Proceedings, Western Section, American Society of Animal Science 1757

Vol. 54, 2003

EVALUATION OF PERENNIAL RYEGRASS STRAW AS A FORAGE SOURCE FOR RUMINANTS

M. J. Fisher[1], D. W. Bohnert[1], C. J. Ackerman[2], C. S. Schauer[1], T. DelCurto[1], A. M. Craig[2], E. S. Vanzant[3], D. L. Harmon[3], and N. F. Schrick[4]

[1]Eastern Oregon Agriculture Research Center, Burns, [2]Oregon State University, Corvallis, [3]University of Kentucky, Lexington, and [4]The University of Tennessee, Knoxville

ABSTRACT: We conducted a 25-d metabolism trial to evaluate digestion and physiological variables in steers offered perennial ryegrass straw containing increasing levels of lolitrem B. Sixteen ruminally cannulated, Angus X Hereford steers (231 ± 2 kg BW) were blocked by weight and assigned randomly to one of four treatments (TRT). Steers were provided perennial ryegrass straw at 120% of the previous 5-d average intake at 0730. Prior to straw feeding (0700), soybean meal was provided (0.1% BW; CP basis) to meet the estimated requirement for degradable intake protein. Mixtures of a low (L) and high (H) lolitrem B straw (<100 and 1550 ppb, respectively) 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). Intake and digestibility of DM and OM, along with ruminal pH, total VFA, and NH_3-N, were not affected by increasing lolitrem B concentration ($P > 0.13$). Ruminal indigestible ADF (IADF) fill increased linearly ($P = 0.01$) and IADF passage rate (%/h) decreased linearly ($P = 0.04$) as lolitrem B level increased. Lolitrem B concentration did not influence serum prolactin or heart rate ($P > 0.41$); however, a quadratic effect ($P = 0.03$) was noted for respiration rate, with the greatest values occurring with the Low Mix and High Mix diets. These data suggest that feeding perennial ryegrass straw containing up to 1550 ppb lolitrem B does not adversely affect nutrient digestion or physiological response variables.

Keywords: Lolitrem B, Perennial Ryegrass, Straw

Introduction

In the Pacific Northwest, grass seed is a major agricultural product (2001 – 2002 Oregon Agriculture and Fisheries Statistics, Oregon Department of Agriculture, Salem, OR). One of the most common is perennial ryegrass (*Lolium perenne*). Burning has been the traditional way of straw disposal following seed harvest; however, the large amount of smoke produced causes adverse impacts upon the environment and can create situations that prove dangerous and/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 (several thousand tons from the state of Oregon) to countries within the Pacific Rim, primarily Japan, Korea, and Taiwan. These countries imported 286,414 tons of Oregon's perennial ryegrass straw during the 2000-2001 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, Japan has expressed concerns relating to impaired health and performance of cattle consuming imported perennial ryegrass straw (Schnitzius et al., 2001). Therefore, the objective of this research was to evaluate the effect of increasing lolitrem B concentration in perennial ryegrass straw on physiological response variables, ruminal fermentation characteristics, and straw intake and digestibility in steers.

Materials and Methods

Sixteen Angus X Hereford, ruminally cannulated steers (231 ± 2 kg BW) were used in a Randomized Complete Block design (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 X 4 m) within an enclosed barn with continuous lighting. Each steer had unrestricted access to fresh water and a trace mineralized salt block (≥ 96.000% NaCl, ≥ 0.200% Mn, ≥ 0.100% Fe, ≥ 0.100% Mg, ≥ 0.050% S, ≥ 0.025% Cu, ≥ 0.010% Co, ≥ 0.008% Zn, and ≥ 0.007% I). In addition, all steers received an intramuscular 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 trial initiation to safeguard against deficiency. Perennial ryegrass straw was provided at 120% of the previous 5-d average intake at 0730, with feed refusals from the previous day determined before feeding. Prior to 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). Mixtures of a low (**L**) and high (**H**) lolitrem B straw (<100 and 1550 ppb, respectively) 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). Nutrient content of straw and SBM is provided in Table 1. Animal protocol for this study was approved by the Institutional Animal Care and Use Committee at Oregon State University.

The experimental period was 25 d with the first 13 d used as an adaptation period. On d 14, rumen fluid (approximately 100 ml) was collected by suction strainer (Raun and Burroughs, 1962; 19-mm diameter, 1.6-mm mesh) at 0 (prior to feeding), 3, 6, 9, 12, and 24 h after straw feeding. Samples were immediately analyzed for pH and sub-sampled by placing 5 ml of rumen fluid in 1 ml of 25% (wt/vol) meta-phosphoric acid and stored (-20°C) for later analysis of NH_3-N and VFA. Frozen samples were prepared for analysis by thawing, centrifuging (15,000 X g; 10 min), and collecting the supernatant. Volatile fatty acids were analyzed as described by Bock et al. (1991) and NH_3-N by a modification (sodium salicylate was substituted for phenol) of the procedure described by Broderick and Kang (1980) using a UV/VIS spectrophotometer (Spectronic 710 Spectrophotometer, Bausch & Lomb, Inc., Rochester, NY).

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

On d 15, reticulo-rumen contents were manually removed (Lesperance et. al., 1960) 4 h post-feeding to determine TRT effects on ruminal IADF fill and passage rate. Ruminal contents were weighed, thouroughly hand mixed, and sub-sampled in triplicate (approximately 400 g). Remaining ruminal contents were then replaced into the appropriate steer. Samples were weighed, dried in a forced-air oven (55°C; 96 h), reweighed for DM, composited by steer, and ground as described previously.

Steers were fitted with fecal bags at 0630 on d 16. Bags were changed once every 24 h for a total fecal collection period of 6 d. Each day, fecal samples were weighed, hand mixed, and a 2.5% sub-sample (wet-weight) collected. Samples 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 later analyzed for DM and OM (AOAC, 1990), N (Leco CN-2000, Leco Corporation, 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 indigestible ADF (**IADF**) as described by Bohnert et al. (2002). Average fecal IADF recovery was 96.4 ± 1.4%. Straw samples were analyzed for lolitrem B and ergovaline as described by Hovermale and Craig (2001).

Physiological variables were measured at 1300 on d 16 through 21 to determine heart rate (**HR**), respiration rate, and rectal temperature. In addition, 10 ml of blood was collected from the jugular vein by venipuncture 4 h post-feeding on d 22 through 25. Blood was immediately transferred to a vacutainer tube and allowed to clot overnight. Samples were then centrifuged (1,500 X g; 15 min) and serum harvested and stored (-20°C) for prolactin analysis as described by Hocket et al. (2000; assay CV = 5.59).

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. Orthogonal contrast statements were: 1) linear effect of increasing lolitrem B concentration; 2) quadratic effect of increasing lolitrem B concentration; 3) L lolitrem B straw vs. H lolitrem B straw. Ruminal pH, NH_3-N, and VFA, collected at fixed timepoints post-feeding, were analyzed using the REPEATED statement with the MIXED procedure of SAS. The model included steer, TRT, block, hour, and TRT X hour. Also, physiological variables and serum prolactin, collected on fixed days, were analyzed using the REPEATED statement with the MIXED procedure of SAS. The model included steer, TRT, block, day, and TRT X day. The same contrasts described above were used to partition TRT effects for ruminal pH, NH_3-N, VFA, physiological variables, and serum prolactin.

Results and Discussion

Neither straw or total DMI was affected by increasing lolitrem B concentration ($P > 0.61$); straw and total feed DMI averaged 19.4 g/kg BW and 21.8 g/kg BW, respectively (Table 2). Similarly, straw and total OM intake was not affected by increasing lolitrem B concentration ($P > 0.60$); with straw and total feed OM intake averaging 18.4 g/kg BW and 20.6 g/kg BW, respectively. Also, intake of N and NDF was not affected ($P > 0.19$) by increasing lolitrem B concentration. Apparent total tract DM, OM, and NDF digestibility did not differ ($P > 0.13$) between diets. This agrees with the findings of Stamm et al. (1994), as they observed no difference ($P > 0.10$) in straw and total DMI or apparent digestibility of DM and NDF in steers consuming tall fescue (*Festuca arundinacea*) straw with an increasing alkaloid (ergovaline) concentration.

No TRT effects were observed for IADF intake or IADF particulate outflow rate ($P > 0.18$); 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 2). It is possible that reticulo-rumen 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 ($P < 0.001$) the frequency of reticular and ruminal contractions in sheep compared with those not receiving lolitrem B. However, the lack of a difference in DM and OM intake as lolitrem B level increased suggests that this did not occur in the current study.

No TRT X hour interactions ($P > 0.08$) were noted for ruminal NH_3-N, pH, total VFA, or molar proportion of acetate, propionate, isobutyrate, butyrate, isovalerate, or acetate:propionate ratio. Therefore, only overall TRT means are discussed. We did observe a TRT X hour interaction (P = 0.01) for molar proportion of valerate; however, after reviewing the data, we concluded that the interaction did not appear physiologically relevant. Consequently, we are only reporting overall TRT means for valerate. Increasing lolitrem B concentration did not affect ($P > 0.15$) ruminal NH_3-N, pH, or total VFA (Table 3). Molar proportions of propionate, isobutyrate, butyrate, valerate, and acetate to propionate ratio, were not affected by increasing lolitrem B level ($P > 0.09$). However, a quadratic influence was observed for both acetate and isovalerate ($P < 0.05$). The greatest molar proportion of acetate occured with the Low Mix and High Mix diets, while the greatest molar proportion of isovalerate occurred with the L and H diets. It is not readily apparent why molar proportions of acetate and isovalerate responded in this manner.

No TRT X day interactions ($P > 0.32$) were noted for serum prolactin, HR, respiration rate, or rectal temperature. Therefore, only overall TRT means are discussed. Alkaloid 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 the Low Mix and High Mix diets (Table 4). 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 of steers as lolitrem B level increased (Table 1). Paterson et al. (1995), in their review of the effects of fescue toxicosis on beef cattle productivity, stated that animal temperature, serum prolactin, and respiration rate are normally increased with increasing ergovaline intake. However, the magnitude of change observed in temperature and respiration rate as lolitrem B level increased in the current study is small (0.5°C and 5 breaths/min, respectively). Also, our 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 may not be related to ergovaline intake. This is supported by the lack of a TRT effect on serum prolactin.

Implications

These data suggest that feeding perennial ryegrass straw containing up to 1550 parts per billion lolitrem B does not adversely affect nutrient digestion or physiological response variables in steers. Therefore, perennial ryegrass straw containing lolitrem B levels similar to those used in the current study has value as a forage source for ruminants. This should provide the grass-seed industry and importers of ryegrass straw with valuable information concerning safe feeding practices for use with ruminant livestock.

Literature Cited

AOAC. 1990. Official Methods of Analysis. 15th Ed. Association of Official Analytical Chemists. Arlington, VA.

Bock, B. J., D. L. Harmon, R. T. Brandt, Jr., and J. E. Schneider. 1991. Fat source and calcium level effects on finishing steer performance, digestion, and metabolism. J. Anim. Sci. 69:2211-2224.

Bohnert, D. W., C. S. Schauer, S. J. Falck, and T. DelCurto. 2002. Influence of rumen protein degradability and supplementation frequency on steers consuming low-quality forage: II. Ruminal fermentation characteristics. J. Anim. Sci. 80:2978-2988.

Broderick, G. A., and J. H. Kang. 1980. Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and in vitro media. J. Dairy Sci. 63:64-75.

Cochran, W. G., and G. M. Cox. 1957. Experimental Design. 2nd Ed. John Wiley and Sons, New York.

Evers, G. W., D. B. Hannaway, S. Griffith, S. Minier, P. Hoagland, M. Runyon, M. H. Hall, I. Jacob, S. W. Johnson, E. Liss, S. Fransen, S. L. Fales, W. Lane, D. M. Ball, M. Chau, J. Matylonek, M. Chaney, J. Cropper, A. Liston, and W. C. Young III. 1996. Perennial Ryegrass (*Lolium perenne L.*) International Forage Fact Sheet. Intl. Forage Species Fact Sheet Series of the Forage Information System. Available: http://www.forages.css.orst.edu/Topics/Species?Grasses/Perennial_ryegrass/International_Fact_Sheet.html. Accessed February 26, 2003.

Goering, H. K., and P. J. Van Soest. 1970. Forage Fiber Analyses (Apparatus, Reagents, Procedures, and Some Applications). Agric. Handbook No. 379. ARS-USDA, Washington, DC.

Hannaway, D., S. Fransen, J. Cropper, M. Teel, M. Chaney, T. Griggs, R. Halse, J. Hart, P. Cheeke, D. Hansen, R. Klinger, and W. Lane. 1999. Perennial Ryegrass. Pacific Northwest Ext. Pub. PNW-503. Available: http://eesc.orst.edu/agcomwebfile/edmat/html/pnw/pnw503/complete.html. Accessed February 26, 2003.

Hockett, M. E., F. M. Hopkins, M. J. Lewis, A. M. Saxton, H. H. Dowlen, S. P. Oliver, and F. N. Schrick. 2000. Endocrine profiles of dairy cows following experimentally induced clinical mastitis during early lactation. Anim. Reprod. Sci. 58:241-251.

Hovermale, J. T., and A. M. Craig. 2001. Correlation of ergovaline and lolitrem B levels in endophyte-infected perennial ryegrass (*Lolium perenne*). J. Vet. Diagn. Invest. 13:323-327.

Lesperance, A. L., V. R. Bohman, and D. W. Marble. 1960. Development of techniques for evaluating grazed forage. J. Dairy Sci. 43:682-689.

McLeay, L. M., B. L. Smith, and S. C. Munday-Finch. 1999. Tremorgenic mycotoxins paxilline, penitrem and lolitrem B, the non-tremorgenic 31-epilolitrem B and electromyographic activity of the reticulum and rumen of sheep. Res. Vet. Sci. 66:119-127.

NRC. 1996. Nutrient Requirements of Beef Cattle – Update 2000. 7th Ed. National Academy Press, Washington, DC.

Paterson, J., C. Forcherio, B. Larson, M. Samford, and M. Kerley. 1995. The effects of fescue toxicosis on beef cattle productivity. J. Anim. Sci. 73:889-898.

Raun, N. S., and W. Burroughs. 1962. Suction strainer technique in obtaining rumen fluid samples from intact lambs. J. Anim. Sci. 21:454-457.

Robertson, J. B., and P. J. Van Soest. 1981. The detergent system of analyses and its application to human foods. Pages 123-158 in The Analysis of Dietary Fiber. W. P. T. James and O. Theander, ed. Marcell Dekker, New York.

Schnitzius, J. M., N. S. Hill, C. S. Thompson, and A. M. Craig. 2001. Semiquantitative determination of ergot alkaloids in seed, straw, and digesta samples using a competitive enzyme-linked immunosorbent assay. J. Vet. Diagn. Invest. 13:230-237.

Smith, B. L., L. M. McLeay, and P. P. Embling. 1997. Effect of the mycotoxins penitrem, paxilline, and lolitrem B on the electromyographic activity of skeletal and gastrointestinal smooth muscle of sheep. Res. Vet. Sci. 62:111-116.

Stamm, M. M., T. DelCurto, M. R. Horney, S. D. Brandyberry, and R. K. Barton. 1994. Influence of alkaloid concentration of Tall Fescue straw on the nutrition, physiology, and subsequent performance of beef steers. J. Anim. Sci. 72:1068-1075.

Tor-Agbidye, J., L. L. Blythe, and A. M. Craig. 2001. Correlation of endophyte toxins (ergovaline and lolitrem B) with clinical disease: Fescue foot and perennial ryegrass staggers. Vet. Human Toxicol. 43:140-146.

Young, B. 2001. 2000-2001 straw export data. Crop and Soil Science News/Notes. Available: http://www.css.orst.edu/newnotes/. Oregon State University Extension Service. 15(6): pp. 6-10. Accessed February 19, 2003.

Table 1. Feedstuff nutrient content (DM Basis)

Item	Low[a] Perennial Ryegrass Straw	High[a] Perennial Ryegrass Straw	Soybean Meal
CP	4.6	5.5	45.6
OM	95	95	90
NDF	63	64	20
ADF	33	34	6
Lolitrem B, ppb	<100	1550	
Ergovaline, ppb	<10	160	

[a]Low and High are indicative of lolitrem B concentration.

Table 2. Effect of increasing lolitrem B concentration on nutrient intake and diet digestibility in steers consuming perennial ryegrass straw

	Treatment[a]					*P*-Value[c]		
Item	Low	Low Mix	High Mix	High	SEM[b]	Linear of LB	Quadratic of LB	Low vs. High
Daily DM Intake, g/kg BW								
Straw	19.7	19.7	19.4	18.9	1.1	0.62	0.81	0.64
Supplement	2.4	2.4	2.4	2.4				
Total	22.1	22.1	21.8	21.3	1.1	0.62	0.81	0.64
Daily OM Intake, g/kg BW								
Straw	18.7	18.7	18.4	17.9	1.1	0.61	0.81	0.63
Supplement	2.2	2.2	2.2	2.2				
Total	20.9	20.9	20.6	20.1	1.1	0.61	0.81	0.63
Daily N Intake, g/kg BW	0.326	0.335	0.341	0.345	0.010	0.20	0.82	0.22
Daily NDF Intake, g/kg BW	12.8	13.0	12.9	12.7	0.7	0.88	0.81	0.89
Total Tract Apparent Digestibility, %								
DM	59.9	61.9	59.2	58.9	0.9	0.19	0.23	0.45
OM	60.6	63.1	60.8	60.3	0.9	0.44	0.14	0.83
NDF	51.6	55.4	52.4	51.9	1.4	0.72	0.16	0.89
IADF Intake, g/kg BW	3.1	3.1	2.9	2.8	0.2	0.19	0.78	0.23
IADF fill, g/kg BW	6.7	7.0	6.6	7.7	0.2	0.01	0.06	<0.01
IADF Passage, %/h	1.93	1.82	1.86	1.51	0.12	0.04	0.31	0.03
IADF Outflow Rate, $g \cdot kg\ BW^{-1} \cdot h^{-1}$	12.9	12.8	12.2	11.6	0.7	0.19	0.78	0.23

[a] Low = 100% low straw (<100 ppb lolitrem B); Low Mix = 67% low straw:33% high straw (1550 ppb lolitrem B); High Mix = 33% low straw:67% high straw; High = 100% high straw.

[b] n = 4.

[c] Linear of LB = linear effect of increasing lolitrem B concentration; Quadratic of LB = quadratic effect of increasing lolitrem B concentration; Low vs. High = low lolitrem B straw vs. high lolitrem B straw.

Table 3. Effect of increasing lolitrem B concentration on ruminal NH_3-N, pH, and VFA in steers consuming perennial ryegrass straw

Item	Treatment[a]				SEM[b]	*P*-Value[c]		
	Low	Low Mix	High Mix	High		Linear of LB	Quadratic of LB	Low vs. High
NH_3-N, mM	2.9	3.0	3.2	3.7	0.59	0.35	0.73	0.36
pH	6.6	6.2	6.6	6.5	0.06	0.16	0.36	0.18
Total VFA, mM	81.7	81.0	80.2	86.7	3.53	0.39	0.33	0.34
Molar proportion, mol/100mol								
Acetate	69.0	70.7	69.2	68.4	0.42	0.10	0.01	0.32
Propionate	17.2	16.8	17.7	17.9	0.41	0.14	0.56	0.28
Isobutyrate	0.66	0.65	0.75	0.72	0.562	0.26	0.87	0.42
Butyrate	11.4	10.2	10.6	11.1	0.46	0.86	0.11	0.71
Isovalerate	1.06	0.83	0.93	1.22	0.107	0.24	0.04	0.30
Valerate	0.72	0.70	0.76	0.70	0.453	0.98	0.70	0.73
Acetate:Propionate ratio	4.0	4.2	3.9	3.9	0.10	0.10	0.24	0.23

[a] Low = 100% low straw (<100 ppb lolitrem B); Low Mix = 67% low straw:33% high straw (1550 ppb lolitrem B); High Mix = 33% low straw:67% high straw; High = 100% high straw.

[b] n = 4.

[c] Linear of LB = linear effect of increasing lolitrem B concentration; Quadratic of LB = quadratic effect of increasing lolitrem B concentration; Low vs. High = low lolitrem B straw vs. high lolitrem B straw.

Table 4. Effect of increasing lolitrem B concentration on serum prolactin and physiological variables in steers consuming perennial ryegrass straw

Item	Treatment[a]				SEM[b]	*P*-Value[c]		
	Low	Low Mix	High Mix	High		Linear of LB	Quadratic of LB	Low vs. High
Serum Prolactin, ng/ml	10.0	5.7	11.2	4.4	4.63	0.60	0.79	0.42
Heart Rate, beats/min	82	77	78	77	4.7	0.55	0.70	0.49
Respirations, breaths/min	29	34	32	29	1.6	0.94	0.03	0.84
Temperature, °C	38.7	38.9	39.2	38.9	0.09	0.05	0.03	0.10

[a] Low = 100% low straw (<100 ppb lolitrem B); Low Mix = 67% low straw:33% high straw (1550 ppb lolitrem B); High Mix = 33% low straw:67% high straw; High = 100% high straw.

[b] n = 4.

[c] Linear of LB = linear effect of increasing lolitrem B concentration; Quadratic of LB = quadratic effect of increasing lolitrem B concentration; Low vs. High = low lolitrem B straw vs. high lolitrem B straw.