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J ANIM SCI 2007, 85:1199-1206.
doi: 10.2527/jas.2005-430 originally published online February 12, 2007

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Physiological and digestive effects of *Neotyphodium coenophialum*-infected tall fescue fed to lambs[1]

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ABSTRACT: The digestive responses and degradation of ergovaline and production of lysergic acid in the rumen of sheep offered *Neotyphodium coenophialum*-infected tall fescue straw at 2 ergovaline levels were investigated. Six crossbred wethers (56 ± 3.0 kg of BW) were used in a randomized crossover design involving 2 treatments, for a total of 6 observations per treatment. The experiment consisted of two 28-d feeding periods with a 14-d washout period between them. The treatments were 1) tall fescue straw containing <0.010 mg of ergovaline/kg (E–), and 2) tall fescue straw containing 0.610 mg of ergovaline/kg (E+). Feed, orts, and feces were measured and analyzed for DM, ADF, and CP, and used to determine digestibilities. Feed and water intake were monitored throughout the feeding periods. Body weight and serum prolactin levels were measured at the beginning and end of each feeding period. Ruminal fluid was sampled 3 times (d 0, 3, and 28) during each 28-d feeding period for determination of ergovaline, lysergic acid, ammonia, and pH. Samples were collected before feeding (0 h) and at 6 and 12 h after feeding. Total fecal and urine collection commenced on d 21 and continued until d 25 of each feeding period. Ruminal ammonia, ruminal pH, and rectal temperature were not influenced by ergovaline concentration ($P > 0.10$). Digestion of DM, ADF, and CP was not different between treatments ($P > 0.10$). Daily water intake was less for the E+ diet (2.95 vs. 2.77 L/d; $P < 0.05$) as was serum prolactin (22.9 vs. 6.4 ng/mL; $P < 0.05$). Ergovaline concentration in ruminal fluid increased over sampling days at each sampling time ($P < 0.05$). Lysergic acid concentration in ruminal fluid increased over time from d 0 to 3 ($P < 0.05$) but was not different between d 3 and 28 ($P > 0.10$). In the E+ treatment, ergovaline was not detectable in the urine, whereas the concentration in the feces was 0.480 mg/kg. Lysergic acid was detected in the diet of the E+ treatment at 0.041 g/kg, lysergic acid in the urine was 0.067 mg/kg and in the feces was 0.102 mg/kg. The apparent digestibility of the alkaloids was 64.2% for ergovaline and –12.5% for lysergic acid. Approximately 35% of dietary ergovaline and 248% of dietary lysergic acid were recovered in the feces and urine. The appearance of lysergic acid in the feces, urine, and ruminal fluid is likely due to microbial degradation of ergovaline in the rumen and further breakdown in the lower digestive tract.

Key words: endophyte, ergovaline, lysergic acid, ruminant, sheep, tall fescue

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J. Anim. Sci. 2007. 85:1199–1206
doi:10.2527/jas.2005-430

INTRODUCTION

In Oregon, approximately 160,000 acres are planted in tall fescue (*Festuca arundinacea*), which is grown primarily for seed production rather than for livestock feed. Upon seed harvest, the stems are left in the field as residue. In the past, residue was eliminated by field burning. Due to increased environmental restrictions, the practice of field burning is no longer allowed in Oregon, resulting in large amounts of tall fescue field residue for disposal. The residue is now baled and marketed as an affordable source of forage that fits well in winter supplemental feeding plans in the United States and in dairy systems in Japan and Pacific Rim countries.

Most health problems associated with consumption of tall fescue by livestock result from the ergopeptide alkaloids produced by the endophyte fungus *Neotyphodium coenophialum*. Of all the ergopeptide alkaloids produced, ergovaline is present in the greatest quantity (Lyons et al., 1986) and is believed to be the primary causative agent of fescue toxicosis (Joost, 1995). Fescue

[1]Research jointly funded by the Oregon Agric. Exp. Sta. and USDA Cooperative Agreement #58-6227-4-0021. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the USDA.

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Received August 9, 2005.

Accepted January 30, 2007.

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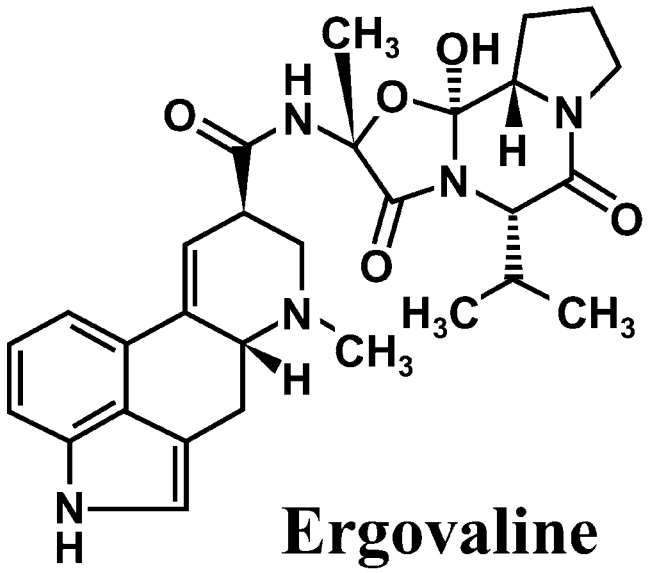

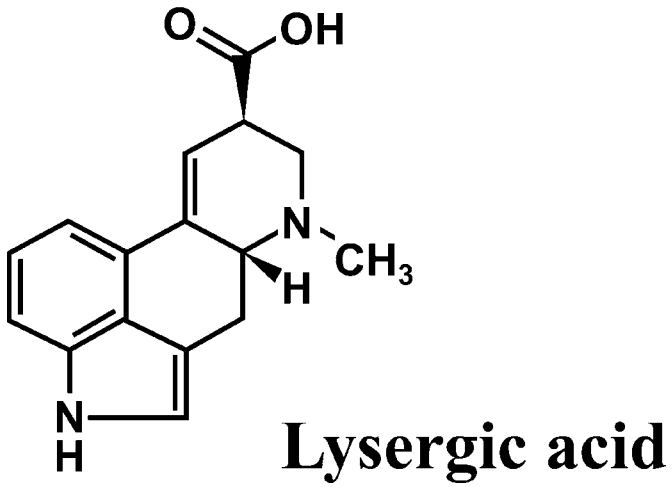

Figure 1. Structure of ergovaline and lysergic acid.

toxicosis is estimated to cost the beef industry $800 million yearly (Strickland et al., 1993).

Hill et al. (2001) reported that the core ring structure of ergopeptide alkaloids, lysergic acid (Figure 1), crossed the ruminal epithelium at a rate greater than any of the other alkaloids tested, and suggested that lysergic acid and not ergovaline may be responsible for fescue toxicosis. However, Hill et al. (2001) did not test all alkaloids present in the endophyte-infected tall fescue, or even ergovaline, for transport across the ruminal epithelium. To our knowledge, the concentrations of lysergic acid in ruminal fluid, urine, or feces have not been compared with dietary concentrations of ergovaline.

The objective of this study was to investigate the digestion of ergovaline and the production of lysergic acid in sheep consuming a *N. coenophialum*-infected tall fescue diet, composed of straw and seed, and containing approximately 0.50 mg of ergovaline/kg.

Table 1. Composition and nutrient content of endophyte-free (E–) and endophyte-infected (E+) tall fescue diets fed to wethers

Item	E– diet	E+ diet
Ingredient	% of DM	
E- straw	90.0	—
E+ straw	—	87.5
Soybean meal	10.0	6.5
E+ seed	—	6.0
Nutrient content		
CP, %	9.1	9.1
ADF, %	57	56
Ergovaline, mg/kg	<0.010	0.610
Lysergic acid, mg/kg	<0.010	0.041

MATERIALS AND METHODS

Animals

Surgical and animal care procedures were approved by the Oregon State University Institutional Animal Care and Use Committee. Six ruminally cannulated, Polypay × Suffolk, crossbred yearling wethers (56 ± 3 kg of BW) were randomly assigned to 1 of 2 treatment groups in a crossover experimental design (Kuehl, 2000). Wethers were individually housed in a partially open barn in metabolism crates for the duration of the study. During the washout period, all wethers were housed indoors as a group. Environmental temperatures were consistent with temperatures in the Pacific Northwest for August through November 2002 (Western Regional Climate Center; average temperature: high 22 ± 6°C, low 9 ± 4°C), with barn temperatures approximately equal to environmental temperatures. For 2 wk before the beginning of the first experimental period, wethers were adapted to the metabolism crates, and voluntary intake was measured.

Diets

The low ergovaline treatment (<0.01 mg/kg of ergovaline; **E**–) consisted of chopped (average length of 12 cm), endophyte-free, tall fescue straw [90% of the diet (DM basis); Table 1]. The target ergovaline level for the high ergovaline treatment (**E+**) was 0.50 mg/kg, which is the lower level of published toxicity values for ergovaline (0.50 to 0.80 mg/kg of intake; Tor-Agbidye et al., 2001). The E+ treatment was formulated to contain chopped (average length of 12 cm), endophyte-infected, tall fescue straw [0.35 mg of ergovaline/kg; 87.5% of the diet (DM basis); Table 1], and endophyte-infected tall fescue seed ground to pass a 2-mm screen with a Wiley mill [3.30 mg of ergovaline/kg; 6% of the diet (DM basis); Table 1]. Posttrial analysis of E+ diet samples showed an average ergovaline concentration of 0.610 mg/kg and an average lysergic acid concentration of 0.041 mg/kg.

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The 2 treatment straws differed in CP (5.5 and 6.5% for E– and E+, respectively), and the addition of seed (16.2% CP) increased the CP content of the E+ diet further. Therefore, soybean meal (**SBM,** 49% CP) was added to both diets to make the diets isonitrogenous and ensure that the protein requirements were fulfilled (Table 1; NRC, 1985).

Experimental Design

Each of the 2 feeding periods was 28 d in length, with a 14-d washout period between feeding periods. Before feeding straw, SBM and the SBM/seed mix were provided (0730; Table 1). Tall fescue straw was then provided at 90% of the previous 5-d average intake (0800). Intake was monitored throughout the trial. Orts were weighed during fecal collections. Diets and orts were sampled on d 0, 3, 28, and each day during the fecal collections (d 21 to 25). Diet and ort samples composited by animal were stored at –20°C until analyzed.

Composited diet and orts samples were analyzed for ergovaline and lysergic acid as outlined in the chemical analysis section. Straw, seed, SBM, orts, and feces were analyzed for DM (AOAC, 1990; method 930.15), ADF (AOAC, 1990; method 973.18), and CP (AOAC, 1990; method 984.13), using wet chemistry techniques, at Dairy One Forage Laboratory (Dairy One, Ithaca, NY). Rectal temperatures were measured daily just after feeding via a handheld digital thermometer (Johnson and Johnson, New Brunswick, NJ) with the probe placed approximately 3 cm into the rectum. Water intake was measured twice daily, before feeding and at 1700, and summed for daily water intake. Wethers were weighed at the beginning and end of each 28-d feeding period.

Blood samples for prolactin analysis were collected before feeding via jugular venipuncture and placed into 10-mL Vacutainer tubes (BD Bioscience, Franklin Lakes, NJ) on d 0, 3, and 28. The tubes were allowed to coagulate at room temperature and were then centrifuged (2,000 × *g*, 10 min). Serum was decanted and frozen at –20°C until prolactin analysis was performed. Serum prolactin was analyzed at the University of Tennessee, as described by Hockett et al. (2000). Intra- and interassay CV were 5 and 7%, respectively.

Ruminal fluid was sampled for ergovaline and lysergic acid analysis on d 0, 3, and 28 of each period before feeding (0 h), and at 6 and 12 h after feeding. Additional ruminal fluid samples were collected at 0, 3, 6, 9, 12, and 24 h after feeding on d 0, 3, and 28 for pH and ammonia analysis. Approximately 60 mL of ruminal fluid was collected with a rumen suction strainer (Bar Diamond, Parma, ID); 9 mL for ammonia analysis was acidified with 3 mL of 3 *M* HCl; two 13-mL samples were used for ergovaline and lysergic acid analysis; and the pH was measured in the remaining ruminal fluid immediately after collection with a high performance combination probe (Corning, Corning, NY). All other ruminal fluid samples were placed on ice immediately after collection for transport to the laboratory and were stored at –20°C.

Collection of total urine and feces commenced on d 21 and continued to d 25. Urine was collected in plastic pans and emptied twice daily (0800 and 1700) and stored in sealed plastic jars at 4°C until composited. Urine was not acidified because only ergovaline and lysergic acid were to be measured, and these alkaloids are relatively stable in basic solutions (A. M. Craig, unpublished observation). Daily urine was composited by sheep, weighed, measured for volume, and a 100-mL subsample of each daily composite was stored at –20°C until analyzed for ergovaline and lysergic acid. Sheep were fitted with fecal bags at 0800 on d 19 of each feeding period for adaptation. Fecal bags were changed twice daily (0800 and 1700) from d 21 to 25. Feces from each animal were composited by day, weighed, and hand-mixed, and a 20% (wet weight) subsample was collected each day at 1700. Subsamples were freeze-dried for 7 d, reweighed to determine DM, ground to pass a 1-mm screen with a Wiley mill, and then composited across days by lamb and stored at room temperature until analysis. A subsample of dried feces was stored at –20°C for ergovaline and lysergic acid determination. Fecal collections were used to estimate digestibility and to calculate excretion and apparent absorption of ergovaline and lysergic acid.

Chemical Analysis

Ergovaline. Feed, fecal, urine, and ruminal fluid samples were analyzed for ergovaline concentration by HPLC, as described by Craig et al. (1994). Briefly, feed and fecal samples were ground with a Wiley mill to pass a 1-mm screen and stored at –20°C until analysis. Approximately 1.0 g (feed and feces) or 6 mL (ruminal fluid) of sample was extracted in chloroform buffered with NaOH (feed and feces) or K_3PO_4 (ruminal fluid), and ergotamine was added as an internal standard (0.667 mg/L). Samples were rotated on a hematology/chemistry mixer (Fisher, Pittsburgh, PA) in the dark for 24 h (feed and feces) or 5 h (ruminal fluid), and then 5 mL of the supernatant was added to an ergosil solid-phase extraction column. The fraction containing ergovaline was collected and evaporated under N_2 gas, reconstituted in 500 μL of methanol, and 20 μL was injected onto the HPLC column. The mobile phase consisted of 30:60 ammonium carbonate (0.2 mg/mL of H_2O):acetonitrile (vol/vol), with the fluorometer excitation and emission wavelengths set at 250 and 420 nm, respectively.

Lysergic Acid. Lysergic acid was determined by HPLC (Lodge-Ivey et al., 2006). Lysergic acid analysis included a purification step and quantification. A 1.0-g sample of dried, ground straw, orts, seed, or feces were extracted by turning overnight on a hematology/chemistry mixer (Fisher) with 10 mL of acetonitrile:water (50:50, vol/vol). Samples were centrifuged (2,000 × *g*, 10 min). The resulting supernatant was transferred

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to disposable glass tubes, and the pH was adjusted to 5.0 to 5.5 with 50% (vol/vol) acetic acid. Ruminal fluid was concentrated (to 6.5 mL) in an ISS-100 centrifugal evaporator (Thermo Forma, Marietta, OH) at high temperature (65°C). The dried pellet was resuspended in 3 mL of water by vortexing and adjusted to pH 5.0 to 5.5 with 50% (vol/vol) acetic acid. Urine was adjusted to pH 5.0 to 5.5 with no other sample preparation. Three milliliters of supernatant was passed through a solid phase extraction (**SPE**) column (Supelco DSC-SCX SPE column, 500 mg/3 mL; Bellefonte, PA). The SPE cartridge was preconditioned with 3 mL of methanol followed by 3 mL of 0.1 *M* HCl and two 3-mL portions of double-distilled water. Caution was used to not allow the SPE cartridge to dry out between additions of each solution. The preconditioning eluents were discarded. The acidified supernatant was loaded onto the SPE cartridge followed by two 3-mL portions of pure water. Lysergic acid was eluted with 0.7 *M* ammonium hydroxide in methanol, evaporated in an ISS-100 centrifugal evaporator (Thermo Forma) at 43°C, and reconstituted in 200 μL of 0.05 *M* phosphoric acid:methanol (50:50). Twenty microliters of sample was injected onto an HPLC column using a mobile phase consisting of 94:6 0.05 *M* phosphoric acid:acetonitrile (vol/vol), and a fluorescence detector set at excitation = 250 nm and emission = 420 nm.

Ammonia. Acidified ruminal fluid samples were thawed, centrifuged (1,000 × *g*, 15 min), and analyzed for ammonia by the phenol-hypochlorite method (Broderick and Kang, 1980) adapted to a 96-well microtiter plate. The microtiter plates were analyzed with a plate reader (ELx808, BioTek Instruments, Winooski, VT) at 650 nm.

Statistical Analysis. Apparent digestibility was calculated as {[DMI × alkaloid (ergovaline or lysergic acid) concentration in the diet] − [fecal output (DM basis) × alkaloid concentration in the feces]}/(DMI × alkaloid concentration in the diet); the data were then expressed as a percentage (vol/vol). Data were analyzed as a crossover design using the MIXED procedure (SAS Inst. Inc., Cary, NC). The statistical model for DMI; apparent digestibility of ADF, CP, and DM; and apparent digestibility of ergovaline and lysergic acid included treatment. The model for rectal temperature, serum prolactin, and water intake included treatment, day, and day × treatment. Ruminal fluid ammonia and pH measured over a 12-h period were analyzed using a model with treatment and day, and sampling time added as a repeated measure. Ergovaline and lysergic acid data for sheep on the E+ treatment were analyzed as a crossover design using the MIXED procedure of SAS. The model included day, sampling time, and the day × sampling time interaction; sampling time was added as a repeated measure, and error covariance was modeled with an autoregressive correlation structure. When the interactions were not significant ($P > 0.05$), main effect means are presented.

RESULTS AND DISCUSSION

To date the cause of fescue toxicosis has not been defined further than the ergot alkaloids present in the grass, with ergovaline being used as an indicator of ergot alkaloid levels. Some attempts have been made to examine total ergot alkaloids (Hill and Agee, 1994; Schnitzius et al., 2001; Gadberry et al., 2003) but with limited success due to a lack of linear response of some alkaloids with the ELISA assay (Schnitzius et al., 2001). Work by Hill et al. (2001) has suggested that lysergic acid may play a greater role in fescue toxicosis than ergovaline, but the role that lysergic acid plays in fescue toxicosis has not been thoroughly examined. It is possible that lysergic acid is more, less, or equally as toxic as ergovaline. To this end, ergovaline and lysergic acid concentrations were measured in an attempt to determine the fate of the alkaloids in the digestive tract.

Ergovaline and Lysergic Acid in the Feed

Ergovaline was determined to be 0.35 mg/kg in the endophyte-infected straw and 3.30 mg/kg in the seed. Lysergic acid was determined to be 0.028 mg/kg in the endophyte-infected straw and 0.132 mg/kg in the seed. To our knowledge these are the first reported values for lysergic acid found in straw and seed used in a feeding trial as measured by HPLC. Other studies have examined strictly ergovaline or total ergot alkaloids as determined with an ELISA test (Hill and Agee, 1994). The lysergic acid HPLC assay used in our study is more precise than the ergot ELISA because the HPLC assay quantifies lysergic acid (Lodge-Ivey et al., 2006). Conversely, the ergot ELISA relies on the binding of an antibody to the core ring structure of the molecule (Figure 1) and tends to be less reproducible, has more day-to-day variation in the results, and has a high binding affinity for lysergol and other ergoline alkaloids, in addition to lysergic acid (Schnitzius et al., 2001). Gadberry et al. (2003) found the ergot ELISA to be a poor indicator of total ergot alkaloids because values corresponded to the ergine (an ergoline alkaloid) levels (as measured by HPLC) and were not related to the ergovaline levels (as measured by HPLC). Taken together, the ergot ELISA should be viewed as an ergoline assay and should not be used to quantify total ergot alkaloids.

Digestive and Physiological Parameters

Ruminal ammonia ($P = 0.90$) and pH ($P = 0.36$) were not different between treatment groups (Table 2). These results are consistent with diets formulated to be isonitrogenous and to contain similar amounts of concentrate ingredients. Daily rectal temperatures were not influenced by alkaloid concentration ($P = 0.40$). These data agree with Fiorito et al. (1991), Stamm et al. (1994), and Matthews et al. (2005) who found no difference in rectal temperature of sheep or cattle on endo-

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Table 2. Digestive and physiological parameters of wethers consuming endophyte-free (E–) and endophyte-infected (E+) tall fescue diets[1]

Item	E–[2]	E+[2]	SEM	*P*-value
Ruminal ammonia, m*M*	4.72	4.70	0.128	0.90
Ruminal fluid pH	6.86	6.97	0.032	0.36
Rectal temperature, °C	38.36	38.41	0.062	0.40
Water intake, L/d	2.95	2.77	0.08	0.04
Serum prolactin, ng/mL	22.9	6.4	5.19	0.02
DMI, kg/d	1.18	1.11	0.15	0.40
DMI, % of BW	2.30	2.16	0.37	0.52
DM digestibility, %	53.8	49.8	2.89	0.48
ADF digestibility, %	49.4	52.2	4.84	0.62
CP digestibility, %	61.4	63.7	1.62	0.89

[1]Values are reported as least squares means, n = 6.
[2]E– contained < 0.01 mg of ergovaline/kg; E+ contained 0.61 mg of ergovaline/kg.

Table 3. Ergovaline and lysergic acid concentrations (ng/mL) by day in ruminal fluid of wethers consuming an endophyte-infected tall fescue diet[1]

Item	Day 0	Day 3	Day 28	SEM
Ergovaline	1.1[a]	4.6[b]	6.5[c]	0.34
Lysergic acid	2.2[a]	6.6[b]	6.8[b]	0.77

[a–c]Within a row, means without a common superscript letter differ ($P < 0.05$).
[1]The diet contained 0.610 mg of ergovaline/kg.

phyte-infected or endophyte-free diets. Other studies have observed differences in daily rectal temperatures in sheep (Aldrich et al., 1993) and cattle (Paterson et al., 1995) consuming endophyte-infected or endophyte-free diets. The variation in observed rectal temperatures in response to endophyte-infected feed between studies could be due to the different ergopeptide alkaloid concentrations used in each study or a difference in ambient temperatures. Early work typically classified treatments as infected vs. noninfected tall fescue with no analysis for ergopeptide alkaloid levels (Fiorito et al., 1991; Paterson et al., 1995). When alkaloids were tested, the treatment levels of ergopeptides varied greatly, ranging from 0.12 mg/kg of ergovaline (Matthews et al., 2005) to 2.43 mg/kg of ergovaline (Aldrich et al., 1993) in the high treatments. Higher ambient temperatures and heat stress can cause a greater change in rectal temperature for animals consuming endophyte-infected diets when compared with endophyte-free diets (Hannah et al., 1990; Gadberry et al., 2003). In this study, animals were not heat stressed, and the alkaloid concentration in the diet was at the lower end of the published toxicity level for sheep (Tor-Agbidye et al., 2001), indicating that the lambs were not exposed to enough alkaloids to affect rectal temperatures.

Apparent DM ($P = 0.48$), ADF ($P = 0.62$), and CP ($P = 0.89$) digestibilities were not different between treatment groups (Table 2), which agrees with Stamm et al. (1994), although it should be noted that DM digestibility was numerically 4 percentage units greater for E– than for E+. Matthews et al. (2005) reported lower DM, ADF, and CP digestibilities in animals consuming an endophyte-infected diet compared with those on an endophyte-free diet. The difference in the effect of ergovaline on nutrient digestibilities could be related to the quality of the different feeds. Matthews et al. (2005) fed a higher quality diet with hay being the source of alkaloids and reported DM digestibilities of 62.3% for E+ and 67.0% for E–. The digestibilities reported by Stamm et al. (1994; 48.8% for E+ vs. 46.0% for E–) or in our study (49.8% for E+ vs. 53.8% for E–), which both used primarily straw as the source of alkaloids, were lower. Daily water intake was less for the E+ diet ($P = 0.04$; Table 2), which is consistent with findings by Fiorito et al. (1991) where endophyte-infected feed caused a drop in voluntary water intake. However, Hannah et al. (1990) and Aldrich et al. (1993) reported no difference in water intake by animals consuming an endophyte-infected diet. Hannah et al. (1990) and Aldrich et al. (1993) maintained lambs at a controlled temperature of 32°C, whereas Fiorito et al. (1991) maintained lambs at a controlled temperature of 21°C; our study had an average high temperature of 22°C. Consumption of large amounts of water due to high environmental temperature may have masked the effect of ergot alkaloids on water consumption in studies conducted under heat stress conditions.

Serum prolactin was decreased from 22.9 ng/mL for animals receiving the E– treatment to 6.4 ng/mL for animals receiving the E+ treatment (Table 2), indicating clinical fescue toxicosis. This result is consistent with previous research (Paterson et al., 1995; Porter, 1995; Gadberry et al., 2003; Matthews et al., 2005), which showed depressed blood (serum or plasma) prolactin levels in animals fed endophyte-infected diets. As demonstrated from our study as well as the current literature, rectal temperature is not a reliable indicator of fescue toxicosis; serum (or plasma) prolactin may be a better parameter for identifying animals exposed to toxins found in endophyte-infected tall fescue.

Ergovaline and Lysergic Acid in Ruminal Fluid

Westendorf et al. (1992) and Hill et al. (2001) suggest that the primary site of degradation and absorption of endophyte alkaloids is the rumen. The concentration of ergovaline in the rumen increased from d 0 to 3 and from d 3 to 28 ($P < 0.001$; Table 3). The concentration of ergovaline was not different from 0 to 6 h ($P > 0.05$) but increased from 6 to 12 h ($P < 0.05$; Table 4). Lysergic acid, measured in ruminal fluid, increased ($P < 0.05$) from d 0 to 3. No difference ($P = 0.80$) in lysergic acid in ruminal fluid was detected between d 3 and 28 (Table 3). Lysergic acid increased from 0 to 6 h ($P < 0.001$), and there was no difference between 6 and 12 h ($P > 0.05$). No carryover effect of ergovaline or lysergic acid in ruminal fluid was detected; i.e., ergovaline and lyser-

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Table 4. Ergovaline and lysergic acid concentration (ng/mL) by time in ruminal fluid of wethers consuming an endophyte-infected tall fescue diet[1]

	Time after feeding, h			
Item	0	6	12	SEM
Ergovaline	3.5[a]	3.9[a]	4.8[b]	0.26
Lysergic acid	3.5[a]	5.7[b]	6.4[b]	0.54

[a,b]Within a row, means without a common superscript letter differ ($P < 0.05$).
[1]The diet contained 0.610 mg of ergovaline/kg.

gic acid in ruminal fluid at 0 h on d 0 of both treatment periods were undetectable. No day × time interaction was detected for ergovaline ($P = 0.40$) or lysergic acid ($P = 0.94$).

Hill et al. (2001) theorized that lysergic acid crossed the ruminal wall at a greater rate than other ergopeptide alkaloids tested, and they speculated that greater transport of lysergic acid was due to the size and polarity of the molecule. Hill et al. (2001) also demonstrated that ergot alkaloids have a higher affinity and greater passage rate for ruminal epithelium than reticular or omasal tissue, implying absorption of ergot alkaloids occurs primarily in the rumen. However, studies examining the absorption rate of alkaloids and metabolites from the hindgut have not been conducted; therefore, it is difficult to state definitively the site of absorption. Westendorf et al. (1992) found that when endophyte-infected seed was incubated with ruminal fluid and then fed to rats, ADG and G:F were improved compared with rats fed nonincubated endophtye-infected seed. The improvements in ADG and G:F were not observed in endophyte-free seed incubated with ruminal fluid, causing the authors to conclude that the improved ADG and G:F were due to detoxification of ergot alkaloids and not from an alteration in the digestibility. Taken together, this implies that the apparent digestibility of ergovaline observed in this study could result from degradation and not necessarily from direct absorption.

Ergovaline and Lysergic Acid in the Urine

As expected ergovaline was undetectable in the urine, whereas lysergic acid was present at approximately 1.4 times (0.214 μmol/d) the amount that was detected in the feed (0.155 μmol/d). Because of its molecular weight and polarity, ergovaline is expected to be excreted in the bile and recovered in the feces, as demonstrated by early work with radiolabeled ergot alkaloids (Eckert et al., 1978). Lysergic acid is a smaller, more polar molecule than ergovaline, and absorbed lysergic acid is expected to be excreted in the urine (Eckert et al., 1978).

Ergovaline and Lysergic Acid in the Feces

The feces of sheep consuming E+ contained 0.102 mg of lysergic acid/kg and 0.480 mg of ergovaline/kg. Ergovaline excreted in the feces was less than the ergovaline consumed by the lambs (Table 5). Lysergic acid excreted in the feces was more than the amount consumed from the diet (Table 5). The apparent digestibility for ergovaline was 64.2% and –12.5% for lysergic acid. The negative apparent digestibility for lysergic acid indicates that lysergic acid was produced by microbes from the degradation of ergot alkaloids present in E+ feed. Lysergic acid may have been poorly absorbed from the digestive tract, although previous research has shown lysergic acid to be absorbed from the rumen at a faster rate than the ergopeptides (Hill et al., 2001). The appearance of lysergic acid in the urine in addition to that recovered in the feces suggests some lysergic acid was absorbed from the gastrointestinal tract including the rumen and that other ergot alkaloids in the diet may have been biotransformed in the liver to lysergic acid (Figure 2).

The appearance of lysergic acid in the feces and urine in amounts greater than in the feed implies that the ergopeptines in the feed were degraded to lysergic acid by ruminal microbial degradation or by degradation in the lower gastrointestinal tract. Research investigating the amount of degradation of ergopeptines in the lower digestive tract has not been conducted. Although ergovaline can account for up to 80% of the alkaloids produced in tall fescue (Lyons et al., 1986), many other ergot alkaloids, such as ergocryptine, ergotamine, and ergonovine, can be found in endophyte-infected tall fescue. All of these ergot alkaloids contain the core ring structure of lysergic acid and vary only in the composition of the side chain. Therefore, they can theoretically

Table 5. Mass balance of ergovaline and lysergic acid in wethers consuming an endophyte-infected tall fescue diet[1]

Item	Ergovaline	Lysergic acid	Combined alkaloids[2]
Intake, μmol/d	1.15 ± 0.20	0.15 ± 0.031	1.37 ± 0.20
Excreted,[3] μmol/d	0.41 ± 0.054	0.38 ± 0.065	0.79 ± 0.092
Urine	ND[4]	0.21 ± 0.082	0.21 ± 0.082
Feces	0.41 ± 0.054	0.17 ± 0.056	0.58 ± 0.088
Apparent digestibility, %	64.2 ± 4.4	–12.5 ± 25	50.4 ± 7.8
Excreted, % of intake	35.4 ± 4.5	248 ± 46.1	60.5 ± 10.7

[1]n = 6; mean ± SD.
[2]Ergovaline plus lysergic acid.
[3]Urine plus feces.
[4]Not detected.

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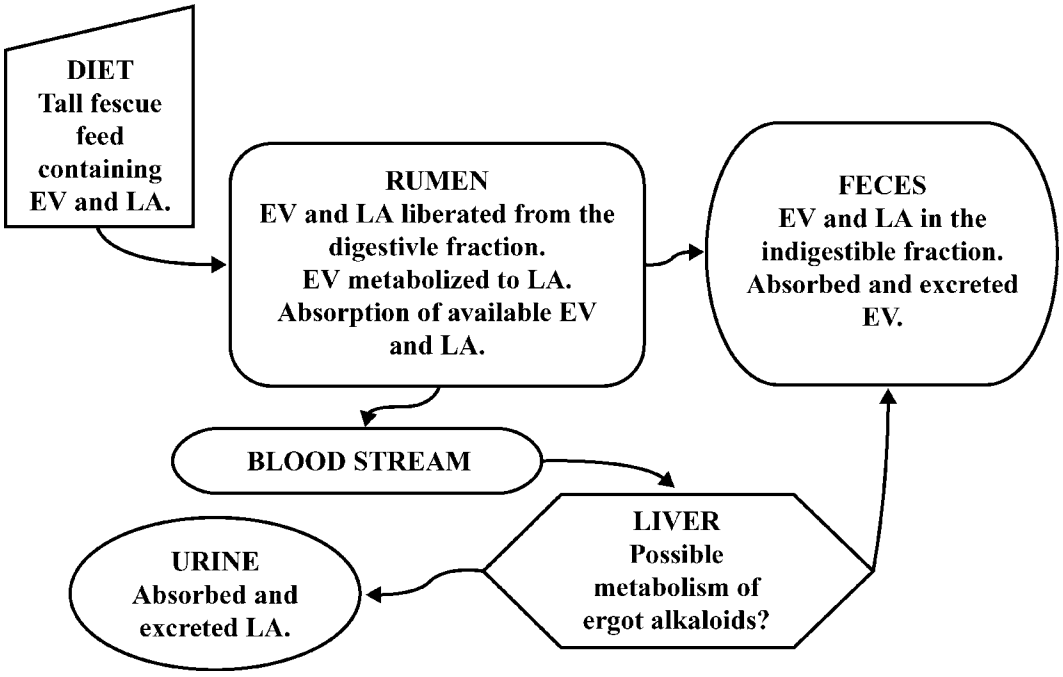

Figure 2. Proposed model for the metabolism, absorption, and excretion of ergot alkaloids. Feed containing ergot alkaloids enters the rumen, where microbial digestion of the plant material liberates the alkaloids into the ruminal fluid. Ergopeptide alkaloids are further degraded by ruminal microbes into the simpler ergoline alkaloids or lysergic acid. Ergoline and lysergic acid are absorbed from the rumen in the greatest quantities, with ergopeptide alkaloids being absorbed in minor quantities. Once absorbed, the alkaloids are rapidly removed by the liver. In the liver, alkaloids are possibly metabolized to improve the body's ability to excrete the compounds. From the liver, large ergopeptide alkaloids flow to the bile for excretion, and smaller ergoline alkaloids, lysergic acid, and metabolites from liver metabolism flow to the kidneys for excretion in the urine. EV = ergopeptide alkaloids, such as ergovaline; LA = ergoline alkaloids, such as lysergic acid.

be degraded to lysergic acid and may account for the additional lysergic acid being excreted.

Our data allow us to propose the following model of ergopeptide degradation and absorption from the digestive tract (Figure 2). Where the alkaloids are liberated from the digestible fraction of the diet, ergovaline is degraded to lysergic acid by microbial action. Alkaloids in the rumen fluid are then absorbed across the rumen wall. Once in the blood stream, the alkaloids flow to the liver. Previous research has shown the rapid disappearance of ergot alkaloids from the blood for sheep (Jaussaud et al., 1998) and goats (Durix et al., 1999) with ergovaline levels falling below the limit of quantification (3.5 ng/mL) within 1 h after an intravenous injection of ergovaline. In the liver, it is possible that there is additional degradation and alteration of the alkaloids. This research has not been conducted in livestock to date, so it is not possible to determine the extent that the liver detoxifies ergot alkaloids. Once processed by the liver, smaller alkaloids such as lysergic acid are excreted via the urine. Larger alkaloids are excreted in the feces via the bile. In addition, the feces would also contain the fraction of alkaloids, both ergopeptides and ergolines, found in the indigestible portion of the feed.

In conclusion, this study is the first to quantify the metabolism of ergovaline to lysergic acid in the ruminant digestive system using HPLC assays for quantification of these alkaloids. It is essential to know the role ergovaline and lysergic acid have in the development of fescue toxicosis, in light of the large amount of tall fescue hay, pasture, and straw being used for livestock production and the high cost of fescue toxicosis to the animal industries. To this end, understanding the metabolism of ergovaline to lysergic acid in the digestive tract will shed light on the possible causative agent in fescue toxicosis and will aid in the management of feeding endophyte-infected fescue straw.

LITERATURE CITED

Aldrich, 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.

AOAC. 1990. Official Methods of Analysis. 15th ed. Assoc. Off. Anal. Chem., Arlington, VA.

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.

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

Durix, A., P. Jaussaud, P. Garcia, Y. Bonnaire, and S. Bony. 1999. Analysis of ergovaline in milk using high-performance liquid

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chromatography with fluorimetric detection. J. Chromatogr. B 729:255–263.
Eckert, H., J. R. Kiechel, J. Rosenthaler, R. Schmidt, and E. Scheier. 1978. Biopharmaceutical aspects: Analytical method, pharmacokinetics, metabolism, and bioavailability. Page 719 in Ergot Alkaloids and Related Compounds. B. Berde, and H. O. Schild, ed. Springer-Verlag, New York, NY.
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.
Gadberry, M. S., T. M. Denard, D. E. Spiers, and E. L. Piper. 2003. Effects of feeding ergovaline on lamb performance in a heat stress environment. J. Anim. Sci. 81:1538–1545.
Hannah, S. M., J. A. Paterson, J. E. Williams, M. S. Kerley, and J. L. Miner. 1990. Effects of increasing dietary levels of endophyte-infected tall fescue seed on diet digestibility and ruminal kinetics in sheep. J. Anim. Sci. 68:1693–1701.
Hill, N. S., and C. S. Agee. 1994. Detection of ergoline alkaloids in endophyte-infected tall fescue by immunoassay. Crop Sci. 34:530–534.
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.
Jaussaud, P., A. Durix, B. Videmann, A. Vigié, and S. Bony. 1998. Rapid analysis of ergovaline in ovine plasma using high-performance liquid chromatography with fluorimetric detection. J. Chromatogr. A 815:147–153.
Joost, R. E. 1995. Acremonium in fescue and ryegrass: Boon or bane? A review. J. Anim. 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.
Lodge-Ivey, S. L., K. Walker, T. Fleischman, J. E. True, and A. M. Craig. 2006. Detection of lysergic acid in ruminal fluid, urine, and in endophyte-infected tall fescue using high-performance liquid chromatography. J. Vet. Diagn. Invest. 18:369–374.
Lyons, P. C., R. D. Plattner, and C. W. Bacon. 1986. Occurrence of peptide and clavine ergot alkaloids in tall fescue grass. Science 232:487–489.
Matthews, A. K., M. H. Poore, G. B. Huntington, and J. T. Green. 2005. Intake, digestion, and N metabolism in steers fed endophyte-free, ergot alkaloid-producing endophyte-infected, or non-ergot alkaloid-producing endophyte-infected fescue hay. J. Anim. Sci. 83:1179–1185.
NRC. 1985. Nutrient Requirements of Sheep. 6th rev. ed. Natl. Acad. Press, Washington, DC.
Paterson, J., C. Forcherio, B. Larson, M. Samford, and M. Kerley. 1995. The effect of fescue toxicosis on beef cattle productivity. J. Anim. Sci. 73:889–898.
Porter, J. K. 1995. Analysis of endophyte toxins: Fescue and other grasses toxic to livestock. J. Anim. Sci. 73:871–880.
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.
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.
Strickland, J. R., J. W. Oliver, and D. L. Cross. 1993. Fescue toxicosis and its impact on animal agriculture. Vet. Hum. Toxicol. 35:454–464.
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. Hum. Toxicol. 43:140–146.
Westendorf, M. L., G. E. Mitchell, Jr., and R. E. Tucker. 1992. Influence of rumen fermentation on response to endophyte-infected tall fescue seed measured by a rat bioassay. Drug Chem. Toxicol. 15:351–364.
Western Regional Climate Center. http://wrcc.dri.edu Accessed Mar. 2007.

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