte-infected or endophyte-free pasture.

Calf gain/day of age at WSW was not affected ($P > 0.57$) by TRT (Table 6). This contradicts other research that has shown a performance difference in nursing young of dams consuming endophyte-infected grass. Eerens et al. (1997b) reported greater ($P < 0.05$) weight gain by lambs of ewes grazing endophyte-free perennial ryegrass/white clover pasture compared with lambs of ewes grazing endophyte-infected pasture. Additionally, Peters et al. (1992) reported that calves of cows grazing endophyte-infected tall fescue had a lower ($P < 0.05$) daily gain than calves of cows grazing endophyte-free tall fescue. In the current study, cows received meadow grass hay during lactation instead of alkaloid-infected straw. This may have contributed to similar calf gain/day of age between TRT.

A linear increase ($P = 0.03$) in milk production was noted as lolitrem B level increased (Table 6). This is contradictory to research conducted by Lean (2001). In a case study, Lean (2001) reported that Holstein-Fresian dairy cows had a 4.6-L reduction in milk production when they consumed perennial ryegrass silage containing a high concentration of ergovaline compared to cows consuming similar perennial ryegrass silage containing a low concentration of ergovaline. Also, in a study with tall fescue, Peters et al. (1992) reported that daily milk consumption by calves nursing cows grazing endophyte-infected tall fescue was 25% lower ($P < 0.05$) than that of calves nursing cows grazing endophyte-free pasture. It is possible that there could have been a stronger ergopeptide influence in the experiments of Peters et al. (1992) and Lean (2001) compared with the current study. In his review, Oliver (1997) suggested that the ergopeptide class of alkaloids influences serum prolactin. Additionally, as reported in Exp. 1, there was no difference in serum prolactin concentration (an indicator of potential milk production) as lolitrem B concentration increased. This suggests that milk production would not be negatively affected by increasing lolitrem B level within

the straw, which is what was observed in the current study. Furthermore, the observed increase in milk production by HIGH may have been the result of removing "stagger" cows from the HIGH TRT which decreased the number of observations used in obtaining pen means.

Implications

Feeding perennial ryegrass straw with greater than 2,000 ppb (dry matter basis) lolitrem B to beef cattle can cause neurological disorders that increase management concerns. However, blending low- and high-lolitrem B straws to obtain a concentration equal to or less than 1,550 ppb (dry matter basis) may be a successful management alternative. This information should provide the grass seed industry, importers of ryegrass straw, and livestock producers with valuable information concerning safe feeding practices for use with ruminant livestock.

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