Vasoconstriction in horses caused by endophyte-infected tall fescue seed is detected with Doppler ultrasonography

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ABSTRACT: The hypotheses that endophyte (Neotyphodium coenophialum)-infected tall fescue (TF) seed causes vasoconstriction in horses in vivo and that ground seed would cause more pronounced vasoconstriction than whole seed were tested. Ten horses each received 1 of 3 treatments: endophyte-free ground (E–G; n = 4 horses) seed, endophyte-positive whole (E+W; n = 3) seed, or endophyte-positive ground (E+G; n = 3) seed. There were two 14-d periods, P1 and P2. During P1, animals were adapted to a concentrate (0.2% BW, as fed, twice daily) and alfalfa cubes. During P2, the seed was mixed into the concentrate portion of the diet and alfalfa cubes were offered ad libitum. Fescue seed was fed in increasing amounts ranging from 0.02% BW on d 1 (averaging 76 ug/kg ergovaline + ergovalinine) to 0.22% BW on d 11 to 14 (averaging 713 ug/kg ergovaline + ergovalinine). The distal palmar artery of the left foreleg of each horse was scanned via Doppler ultrasonography for 4 d during each period, with 5 replicate scans performed on each scanning day. The measurements taken at each scan included artery luminal diameter, area, and circumference, peak systolic velocity, end diastolic velocity and blood flow variables. Animal temperature, heart rate, and respiration rate and ambient temperature and humidity were also recorded. Blood samples were taken on each scanning day to measure inflammatory cytokine mRNA abundances, and blood samples were collected on d 0, 4, 8, and 14 of P2 to measure prolactin concentrations. Consumption of E+G TF seed caused decreased artery lumen diameter (P = 0.0033), area (P = 0.0406), and circumference (P = 0.0480) compared with E–G seed, and E+W seed produced an intermediate response. Blood flow volume was reduced (P < 0.05) during P2 in horses receiving E+G seed compared with horses receiving E–G seed. Other ultrasound variables were not different (P > 0.05) among treatment groups, and neither were cytokine mRNA or prolactin concentrations. Treatment did not alter (P > 0.05) animal temperature, heart rate, or respiration rate, and neither ambient temperature nor relative humidity was consistently correlated with any response variable measured. Taken together, these data confirm that consumption of E+G fescue seed caused vasoconstriction in horses, which could be readily measured by Doppler ultrasonography. Use of Doppler ultrasound to monitor the diameter of the palmar artery of horses grazing endophyte-infected (E+) fescue pastures may provide a convenient and satisfactory biomarker to determine premonitory signs of fescue toxicosis.

Key words: Doppler ultrasonography, equine, fescue toxicosis, horse, tall fescue, vasoconstriction


1The authors are grateful to the students and farm staff who assisted with animal care and handling, to Donald L. Thompson, Louisiana State University, for prolactin RIA, and to Angela Schorgendorfer, University of Kentucky, for statistical analyses. This research was funded through a Specific Cooperative Agreement (#58-644-8-290) with the USDA-ARS, Forage Animal Production Unit, the University of Kentucky College of Agriculture, and the Departments of Veterinary Science and Animal and Food Sciences, Lexington, KY. It is publication 12-14-078 of the University of Kentucky Agricultural Experiment Station.

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Received September 12, 2012.

Accepted January 14, 2013.
INTRODUCTION

Tall fescue [Lolium arundinaceum (Schreb.) Darbysh.] is a perennial grass that covers approximately 15 million ha in the eastern half of the United States (Hannaway et al., 2009) and can be infected with an endophytic fungus, Neotyphodium coenophialum (Burns and Chamblee, 1979; Bacon, 1995). In horses the most frequently reported problems associated with endophyte-infected (E+) fescue consumption are in late pregnancy and include extended gestation, thickened placenta, dystocia, and agalactia (Monroe et al., 1988; Cross et al., 1995). In addition, changes in cytokines have been implicated in fescue toxicosis in mares (Fitzgerald et al., 2007). Fescue toxicosis in cattle is associated with vasoconstriction, poor growth rates, and increased body temperature in warmer climates, and lameness and gangrenous sloughing of the hooves and tips of the ears in colder climates (Schmidt and Osborn, 1993; Strickland et al., 1993; Oliver, 1997) as well as decreased reproductive performance (Porter and Thompson, 1992). Ergot alkaloids in E+ fescue have been shown to cause vasoconstriction in vitro in both cattle (Solomons et al., 1989; Oliver et al., 1993; Klotz et al., 2008) and horses (Abney et al., 1993; Klotz and McDowell, 2010). Aiken et al. (2007), using in vivo color Doppler ultrasonography, demonstrated that cattle fed a diet of chopped alfalfa hay containing E+ seed had significant constriction of the caudal artery, decreased arterial blood flow, and decreased heart rates compared with cattle fed endophyte-negative (E−) fescue seed. Peripheral vascular effects of E+ fescue have not been examined in vivo in horses; therefore, these experiments were designed to test the hypothesis that consumption of E+ tall fescue seed by horses causes vasoconstriction that can be measured by Doppler ultrasonography and to compare the effects of whole vs. ground E+ fescue seed. Doppler ultrasonography may be a sensitive and useful tool to identify vascular changes associated with fescue toxicosis in horses.

MATERIALS AND METHODS

Horses used in this study were Thoroughbred, Quarter Horse, or mixed breeds and were used in protocols that were approved by the University of Kentucky Animal Care and Use Committee using guidelines set forth by the Federation of Animal Science Societies (FASS, 2010).

Preliminary Studies: Development of the Model

Three preliminary studies were conducted to 1) determine a good candidate vessel for detecting vasoconstriction in horses, 2) compare automated measurements calculated by the ultrasound machine (Sonosite Titan with an 11 mm convex array 5/8 MHz transducer; Bothell, WA) with manual trace measurements on the same variables, and 3) confirm the ability to detect vasoconstriction in the candidate vessel using Doppler ultrasonography.

To determine a satisfactory vessel for study, maxillary, caudal, and palmar arteries were scanned in both real-time B mode, color Doppler, and pulsed wave Doppler on 6 different horses on each of 3 d. Desirable criteria for selecting the focal vessel for further study were that it was sufficiently straight to minimize flow artifacts due to vessel branching and/or turning, there were good landmarks to allow for repeated scanning of the same area both within and between animals, it was readily accessible to the sonographers, and that scanning was well tolerated by the animals. Different vessels required different machine settings, but once a focal vessel was selected, uniform machine settings were used for all animals. This study resulted in the palmar artery, just proximal to the fetlock joint, metacarpal zone 3A (Sande et al., 1998), being selected as the focal vessel based on the criteria listed above (Fig. 1).

To compare manual vs. machine measurements and ability to detect vasoconstriction in the candidate vessel (distal plamar artery), ultrasonographic scans were performed in both transverse and longitudinal views of the vessel. B-mode images were first used to locate vessels and to determine vascular and tissue landmarks. Then color Doppler images were used to measure the cross-sectional area of the artery in a frozen image. To minimize variability due to changes in vessel luminal size between systole and diastole, only images at peak systole were used. Manual measurements were made by using the machine touch pad and on-screen cursor to trace around the artery cross-sectional lumen area. Then automated measurements were made using the machine touch pad and ellipse feature. A longitudinal view of the artery was then established and a Doppler spectral tracing obtained. On the frozen image, manual measurements were made by moving the on-screen caliper with the touchpad and manually tracing the wave form to obtain peak systolic velocity (PSV; cm/sec), end diastolic velocity (EDV; cm/sec), the ratio of PSV:EDV, and the resistivity index (RI), defined as (PSV – EDV)/PSV (Kremkau, 2006). For automated measurements, 1 caliper was placed at peak systole of the wave form and a second caliper was placed at end diastole on the wave form, and PSV, EDV, PSV:EDV Ratio, and RI were computed by the machine. All manual or machine automated measurements were compared among 5 horses with 3 replicate scans on each horse on each of 5 d. Manual vs. automated trace measurements were compared for equal variances using t tests, and correlations between manual vs. automated trace measurements were determined with
Pearson correlation coefficients (SAS Inst. Inc., Cary, NC). As a result, the automated and manual measurements were highly correlated \((P < 0.0001; \text{Table 1})\) and there were no differences in variances \((P > 0.05)\) between any of the measurements. Therefore the less time-consuming automated measurements were used in all subsequent scans.

To confirm the ability to observe vasoconstriction with Doppler ultrasound, xylazine HCL (0.2 mg/kg; Lloyd Laboratories, Shenandoah, IA) was administered to 6 different horses on 1 to 3 separate days each. Four to six cross-sectional scans of the distal palmar artery were performed over approximately 10 to 15 min immediately before and again immediately after xylaxine administration. Color Doppler images at peak systole were used to obtain the mean diameter of the vessel lumen (defined as the average of the longer and shorter axes of the elliptical shape of the vessel, taken at right angles to each other) and the area and circumference (area and circumference were obtained by the machine ellipse feature). Use of Doppler ultrasonography to detect vasoconstriction of the palmar artery caused by a pharmacological agent was confirmed (Fig. 2). Administration of xylazine caused an observable decrease in vessel circumference, diameter and, to a lesser degree, area. Because xylazine caused a very rapid and transient vasoconstriction, only measurements in the transverse view of the artery were obtained.

**Main Experiments: Effect of Endophyte-Infected Fescue Seed on Vasoconstriction, Prolactin, and Cytokine mRNA Concentrations**

The hypotheses that endophyte \((Neotyphodium coenophialum)\) -infected fescue seed causes vasoconstriction in horses in vivo and that ground seed would be more effective than whole seed were tested. Eleven horses received either E– ground (E–G), E+ whole (E+W), or E+ ground (E+G) fescue seed in a randomized block design. The first block was from October 13 to November 6 and consisted of 3 mature geldings, the second block was from November 1 to November 20 and consisted of 3 mature mares, and the third and fourth blocks occurred simultaneously, from November 29 to December 18, and consisted of three 2-yr-old fillies and 2 mature mares, respectively. Horses within blocks were randomly assigned to receive 1 of the 3 fescue seed treatments. Ergovaline and ergovalinine concentration were determined by HPLC florescence using the procedure of Yates and Powell (1988) as modified by Aiken et al. (2009). The E+ seed (“Rendition” tall fescue; Smith Seed, Halsey, OR) contained 4.93 mg/kg ergovaline and 3.42 mg/kg ergovalinine, and the E– seed (“Bull” tall fescue; DLF International Seeds, Halsey, OR) was certified endophyte free and tested negative for both ergovaline and ergovalinine. Seed was ground by passing it through a 2 mm screen in a Wiley Mill. Animals were housed in individual 4 by 16 m runs with individual

### Table 1. Correlation coefficients for manual vs. automated trace measurements

<table>
<thead>
<tr>
<th></th>
<th>Automated</th>
<th>Manual measurements</th>
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<tr>
<td></td>
<td>Area</td>
<td>Area 0.9796</td>
</tr>
<tr>
<td></td>
<td>PSV</td>
<td>0.9881</td>
</tr>
<tr>
<td></td>
<td>EDV</td>
<td>0.9631</td>
</tr>
<tr>
<td></td>
<td>S:D</td>
<td>0.9398</td>
</tr>
<tr>
<td></td>
<td>RI</td>
<td>0.9503</td>
</tr>
</tbody>
</table>

1All correlations were significant at \(P < 0.0001\).
2PSV, peak systolic velocity, cm/sec.
3EDV, end diastolic velocity, cm/sec.
4Ratio of PSV:EDV.
5RI, resistivity index: (PSV – EDV)/PSV.

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**Figure 1.** Diagram of the left foreleg of a horse. The area scanned for the Doppler ultrasound studies of the distal palmar artery (arrow) was just proximal to the fetlock joint. See online version to view figure in color.
sheds containing covered feeding areas. In addition, animals were placed in dry lots daily for exercise, either conningled in small groups or individually, depending on the temperament of the animal. Water was available ad libitum in the run-in sheds and in the turn-out paddocks. Parts of this study were reported previously in abstract form (Moore et al., 2008).

Each experimental block was divided into 2 periods (P1 and P2) of 14 d each. Period 1 was an adaptation period during which animals received a commercially available textured concentrate (McCauley Brothers Top Ten feed; McCauley Brothers Inc., Versailles, KY; 0.2% BW, as fed, twice daily) and were offered alfalfa cubes in increasing amounts until the amount offered exceeded the amount consumed. Period 2 was the treatment period during which the fescue seed was mixed into the concentrate portion of the diet, and alfalfa cubes were offered ad libitum. Seed was mixed with the grain in twice per day feedings, in increasing amounts ranging from 0.02% BW on d 1 to 0.22% BW on d 11 to 14. The concentration of ergovaline + ergovalinine in the total diet was calculated from the measured feed intakes of the concentrate and hay cubes (Table 2). To image the distal palmar artery, in gray scale M mode, the ultrasound probe was placed in the palmar aspect at metacarpal zone 3A (Sande et al., 1998), and landmarks that included the superficial digital flexor tendon and the deep digital flexor tendon were noted. The probe was then moved laterally on the limb until the distal palmar artery and vein were readily recognized (Sande et al., 1998; Denoix, 2000). Locations of small branches of the palmar vein were used to confirm placement of the probe in the same location for repeated scans in individual animals. For each scan, the artery was imaged in the transverse view using the color Doppler mode and then in the longitudinal view using the duplex color Doppler and Doppler spectral modes. No sedation was given to avoid potential confounding effects on vasomodulation, heart, or respiration rates. Measurements recorded in each view are shown in Table 3.

Each horse was scanned on 4 separate days during P1 and on 4 separate days during P2. Scans were performed on Tuesday and Thursday mornings immediately after the horses finished their morning meal. Period 1 began on a Tuesday morning, and the first scanning day was the next Thursday (d 3 of P1). Period 2 began on a Tuesday morning, and animals were first fed seed treatments in the afternoon feeding that day (d 1 of P2). The first scanning day of P2 was the next Thursday (d 3 of P2), approximately 40 h after first seed treatment feeding. The final treatment feeding was in the afternoon on d 14 of P2, and the final ultrasound scanning occurred the next morning, approximately 15 h after the final treatment feeding.

The sonograms were performed by the same 2 people throughout, and the sonographers were blinded to the seed feeding treatments of each horse. Five replicate scans were performed on each horse on each scanning day and were performed in approximately 15 to 20 min per horse. Animal heart rate (via manually feeling heart beats through the chest wall or by palpation of the digital pulse), respiration rate (visual observation), and rectal temperature (use of a digital thermometer) as well as ambient temperature and relative humidity (digitally recorded at the time of the scans and within 2 feet of the horse) were recorded after scans 3 and 5 on each day.

To determine if E+ fescue seed caused a change in inflammatory cytokine mRNA production, blood samples were taken from each horse on each scanning day. Jugular venous blood samples (2.5 mL) were collected on each scanning day into PreAnlytiXPAXgene Blood

Table 2. Amount of fescue seed offered to each horse on each day in Period 2 and concentration of alkaloids in the ration of each day for horses receiving endophyte positive fescue seed

<table>
<thead>
<tr>
<th>Treatment day</th>
<th>Fescue seed, % of BW</th>
<th>Ergovaline + ergovalinine, ng/g DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.02</td>
<td>76</td>
</tr>
<tr>
<td>2</td>
<td>0.08</td>
<td>337</td>
</tr>
<tr>
<td>3</td>
<td>0.12</td>
<td>400</td>
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<tr>
<td>4 to 9</td>
<td>0.16</td>
<td>556</td>
</tr>
<tr>
<td>10</td>
<td>0.19</td>
<td>668</td>
</tr>
<tr>
<td>11 to 14</td>
<td>0.22</td>
<td>713</td>
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RNA tubes (vacuum tubes containing 6.9 mL Hemogard; Quigen, Balencia, CA) with subsequent isolation of RNA, as described by Breathnach et al., 2006. The corresponding cDNA was analyzed by real-time PCR for the cytokines IL-1, IL-10, interferon-γ (INFγ), and tumor necrosis factor α (TNFα) as described by Breathnach et al. (2006). In addition, a jugular venous blood sample was collected from each horse on d 0, 4, 8, and 14 of P2 to measure serum prolactin concentrations. Prolactin was measured by RIA as described by Colborn et al. (1991). Blood (10 ml) was collected into vacuum tubes and allowed to separate at room temperature. It was then centrifuged (25 min at 2500 x g), and serum was stored frozen (-20 oC) until analysis.

**Statistical Analyses**

Data were analyzed in the Mixed Procedure of SAS using the model Block, Treatment, Day, and Treatment × Day interaction. There was no effect of block for any of the variables measured, so data were combined across blocks and compared using the model Treatment, Day, and Treatment × Day interaction in the Mixed Procedure of SAS for repeated measures, with Day being the days of ultrasound scans. Replicate ultrasound scans were treated as subsamples, and the covariance structure used was compound symmetry with variance components. Using the variance component structure allowed for different variance estimates among treatment groups. When the interaction term was significant, pairwise comparisons were used to determine differences by Day. The relationship of ambient temperature and relative humidity to response variables were tested using Pearson correlation coefficients.

<table>
<thead>
<tr>
<th>Table 3. Physiological response variables recorded with each Doppler ultrasound scan of the palmar artery (5 replicates per horse on each day scanned)</th>
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<tbody>
<tr>
<td>In transverse view</td>
</tr>
<tr>
<td>Diameter, long axis of ellipse, cm</td>
</tr>
<tr>
<td>Diameter, short axis of ellipse, cm</td>
</tr>
<tr>
<td>Mean diameter, cm</td>
</tr>
<tr>
<td>Area, cm²</td>
</tr>
<tr>
<td>Circumference, cm</td>
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<td></td>
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</table>

1In the longitudinal view, the measurements were made with a gate width of 3 mm, a correction angle of –60°, a pulse repetition frequency of 18, and both flow sensitivity and wall filters set to low. Measurements were taken over 3 consecutive cardiac cycles.

**RESULTS**

**Effect of Endophyte-Infected Fescue Seed on Vasoconstriction, Prolactin, and Cytokine mRNA Concentrations**

One filly assigned to receive E+G seed in block 4 was injured during P1 (unrelated to the present study) and was subsequently removed from the experiment, leaving 4 animals receiving E–G seed, 3 receiving E+W seed, and 3 receiving E+G seed.

In P2, horses fed E+G seed had a marked reduction in artery lumen diameter ($P = 0.0033$; Fig. 3 and 4), circumference ($P = 0.0480$), and area ($P = 0.0406$) compared with those fed E–G seed. Horses fed E+G seed had reduced ($P < 0.05$) artery diameter, circumference, and area at the first scanning day of the treatment period, which averaged about 40 h after first fescue seed feeding, and when they received an average of 337 µg/kg ergovaline + ergovalinine (Fig. 3; Table 2). In addition, those horses maintained palmar artery constriction for at least 15 h after final seed feeding (Fig. 3). Horses fed E+W seed had lumen diameter, circumference, and area that were intermediate between the other 2 groups but tended to more closely resemble those in the E–G treatment group. The blood flow variables obtained in the longitudinal view with spectral Doppler ultrasound were highly variable between and within horses on different scanning days, and there were no overall treatment by day interactions in those measurements. However, if E+G vs. E–G were compared for only P2, flow volume was reduced ($P < 0.05$) on each day of the...
treatment period. Taken together, these data confirm that consumption of E+G fescue seed caused constriction of the palmar artery in horses, which could be readily measured by Doppler ultrasonography.

There were no treatment effects on pulse, respiration rate, or rectal temperatures (Table 4), and neither ambient temperature nor relative humidity (Fig. 5) was consistently correlated with any response variable measured. Serum prolactin concentrations varied widely, ranging from below assay sensitivity (0.04 ng/mL) to 2.85 ng/mL, and there were no differences in serum prolactin concentrations among treatment groups ($P > 0.10$). Likewise, cytokine mRNA abundance varied widely within and among animals, and there were no differences among the treatment groups.

**DISCUSSION**

An in vivo model demonstrating that E+ fescue seed causes peripheral vasoconstriction in horses was established, and feeding ground fescue seed was more effective in causing vasoconstriction than was feeding whole seed, presumably by making the alkaloids more available for absorption in the digestive tract. The vasoconstrictive effects of ergot alkaloids are well documented in cattle (Oliver et al., 1993; Aiken et al., 2007, 2009; Klotz et al., 2008) and sheep (Aiken et al., 2011) and have been reported in in vitro assays for horses (Abney et al., 1993; Klotz and McDowell, 2010). In the latter in vitro study, biopsied palmar arteries and veins were exposed to the ergot alkaloids ergotamine, ergonovine, ergocryptine, ergocristine, ergocornine, and lysergic acid as well as norepinephrine and 5-hydroxytryptamine. All alkaloids except lysergic acid produced contractile response, as did norepinephrine and 5-hydroxytryptamine. Interestingly, the palmar artery responded most strongly to ergocornine and the palmar vein responded most strongly to ergonine, suggesting apparent differences in responses in different vessel types.

Changes in physiological responses in vivo have been reported in horses consuming E+ fescue and may be due to peripheral vasoconstriction and hence lessened ability of the animals to dissipate heat effectively. Putnam et al. (1991) made the observation that pregnant mares grazing E+ fescue pasture vs. E– pasture had increased sweating, but there were no differences between the groups in rectal temperatures or apparent effort to avoid midday heat. Vivrette et al. (2001) reported that when horses were exercised by riding in high ambient temperatures, there were increased respiration rates, heart rates, and skin temperatures in the horses grazing E+ vs. E– fescue pastures. However, there were no differences in sweating scores between the 2 groups of animals. Horses consuming E+ fescue seed had greater respiration rates after anaerobic exercise (Webb et al., 2010) but not after light exercise (Webb et al., 2008). Parks et al. (2009) reported that horses on E+ treatment had higher rectal temperatures after exercise than did horses on E– treatment, but treatment did not affect heart rate before, during, or after exercise. Apparent discrepancies in these reports are likely due to

<table>
<thead>
<tr>
<th>Tmt$^1$</th>
<th>P$^2$</th>
<th>Rectal temperature, °C</th>
<th>Heart rate, beats/min</th>
<th>Respirations, breaths/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>E–G</td>
<td>P1</td>
<td>38.6 (0.04)$^3$</td>
<td>41.6 (0.90)</td>
<td>15.6 (0.59)</td>
</tr>
<tr>
<td></td>
<td>P2</td>
<td>37.8 (1.00)</td>
<td>40.9 (1.42)</td>
<td>14.9 (0.55)</td>
</tr>
<tr>
<td>E+W</td>
<td>P1</td>
<td>37.6 (0.05)</td>
<td>40.7 (1.08)</td>
<td>15.6 (0.64)</td>
</tr>
<tr>
<td></td>
<td>P2</td>
<td>37.5 (0.03)</td>
<td>39.5 (0.64)</td>
<td>14.3 (0.46)</td>
</tr>
<tr>
<td>E+G</td>
<td>P1</td>
<td>37.9 (1.51)</td>
<td>40.4 (0.57)</td>
<td>15.4 (1.46)</td>
</tr>
<tr>
<td></td>
<td>P2</td>
<td>38.4 (0.03)</td>
<td>40.3 (0.93)</td>
<td>14.5 (0.79)</td>
</tr>
</tbody>
</table>

$^1$E–G = endophyte negative seed, ground; E+W = endophyte positive seed, whole; E+G = endophyte positive seed, ground.

$^2$P1 = period 1; P2 = period 2.

$^3$Mean (SEM).

**Figure 4.** Marked constriction of the palmar artery of a horse during Period 1 (a) and during Period 2 (b and c). The measurements 0.55 and 0.3 cm indicate vessel lumen diameter. Image b was taken immediately after the horse walked into the stocks for examination (artery is circled), and image c was taken 10 min later. The vessel did not return to pretreatment size at any time during the treatment period. Parts of the palmar vein can be seen in a and c (blue areas).
a variety of different variables in the experiments, such as levels of ergot alkaloids in the various feeds, ambient temperature and humidity, and the relative athletic fitness of the animals.

Although variables associated with cardiac cycles (Table 3) were highly variable, blood flow volume was lowered for E+G vs. E–G treated animals in P2. Likewise, heifers consuming E+ fescue seed had reduced blood flow volume compared with heifers consuming E– seed and remained lower longer when alkaloid concentrations were greater (Aiken et al., 2007, 2009). Those authors concluded that the vasoconstrictive response to E+ TF seed was the primary contributor to reduced flow rates.

No changes in the cytokines IL-1, IL-10, INFγ, and TNFα mRNA were detected among treatment groups in this study. However, Fitzgerald et al. (2007) reported that, for mares on E+ vs. E– pastures, serum INFγ mRNA was increased from March through mid April and IL-1β mRNA was increased from mid April to July, but there were no differences in TNFα or IL-5 mRNA expression at any time evaluated. In addition, Filipov et al. (1999) reported that TNFα and IL-6 were increased in macrophages from mice treated with ergotamine tartrate but that IL-7 β, IL-2, IL-4, and INFγ were not affected. Clearly the role of cytokines in fescue toxicosis, if any, requires further investigation. Although a decrease in serum prolactin concentrations is common in late pregnant mares exposed to E+ TF, no differences were detected in this study. Cytokines may have an inhibitory effect (IL-1), a stimulatory effect (IL-6), or both (TNFα and INFγ) on prolactin secretion (Mandrup-Poulsen et al., 1995). Therefore, it is consistent in this study that neither cytokines nor prolactin were affected by treatment. Prolactin concentrations were generally low (less than 3 ng/mL) and the experiment was performed in the fall and winter (October through December) when prolactin concentrations are less than in the spring and summer (Johnson, 1986; Thompson et al., 1986). In addition, the different ages and sexes of horses used in this study (mature nonpregnant mares, 2-yr-old fillies, and geldings) likely contributed to the low concentrations and high variability of prolactin concentrations.

Although it is well documented that E+ fescue has detrimental health effects in horses, particularly in pregnant mares, a minimum dose of ergot alkaloids necessary to cause those effects is not known. Current studies in our laboratory include dose–response studies to determine the minimal amounts of ergovaline/ergovaline necessary to cause peripheral vasoconstriction in horses. In addition, we are examining the effects of E+ fescue seed on vasomodulation of uterine arteries in pregnant mares as well as blood flow to the corpus luteum in cycling mares. This model of using Doppler ultrasound to monitor vasoconstriction of the palmar artery of mares on E+ fescue pastures may, for the first time, provide veterinarians, farm managers, and research scientists with a convenient and satisfactory response variable to determine premonitory signs of fescue toxicosis. With such a biomarker, decisions can be made to treat affected pregnant mares and/or remove them from undesirable pastures before problems associated with fescue toxicosis at parturition occur.

**LITERATURE CITED**


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**Figure 5.** Ambient air temperature and relative humidity in the barn beside the horse (solid lines, means ± SEM during the scan times) and daily maximum (short dashes) and minimum (long dashes) data on those scan days. Daily maximum and minimum data were obtained from a nearby meteorological weather station (Bluegrass Airport, Lexington, KY, 10 km from the research station site). Ambient temperature or relative humidity were not correlated with any response variable measured.


