Scientific Article

Effects of initial and extended exposure to an endophyte-infected tall fescue seed diet on faecal and urinary excretion of ergovaline and lysergic acid in mature geldings

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Abstract

AIM: To determine the amount of ergovaline and lysergic acid retained or excreted by geldings fed endophyte-infected seed containing known concentrations of these alkaloids, and the effects of exposure time on clinical expression of toxicosis.

METHODS: Mature geldings (n=10) received diets containing either endophyte-free (E–) or endophyte-infected (E+) tall fescue seed during three experimental phases. The first phase (Days –14 to –1) was an adaptation phase, to allow all horses to adapt to a diet containing E– tall fescue seed. The second (Days 0 to 3) was the initial exposure phase to E+ tall fescue seed, used for the delivery of ergovaline and lysergic acid at 0.5 and 0.3 mg/kg of diet, respectively, to test the initial effects of exposure on routes and amounts of elimination of alkaloid. During this phase, half the geldings were exposed to an E+ diet while the rest served as controls by remaining on the E– diet. Once assigned to treatments, geldings remained on the same diet through the third phase (Days 4 to 21), which served as the extended exposure phase. Total outputs of faeces and urine were collected within each phase, to determine retention of ergovaline and lysergic acid and nutrient digestibility. Serum was collected weekly and analysed for activities of enzymes and concentrations of prolactin. Bodyweights (BW) and rectal temperatures were recorded weekly.

RESULTS: BW, rectal temperature, enzyme activities and concentrations of prolactin in serum, and nutrient digestibility were not affected by treatment. Total intake of ergovaline by geldings on the E+ diet was 3.5 and 3.6 (SE 0.20) mg/day, and 2.1 and 2.3 (SE 0.11) mg/day were not accounted for in initial and extended phases, respectively. Lysergic acid was excreted in the urine (4.0 and 4.9 (SE 0.97) mg/day) and faeces (2.5 and 2.7 (SE 0.35) mg/day) at greater amounts than that consumed (2.0 and 1.9 (SE 0.09) mg/day) during the initial and extended exposure phases, respectively. Animals exposed to E+ seed for a period of 20 days appeared to excrete more (1.5 *vs* 1.2 mg/day; SE 0.08; p=0.03) ergovaline in the faeces than those exposed for only 4 days.

CONCLUSIONS: Exposure time to the ergot alkaloids had a limited effect on the route of elimination or the amounts of ergovaline or lysergic acid excreted by horses. The primary alkaloid excreted was lysergic acid, and urine was the major route of elimination. These data will aid future research to improve animals' tolerance to toxic endophyte-infected tall fescue.

KEY WORDS: *Horse, tall fescue, ergovaline, lysergic acid, endophyte, toxicosis*

Introduction

Tall fescue (*Festuca arundinacea* Schreb.) is a perennial, cool-season grass commonly used for forage and turf purposes (Sleper and Buckner 1995). Despite its high nutritive value, consumption of tall fescue by livestock results in a decrease in both reproductive and growth performance due to the presence of ergot alkaloids produced by an endophytic fungus, *Neotyphodium coenophialum,* in seed (Bacon et al 1977). In Kentucky alone, more than 96,000 horses and/or ponies graze tall fescue (Anonymous 1999), resulting in exposure to ergot alkaloids, e.g. ergovaline and lysergic acid, that have been linked to impaired animal performance and signs of fescue toxicosis. The general signs of fescue toxicosis exhibited by horses include weight loss, increased sweating (Cross et al 1995), and decreased nutrient digestibility (Aiken et al 1993). Further, prolonged gestation, agalactia, increased mortality of foals and mares, dystocia, tough and thickened placentae, weak and dysmature foals, reduced concentrations of progesterone and

ANOVA	Analysis of variance
Alk-P	Alkaline phosphatase
AST	Aspartate aminotransferase
BW	Bodyweight(s)
CK	Creatine kinase
CP	Crude protein
CS	Compound symmetry
CV	Coefficient(s) of variation
DM	Dry matter
DMD	Dry matter digestibility
E+	Endophyte-infected
E–	Endophyte-free
ELISA	Enzyme-linked immunosorbent assay
HPLC	High-performance liquid chromatography
NDF	Neutral detergent fibre
SE	Standard error
SPE	Solid-phase extraction

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prolactin in serum, and increased concentrations of oestradiol 17-β in serum, are all signs commonly associated with pregnant mares consuming E+ tall fescue (Porter and Thompson 1992). Although the reproductive implications of toxic E+ tall fescue for pregnant mares have received much attention (Putnam et al 1991; Brendemuehl et al 1994; Arns et al 1997), limited research has investigated the metabolic fate of ergot alkaloids and/or their metabolites in horses. Ergot alkaloids have been detected in ruminal and abomasal fluid (Westendorf et al 1993; Craig et al 1994), urine and biliary fluid (Stuedemann et al 1998), serum (Savary et al 1990; Bony et al 2001), milk (Durix et al 1999), and faeces (Westendorf et al 1993) of sheep, horses and cattle consuming E+ tall fescue; urinary excretion was the primary route of elimination in cattle (Stuedemann et al 1998). More recently, it has been suggested that ergovaline is not the primary toxin absorbed by the gastrointestinal tract but lysergic acid may be (Hill et al 2003). Discrepancies in understanding of the fate of ergot alkaloids in grazing animals and the limited research evaluating the effects of exposure time warrant further investigation. Complicating provision of a solution to toxicosis is the lack of a defined threshold level of toxicity for the ergot alkaloids in horses. Thus, the objectives of this study were to determine the amount of ergovaline and lysergic acid retained or excreted by geldings fed E+ seed containing known concentrations of these alkaloids, and the effects of exposure time to them on rectal temperature, serum prolactin concentrations and selected serum enzyme profiles.

Materials and methods

The research was conducted at the University of Kentucky Coldstream Agricultural Research Farm in Lexington, Kentucky, in June and July 2003, and was approved by the University of Kentucky Animal Care and Use Committee, Lexington KY, United States of America.

Animals

Mature geldings (n=10) of mixed breeding were randomly assigned to one of two treatments. One group (n=5) was fed a diet of E– tall fescue seed and the other group (n=5) was fed a diet of E+ tall fescue seed containing 0.5 mg ergovaline and 0.3 mg lysergic acid/kg diet/day. Once assigned to treatments, geldings remained on that treatment throughout the study. The concentration of alkaloids fed was based on the results of a 4-year survey undertaken on a horse farm in Kentucky which reported an average forage concentration of ergovaline of 0.5 mg/kg dry matter (DM) during summer and autumn, when clinical signs of fescue toxicosis typically occur (Long et al 2004); a concentration of 0.5 mg/kg diet is also the reported threshold level for horses in which clinical signs of fescue toxicosis become evident (Hovermale and Craig 2001). Horses were housed individually in free stalls measuring 4.9 x 4.9 m and had access to water and a trace mineral salt block (Ranch House; United Salt Corp, Houston TX, USA) *ad libitum*. The composition of the diet is provided in Table 1. Ground (0.5–1 mm) E– and E+ tall fescue seed were mixed with a commercial sweet feed (16% crude protein; CP) formulated for horses (Farmer's Feed Mill, Lexington KY, USA). Molasses was added to the mixture (5.6% of diet) to increase palatability. Timothy (*Phleum pratense*) hay was used as the source of forage and fed at 1.3% of BW. A concentrate mixture containing E– or E+ tall fescue seed was offered at 0.7% of BW, at 0800 h daily. Equal quantities of hay were fed in the morning after the concentrate had been consumed and again at 1700 h. All refusals of hay were collected, weighed, and stored for later analysis for determination of digestibility.

Table 1. Composition of endophyte-free and endophyte-infected tall fescue seed diets containing known concentrations of ergovaline (0.5 mg/kg diet) and lysergic acid (0.3 mg/kg diet).

	% of Diet	Average DMI (kg/day)
Feed		
Timothy hay	62.8	3.8
Tall fescue seed diet	37.2	2.2
Seed	17.1	1.0
Sweet feed[a]	14.5	0.9
Molasses	5.6	0.3
	Nutrient composition (%)	
Nutrient	Timothy hay	Concentrate-seed mixture
Crude protein	5.3	16.0
NDF	70.8	25.0
ADF	43.4	10.4
TDN	56.0	75.0

[a] Primary ingredients = crimped oats, oats, cracked corn, molasses, soybean meal, ground extruded soybeans, ground oats, linseed meal, distillers' dried grains with solubles, and dehydrated alfalfa meal
DMI = dry matter intake; NDF = neutral detergent fibre; ADF = acid detergent fibre; TDN = total digestible nutrients

Parameters recorded and measured

The experiment was divided into three phases to evaluate effects of exposure time to alkaloid-containing tall fescue seed (E+). The first phase (adaptation phase; Days –14 to –1) served as a dietary adaptation period in which all horses were adjusted to consuming a forage-based diet containing a concentrate mixture of E– tall fescue seed. During this time, basal concentrations of ergovaline and lysergic acid in urine and faeces, as well as baseline concentrations of prolactin and enzyme profiles in serum, BW, and rectal temperatures were determined. The second phase (initial exposure phase; Days 0 to 3) included initial exposure of one group of animals (n=5) to E+ seed containing known concentrations of ergovaline (0.5 mg/kg diet) and lysergic acid (0.3 mg/kg diet), followed by a final phase (extended exposure phase; Days 4 to 21) consisting of continuous exposure to ergovaline and lysergic acid at the same levels as the initial exposure phase.

Blood, via jugular venepuncture, was collected (BD Vacutainer – Serum Collection Tubes; Becton, Dickinson & Company, Franklin Lakes NJ, USA) and rectal temperatures were recorded at 0800 h daily for a 4-day period within each phase (Days –4 to –1 in the adaptation phase, Days 0 to 3 in the initial exposure phase, and Days 18 to 21 in the extended exposure phase). Blood samples were centrifuged at 1,500g for 20 min, and the serum from individual geldings was decanted and stored at –20°C for later analysis of creatine kinase (CK), alkaline phosphatase (Alk-P), and aspartate aminotransferase (AST) activities. Additional blood samples were collected on Days 0, 7, 14 and 21, for analysis of serum prolactin, and rectal temperatures were taken using a handheld digital thermometer (Vicks Speed-Read; Proctor & Gamble Co, Cincinnati OH, USA), accurate to within 0.1°C. BW were determined at the beginning of each phase and at the end of the experiment.

Total collections of faeces and urine were made using nappies (Stablemaid Horse Hygiene and Waste Management, Bendigo, Victoria, Australia). These devices were fitted to each horse and were capable of collecting both urine and faeces in the same vessel while maintaining them in separate compartments. Nappies were emptied twice daily (morning and evening), or three times daily if needed. A sub-sample (2%) of faeces from each collection time was obtained and pooled for a resultant 24-h collection. Urine was pooled in a plastic screw-cap container assigned to each horse. Following each collection, the volume and weight of urine were recorded, and 100-ml aliquots were taken daily after the final collection. Feed intake was recorded daily during collections of faeces and urine within each phase. Faecal and urine samples, and hay and concentrate offered and refused, were pooled by animal during each phase for a total 4-day composite. Samples were stored at –20°C until later analysis of alkaloid content and apparent digestibilities of DM, CP and neutral detergent fibre (NDF).

Analysis of samples

Analysis of feed and serum

Hay, the seed mixture, feed refusals, and faeces were analysed for DM, CP and NDF, by Dairy One Inc (Forage Testing Laboratory, Ithaca NY, USA). Concentrations of prolactin in serum were analysed using radioimmunoassay, as described by Colborn et al (1991), with an assay sensitivity of 0.2 ng/ml and average inter- and intra-assay coefficients of variation (CV) of 7.0%. Activities of CK, Alk-P, and AST in serum were determined using liquid reagent kits obtained from Pointe Scientific Inc (Lincoln Park MI, USA). Samples were processed through the kits using a Konelab 20XT clinical blood analyser (Thermo Electron Corp, Waltham MA, USA). Standard curves for quantification were prepared by serial dilution of control sera supplied by Pointe Scientific Inc, and a Chem Track Plus Level II standard (Fisher Scientific, Hampton NH, USA). The inter- and intra-assay CV for AST, CK, and Alk-P were 5.0 and 3.5, 4.0 and 2.6, and 5.5 and 0.8%, respectively.

Extraction and quantification of ergovaline and lysergic acid

Concentrations of ergovaline in hay, concentrate, faeces and urine were analysed, as described by Craig et al (1994). For extraction and analysis of lysergic acid, feed and faecal samples were prepared using a 1-g sample size (to pass a 0.5-mm screen) mixed with 10 ml water:acetonitrile (1:1 v/v). Tubes were sealed and rotated on a haematology/chemistry mixer (Fisher, Pittsburgh PA, USA) for 16 h under darkness at room temperature. The sample-water:actonitrile mixture was separated by centrifugation for 10 min at 2,000 rpm. A 5-ml aliquot of the supernatant was transferred to a disposable glass tube and adjusted to a pH of 5.0–5.5 using 10% acetic acid. The pH of urine samples (3.0 ml) was similarly adjusted, and then these were centrifuged at 2,000 rpm for 10 min, and the supernatant transferred to disposable tubes.

Lysergic acid was extracted and purified from the resulting supernatants using strong cation-exchange solid-phase extraction (SPE) cartridges (Discovery DSC-SCX SPE; Supelco, Bellfonte PA, USA) on a vacuum manifold (Alltech, Deerfield IL, USA). The SPE cartridge was preconditioned with 3 ml methanol, followed by 3 ml 0.1 M HCl and two 3-ml portions of pure water. The preconditioning eluents were discarded. The acidified supernatant was applied to the SPE cartridge, followed by two 3-ml portions of pure water. Lysergic acid was eluted from the SPE cartridge using a 3-ml portion of methanol:ammonium hydroxide (95:5). The fraction was collected in a 12 x 75 glass test tube and concentrated to dryness using a Savant ISS-100 centrifugal evaporator (Thermo Electron Corporation, Waltham MA, USA). The residue was reconstituted in 200 µl methanol:0.05 M phosphate buffer at pH 8.5 (50:50). Reconstituted samples were placed in an ultrasonic bath for 30 sec, then transferred to 1.7-ml microcentrifuge tubes, centrifuged at 10,000 rpm for 10 min, and the supernatant transferred to high-performance liquid chromatography (HPLC) vials.

Analyses using HPLC were carried out using a guard column hand-packed with Pellicular C18 material (Alltech Associates Inc, Deerfield IL, USA), and a Luna C18 analytical column (150 x 3.0 mm inside diameter, 5 µm particle size; Phenomenex, Torrance CA, USA) eluted at 1 ml/min under isocratic conditions using a 94:6 ratio of 0.05 M phosphate buffer (pH 8.5):acetonitrile.

Statistical analysis

Changes in concentrations of prolactin in serum from baseline values (Day 0 prior to exposure to ergovaline) were analysed as repeated measures using the MIXED models procedure of SAS (SAS Institute Inc, Cary NC, USA). Treatment, day and treatment-x-day were included in the model, using the compound symmetry (CS) variance structure for the residual covariance based on Akaike's information criterion values. Serum enzyme activities, nutrient digestibility, BW and rectal temperatures were analysed using one-way analysis of variance (ANOVA) using the MIXED models procedure of SAS, comparing all three phases. Treatment, phase, and treatment-x-phase were included in the model using the CS variance structure. Retention of ergovaline and lysergic acid and their outputs in faeces and urine were analysed using one-way ANOVA using the MIXED models procedure of SAS, comparing only the initial and extended exposure phases for geldings receiving an E+ diet, and treatment, phase and treatment-x-phase were included in the model using a CS variance structure. Power analysis of BW and alkaloid excretion data indicated that for a power of 0.8, the sample size would need to be between five and 500 animals, depending on which response the calculations were performed. As such, readers are cautioned that some data reported as being similar may in fact have differed with larger sample sizes. However, logistics of the study prevented larger sample sizes.

Results

Bodyweight, rectal temperature, and serum profiles

Geldings were in less than optimum body condition upon arrival at the research facilities, as gains in BW were observed in all of them throughout the experiment (Table 2). Rectal temperatures of geldings were not affected by diet. An effect of phase was detected ($p=0.04$), and the highest rectal temperatures were observed during the initial exposure phase for all horses. Rectal temperatures during the adaptation phase did not differ from either the initial or extended-exposure phases (Table 2).

Concentrations of prolactin in serum were not affected by treatment ($p=0.28$; Figure 1). In addition, neither initial nor extended exposure to E+ tall fescue seed affected prolactin concentrations ($p=0.41$). However, a day effect was observed ($p<0.001$) in which concentrations of prolactin in serum decreased in both treatment groups over the course of the experiment. Serum enzyme profiles are presented in Table 3. Activities of CK and Alk-P did not differ between groups, and no effect of exposure time was detected. Activities of AST tended to be greater ($p=0.09$) for geldings on

Table 2. Bodyweight (kg) and rectal temperature (°C) of gelding horses assigned to either an endophyte-free (E–) or endophyte-infected (E+) tall fescue seed diet containing known concentrations of ergovaline (0.5 mg/kg diet) and lysergic acid (0.3 mg/kg diet), averaged over 4-day periods during an adaptation phase (Days –4 to –1), initial exposure phase (Days 0 to 3), and an extended exposure phase (Days 18 to 21).

	Phase						
	Adaptation[a]		Initial exposure		Extended exposure		
Parameter	E–	E+	E–	E+	E–	E+	SE
Bodyweight	390.9	397.3	392.2	391.3	405.2	403.1	17.8
Temperature[b]	38.5	38.6	38.6	38.7	38.4	38.3	0.27

[a] There was no E+ fescue fed during the adaptation phase, but the animals were listed as two groups to illustrate that there were no pre-existing differences between treatment groups
[b] Phase effect (initial exposure phase > extended exposure phase; p=0.04)
SE = standard error

Table 3. Activities (U/L) of creatine kinase (CK), alkaline phosphatase (Alk-P) and aspartate aminotransferase (AST) in the serum of gelding horses assigned to either an endophyte-free (E–) or endophyte-infected (E+) tall fescue seed diet containing known concentrations of ergovaline (0.5 mg/kg diet) and lysergic acid (0.3 mg/kg diet), averaged over 4-day periods during an adaptation phase (Days –4 to –1), initial exposure phase (Days 0 to 3), and an extended exposure phase (Days 18 to 21).

	Phase						
	Adaptation[a]		Initial exposure		Extended exposure		
Enzyme	E–	E+	E–	E+	E–	E+	SE
CK	239.2	228.2	311.9	231.8	293.9	231.3	27.2
Alk-P	222.1	213.6	247.2	233.4	207.4	210.4	29.6
AST[b]	329.8	228.9[x]	402.6	269.6	407.9	326.6[y]	41.3

[a] There was no E+ fescue fed during the adaptation phase, but the animals were listed as two groups to illustrate that there were no pre-existing differences between treatment groups
[b] Phase effect (extended phase > adaptation phase; initial exposure phase > adaptation phase; p=0.03)
[x, y] Within E+ treatment, means differed (p=0.03)
SE = standard error

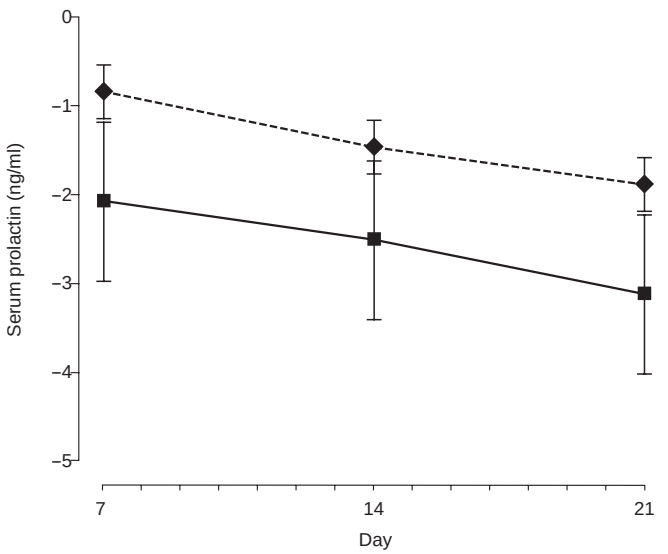

Figure 1. Concentrations of prolactin in serum (ng/ml) from baseline values for geldings receiving either an endophyte-free (--◆--) or endophyte-infected (—■—) tall fescue seed diet containing known concentrations of ergovaline (0.5 mg/kg diet) and lysergic acid (0.3 mg/kg diet). Day effect p<0.001.

the E– diet during the initial exposure phase compared with those on the E+ diet. Within the E+ treatment group, activities of AST were greater (p=0.03) during the extended exposure phase compared with the adaptation phase.

Measures of digestibility

Digestibility of DM, CP and NDF of diets by treatment and phase are shown in Table 4; there were no significant effects of treatment. Although inclusion of ergovaline and lysergic acid in the diet via E+ tall fescue seed had no effect on DM digestibility (DMD), geldings within each treatment group digested less of their diet during the initial exposure phase compared with the adaptation phase (p=0.02). CP digestibility was similar across all phases and treatments. There was a tendency for CP digestibility to be higher during the dietary adaptation phase (62.5%) than the initial exposure phase (56.0%) for geldings fed the E+ diet (p=0.06).

Ergovaline and lysergic acid

Retention of ergovaline and lysergic acid and their outputs in faeces and urine are shown in Table 5. Ergovaline and lysergic acid were not detected (<0.01mg/kg 'or L') in the faeces and urine of geldings receiving an E– tall fescue seed diet for the duration of the study. The intake of ergovaline by geldings consuming the E+ tall fescue seed diet was similar (p=0.67) during the initial and extended exposure phases, indicating delivery of E+ tall fescue seed and alkaloids was equal between treatment phases. Because ergovaline was not detected in the urine, faecal excretion was the only evident route for elimination (34.3 and 41.7% of total intake during the initial and extended exposure phases, respectively), and greater (p=0.03) faecal losses occurred during the extended exposure phase (1.5 mg/day) compared to the initial

Table 4. Nutrient digestibility (%) of diets consumed by gelding horses assigned to either an endophyte-free (E–) or endophyte-infected (E+) tall fescue seed diet containing known concentrations of ergovaline (0.5 mg/kg diet) and lysergic acid (0.3 mg/kg diet), averaged over 4-day periods during an adaptation phase (Days –4 to –1), initial exposure phase (Days 0 to 3), and an extended exposure phase (Days 18 to 21).

	Phase						
	Adaptation[a]		Initial exposure		Extended exposure		
Digestibility	E–	E+	E–	E+	E–	E+	SE
DM	34.5[w]	38.9[y]	28.0[x]	32.1[z]	31.4	36.2	4.3
CP	56.1	62.5	57.3	56.0	58.9	57.4	2.5
NDF	32.1	38.9	26.6	31.9	31.6	35.9	3.8

[a] There was no E+ fescue fed during the adaptation phase, but the animals were listed as two groups to illustrate that there were no pre-existing differences between treatment groups
[w, x] Within E– treatment, means differed (p=0.05)
[y, z] Within E+ treatment, means differed (p=0.02)
SE = standard error; DM = dry matter; CP = crude protein; NDF = neutral detergent fibre

Table 5. Faecal and urinary concentrations of alkaloids and alkaloid balance (mg/day) of geldings after short-term and extended exposure to an endophyte-free (E–) or endophyte-infected (E+) tall fescue seed diet containing known concentrations of ergovaline (0.5 mg/kg diet) and lysergic acid (0.3 mg/kg diet), averaged over 4-day periods during the initial exposure phase (Days 0 to 3) and extended exposure phase (Days 18 to 21).

	Phase					
	Initial exposure		Extended exposure			
Parameter	E–	E+	E–	E+	SE	P-value[a]
Ergovaline[b]						
Intake	ND	3.5	ND	3.6	0.20	0.67
Faeces	ND	1.2	ND	1.5	0.08	0.03
Urine	ND	ND	ND	ND	–	–
Retained[c]	ND	2.3	ND	2.1	–	–
Lysergic acid[b]						
Intake	ND	2.0	ND	1.9	0.09	0.35
Faeces	ND	2.7	ND	2.5	0.35	0.17
Urine	ND	4.0	ND	4.9	0.97	0.38
Retained[c]	ND	–4.7	ND	–5.5	–	–

[a] Compared initial *vs* extended exposure phase for geldings fed an E+ diet
[b] Values for the adaptation phase were <0.01 mg/kg 'or L'
[c] Retained = intake – (faeces + urine)
SE = standard error; ND = below detectable limit of 0.01 mg/kg 'or L'

exposure phase (1.2 mg/day). As a result, those exposed to an E+ tall fescue seed diet tended (p=0.09) to retain and/or metabolise more ergovaline during the initial exposure phase (65.7%) than the extended exposure phase (58.3%).

Although the diet was formulated to deliver a known concentration of ergovaline to horses on the E+ tall fescue seed diet, intakes of lysergic acid were constant as well (Table 5), thus verifying that delivery of E+ tall fescue seed was constant between treatment phases. Contrary to ergovaline, the primary route for elimination of lysergic acid was the urine. Although there was no difference (p=0.38) between the two exposure phases to E+ tall fescue seed, lysergic acid recovered in the urine was more than 200% of that consumed. Additionally, concentrations of lysergic acid in the faeces were, on average, 134% of total intake, and no differences were observed between phases (2.7 *vs* 2.5 mg/day for the initial and extended exposure phases, respectively).

Discussion

Geldings in the present study exhibited no clear signs of fescue toxicosis as a result of consuming an E+ tall fescue seed diet of approximately 1 kg/gelding/day, delivering 0.5 and 0.3 mg/kg ergovaline and lysergic acid/day, respectively (Table 1). Bony et al (2001) observed signs of fescue toxicosis, i.e. excessive sweating and prostration, in horses administered 15 µg/kg BW ergovaline intravenously, and decreased reproductive efficiency was exhibited by pregnant mares grazing E+ tall fescue pastures containing an estimated concentration of ergovaline of 1.2 mg/kg DM (Brendemuehl et al 1994). However, pregnant mares consuming approximately 2.5 kg/mare/day tall fescue seed, containing either 0.16 or 0.32 mg ergovaline/kg, for a 28-day period exhibited no clear signs of fescue toxicosis (Arns et al 1997). BW of all geldings increased in our study, which was expected as the horses arrived

in below-optimum body condition. Research has suggested a diet high in ergovaline will increase sweating in horses (Cross et al 1995). In our study, a rise in rectal temperatures was observed in horses exposed to an E+ seed diet during the initial exposure phase; however, this was also noted in horses not exposed to the ergot alkaloids. The average ambient temperature during the initial exposure phase was also greater (27.1°C) than that observed during the adaptation (24.8°C) and extended exposure phases (22.1°C) and may have contributed to the higher rectal temperatures observed in all animals during that phase.

The increased ambient and rectal temperatures exhibited by all geldings may have contributed to decreased DMD observed during the initial exposure phase. All feedstuffs were purchased prior to the start of the trial and rations remained the same for the duration of the study. Variation associated with sampling and analysis may have contributed to the differences in DMD for one or both of the treatment groups. Digestibility of CP tended to decrease in horses fed the E+ diet when initially exposed to the diet. This was likely an artefact in the data as a result of low numbers of animals, and did not appear to be alkaloid-driven given the lack of any apparent effects during the extended exposure phase. However, similar results have been reported for horses consuming tall fescue hay (Redmond et al 1991; McCann et al 1992b), in which nutrient digestibility of DM, CP, and NDF tended to decrease with increasing infection levels of endophyte.

Although concentrations of prolactin in serum decreased over the duration of the trial, concentrations were similar between groups. The lack of difference observed may be explained in part by the high CV associated with horses receiving the E+ diet (CV of 4.3 *vs* 0.7 for the E– diet). However, the results agree with those found by McCann et al (1992b), when yearling geldings received either an E– or E+ tall fescue hay diet for a 5-month period; concentrations of prolactin decreased in both groups over the course of the experiment but did not differ between the two groups. However, contradictory results were observed by Arns et al (1997) and McCann et al (1992a), in which concentrations of prolactin in serum were suppressed in pregnant mares consuming a diet containing ergovaline compared with a diet containing no ergovaline. Contradictory findings, for mares and geldings, suggest concentrations of prolactin in serum may be a better indicator of fescue toxicosis in mares than in geldings.

To further assess the clinical condition of the animals in our study, the activities of CK, Alk-P and AST in serum, which are indicative of muscle and/or liver damage (Tannant 1997), were analysed. Activities of CK and Alk-P were similar to those reported in the literature for horses with low physical activity (Tannant 1997; Edwards et al 2003; Williams et al 2004), suggesting no subclinical intoxication as a result of consuming an E+ tall fescue seed diet. Activities of AST increased in all horses throughout the experiment but treatment had no effect on them, except during the initial exposure phase when horses receiving the E– tall fescue seed exhibited higher activities than those receiving the E+ diet. Although activities of AST in serum increased over the course of the study, which may be attributed to the gain in body condition exhibited by all horses, they were within normal ranges for healthy horses (Tannant 1997; Williams et al 2004). This suggests little to no tissue damage occurred in these horses as a result of initial or extended exposure to ergovaline and lysergic acid.

Bony et al (2001) injected geldings with 15 µg ergovaline/kg BW and observed a plasma clearance rate of 0.02 L/min/kg BW. However, because only activities in plasma were evaluated, it remains unclear as to whether ergovaline was sequestered in tissues, metabolised for example to lysergic acid, or actually eliminated from the body. Ergovaline was not detected in the urine of geldings receiving the E+ tall fescue seed diet in the present study. Further, faeces contained 35–40% of the total ergovaline consumed, indicating the faeces as an important route of excretion for intact ergovaline in the horse. The remaining 60–65% of the ergovaline was apparently retained or metabolised to another form. Apparent differences in the metabolism and/or elimination of the ergot alkaloids discussed in the literature (Westendorf et al 1993; Stuedemann et al 1998; Hill et al 2001) may be a result of different techniques used to determine their concentrations, or differences between species in digestion and/or metabolism, i.e. foregut fermentation *vs* hindgut fermentation in cattle and horses, respectively. Currently, primary methods for quantification of ergot alkaloids include HPLC (Craig et al 1994; Jaussaud et al 1998), which is capable of quantifying individual alkaloids, and a competitive enzyme-linked immunosorbent assay (ELISA; Hill and Agee 1994) that determines total alkaloid levels within a given matrix. In addition, HPLC mass spectrometry has been used to identify and quantify individual alkaloids (Yates et al 1985; Lehner et al 2004) and may soon play a role in determining the fate of ergot alkaloids.

Recent research has suggested that lysergic acid may play a more important role in fescue toxicosis than originally thought (Hill et al 2003). Those authors evaluated the transport of ergot alkaloids across ruminal and omasal tissues, using parabiotic chambers. Lysergic acid was the only ergot alkaloid reported to be transported across those tissues, as measured by ELISA. From that, the authors concluded that lysergic acid, not ergovaline, was the primary toxin causing fescue toxicosis. However, in a previous study other ergot alkaloids, e.g. ergonovine, ergotamine and ergocryptine, were transported across ruminal and omasal tissues but not to the same degree as lysergic acid and lysergol (Hill et al 2001). The findings in the current study suggest lysergic acid was absorbed by the animal and excreted in the faeces and urine in greater amounts than those ingested (133% and 200%, respectively). The site of transport of the ergot alkaloids in the horse has yet to be determined. We speculate that metabolism of ergovaline, and other ergot alkaloids not measured in this study, to lysergic acid in the stomach, small intestine, hindgut, or hepatic tissues, may explain the excess levels of lysergic acid excreted by these animals compared with intake. The ability to quantify other ergot alkaloids and metabolites would provide a better understanding of the toxicant metabolism and excretion in horses. There appeared to be little adaptation of metabolic processes for removal or routes of elimination of alkaloids due to continued exposure to ergovaline or lysergic acid via E+ tall fescue seed, as evidenced by the similar excretion profiles in the initial and extended exposure phases.

The primary objectives of the current study were to determine the route of elimination of ergot alkaloids, and whether exposure time had any effect on the animal's ability to eliminate ergovaline or lysergic acid. Concentrations of ergot alkaloids detected in the urine and faeces did not change with increasing exposure time, thus the route for elimination of ergovaline and lysergic acid was similar between the initial and extended exposure phases; this suggests the animal eliminates these compounds similarly regardless of exposure time. Faecal and urinary concentrations of ergovaline suggest that metabolism or storage of ergovaline occurs. The negative balance of lysergic acid suggests that ergovaline and other alka-

loids are eliminated via biotransformation to lysergic acid. Along with the need to establish more satisfactory laboratory techniques to measure individual alkaloids and metabolites, further research is needed to understand degradation of these compounds in both fore- and hind-gut fermenting animals such as cattle and horses. Delineation of the fate of ergovaline and lysergic acid will aid in the development of management protocols to enhance tolerance to toxic E+ tall fescue in horses and other herbivores.

Acknowledgements

The authors would like to thank Danica Kubley for management and care of the horses at Coldstream Agricultural Research Farm. We would also like to thank all the student workers for their time and help with collection of data and daily chores needed for the proper maintenance and care of the horses and facilities; and Dr Don Thompson at the Louisiana State University for generously analysing all serum prolactin samples. The research reported here was conducted through a cooperative agreement (SCA No. 58-6402-2-0025; CLS and LPB) with the Department of Agronomy, University of Kentucky and the USDA-ARS, Forage-Animal Production Research Unit, Lexington KY, USA.

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Submitted 10 October 2005

Accepted for publication 10 May 2006