Effects of short-term early gestational exposure to endophyte-infected tall fescue diets on plasma 3,4-dihydroxyphenyl acetic acid and fetal development in mares¹,²

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ABSTRACT: Consumption of wild-type (toxic) endophyte-infected tall fescue (E+) by horses during late gestation is known to adversely affect pregnancy outcome; however, little is known of the potential disruptive consequences of E+ consumption by mares during the critical phases of placentation and fetal development in early pregnancy. The objective of this study was to evaluate the detrimental effects of feeding E+ to mares during early gestation. Mares (n = 12) paired by stage of gestation (d 65 to 100) were assigned to diets (six per diet) consisting of endophyte-free (E−) or E+ tall fescue seed (50% E− or E+ tall fescue seed, 45% sweet feed, and 10% molasses fed at 1.0% of BW/d). Mares also had ad libitum access to E+ or E− annual ryegrass hay, and were fed diets for 10 d. Following removal from the tall fescue diet on d 11, mares were placed on common bermudagrass pasture and monitored until d 21. Morning and evening rectal temperatures were recorded and daily blood samples were collected for progesterone and prolactin (PRL) analyses, whereas samples for 3,4-dihydroxyphenyl acetic acid (a catecholamine metabolite) analysis were collected on alternate days. For clinical chemistry analysis, blood samples were collected on d 0, 5, 10 and 21. Daily urine samples were collected for ergot alkaloid analysis, and ultrasonography was performed for presence of echogenic material in fetal fluids. Rectal temperatures (E+ 37.76 ± 0.03; E− 37.84 ± 0.03°C) and serum PRL concentrations (E+ 14.06 ± 0.76; E− 12.11 ± 0.76 ng/mL) did not differ (P = 0.96) between treatments. Measuring the change in basal serum concentration from d 0 over time, progesterone concentrations did not differ (−0.64 ± 1.49 and −0.55 ± 1.47 ng/mL for E+ and E− mares, respectively). There was no negative pregnancy outcome, and ultrasonography indicated no increase in echogenic material in fetal fluids. Plasma 3,4-dihydroxyphenyl acetic acid concentrations decreased (P < 0.05) in E+ compared with E− mares (2.1 ± 0.14 and 4.4 ± 0.43 ng/mL, respectively). Urinary ergot alkaloid concentration was greater (P < 0.01) in mares consuming E+ compared with E− mares (2.1 ± 0.14 and 4.4 ± 0.43 ng/mL, respectively). Although no fetal loss was observed during the current study, elevated concentrations of urinary ergot alkaloid were consistent with depressed endogenous catecholamine activity, suggestive of an endocrine disruptive effect of hypothalamic origin.

Key Words: Catecholamine, Equine Pregnancy, Ergot Alkaloid, Tall Fescue


Introduction

Tall fescue (Lolium arundinaceum (Schreb.)) is an economically important cool-season perennial grass grown extensively throughout the United States, especially in the southeast. It has been reported that a sizeable acreage of tall fescue is grazed by horses, of which >75% is infected with the endophyte Neotyphodium coenophialum (Glen et al., 1996; McCluskey et al., 1999). Ergot alkaloids present in wild-type endophyte-infected tall fescue (E+) are known to cause physiological aberrations in livestock grazing contaminated pastures (Porter and Thompson, 1992; Thompson and Stuedemann, 1993). Some of these compounds act as dopaminergic agonists at the hypothalamic–pituitary axis level and interfere with normal metabolic and reproductive performance (Strickland et al., 1994; Browning et al., 1997).
Fescue toxicosis is a serious clinical disorder in late gestation horses and is characterized in the most severe cases by poor pregnancy outcome (prolonged gestation, agalactia, thickened placentas, dysmature foals, and, in extreme cases, foal and mare mortality; Cross et al., 1995). The adverse effects of E+ consumption by horses during late gestation have been well documented (Monroe et al., 1988; Putnam et al., 1991). However, the potential disruptive effects of E+ consumption by mares during the critical phases of placentation and early fetal development are poorly understood. Moreover, horses do not benefit from pregastric metabolism of alkaloids as do ruminants (Wachenheim et al., 1992), which may make the equine species more at risk to larger doses of alkaloids when consuming wild-type E+ forage.

Early embryonic and fetal development is a highly sensitive and vulnerable developmental process. Exposure to plant toxicants during pregnancy can disrupt normal maternal hormone concentrations and affect embryo survival and fetal development in domestic species (McEvoy et al., 2001). There are two conflicting reports on the potential detrimental effects of toxic tall fescue consumption on embryo survival during early pregnancy in the horse (Brendemuehl et al., 1994; Arns et al., 1997). However, these studies did not investigate the effects of E+ consumption by mares during the critical phases of placentation and fetal development. Consequently, the current study examined the effects of acute toxic E+ exposure on fetal survival from d 65 of gestation. To this end, pregnant mares were acutely exposed to ergot alkaloid-containing diets to explore the fetotoxic potential during early gestation.

Materials and Methods

Experimental Design

Twelve pregnant (65 to 100 d gestation) mares of light horse breeds (quarter horse and Thoroughbred) were paired by stage of gestation and randomly assigned to one of two diets containing either endophyte free (E−) or E+ tall fescue seed and hay, respectively. The mean gestational age of mares in each treatment group was 83.8 ± 5.2 and 81.2 ± 5.2 d for the control (E−) and treated (E+) mares, respectively. Mares were housed individually in 3.5-× 3.5-m stalls erected under a parabola shaped barn for protection from the midsummer heat. Stalls contained buckets and hay bags for ad libitum access to water and hay, respectively. Mares were placed in stalls 2 d before commencing the study, maintained on a commercial sweet feed (1% BW/d), Marshall annual ryegrass (Lolium multiflorum) hay, and water to allow them time to adjust to their new diet and environment. After adaptation, mares were placed on treatment diets while housed for 10 d (beginning July 19, 2002) and were given access to tree-shaded dry lots (approximately 0.5 ha) for 6 h/d for exercise. Following removal from their respective fescue diets, mares were turned out onto bermudagrass (Cynodon dactylon) pasture for the remaining 11 d of the study. The study was 21 d long. One mare in the E− group developed an allergic reaction of unknown etiology on d 3 and was excluded from further data analysis from that point onward. This study followed the FASS (1999) Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching and was approved by the Mississippi State University Institutional Animal Care and Use Committee (Protocol No. 02-046).

Daily Procedures

Rectal temperatures (RT) were recorded daily between 0700 to 0800 and again between 2100 to 2200. Blood samples for progesterone and prolactin (PRL) analyses were collected daily for the first 12 d of the study and one time on d 21 between 0700 to 0800. Immediately following the morning RT and blood sampling, mares were fed the morning portion of the treatment diet and hay. After the morning feeding (30 to 60 min), mares were placed in stocks where urine samples were obtained and fetal well being was monitored by transrectal ultrasonography. Fetal ultrasonography was performed with an Aloka 500 with a 7.5-MHz probe (Aloka Co. Ltd., Wallingford, CT) for detection of fetal heartbeat and changes in the echogenicity of fetal fluids (Riddle and LeBlanc, 2003). Ultrasound examinations were performed by the same individual over the course of the study and all settings (i.e., gain, contrast, brightness, and focal zones) remained constant across the examination period. Following these procedures, mares were turned out onto dry lots for exercise (1000 to 1600). Evening portions of treatment diets were administered between 1600 to 1700 daily. Refusals were weighed and noted but this was a rare event.

Diets

Two lots of tall fescue seed were obtained from Pennington Seed (Lebanon, OR), of which one was certified as positive for the endophyte Neothyphodium coenophialum (Plantation positive) and the second as endophyte negative (Jespur minus) by the Oregon State Department of Agriculture. Mares were fed one of two diets consisting of either E− or wild-type E+ tall fescue seed. Additionally, mares had ad libitum access to hay. The concentrate portion of the diet was formulated and fed to meet recommendations for maintenance (NRC, 1989), and consisted of fescue seed, a commercial 10% CP sweet feed (All-Stock Sweet 10, Country Acres Feed Co., Brentwood, MO; Table 1), and molasses, producing a 50:40:10 seed, sweet feed, molasses ratio. Body weights of mares were recorded 2 d before starting the study, and the concentrate portion of the diet was fed at 1.0% of the mare’s BW (i.e., tall fescue seed fed at 0.5% BW). Concentrate and hay fed to mares were analyzed for CP, NDF, ADF, and ash using AOAC (2003) methods (Table 1). Ergot alkaloid content of E+ feed
was measured at 271 ppb as determined by a specific ELISA ergot alkaloid assay (Hill et al., 2000), which was equivalent to the E+ mares receiving 1.36 μg/kg BW of ergot alkaloids from the seed diet each day. Ergot alkaloids were undetectable in the E− seed diet. Mares in the E+ group were fed E+ tall fescue hay, whereas E− mares were provided with E− annual ryegrass hay. The tall fescue hay was harvested from a pasture at the Mississippi Agricultural and Forestry Experiment Station in Starkville, of which greater than 90% was infected with the wild-type endophyte and contained ergot alkaloid concentrations of 45 ppb as determined by a specific ELISA assay (Hill et al., 2000). Annual ryegrass hay, which was substituted in the absence of E− tall fescue hay, was harvested at the same location and tested negative for ergot alkaloid content. Whereas the annual ryegrass hay differed in some analytical components from endophyte-infected tall fescue hay (Table 1), overall they were similar in nutritional value. Mares were individually offered their concentrate ration (0.5% of BW twice daily for a total of 1.0% BW/d), which was split equally between the morning (0900 to 1000 h) and the evening (1600 to 1700 h) feedings.

### Blood Sampling

Blood samples were collected between 0700 and 0800 h daily by jugular venipuncture from d 0 to 10, and on d 12 and 21 for serum progesterone and PRL analyses. Blood samples were obtained every other day from d 0

| Table 2. Mean values of serum analytes for pregnant mares fed endophyte-free (E−) or wild-type endophyte-infected (E+) tall fescue seed diets |
|----------------|----------------|
| Variable<sup>a</sup> | E− fed mares (n = 6)<sup>b</sup> | E+ fed mares (n = 6) | P > F | Reference range<sup>d</sup> |
| Albumin, g/100 mL | 3.15 | 3.18 | 0.93 | 2.3–3.8 |
| Albumin:globulin ratio | 0.98 | 0.99 | 0.89 | 0.6–1.6 |
| Alkaline phosphatase, U/L | 192.82 | 199.53 | 0.48 | 50–250 |
| ALT (SGPT) U/L | 7.12 | 7.79 | 0.37 | 5–25 |
| AST (SGOT) U/L | 270.12 | 297.21 | 0.14 | 180–380 |
| Amylase, U/L | 2.00 | 2.11 | 0.40 | 25–600 |
| Blood urea N:creatinine ratio | 10.65 | 13.11 | 0.01 | — |
| Cholesterol, mg/100 mL | 85.47 | 79.63 | 0.004 | 50–140 |
| CPK, U/L | 339.29 | 317.11 | 0.77 | 100–300 |
| Creatinine, mg/100 mL | 1.61 | 1.41 | 0.003 | 1.0–2.2 |
| GGTP, U/L | 10.94 | 11.79 | 0.34 | 1–35 |
| Globulin, g/100 mL | 3.23 | 3.33 | 0.55 | 2.2–4.4 |
| Glucose, mg/100 mL | 80.59 | 79.53 | 0.33 | 60–100 |
| Lipase, U/L | 16.29 | 17.11 | 0.40 | 1–100 |
| Total bilirubin, mg/100 mL | 0.99 | 1.19 | 0.24 | 0.8–3.2 |
| Total protein, g/100 mL | 6.38 | 6.51 | 0.37 | 5.8–7.5 |
| Triglycerides, mg/100 mL | 36.64 | 26.42 | 0.03 | 5–80 |
| Urea nitrogen, mg/100 mL | 17.71 | 18.11 | 0.55 | 8–26 |

<sup>a</sup>Data from four individual bleeds collected on d 0, 5, 10, and 21 of the study. Abbreviations: ALT and AST = alanine and aspartate aminotransferase, respectively; CPK = creatinine phosphokinase; GGTP = γ-glutamyl transferase.

<sup>b</sup>E− and E+ seed diets (fed at a rate of 0.5% seed, 0.4% sweet feed [10% CP], and 0.1% molasses BW/d) were supplemented with annual ryegrass hay and wild-type E+ tall fescue hay, respectively.

<sup>c</sup>One mare in the E− group developed an allergic reaction of unknown etiology on d 3 and was excluded from further data analysis from that time point onward.

<sup>d</sup>Reference ranges from Myer et al. (1992) and The Diagnostic Laboratory, College of Veterinary Medicine, Mississippi State University.
to 10 and on d 12 and 21 for plasma concentration analysis of epinephrine, norepinephrine, and the catecholamine metabolite 3,4-dihydroxyphenyl acetic acid (DOPAC, a catecholamine metabolite). On d 0, 5, and 10 of the study, blood samples were collected for blood chemistry analyses, blood cell counts, and differential totals. Blood chemistry analyses (serum analytes and mineral content) were performed by Antech Diagnostics (Memphis, TN). Analyses included: glucose, urea nitrogen, creatinine, total protein, albumin, total bilirubin, alkaline phosphatase, cholesterol, calcium, phosphorus, sodium, potassium, chloride, globulin, lipase, amylase, triglycerides, creatinine phosphokinase, γ-glutamyl transferase P, magnesium, and osmolality. Antech also performed the complete blood count parameters, which included hemoglobin, hematocrit, red and white blood cell, basophil, eosinophil, lymphocyte, monocyte, neutrophil and platelet counts, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration. Reference ranges for analysis were based on those from Jain (1986), Myer et al. (1992), and The Diagnostic Laboratory, College of Veterinary Medicine, Mississippi State University.

Prolactin and Progesterone Concentration Analyses

Serum progesterone and PRL were analyzed by RIA. Concentrations of serum PRL were determined by a previously described and validated heterologous prolactin RIA (Colborn et al., 1991) with modifications (Fitzgerald and Davison, 1997). The assay employed an antiporcine PRL antiserum (LSU R4 EPRL, supplied by D. Thompson, Jr., Louisiana State University, Baton Rouge) and highly purified equine PRL (AFP-7730B, supplied by A. F. Parlow, Harbor-UCLA, Torrance, CA), which was used for both radioiodination and standards, respectively. The sensitivity of the assay was 0.3 ng/mL; the intra- and interassay CV were 5.6 and 12.2%, respectively. Serum progesterone concentrations were measured with a commercially available RIA kit (DSL 3900, Diagnostic System Laboratories, Webster, TX) according to manufacturer instructions. The intra- and interassay CV for the progesterone assay were 4.8 and 12.0%, respectively.

Catecholamine Extraction and HPLC Analysis

Venous blood for the analysis of DOPAC was collected in chilled 10-mL EDTA Vacutainer vials that contained 240 µL of reduced glutathione solution (6 g/100 mL, pH 7.4). Samples were kept on ice and processed within 30 min of collection according to Jimenez et al. (1998). A minimum of four 1-mL aliquots of plasma from each sample was frozen immediately and kept at −80°C until analysis.

Activated acid-washed alumina (Bio Analytical Systems Inc., West Lafayette, IN) was used for catecholamine extraction according to previously reported procedures (Jimenez et al., 1998) with modifications aimed to enhance the recovery of DOPAC. Briefly, to each reaction tube on ice, the following were sequentially added: 50 mg of acid-washed alumina, 2 mL of plasma, 1 mL of Tris buffer (pH 8.7), and 20 µL of internal standards (2 ng each; dihydroxy-benzylamine and epinephrine). The tubes were vortexed briefly and incubated for 15 min at room temperature on a rocker platform. Following centrifugation (10,000 × g for 1 min), the supernatant was removed and the alumina was rinsed three times with a 1-mL wash solution (1.1 g of Na2EDTA/L water, pH 7.0). After decanting the supernatant from the last wash, the excess wash solution was removed with a Pasteur pipette. To elute the catecholamines, 200 µL of 0.2N perchloric acid was added to the alumina and incubated for 5 min at room temperature (−21°C) while vortexing at the beginning, middle, and end of the incubation period. Finally, reaction tubes were centrifuged (10,000 × g) for 1 min, the supernatant was transferred into microcentrifuge tubes, which were centrifuged (10,000 × g) for 3 min at 4°C, and the clear supernatant was divided into two aliquots. One aliquot was transferred into HPLC vials for analysis; the other aliquot was transferred into a clean microcentrifuge tube and stored at −80°C.

Plasma catecholamines were analyzed by HPLC with electrochemical detection by a procedure similar to that described by Seegal et al. (1986). Briefly, the eluate was injected into a C18, 5-µm base deactivated reverse-phase column. The mobile phase (final pH 3.94) consisted of NaH2PO4 (83.8 mM), octyl sodium sulfate (2.6 mM), EDTA (0.09 mM), and triethyamine (35 µL/L) with methanol (20%) as an organic modifier, and it was pumped at a flow rate of 1.0 mL/min. The detector (Waters 464; Waters Corp., Milford, MA) contained a glossy carbon flow-through detector cell and an Ag/AgCl electrode set at 850 mV. Following collection, data were analyzed with the Breeze software (Waters Corp.) and corrected for dihydroxy-benzylamine/epinephrine recovery. Under these conditions, the norepinephrine and epinephrine peaks have interfering peaks, whereas the dopamine peak was frequently below the limit of detection, a condition also reported for horses by Nagata et al. (1999). Consequently data presented is for DOPAC values only.

Urine Sampling and Urinary and Forage Ergot Alkaloid Analysis

Urine was collected following cleaning of the perineal area with Betadine scrub solution (Medline, Mundelein, IL) and passing a sterile catheter into the urethra of the mare guided by a sterile rectal sleeved arm with sterile lubricant and collection in a sterile 50-mL vial. Urine samples, along with tall fescue seed diets, were analyzed for ergot alkaloid content by competitive ELISA using an ergot alkaloid monoclonal antibody 15F3.E5 specific to the lysergic moiety of all ergot alkaloids and expressed as nanograms of creatinine per milli-
Tall fescue toxicosis and equine pregnancy

Figure 1. Serum concentrations of prolactin in mares fed endophyte-free (○, E−) or wild-type endophyte-infected (■, E+) tall fescue seed and forage in their daily ration. Prolactin concentrations did not differ between the E− and E+ tall fescue treatment groups during the dietary treatment phase of the study (d 0 to 10). However, an increase (P < 0.05) in serum prolactin concentrations was observed on d 21 in the E+ treatment group, 11 d after withdrawal of the E+. One mare in the E− group developed an allergic reaction of unknown etiology on d 3 and was excluded from further data analysis from that time point onward.

Figure 2. Serum concentrations of progesterone in mares fed endophyte-free (○, E−) or wild-type endophyte-infected (■, E+) tall fescue seed and forage in their daily ration. Mean systemic concentrations of progesterone were greater (P < 0.05) in mares consuming E+ diets, but changes in serum progesterone concentrations over time did not differ between treatment groups. One mare in the endophyte-free group developed an allergic reaction of unknown etiology on d 3 and was excluded from further data analysis from that time point onward.

Statistical Analysis
The physiological variables and blood chemistry changes from pretreatment levels were analyzed for treatment and time using repeated-measures ANOVA. The MIXED procedure in SAS (SAS Inst., Inc., Cary NC) was employed with pairs and individual mares considered random effects. Blood chemistry and other physiological variables studied (blood hormone, urine ergot alkaloid content) were also analyzed with repeated-measures analysis. Significance was set at P < 0.05. When significant differences were found, treatment means were separated using Fisher’s protected LSD post hoc test.

Results

Body Weight and Rectal Temperatures

Mean BW for mares consuming E+ and E− diet treatments on d −2 were 536.8 ± 10.26 and 532.9 ± 13.3 kg, respectively, which did not change significantly over the dietary treatment phase of the study. There were no differences in rectal temperatures between the treatment groups (E+, 37.7 ± 0.01°C; E−, 37.8 ± 0.02°C). However, there was a difference (P < 0.01) between the morning (0700 to 0800 h) and evening (2100 to 2200 h) rectal temperatures regardless of treatment (E−, 37.8 ± 0.02 and 37.9 ± 0.03°C and E+, 37.7 ± 0.02 and 37.8 ± 0.02°C, respectively).

Endocrine Measurements

Serum PRL concentrations were not different between treatment groups during the tall fescue dietary phase of the study (d 0 to 10), with mean concentrations of 14.06 ± 0.76 ng/mL and 12.11 ± 0.76 ng/mL for mares in the E+ and the E− treatment groups, respectively. However, there was an increase (P < 0.05) in serum PRL on d 21 in mares that had consumed the E+ tall fescue, which was 11 d after removal from E+ tall fescue seed diets (Figure 1).

Serum progesterone concentrations differed (P < 0.05) between the two treatment groups, with mean concentration values of 24.26 ± 6.79 and 13.79 ± 1.65 ng/mL for mares fed E+ and E−, respectively. Although the systemic concentrations of progesterone differed (P < 0.05) between groups, there was no change in progesterone values over time from pretreatment values (Figure 2). The E+ group had a mean change in serum progesterone of −0.64 ± 1.49, and the E− group had a mean change of −0.55 ± 1.47 ng/mL.

DOPAC

Mares that were fed the E+ diet manifested a decline (P < 0.05) in plasma DOPAC concentrations when com-
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Figure 3. Plasma concentrations of 3,4-dihydroxyphenyl acetic acid (DOPAC), expressed as ng/mg creatinine, in mares fed endophyte-free (○, E−) or wild-type endophyte-infected (■, E+) tall fescue seed and forage in their daily ration. Values were significantly ($P < 0.05$) lower in mares consuming E+ diets on d 4, 6, 8, and 10 of the dietary treatment phase of the study. Following the return of mares to common bermudagrass pastures, DOPAC values increased markedly, but did not differ between treatment groups. One mare in the E− treatment group developed an allergic reaction of unknown etiology on d 3 and was excluded from further data analysis from that time point onward.

pared with the mares fed the E− diet (Figure 3). While on treatment diets, the mares consuming E+ had a mean plasma DOPAC concentration of $2.1 \pm 0.14 \text{ ng/mL}$, whereas mares consuming E− had a mean concentration of $4.4 \pm 0.43 \text{ ng/mL}$. Posttreatment phase DOPAC concentrations were elevated ($P < 0.001$) from treatment phase values for both the E− and the E+ treatment groups ($11.07 \pm 1.37$ and $7.80 \pm 1.03 \text{ ng/mL}$, respectively); however, there was no difference in the posttreatment phase values between the two treatment groups.

**Urinary Ergot Alkaloid Concentrations**

Mares fed the E+ diet had greater ($P < 0.01$) urinary ergot alkaloid concentrations compared with mares that received the E− diet (Figure 4). Mares consuming E+ had a mean urinary ergot alkaloid concentration of $532.12 \pm 52.51 \text{ ng/mg creatinine}$, whereas mares fed E− had a mean concentration of $13.36 \pm 2.67 \text{ ng/mL}$ creatinine. Urinary concentrations of ergot alkaloids decreased rapidly when the mares were removed from the E+ tall fescue diets and were undetectable in the urine within 48 h of removal.

**Blood Chemistry**

Serum analytical values of mares on both diets were within the established normal physiological ranges and did not differ over time (Table 2). However, there were some differences for mean serum values between treatment groups, with the BUN:creatinine ratio being greater ($P < 0.01$) in the mares fed E+ diets, whereas creatinine ($P < 0.003$), triglycerides ($P < 0.03$), and cholesterol ($P < 0.004$) were decreased. Dietary treatments had no effect on serum mineral values, all of which fell within normal physiological ranges (Table 3). Hemogram values were not affected by dietary treatment over time (Table 4); however, the monocyte count was depressed ($P < 0.04$) in E+ mares.

**Ultrasoundography**

Ultrasoundography was used to monitor changes in fetal heart rate and echogenic activity of fetal (allantoic and amniotic) fluids as a means of evaluating fetal well being (Riddle and LeBlanc, 2003). Fetal activity and heartbeat, when detectable, appeared normal in both the mares consuming E− and E+ diets throughout the course of the study. Ultrasound imaging revealed no increase in echogenic material in fetal fluids in either group of mares. All fetuses remained viable, and no fetal loss was associated with either of the dietary treatments. In the E+ group, one mare showed distinct signs of mammary development by d 3, from which a dilute milky secretion could be expelled from the teats. A second mare in the E− group had signs of a swollen udder on d 4, but the swelling had regressed the following day.
Table 3. Mean value of serum minerals for pregnant mares fed endophyte-free (E−) or wild-type endophyte-infected (E+) tall fescue diets

<table>
<thead>
<tr>
<th>Variable</th>
<th>E− fed mares (n = 6)</th>
<th>E+ fed mares (n = 6)</th>
<th>P &gt; F</th>
<th>Reference range4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca, mg/100 mL</td>
<td>11.73</td>
<td>11.72</td>
<td>0.95</td>
<td>10.8–13.5</td>
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<tr>
<td>Cl, mEq/L</td>
<td>107.82</td>
<td>108.32</td>
<td>0.94</td>
<td>97–108</td>
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<tr>
<td>Mg, mEq/L</td>
<td>1.52</td>
<td>1.49</td>
<td>0.80</td>
<td>1.2–2.3</td>
</tr>
<tr>
<td>P, mEq/L</td>
<td>4.09</td>
<td>4.22</td>
<td>0.45</td>
<td>2.0–5.0</td>
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<tr>
<td>Na, mEq/L</td>
<td>140.00</td>
<td>139.63</td>
<td>0.56</td>
<td>132–146</td>
</tr>
<tr>
<td>Calculated osmolality, mOsm/L</td>
<td>278.82</td>
<td>278.00</td>
<td>0.25</td>
<td>280–300</td>
</tr>
</tbody>
</table>

aData from four individual bleeds collected on d 0, 5, 10, and 21 of study.

bE− and E+ seed diets (fed at a rate of 0.5% seed, 0.4% sweet feed [10% CP], and 0.1% molasses BW/d) were supplemented with annual ryegrass hay and wild-type E+ tall fescue hay, respectively.

cOne mare in the E− group developed an allergic reaction of unknown etiology on d 3 and was excluded from further data analysis from that time point onward.

dReference ranges from Myer et al. (1992) and The Diagnostic Laboratory, College of Veterinary Medicine, Mississippi State University.

Discussion

Pregnancy loss due to placentitis (Giles et al., 1993) and plant toxins (Panter et al., 1992; McEvoy et al., 2001) has a severe, negative economic impact on the equine industry annually. Many forages contaminated with poisonous plants grazed by livestock, including grasses infected with fungal contaminants, are known to be fetotoxic and cause fetal abnormalities, embryonic or fetal death, abortion, prolonged gestation, or premature parturition (Panter et al., 1992; McEvoy et al., 2001). More specifically, a number of reports have documented evidence of adverse effects following wild-type E+ tall fescue consumption by pregnant mares in late gestation (Putnam et al., 1991; Porter and Thompson, 1992; Cross et al., 1995; Porter, 1995; Ryan et al., 2001), but the effects of these forages on fetal survival during placentation is poorly understood. This study was de-

Table 4. Hemogram values for pregnant mares fed endophyte-free (E−) or wild-type endophyte-infected (E+) tall fescue seed diets

<table>
<thead>
<tr>
<th>Variable</th>
<th>E− fed mares (n = 6)</th>
<th>E+ fed mares (n = 6)</th>
<th>P &gt; F</th>
<th>Reference range4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematocrit, %</td>
<td>36.42</td>
<td>35.98</td>
<td>0.69</td>
<td>32.0–50.0</td>
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<tr>
<td>Hemoglobin, g/100 mL</td>
<td>12.91</td>
<td>12.72</td>
<td>0.61</td>
<td>11.0–17.0</td>
</tr>
<tr>
<td>MCH</td>
<td>16.46</td>
<td>16.54</td>
<td>0.91</td>
<td>12–19</td>
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<td>MCHC</td>
<td>35.46</td>
<td>35.36</td>
<td>0.52</td>
<td>31–39</td>
</tr>
<tr>
<td>MCV</td>
<td>46.47</td>
<td>46.68</td>
<td>0.91</td>
<td>34–58</td>
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<tr>
<td>RBC, 1 × 1012/L</td>
<td>7.87</td>
<td>7.77</td>
<td>0.82</td>
<td>7.00–12.00</td>
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<td>WBC, 1 × 109/L</td>
<td>5.49</td>
<td>5.51</td>
<td>0.96</td>
<td>5.5–12.5</td>
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<tr>
<td>Platelet count, 1 × 1012/L</td>
<td>182.41</td>
<td>173.89</td>
<td>0.75</td>
<td>100–400</td>
</tr>
<tr>
<td>Differential absolute, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bands</td>
<td>0</td>
<td>0</td>
<td></td>
<td>0–300</td>
</tr>
<tr>
<td>Basophils</td>
<td>4.71</td>
<td>4.71</td>
<td>0.19</td>
<td>0–290</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>227.24</td>
<td>175.00</td>
<td>0.07</td>
<td>0–1,000</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>1,569.40</td>
<td>1,646.50</td>
<td>0.74</td>
<td>1,500–7,700</td>
</tr>
<tr>
<td>Monocyte</td>
<td>277.29</td>
<td>223.05</td>
<td>0.04</td>
<td>0–1,000</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>3,410.60</td>
<td>3,466.00</td>
<td>0.95</td>
<td>2,600–7,500</td>
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</table>

aData from four individual bleeds collected on d 0, 5, 10, and 21 of study. Abbreviations: MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; RBC = red blood cells; WBC = white blood cells.
bE− and E+ seed diets (fed at a rate of 0.5% seed, 0.4% sweet feed [10% CP] and 0.1% molasses BW/d) were supplemented with annual ryegrass hay and wild-type E+ tall fescue hay, respectively.
cOne mare in the E− group developed an allergic reaction of unknown etiology on d 3 and was excluded from further data analysis from that time point onward.
dReference ranges from Jain (1986), Myer et al. (1992), and The Diagnostic Laboratory, College of Veterinary Medicine, Mississippi State University.
signed to investigate the effects of toxic E+ tall fescue consumption on fetal well-being and pregnancy outcome in the mare during the critical phase of placentation and early fetal development.

Ultrasonography failed to identify any abnormal fetal activity or premature increase in echogenic material in fetal fluids of mares in the course of this study. Others have reported that consumption of E+ tall fescue grass during early pregnancy (between d 14 and d 21 of gestation) resulted in an increased (30% E+ vs. 7.7% E−) incidence of embryonic death, which was characterized by a significant decrease in vesicular height and irregular shape of embryos compared with viable embryos at the same stage of gestation (Brendemuehl et al., 1994). In contrast, a subsequent study failed to confirm these findings when mares were fed E+ tall fescue seed at similar stages of gestation (Arns et al., 1997). Pregnancy well being was evaluated by monitoring heart-beat and rapid alterations in the echoic nature of fetal fluids. During this stage of gestation (d 30 to 85), fetal fluids are normally devoid of echoic material, the appearance of which only begins to change as pregnancy advances beyond d 85 (Riddle and LeBlanc, 2003). The presence of hyperechoic fetal fluids, which is associated with an increased presence of squamous epithelial cells, was a consistent finding and was characteristic of fetal demise in mares experiencing early fetal loss (40 to 100 d gestation) in central Kentucky during the mare reproductive loss syndrome epidemic in the spring of 2001 and 2002 (Riddle and LeBlanc, 2003). Moreover, a preponderance of mare reproductive loss syndrome-related pregnancy losses occurred between 45 and 80 d of gestation (Morehead et al., 2002), which was one criterion in the current study for investigating the effects of E+ tall fescue consumption by pregnant mares during this stage of gestation, although none was identified.

Rectal temperatures appeared to be unaffected by exposure to the E+ diet. Others have reported no dramatic changes in core temperatures in the horse (Monroe et al., 1988; Putnam et al., 1991), while cattle show a marked elevation in temperature on exposure to ergot alkaloids (Rhodes et al., 1991; Al-Haidary et al., 2001).

Blood chemistry measurements remained within the normal ranges for the equine although there was an increase in the blood urea nitrogen:creatinine ratio and a decline in creatinine content in animals consuming the E+ diet. Similar blood analytes measured in cattle grazing E+ tall fescue showed significant changes in some measurements (i.e., globulin, albumin:globulin ratio, cholesterol, creatinine) compared with control animals, although most were within the normal range for the bovine (Oliver et al., 2000). In another study, Schultze et al. (1999) reported that cattle exposed to E+ tall fescue pasture had decreased activities of alkaline phosphatase and alanine transferase and increased creatinine and albumin:globulin ratios, which is consistent with the findings of Oliver et al. (2000). Creatinine also was decreased in mares on E+ diets in this study, but values were regarded as still within the normal range. However, both of these studies used cattle that were exposed to the E+ forage for several months compared with the short-term (10 d) exposure in the current study. Under these conditions, the E+ consumption caused alterations of blood analytes that fell within the normal range for the bovine. Serum mineral values were also monitored in the cattle study, but the only significant effect on mineral values observed was a reduced copper concentration for cattle on E+ tall fescue pastures. No marked changes in serum mineral values were observed in the either group of mares. Whole blood cell counts and hemogram analysis of blood samples from the mares also failed to show any marked changes in response to acute ergot alkaloid exposure. Although there were some differences in blood chemistry and blood cell count values between the two treatment groups of mares, these changes were within established normal ranges for the equine and thus may not be physiologically relevant.

Progesterone is an essential hormone for establishment and maintenance of early pregnancy in the horse (Ginther, 1992) with peak concentrations being attained around 100 d of gestation (Holtan et al., 1991). Although mean serum progesterone concentrations were significantly greater in the mares consuming E+ tall fescue seed diets, no change in values was observed over time in either group of mares in the current study. Differences in systemic progesterone profiles of mares between d 20 and 180 of gestation have been reported, which was attributed to the varying numbers of secondary corpora lutea on the ovaries of individual mares (Squires, et al., 1974; Schwab et al., 1990). Numbers of secondary corpora lutea were not accessed in the current study, but one mare in the E+ group had much higher serum progesterone concentrations compared with others within the same group. In a similar study, Brendemuehl et al. (1994) reported no differences in plasma progesterone profiles between mares on E− and E+ tall fescue pastures with viable pregnancies. In contrast, mares on E+ tall fescue pasture experiencing embryonic demise had decreased plasma progesterone concentrations. Arns et al. (1997) observed increased serum progesterone values in mares fed E+ tall fescue seed during a comparable stage of gestation. However, several studies have documented a decrease in systemic progesterone values in mares exposed to E+ tall fescue during late gestation (Monroe et al., 1988; Redmond et al., 1991; Boosinger et al., 1995). We did not observe similar changes in serum progesterone in our early gestation exposure paradigm.

Serum PRL is reported to be one of the most consistent physiological markers of tall fescue toxicosis in domestic livestock and horses (Porter and Thompson, 1992; Cross et al., 1995) and the most pronounced and extensively studied consequence of fescue toxicity is the inhibitory effect of ergot alkaloids on PRL secretion by the pituitary gland (Oliver, 1997). In the current study, mean serum PRL concentrations did not differ between
treatment groups during the experimental dietary phase of the study. However, serum values began to rise markedly within 48 h of removal from the diets in both groups of mares, but the magnitude of the increase was greater by d 21, 10 d after removal, in the mares fed E+ tall fescue diets. Schwab et al. (1990) measured plasma PRL in pregnant mares undergoing pregnancy loss in early gestation (d 28 to 44), but observed no differences between these mares and those maintaining their pregnancy. This observation is consistent with PRL concentrations observed in the current study. However, the lack of an effect is unlikely due to low levels of ergot alkaloid contamination as evidenced by the elevated concentrations measured in the urine, but the short duration of exposure to endophyte-infected feed may have been a factor. Boosinger et al. (1995) observed no change in plasma PRL values in a group of mares in late gestation upon removal from E+ tall fescue pastures on d 300 of gestation, although they did observe considerably improved pregnancy outcome in the same mares. In contrast, mares maintained on E+ tall fescue pasture before parturition had significantly reduced PRL values associated with negative pregnancy outcomes (Boosinger et al., 1995). Thus, it seems that stage of gestation in the horse rather than duration of exposure might be the critical factor in terms of the negative impact of ergot alkaloids on PRL secretion. The increase in PRL in the posttreatment phase of the current study is puzzling because plasma DOPAC concentrations follow a similar pattern. Changes in systemic PRL and DOPAC values under normal physiological conditions are negatively correlated. By d 4 of the study, DOPAC values had decreased in mares consuming E+ tall fescue seed diets and remained depressed until mares were removed from the E+ tall fescue seed diet. Within 48 h of removal from E+ tall fescue seed diets, plasma DOPAC concentrations increased markedly over experimental phase values in a pattern consistent with that of PRL. Whether increased daylight exposure and/or more exercise impacted hypothalamic–pituitary neuroendocrine activity upon returning mares to free-range pasture, since both groups responded in a similar manner, is open to speculation.

Endophyte-infected tall fescue produces pharmacologically active ergot alkaloid compounds (Strickland et al., 1992) that are known to interact with dopamine receptors where they act as partial agonists (Strickland et al., 1994; Larson et al., 1995). One function of dopamine secretion, from the hypothalamus in particular, is its role as a PRL-inhibiting factor (Ben-Jonathan et al., 1989). The wild-type E+ tall fescue-derived ergot alkaloids act as dopamine receptor agonists and bind to dopamine receptors of the anterior pituitary and cause inhibition of PRL secretion. They can also interfere with endogenous dopamine production via an autoreceptor-mediated downregulation (Cooper et al. 1996), and observed changes in endogenous PRL and DOPAC in the current study may be a consequence of endocrine disruptive effects caused by exposure to exogenous ergot alkaloids. It has been shown in vitro that ergot alkaloids decrease PRL secretion by rat pituitary cells (Strickland et al., 1994), and that removal of dopamine treatment from pituitary infusion studies results in an increase in PRL secretion (Fagin and Neill, 1981). Moreover, direct effects of ergot alkaloid compounds on normal secretory patterns of dopamine and its metabolites in brain tissue (i.e., hypothalamus) in rodents (Filipov et al., 1999) and in cattle (Schillo et al., 1988) have been demonstrated. Both of these studies demonstrate a central nervous system effect of ergot alkaloids on the brain dopaminergic activity. Human subjects treated with bromocriptine, an ergot alkaloid similar to the ones found in wild-type E+ tall fescue that also decrease hypothalamic dopamine metabolism, experienced marked decreases in plasma DOPAC values (Mercuro et al., 1985), which in part suggests decreased hypothalamic function. Thus, the decrease in DOPAC plasma values in the current study may be due to a decline in either central or peripheral sources of dopamine. Although in other species a significant portion of plasma DOPAC is of central origin (Fekete et al., 1980; Amin et al., 1995), the source of plasma DOPAC under these conditions in the horse is as yet unknown. The removal of E+ tall fescue from the mares’ diet may account, in part, for the rapid and dramatic rebound in endogenous PRL and DOPAC values observed because both treatment groups showed approximately a threefold increase following return to pasture. Another source of plasma DOPAC is the sympathetic nervous system, which is activated by various stimuli, such as stress, with the net result being an increase in plasma DOPAC concentrations (Harikai et al., 2002). The fact that plasma DOPAC concentration was decreased by E+ consumption in our study suggests that the sympathetic nervous system was not involved.

In the current study, urinary ergot alkaloid concentration was measured as a means of assessing ergot alkaloid load using an ELISA employing a monoclonal antibody that is specific to the lysergic moiety of all ergot alkaloids (Hill et al., 1994). Urinary ergot alkaloid excretion gives an accurate assessment of whether animals are consuming E+ tall fescue by measuring total ergot alkaloid content (Hill et al., 2000). This is the first study to report urinary ergot alkaloid concentration in mares following exposure to wild-type E+ tall fescue feed. Currently, little is known regarding the absorption and metabolism of ergot alkaloids in the equine. Others have reported that urinary ergot alkaloid concentration rises rapidly within 48 h of exposure to wild-type E+ tall fescue pastures in beef steers and that concentrations clear the urine equally rapidly on removal from the wild-type E+ tall fescue pasture (Stuedemann et al., 1998; Hill et al., 2000). These findings are consistent with the pattern of urinary ergot alkaloid excretion observed in the current study. Moreover, the excretory pattern mirrored the endocrine profile for DOPAC, which is suggestive of the agonistic effects of ergot alkaloid on dopamine receptor systems.
Implications

The findings of this study indicate that ergot alkaloids are absorbed very rapidly from the equine digestive tract and that they translate into detectable changes in plasma catecholamine metabolite 3,4-dihydroxyphenyl acetic acid concentrations, even after short-term exposure to wild-type endophyte-infected tall fescue. Although no fetal loss was observed during the current study, the elevated concentrations of urinary ergot alkaloid content significantly decreased endogenous catecholamine activity. Thus, studies on exposure of mares to ergot alkaloids from early gestation onward are necessary to determine the effect of prolonged disruption of catecholamine secretion on normal fetal development and survival in horses. Understanding the bioavailability and biotransformation of ergot alkaloids in the equine is important for the development of therapeutic strategies to ameliorate the effects of tall fescue toxicosis.

Literature Cited


Tall fescue toxicosis and equine pregnancy


