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EFFECTS OF FEEDING *NEOTYPHODIUM COENOPHIALUM* -INFECTED TALL FESCUE STRAW ON LAMB PERFORMANCE

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ABSTRACT: An experiment was conducted to investigate the digestion responses and degradation of ergovaline and production of lysergic acid in the rumen of sheep offered *Neotyphodium coenophialum*-infected tall fescue straw at two ergovaline levels. Six crossbred wethers (56 +/- 3 kg BW) were randomly assigned to one of two treatment groups in a cross over design. Each experimental period consisted of 28 d feeding periods with a 14 d wash out between treatment periods. During the wash out period all animals received a diet devoid of ergovaline. Treatments were 1) <10 ppb ergovaline (E-) and 2) 500 ppb ergovaline (E+). Diets were isonitrogenous. Ergovaline levels were achieved with a combination of tall fescue straw and *Neotyphodium coenophialum*-infected tall fescue seed (>3,300 ppb ergovaline). Rumen ammonia, rumen pH, and rectal temperature were not influenced by alkaloid concentration ($P<0.10$). Digestion of DM, ADF and CP were not different between treatments ($P<0.10$). Water intake was reduced by treatment ($P<0.05$). Feed intake and body weight were not changed by ergovaline intake ($P>0.10$). Serum prolactin was reduced by 27% with ergovaline intake ($P<0.05$). Rumen fluid was sampled 3 times (d 0, 3, 28) during the 28-day period for ergovaline and lysergic acid. Samples were collected at time 0 (prior to feeding), 6, and 12 h post feeding. Ergovaline concentration in rumen fluid expressed as a percent of intake increased over sampling time and sampling day ($P<0.05$). Lysergic acid concentration in rumen fluid expressed as a percent of intake increased over time from d0 to d3 ($P<0.05$) but was not different on d28 between time ($P>0.10$). Ergovaline and lysergic acid detected in the feces was 35.82% +/- 4.45 and 112.53% +/- 24.88 of intake, respectively. The appearance of lysergic acid in the feces and rumen fluid is likely due to the degradation of ergovaline in the rumen due to microbial degradation and further hydrolysis in the lower digestive tract.
Key words: Sheep, Ruminants, Endophyte, Ergovaline, Tall fescue

Introduction

There is a long history of problems associated with feeding tall fescue (Strickland et al., 1993; Bacon, 1995; Tor-Agbidye et al., 2001). These problems are associated with the endophyte fungus *Neotyphodium coenophialum,* which infects varieties of tall fescue in a symbiotic fashion. The endophyte produces ergopeptine alkaloids, with ergovaline being produced in the greatest quantity. Tall fescue toxicosis is estimated to cost the beef industry $800 million dollars a year from reduction in reproductive performance and reduced gains. In Oregon grass seed production is the fourth largest agriculture commodity and approximately 160,000 acres are planted in tall fescue mainly used for grass seed production (National Ag Statistic Service, 2002). After the seed is harvested, straw is left as a field residue. In the past, straw was eliminated by field burning, but increased restrictions on burning have left producers looking for other avenues to dispose of straw. The main market for this straw is Japan and other Pacific Rim countries as forage for livestock. Oregon currently exports 65% (600,000 tons) of the straw to Japan, 34% to Taiwan, 1% to Korea and 60,000 ton is used domestically in the US. For many years ergovaline has been believed to cause fescue toxicosis (Joost, 1995). However, recent work by Hill et al. (2001) implies the core ring structure of ergopeptine alkaloids, lysergic acid (Figure 1), crosses the rumen wall at a higher rate than any of the other alkaloids. To date no one has quantified lysergic acid in the feed or measured the metabolism of lysergic acid in relation to ergovaline levels in the diet. This study assessed the metabolic fate of ergovaline and lysergic acid.

Materials and Methods

Animals, Experimental Design, and Diets. Six ruminally cannulated Poly Pay x Suffolk crossbred wethers (56 +/- 3kg BW) were randomly assigned to one of two treatment groups in a cross over design (Kuehl, 2000). Surgical and animal care procedures were approved by the Oregon State University Institution of Animal Care and Use Committee. Wethers were individually housed in metabolism crates within a barn during the duration of the study. Treatment periods were d 28 with total fecal collections on d 21 to 25. A 14d washout period was allowed between treatment periods. A two-week adaptation to the metabolism crates and voluntary intake was allowed before the first feeding period. Environmental temperatures were consistent with temperatures in the Pacific Northwest during August through November. Treatments were <10 ppb **(E-)** ergovaline and 500 ppb **(E+)** ergovaline. For both treatments chopped tall fescue straw was used. E- consisted of straw (95% AF) with <10 ppb ergovaline. E+ contained straw (88.5% AF) with 350 ppb ergovaline, the remainder of ergovaline was provided by endophyte infected tall fescue seed (>3,300 ppb ergovaline) (Table 1). The two treatment straws differed in CP and the addition of seed increased the CP of E+, therefore soybean meal (SBM) was added to ensure diets were isonitrogenous and CP requirements were fulfilled (NRC, 1985). Tall fescue straw was provided at 90% of previous 5-d average intake at 0800, with feed refusals from the previous day determined prior to feeding. Prior to feeding straw (0730), SBM and

SBM/seed mix was provided (SBM: 10% and 7% of intake; seed 4.5% of intake on an as fed basis). Rectal temperatures were taking daily just after feeding via a handheld digital thermometer with probe placed approximately 3 cm into the rectum. Water intake was measured twice a day, first prior to feeding and then at 1700 and summed for daily water intake. Wethers were weighed at the start and end of each feeding period.

Rumen fluid was sampled on d 0, 3, and 28 of each period in a time course at 0 (prior to feeding), 6, and 12 h after feeding for ergovaline and lysergic acid analysis. Additional samples were collected at 0, 3, 6, 9, 12, and 24 hr for pH and ammonia analysis, pH measurements of rumen fluid were taken immediately after collection with a high performance combination probe (Corning, New York, 14831). Approximately 60 ml of rumen fluid was collected with a rumen suction strainer and aliquoted in the following fashion, 9 ml for ammonia was added to 25% HCl acid (3 ml); 13 ml for each ergovaline and lysergic acid, remaining rumen fluid was used for pH measurement. Rumen fluid was place on ice immediately after collection then frozen and stored at -20°C. Blood samples for prolactin analysis was collected prior to feeding via jugular venipuncture with 10 ml vacutainers, tubes were allowed to coagulate at room temperature, centrifuged and serum was decanted and frozen for later analysis. Sheep were fitted with fecal bags at 0800 on d 19 of each treatment period for adaptation. Collection of total fecal samples commenced on d 21 to 25. Bags were changed twice in a 24 hr period, 0800 and 1700. The feces for each time was composited, weighed, hand mixed and 20% subsample (wet weight) was collected each day at 1700. Samples were dried in a freeze-drier for 7 days, reweighed for DM, ground, and composited by lamb. Fecal collections were used to estimate digestibility and calculate ergovaline and lysergic acid absorption. Intake and orts were monitored through out the trial; however official measurements were taken on d 0, 3, 28 and each day during fecal collections. Diet grab samples collected on each sampling day and each fecal collection day were composited for analysis. Orts were collected during fecal collection and were individually analyzed for ergovaline, lysergic acid, DM, ADF and CP. Straw and ort samples ground to pass through a 1mm Wiley mill screen and were stored at -20°C until analyzed.

Laboratory Analysis. Feed, fecal, and rumen fluid samples were tested for ergovaline concentration by high performance liquid chromatography (HPLC) as described by Craig et al. (1994). Briefly, feed samples were with a Wiley mill to pass a 1mm screen. Approximately 1.0g (feed and feces) or 6 ml (rumen fluid) of sample were extracted in chloroform with ergotamine added as an internal standard (1µl/ml ergotamine), chloroform was buffered with NaOH (feed and feces) or KPO_4 (rumen fluid). Samples were rotated in the dark for 24 hours (feed and feces) or 5 h (rumen fluid), 5ml of the supernatant were eluted from an ergosil column. The ergovaline containing elute was then evaporated and residue was suspended in 500µl methanol and injected onto the HPLC. HPLC conditions were as follows: 30:60 ammonium carbonate (0.2mg/ml):acetonitrile mobile phase, injection volume was 20 µl, and the fluorometer setting was 250 nm excitation and 420 nm emission

Lysergic acid was determined by HPLC. Briefly, ground samples were extracted with 10 ml acetonitrile:water (50:50 v/v) for overnight, centrifuged 2000 rpm for 10 minute. Rumen fluid was concentrated (6.5 ml) in the rotovap on high temperature (65°C) the pellet resuspended in 3 ml water by vortexing. Supernatant pH was adjusted to 5.0 to 5.5 with 50% acetic acid. Three ml of supernatant was passed through a solid phase extraction column (Supelco DSC-SCX SPE column, 500 mg/3ml; Bellefonte, PA. 16823). Lysergic acid was eluted, evaporated, suspended in 200 µl of 0.05 M phosphoric acid:methanol (50:50) and placed in HPLC vials. Samples were injected onto HPLC under the following conditions: mobile phase was 94:6 0.05M phosphoric acid:acetonitrile, injection volume was 20 µl, and the fluorometer setting was 250 nm excitation and 420 nm emission.

Acidified rumen fluid samples were thawed, centrifuged (1,000 x g) for 15 minutes and analyzed for ammonia by the phenol-hypochlorite method Broderick and Kang 1980 using 96-well microtiter plate reader attached to UV/Vis Spectrometer (Elx808; Bio-Tek instruments, Winooski, VT. 05404). Straw, SBM and feces were analyzed for DM, ADF, and CP by NIR and seed was analyzed by wet chemistry at Dairy One Forage Laboratory (Dairy One, Ithaca, NY. 14850). NIR is recognized by the Association of Official Analytical Chemists (AOAC) as an official method of analysis. Serum prolactin was analyzed as described by Hockett et al. (2000) by the University of Tennessee (assay CV = 5%).

Statistical Analysis. Data was analyzed as a crossover by SAS GLM procedure. Animal, treatment (TRT), period, day and day X TRT were included in the model. Ruminal pH and NH_3, serum prolactin and physiological variables were analyzed using the REPEATED statement with the MIXED procedure of SAS.

Results and Discussion

No clinical signs of tall fescue toxicosis were observed during the treatment periods. Rumen ammonia and pH was not different between treatment groups ($P = 0.896$ and $P = 0.355$ respectively) at any time point. These results are consistent with diets formulated to be isonitrogenous. Contrary to some other published studies (Aldrich et al., 1993; Paterson et al., 1995), daily rectal temperatures were not influenced by alkaloid concentration ($P = 0.395$). However, these results are similar to findings by Stamm et al. (1994) where no difference in rectal, tail and ear temperature was detected. The variation in observed rectal temperatures in response to E+ feed between studies could result from the different alkaloid levels of alkaloid used in each study. Consistent with finding by Stamm et al. (1994) and Aldrich et al. (1993) apparent digestibility of DM, ADF, and CP was not different between treatment groups (see Table 2). Aldrich et al. (1993) also reported no difference in water disappearance; however, in this study water intake was decreased by E+ diet ($P < 0.05$) which is consistent with

findings by Fiorito et al. (1991) where E+ feed caused a drop in voluntary water intake. Serum prolactin was decreased by 27% (Table 2) indicating subclinical fescue toxicosis. This result is consistent with previous research (Stamm et al., 1994; Porter, 1995; Paterson et al., 1995) which shows depressed blood (serum or plasma) prolactin levels of animals on E+ diets.

Ergovaline to lysergic acid ratio in the feed was 3.00:1 while in the feces the ratio was 0.94:1 (Table 4). Ergovaline released in the rumen, as a percentage of intake, increased over time within a sampling day and over the treatment period (Table 3). Lysergic acid liberated in the rumen, as a percentage of intake, increased from d 0 to d 3 but no difference was detected between d 3 and d 28 (Table 3). Hill et al. (2001) theorized that lysergic acid crosses the ruminal wall at a greater rate than other ergopeptine alkaloids because it is more polar and can be transported across tissue easier. This implies that the apparent digestibility of ergovaline results from degradation not from direct absorption. No carryover effect of ergovaline or lysergic acid in the rumen fluid was detected; concentration of ergovaline and lysergic acid at 0hr on d0 of both treatment periods was undetectable (data not shown).

The appearance of lysergic acid in the feces implies the ergot alkaloids in the feed were degraded to lysergic acid by rumen microbial digestion and degradation in the lower gastrointestinal tract.

Implications

This study is the first reported attempt to quantify the metabolism of lysergic acid in the ruminant digestive system using a HPLC assay. Results of this study may lead to a better understanding of ergovaline metabolism and the causes of fescue toxicosis.

Literature Cited

Alrich, C. G., M. T. Rhodes, J. L. Miner, M. S. Kerley, and J. A. Paterson. 1993. The effects of endophyte-infected tall fescue consumption and use of a dopamine antagonist on intake, digestibility, body temperature, and blood constituents in sheep. J. Anim. Sci. 71:158-163

Bacon, C. W. 1995. Toxic endophyte-infected tall fescue and range grasses: historical perspectives. J. Animal Sci. 73:861-870

Broderick, G. A. and J. H. Kang-Meznarich. 1980. Effects of incremental urea supplementation on ruminal ammonia concentration and bacterial protein formation. J. Dairy Sci. 63:64-75

Cheeke, P.R. 1995. Endogenous toxins and mycotoxins in forage grasses and their effects on livestock. J. Animal Sci. 73:909-918

Craig, A. M., D. Bilich, J. T. Hovermale, R. E. Welty. 1994. Improved extraction and HPLC methods for ergovaline from plant material and rumen fluid. J. Vet. Diagn. Invest. 6:348-352

Fiorito, I. M., L. D. Bunting, G. M. Davenport, and J. A. Boling. 1991. Metabolic and endocrine responses of lambs fed *Acremonium coenophialum*-infected or noninfected tall fescue hay at equivalent nutrient intake. J. Anim. Sci. 69:2108-2114.

Hill, N. S., F. N. Thompson, J. A. Stuedemann, G.W. Rottinghaus, H. J Ju, D. L. Dawe, and E. E. Hiatt III. 2001. Ergot alkaloid transport across ruminant gastric tissues. J. Anim. Sci. 79:542-549.

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.

Joost, R. E. 1995. *Acremonium* in fescue and ryegrass: Boon or bane? A review. J. Animal Sci. 73:881-888

Kuehl, R. O. 2000. Design of experiments: statistical principles of research design and analysis. 2nd Ed. Brooks/Cole Publishing Co. Pacific Grove, CA. 93950.

National Ag Statistic Service web page 2002-2003. Available: www.nass.usda.gov/or/annsum2003.htm

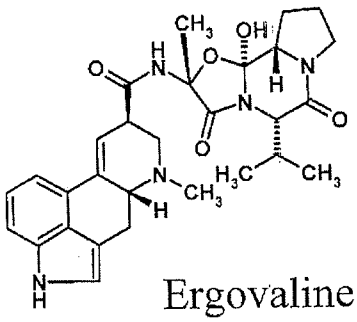
Paterson, J., C. Forcherio, B. Larson, M. Samford and M. Kerley. 1995. The effect of fescue toxicosis on beef cattle productivity. J. Animal Sci. 73:889-898.

Porter, J. K. 1995. Analysis of endophyte toxins: fescue and other grasses toxin to livestock. J. Animal Sci. 73:871-880

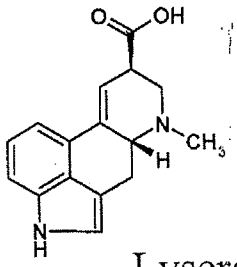
Stamm, M. M., T. DelCurto, M. R. Horney, S. D. Brandyberry, and R. K. Barton. 1994. Influence of alkaloid concentration of tall fescue straw on the nutrition, physiology, and subsequent performance of beef steers. J. Animal Sci. 72:1068-1075

Strickland, J. R., J. W. Oliver, and D. L. Cross. 1993. Fescue toxicosis and its impact on animal agriculture. Vet. Human Toxicol 35:454-464

Tor-Agbidye, J., L.L. Blythe, A.M. Craig. 2001. Correlation of endophyte toxins (ergovaline and Lolitrem B) with clinical disease: fescue foot and perennial ryegrass staggers.



Ergovaline



Lysergic acid

Figure 1 Structure of ergovaline and lysergic acid

Table 1 Feedstuff nutrient content (DM basis)

Item	E- straw	E+ straw	Soybean meal	E+ seed
CP, %	5.5	6.5	49	16.2
NDF, %	68.0	73.0	N/A	41.7
EV[a], ppb	<10	350	N/A	>3,300
LA[a], ppb	<10	142	N/A	660

[a]EV = ergovaline; LA = Lysergic acid

Table 2 Least square means for the physiological parameters during treatment

	E-[a]	E+	SE
Rectal temperature, °C[b]	38.36	38.41	0.0615
Water Intake, L/d[c]	2.95	2.77	0.590
Rumen, pH[d]	6.86	6.97	0.0322
Rumen NH_3, mMol[e]	4.72	4.70	0.128
Prolactin, ng/ml[f]	22.9	6.4	5.19

[a] E- = <10 ppb ergovaline, E+ = 500 ppb ergovaline; [b] P = 0.395; [c] P = 0.0398; [d] P = 0.355; [e] P = 0.896; [f] P = 0.023

Table 3 Least square means for digestibility of DM, ADF and CP in treatment groups[a]

	Treatment[b]		
	E-	E+	SE
DM	53.8	49.8	2.89
ADF	49.4	52.2	4.84
CP	61.4	63.7	1.62

[a] Values expresses as percent on a dry matter basis
[b] E- = <10 ppb ergovaline, E+ = 500 ppb ergovaline

Table 4 Ergovaline and lysergic acid content of rumen fluid of animals consuming E+ diet presented as a percentage of intake[a]

	Time	Day 0	3	28	SE
EV[e]	0 hr	0.00[b]	5.12[c]	7.60[d]	0.451
	6 hr	1.42[b]	5.33[c]	7.25[d]	0.511
	12 hr	2.67[b]	6.20[c]	8.42[d]	0.601
LA[e]					
	0 hr	0.00[b]	29.5[c]	27.4[c]	3.01
	6 hr	13.4[b]	37.6[c]	42.3[c]	5.94
	12 hr	21.6[b]	30.1[b]	41.6[b]	8.32

[a] Calculated as amount in rumen fluid/amount in feed
[b,c,d] Within a row, means without a common superscript letter differ (P<0.05)
[e] EV = ergovaline; LA = Lysergic acid

Table 5 Ergovaline and lysergic acid in the diet and feces of animals consuming E+ diet

	EV[ab]	SD	LA[ab]	SD	EV:LA[b]
Diet[c]	0.60	0.07	0.20	0.04	3.00
Feces[d]	0.48	0.04	0.51	0.15	0.94

[a] Actual means, n = 6
[b] EV = ergovaline; LA = Lysergic acid
[c] mg/kg of Intake
[d] mg/kg of Fecal output