Ergot alkaloids induce vasoconstriction of bovine uterine and ovarian blood vessels

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ABSTRACT: Fescue toxicosis is a syndrome that impairs growth and reproduction in cattle grazing endophyte-infected tall fescue [*Lolium arundinaceum* [(Schreb.].) Darbysh)] in the United States, resulting in approximately $1 billion in annual economic loss in species that utilize this forage resource. Approximately 90% of tall fescue contains an endophytic fungus (*Epichloë coenophiala*) that produces ergot alkaloids. Ergot alkaloids cause vasoconstriction and reduced blood flow to the extremities; however, it remains unknown how blood flow to the reproductive organs is affected in cattle. Therefore, the objective of this study was to determine if ergot alkaloids from endophyte-infected tall fescue reduce blood flow to the reproductive organs, thus hindering reproductive function. Angus heifers (n = 36) naïve to ergot alkaloids were placed in Calan gates and randomly assigned to receive either endophyte-infected fescue seed (E+) or noninfected fescue seed (E−; control) in a total mixed ration for 63 d. Weekly measurements were taken to monitor heifer growth and response to ergot alkaloid exposure. Reproductive measurements, including ovarian structures, uterine and ovarian vessel diameter, and hormone concentrations were determined after heifers were synchronized using the standard CO-Synch + 7 d CIDR protocol to ensure all measurements were collected at the same stages of the estrous cycle (0, 4, 10, and 17 d). Data were analyzed using repeated measures in PROC MIXED of SAS. Average daily gain was decreased for the E+ group (0.8 kg/d) compared to control heifers (1.0 kg/d). Body condition scores tended to be greater in control heifers compared to the E+ group (P = 0.053). Additionally, hair coat and hair shedding scores were greater in E+ heifers compared to controls (P < 0.05). Heart rate, rectal temperature, respiration rate, and blood pressure did not differ between treatments (P > 0.05). Vasoconstriction was observed in the caudal artery, but not the caudal vein, in heifers consuming the E+ fescue seed (P < 0.05). No differences were observed in antral follicle counts, corpus luteum area or circulating progesterone concentrations in E+ heifers compared to controls (P > 0.05). There was a significant decrease in the diameter of arteries and veins servicing the ovary and uterus on day 10 and 17 of the estrous cycle. Reduction in blood flow to the reproductive organs during critical times in the estrous cycle may contribute to the reduced ovarian function and pregnancy rates associated with fescue toxicosis.

Key words: fescue toxicosis, ergovaline, reproductive tract

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INTRODUCTION

Reproductive success of beef cattle is very important for the sustainability and profitability of cow-calf operations, which make up a large portion of the beef production systems in the southeastern United States. These systems are mainly small and pasture-based, so optimizing on-farm resources such as land and forages for year-round utilization is essential to overall farm economics. The most prominent pasture species in the southeastern United States is Kentucky-31 tall

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fescue (*Lolium arundinaceum [Schreb.] Darbysh*), a cool-season perennial grass that is known for its persistence and durability (White et al., 1992). These attractive qualities are largely due to an endophytic fungus, *Epichloë coenophiala*, which naturally infects the grass and produces ergot alkaloids, metabolites that have shown to cause adverse effects on livestock performance cumulatively entitled as fescue toxicosis (Aiken and Strickland, 2013). Fescue toxicity symptoms include decreased feed intake and reduced weight gain, elevated respiration rates and salivation, increased body temperatures (Strickland et al., 2011; Klotz, 2015a), and vasoconstriction to the extremities (Klotz et al., 2016). Studies have also shown ergot alkaloids to cause issues with reproductive performance, including decreased pregnancy rates and reduced circulating hormone concentration, such as luteinizing hormone, prolactin, and progesterone (reviewed by Klotz, 2015a, 2015b). The economic losses in the United States due to issues associated with fescue toxicosis in equids and ruminants approach $1 billion annually (estimated by Strickland et al., 2011), so developing a better understanding of how ergot alkaloids negatively affect cattle physiology along with agronomic advancements is essential for improving productivity and profitability of cattle grazing endophyte-infected tall fescue. Thus, the objective of the current study was to determine if chronic exposure of ergot alkaloids from endophyte-infected tall fescue reduces systemic blood flow to the reproductive organs in heifers, thus hindering reproductive function.

MATERIALS AND METHODS

Purebred Angus heifers (n = 36) naïve to ergot alkaloid exposure were purchased from a ranch in Montana and delivered to the Butner Field Research Laboratory in Bahama, NC, in April of 2014. Heifers were acclimated to the working facility for conditioning purposes once per week for 4 wk prior to the treatment period (Fig. 1). Two weeks prior to the start of the treatment period (May 19, 2014), heifers were evaluated to assess growth and reproductive status, via ultrasonography. Heifers averaged 13.3 ± 0.1 mo of age, 342.4 ± 1.5 kg, 5.3 ± 0.03 BCS and were 60.0 ± 0.6% of mature body weight during this initial exam. Furthermore, all 36 heifers displayed various stages of follicular development and/or the presence of corpus luteum on the ovary prior to the start of the treatment indicating that these heifers achieved puberty and cyclicity. Heifers were then blocked by BW and housed in a free-stall barn and transitioned to Calan gates (American Calan, Northwood, NH) for individual

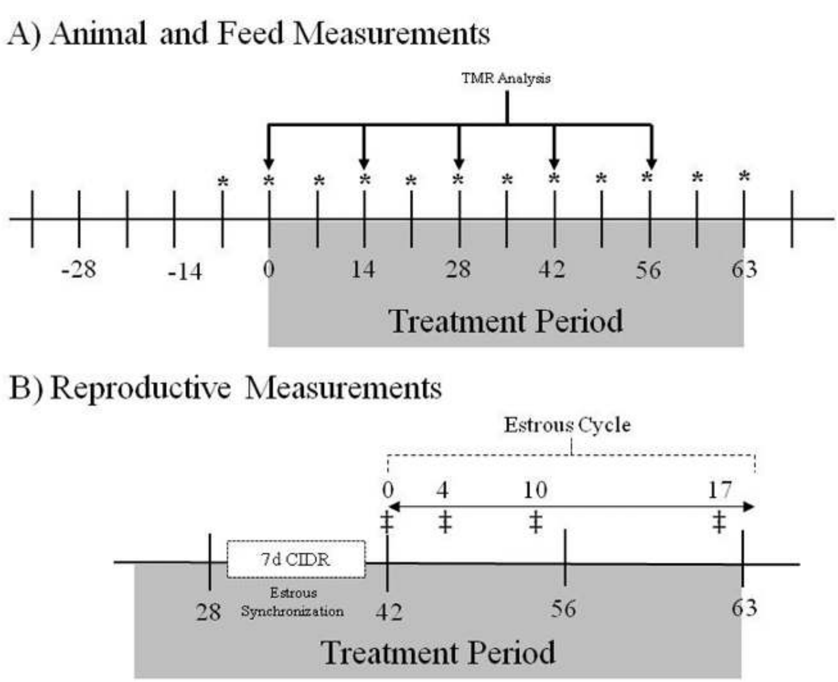

Figure 1. Experimental time line for (A) animal and feed and (B) reproductive measurements used for beef heifers consuming endophyte-infected (E+) and noninfected fescue (E−) seed. (A) Samples of TMR were collected biweekly and tested for nutrient content and ergot alkaloid analysis. Weekly animal measurements (*) included blood sampling, animal weights, body condition scores, respiration and heart rates, hair coat and shedding scores, blood pressure, and rectal temperatures. (B) Reproductive measurements (‡) via Doppler ultrasound consisted of ovarian mapping and characterization of the uterine and ovarian vessel characterization.

feeding. All methods described in this study were approved by the Institutional Animal Care and Use Committee at North Carolina State University (IACUC #13-093).

Barn temperatures were collected daily and ambient temperature and humidity were recorded from the National Weather Service station at Durham 11 W, approximately 40 km from the research station. The temperature-humidity index (**THI**) was calculated using the formula: THI = $T_{db} - [0.55 - (0.55 \times RH/100) \times (T_{db} - 58)]$ where T_{db} represents dry bulb temperature (°F) and RH represents relative humidity (Mader et al., 2002). Daily high ambient temperatures and overall ambient temperatures at 1:00 p.m. remained above the 21 °C thermoneutral housing temperature (Eisemann et al., 2014), and were generally above the ambient temperature stress threshold of 25 °C (Hahn, 1999), above which performance is significantly impacted (Supplementary Fig. 1). Daily low ambient temperatures were generally below the stress threshold, potentially allowing for nighttime recovery from heat stress. However, daily low temperatures recorded in the barn were generally above 21 °C (Supplementary Fig. 1); therefore, these heifers were less likely to experience nighttime recovery while in the barn. The THI in the barn fluctuated above and below the recovery threshold of 75 throughout the study, and rose above the emergency threshold of 85 on multiple days during the middle and end of the trial period (Supplementary Fig. 2).

Treatments

The basal diet consisted of a corn silage total mixed ration (**TMR**) made up of 65.6% corn silage, 9.4% ground fescue seed, and 25% concentrate on a dry matter (DM) basis. The concentrate consisted of 15.2% corn, 8.3% soybean meal, 1.0% limestone, and 0.5% trace mineral salt on a DM basis. Rumensin premix was included to give a final diet concentration of 22 mg/kg, and 4 g Vitamin A, D, and E premix was added to give final diet concentrations of 2,200, 250, and 15 IU/kg diet DM, respectively. Diets were formulated according to National Research Council (1996) requirements for 1 kg/d ADG, and were limit fed at 2% of body weight to equalize DMI. The amount of feed provided per animal was adjusted weekly in response to the increase in heifer BW. This feeding method ensured that the heifers consumed the ground fescue seed treatment, thus refusals rarely occurred. If feed was refused, the amount of refusal was weighed prior to the next day's feeding. Cattle were provided ad libitum access to fresh water.

Following the transition period, heifers were randomly assigned to receive either endophyte-infected fescue seed (E+, 500 μg ergovaline + ergotamine per kg, Southern States KY-31, Southern States Cooperative, Richmond, VA) or noninfected fescue seed (E−, 0 μg ergovaline per kg, Pennington KY-31, Pennington Seed, Inc., Madison, GA) mixed into the corn silage TMR for a 63-d treatment period (Fig. 1). Fescue seed, both E+ and E−, was ground using a hammer mill with a 1.1 cm screen (Meadow Mills, North Wilksboro, NC) prior to being added to the TMR. The TMR and TMR components were sampled biweekly and composited into 2 subsamples for nutrient content analysis by the North Carolina Department of Agriculture and Consumer Service Feed Laboratory as reported by Eisemann et al. (2014; Table 1). Fescue seed was analyzed for alkaloid concentration prior to the experiment (Table 2; University of Missouri Veterinary Medical Diagnostic Laboratory, Columbia, MO) using HPLC according to Rottinghaus et al. (1993). The E+ seed contained a mixture of ergot alkaloids, with ergovaline and ergotamine being the most bioactive (Klotz, 2015b), while the E− seed contained only a trace level of ergovaline. A dietary concentration of 500 μg/kg ergovaline + ergotamine was chosen to induce toxicosis consistent with moderate environmental exposure (Liebe and White, 2018).

Animal Measurements

Baseline measurements were collected 1 wk prior to initiation of treatments. Weekly physiological measurements were collected to evaluate heifer response to ergot alkaloid exposure. Measurements included BW, BCS (1 to 9 scale; Kunkle et al., 1998), respiration rate, and rectal temperature using a digital thermometer (Lumniscope, Graham-Field Health Products, Atlanta, GA). Hair coat score (**HCS**) was evaluated on a scale from 1 to 5 using a modification of the technique described by Olson et al. (2003) where 5 represents a heavy, long hair coat and 1 represents a short slick hair coat. A hair shedding score (**HSS**) was also given using a 1 to 5 scale, 5 representing a hair coat that had not been shed and 1 representing a completely shed hair coat (Gray et al., 2011). Hair coat scores were independent of shedding and characterized the hair present after shedding. To evaluate skin surface temperatures, an 18 × 20 cm rectangle was shaved with electric clippers (#10 blades, Oster Professional Care) posterior to the left scapula. This area was clipped weekly following capture of an image by a thermal imaging camera (Fluke Ti45FT IR Flexcam, Fluke Corporation,

Table 1. Nutritive value (DM basis) of total mixed ration and fescue seed fed to beef heifers for 63 d

Composite sampling		Days 0–42				Days 43–63			
Item	Concentrate[1]	E− seed[2]	E+ seed[3]	E− TMR[4]	E+ TMR[4]	E− seed[2]	E+ seed[3]	E− TMR[4]	E+ TMR[4]
DM (%)	91.4	91.5	91.9	43.6	43.7	91.4	91.5	45.7	43.5
CP (%DM)	22.8	14.5	14.6	10.2	11.0	14.7	13.8	9.7	9.4
NDF (%DM)	7.8	27.8	31.5	31.0	31.0	28.4	28.1	28.5	31.7
ADF (%DM)	4.1	13.4	14.2	19.6	18.1	12.8	13.0	16.7	17.6
TDN[5] (%DM)	79.5	81.8	81.2	78.0	79.4	82.3	82.1	80.7	79.8
Fat (%DM)	2.9	1.4	1.8	3.2	2.9	1.9	1.2	3.1	2.9
Ash (%DM)	8.6	4.8	5.0	4.2	4.1	4.6	4.7	3.8	3.7
Ca (%DM)	1.8	0.2	0.2	0.5	0.5	0.2	0.2	0.4	0.4
P (%DM)	0.4	0.4	0.4	0.3	0.3	0.4	0.4	0.3	0.3
S (%DM)	0.2	0.2	0.2	0.1	0.1	0.2	0.2	0.1	0.1
Mg (%DM)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Na (%DM)	0.4	0.0	0.0	0.1	0.1	0.0	0.0	0.1	0.1
K (%DM)	1.1	0.6	0.5	0.9	0.9	0.5	0.5	0.8	0.8
Cu (ppm)	55	6	7	21	18	6	5	17	15
Fe (ppm)	156	155	76	380	375	78	85	447	426
Mn (ppm)	49	78	61	48	48	53	93	48	46
Zn (ppm)	93	33	34	46	44	33	30	40	36

[1]Concentrate composition (DM basis): 15.2% corn, 8.3% soybean meal, 1% limestone, 0.5% TM salt, 1.8 g/kg Rumensin (Elanco, Indianapolis, IN) 90, 5.4 g/kg Vitamin A, D, and E.

[2]E−: noninfected fescue seed (Southern States KY-31).

[3]E+: endophyte-infected fescue seed (Pennington KY-31)

[4]TMR composition (DM basis): 65% corn silage, 25% concentrate, 10% fescue seed.

[5]TDN = 92.5135 − (0.7965 × ADF).

Table 2. Ergot alkaloid concentration of tall fescue seed

Seed type	E−[1]	E+[2]
Ergosine (µg/kg)	0	1,430
Ergotamine (µg/kg)	0	1,405
Ergocornine (µg/kg)	0	960
Ergocryptine (µg/kg)	0	1,940
Ergocristine (µg/kg)	0	665
Ergovaline (µg/kg)	45	3,910
Total (µg/kg)	45	10,310

Lots of fescue seed were subsampled and analyzed via HPLC at the University of Missouri Veterinary Medical Diagnostic Laboratory (Columbia, MO; Rottinghaus et al., 1993).

[1]E−: noninfected fescue seed (Pennington KY-31).

[2]E+: endophyte-infected fescue seed (Southern States KY-31).

Everett, WA; Huntington et al., 2012; Eisemann et al., 2014). The highest, lowest, and average skin temperatures were recorded in the 18 × 20 cm rectangle using thermal imager software (SmartView 3.5, Fluke Corporation, Everett, WA). Heart rate (**HR**) and caudal systolic and diastolic blood pressure (**BP**) were collected in triplicate with a 16 to 24 cm BP cuff (Lifesource A&D Engineering, Inc., San Jose, CA) placed around the tail at the third caudal vertebra. Due to animal movement, caudal systolic and diastolic BP values were sometimes variable, so outliers within the triplicate exceeding 15% variance were removed from the data set prior to analysis. Caudal vein and artery diameters were measured via Doppler ultrasonography (M-Turbo, SonoSite Inc., Bothell, WA). Weekly blood samples were collected via jugular venipuncture with 20-gauge needles into sterile silicone-coated glass vacutainer serum tubes without additive (Becton Dickinson, Franklin Lakes, NJ) for progesterone and prolactin analysis, while sterile glass vacutainer serum tubes with liquid K3-EDTA were used for hematocrit analysis.

To ensure that all reproductive measurements were taken at the same stage of the estrous cycle, heifers were synchronized using the standard CO-Synch + 7 d CIDR protocol during the treatment period. All heifers received an injection of gonadotropin-releasing hormone (GnRH; 100 µg) and a controlled internal drug release insert (CIDR; 1.38 mg progesterone, Zoetis, Parsippany, NJ) on day 32 of the study. The CIDR remained in the heifers for 7 d. On day 39 of the treatment period, CIDR were removed and heifers received 5 mL of prostaglandin F2α (Lutalyse, 5 mg/mL, Zoetis, Parsippany, NJ). Heifers were observed for estrus and all heifers received a second injection of GnRH (100 µg) on day 42 of the study to ensure that ovulation occurred when desired. Response rate, of 100%, to the estrous synchronization program was confirmed through the disappearance of large follicles and progesterone concentrations < 1 ng/mL on day 42 of the treatment period. Uterine and ovarian characteristics, antral follicular counts, and luteal area were observed via transrectal ultrasound on day 42, 46, 52, and 59 of the treatment period, representing day 0, 4, 10, and 17 of the estrous cycle. Uterine and ovarian vein and artery vessel area were measured via Doppler ultrasonography (M-Turbo, SonoSite Inc., Bothell, WA). Since ovarian activity impacts blood flow (Acosta et al., 2003), uterine and ovarian vein and artery measurements were collected on the vessels ipsilateral to the corpus luteum. For anatomical constancy between heifers, uterine vein and arterial measurements were recorded at the bifurcation of the uterus, and ovarian vein and arterial measurements were collected at the hilus of the ovarian vessels ipsilateral to the corpus luteum. Serum concentrations of progesterone were analyzed on 42, 46, 52, and 59 of the treatment period, representing day 0, 4, 10, and 17 of the estrous cycle. Progesterone concentrations were determined using Immuchem Coated Tube Progesterone 125I RIA assays (ICN Parmaceuticals, Inc., Costa Mesa, CA) and were counted for 1 min using the Cobra II Auto Gamma Counter (Packard Instrument Company, Meriden, CT). The intra- and inter-assay coefficients of variation were 4.8% and 6.9%, respectively. Serum concentrations of prolactin were analyzed on day 0, 14, 28, 42, and 56 of the treatment period. Sample concentrations were determined using a commercially available Bovine Prolactin ELISA kit (MyBioSource, San Diego, CA). This assay was validated through a series of spike-and-recovery experiments as well as linearity-of-dilution experiments, and the results were in accordance with the expectations based on manufactures recommendations. A total of 5 assays were completed and a control sample was included in each assay. The inter-assay coefficient of variation based on the duplicate sample controls was 8.8%, and the intra-assay coefficient of variation was 9.6%.

Statistical Analyses

Data were analyzed using repeated measures in PROC MIXED of SAS with least squared means (SAS Institute, 1999). The model included treatment and sample date, and the experimental unit was animal within each treatment. Terms with a significance value of $P > 0.20$ were removed from the complete model in a stepwise manner to derive the final reduced model for each variable. P values of ≤0.05 represented significant differences, and P values of $0.05 \le P \le 0.10$ were defined as tendencies.

RESULTS AND DISCUSSION

Animal Feed Intake and Performance

Daily intake for heifers on the E+ diet was not different compared to heifers on the E− diet (18.1 and 18.5 kg/d, respectively; $P > 0.05$). However, heifers consuming the E− diet had a greater average daily gain (ADG) compared to heifers on the E+ diet (1.05 and 0.81 kg/[hd * d], respectively; $P = 0.001$). Although reduced intake has been seen in cattle consuming E+ tall fescue primarily due to the presence of ergot alkaloids (Matthews et al., 2005; Panaccione et al., 2006; Drewnoski et al., 2009), the designed feeding regiment in the current study controlled daily intake so that the changes in reproductive processes were reflective of ergot alkaloid exposure and not cofounded by reduced intake.

The reduction in ADG lead to significantly lower BW by the end of the treatment period for the heifers fed the E+ treatment (414.3 ± 3.6 and 399.3 ± 3.5 kg for E− and E+ treatments, respectively; $P = 0.0033$). Furthermore, there was a treatment × day interaction on heifer BW (Fig. 2). Body weight of heifers consuming the E+ diet started to decrease after 21 d of exposure to the ergot alkaloids (Fig. 2). Although all heifers had acceptable BCS throughout this study, BCS tended to decrease in heifers consuming the E+ diet compared to heifer consuming the E− diet (5.25 vs. 5.15, respectively; $P = 0.054$; Table 3). In addition, heifers consuming the E+ diet exhibited a negative (−0.025) change in BCS compared to heifer consuming the E− diet that exhibited a positive (0.338) change in BCS throughout the treatment period ($P = 0.0001$). When considering the interaction between treatment and sample time, heifers consuming the E− diet had a higher BCS than heifers consuming the E+ diet after 49 d of exposure to the ergot alkaloids ($P < 0.05$). It is well established that temperatures greater than 25 °C are associated with decreased feed intake and growth performance (Hahn et al., 1992). The combination of elevated THI (Supplementary Fig. 2) and chronic exposure to ergot alkaloids may have attributed to the gradual decrease in ADG, BW, and BCS throughout the treatment period.

Numerous studies have reported that cattle consuming E+ tall fescue have retained and rough hair coat (reviewed by Strickland et al., 2011). The mechanism for this physiological effect is not well understood; however, retained hair coats in cattle consuming E+ tall fescue exacerbate heat stress during the summertime, thereby worsening the severity of "summer slump" (Bond et al., 1984). As expected, HCS and HSS decreased throughout the treatment period in response to environmental cues, such as increased ambient temperature and day length, (Fig. 3). Additionally, both HCS and HSS were higher ($P = 0.0020$ and 0.0069, respectively; Table 3; Fig. 3) for heifers consuming the E+ diet (3.7 and 3.5 for HCS and HSS, respectively) compared to heifers consuming the E− diet (3.1 and 2.9 for HCS and HSS, respectively). While heifers consuming the E+ diet had greater HCS and HSS, exposure to alkaloids did not affect the rectal temperatures compared to heifers consuming the E− diet ($P = 0.9750$; Table 3). Under the influence of E+ tall fescue, previous studies have reported elevated rectal temperatures between 0.4 and 1.2 °C compared to controls (Burke et al., 2001; Parish et al., 2003; Burke et al., 2006). Similar to rectal temperature, there was no difference in skin surface temperature between treatments (36.2 and 36.1 °C, respectively; $P = 0.4459$; Table 3). Eisemann et al. (2014) fed diets containing E+ or E− fescue seed to steers (approximately 285 ppb ergovaline for E+) and recorded skin surface temperatures using similar methodology. The steers consuming E+ fescue seed had higher skin surface temperatures than those on the E− diet, suggesting that vasodilation occurred in order to transfer a greater amount of core body heat to the skin surface. While the concentration of ergot alkaloids fed in the current study was consistent with previous studies (Aldrich et al., 1993; Eisemann et al., 2014), the lack of difference observed between treatments in skin surface temperatures was unexpected, especially since

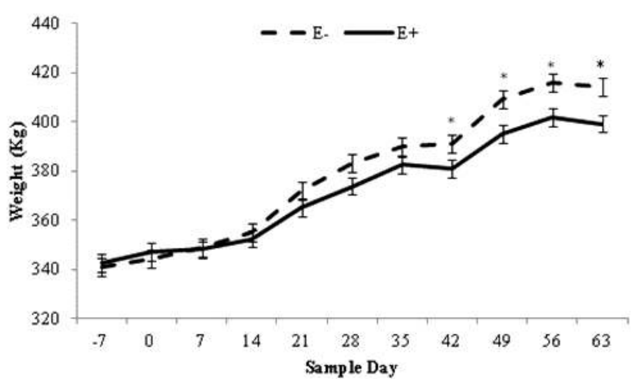

Figure 2. Change in body weight (kg) of beef heifers consuming endophyte-infected (E+) and noninfected fescue (E−) seed throughout the treatment period. *Effect of treatment ($P < 0.0001$); treatment × day interaction ($P < 0.0001$).

Table 3. Physiological parameters in beef heifers chronically exposed to either endophyte-infected (E+) and noninfected fescue (E−) seed for 63 d

				Seed	Time	Interaction
Item	E−[1]	E+[2]	SEM	*P*-value	*P*-value	*P*-value
BCS[3]	5.25*	5.15*	0.03	0.054	<0.0001	0.067
HCS[4]	3.1[a]	3.7[b]	0.12	0.0020	<0.0001	0.0004
HSS[5]	2.9[a]	3.5[b]	0.16	0.0069	<0.0001	<0.0001
Rectal temperature, °C	39.2	39.2	0.05	0.9750	<0.0001	0.5379
Surface temperature, °C	36.1	36.2	0.17	0.4459	<0.0001	0.0127
Caudal artery area, mm^2	35.5[a]	33.6[b]	0.56	0.0216	0.0017	0.031
Caudal vein area, mm^2	44.0	42.3	0.74	0.1223	<0.0001	0.6155
Systolic pressure, mmHg	125.4	121.8	2.5	0.3049	0.1154	0.3985
Diastolic pressure, mmHg	68.0	65.1	2.3	0.3872	0.1799	0.1743
Hematocrit[6]	34.1	34.4	0.48	0.7077	<0.0001	0.2925
Heart rate[7]	71.9	76.4	1.9	0.1111	<0.0001	0.6324
Respiration rate[8]	59.5	60.3	1.0	0.6054	<0.0001	0.1279

[a,b]Means with differing superscripts indicate $P < 0.05$.
[1]E−: noninfected fescue seed (Southern States KY-31).
[2]E+: endophyte-infected fescue seed (Pennington KY-31).
[3]BCS = body condition scores (1–9 scale).
[4]HCS = hair coat scores (1–5 scale).
[5]HSS = hair shedding scores (1–5 scale).
[6]Hematocrit is represented by the percentage of packed red cells in blood.
[7]Heart rate represented by heart beats per minute.
[8]Respiration rate represented by breaths per minute.
*Indicate a tendency at $0.05 < P < 0.1$.

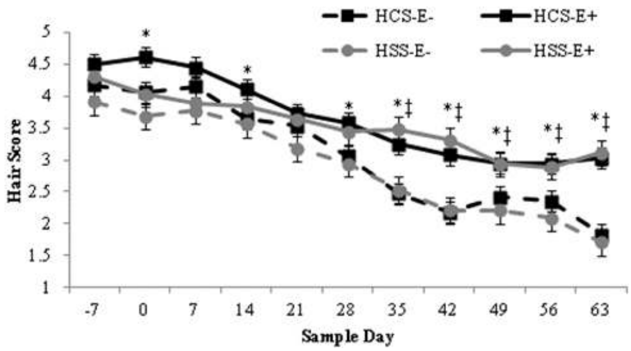

Figure 3. Changes in hair coat score (HCS, black lines) and hair shedding score (HSS, gray lines) in beef heifers chronically exposed to either endophyte-infected (E+) or noninfected fescue (E−) seed. As expected, HCS and HSS decreased throughout the treatment period. *Effect of treatment (P = 0.002); treatment × day interaction (P = 0.0004) for HCS. ‡Effect of treatment (P = 0.0069); treatment × day interaction (P < 0.0001) for HSS.

ambient and barn temperatures were above 25 °C during the treatment period, which has been shown to elevate core body temperatures of cattle (Scharf et al., 2011). In contrast to previous studies, heifers in the current study were housed in a covered barn and were not directly influenced by sunlight, thereby reducing the possibility of solar heat-induced stress, which may explain the discrepancies in body temperatures observed in this study.

While previous studies have shown that cattle consuming E+ tall fescue have elevated respiration rates to counteract increased body temperatures (Browning et al., 1998; Burke et al., 2001), no difference in respiration rate was observed in heifers consuming the E+ diet compared to heifers consuming the E− diet (P = 0.6054; Table 3). In addition to respiration rate, evaluating hematocrit can provide a means to analyze the oxygen carrying capacity of an animal's circulatory system and level of dehydration (Nordenson, 2006), both of which may be impacted following chronic exposure to ergot alkaloids. Reports of hematocrit values in cattle range from 41% to 85% (Reeves et al., 1962; Kirk and Davis, 1970) depending on diet, elevation, and time of year. Hematocrit values in the current study ranged from 33.1% to 35.5% packed cells for the E− group and 32.9% to 35.1% for the E+ group, lower than the ranges reported in the literature, and there was not a significant interaction between treatment and sampling date (P = 0.2925; Table 3). Previous studies have shown that cattle consuming E+ tall fescue have elevated HR (Walls and Jacobsen, 1970; Bond et al., 1984); however, no difference in HR was observed in heifers consuming the E+ diet compared to heifers consuming the E− diet (P = 0.1111; Table 3). Walls and Jacobsen (1970) found increased HR in a heat-stressed environment versus a thermoneutral setting (76 beats per min in 34.8 °C and 59.3 beats per min in 15 °C), and the addition of E+ tall fescue extracts to the diet resulted in greater HR at 15 °C (69 beats per min vs. 61 beats per min for E+ and E−, respectively). Additionally, cattle consuming E+ fescue seed have previously shown reduced BP as compared to animals consuming E− fescue seed in thermoneutral conditions (Eisemann et al., 2014); however, studies involving heat stress have reported increased BP due to dietary ergot alkaloids as well as acute responses to ergotamine tartrate or ergonovine maleate injection (Browning and Leite-Browning, 1997; Browning, 2000). In the current study, both systolic and diastolic BP did not differ between heifers consuming the E+ diet compared to heifers consuming the E− diet (P > 0.05; Table 3); however, there was an initial increase in both systolic and diastolic BP in heifers consuming the E+ diet following the first week of ergot alkaloid exposure before decreasing below the E− group throughout the rest of the treatment period. Average daily high barn temperature was 27 °C and overall average THI was 72 (Supplementary Figs. 1 and 2). Although the barn temperature exceeded the thermoneutral housing threshold of 21 °C (Eisemann et al., 2014), the average THI did not exceed the threshold of 75 for thermal stress-limiting measures (Hahn, 1999). While the addition of the ergot alkaloids was expected to reduce the ability of the heifers to tolerate the hot and humid conditions, the barn environment in combination with ergot alkaloid concentration may not have been extreme enough to elicit physiological responses, such as increased rectal and surface temperature, hematocrit, and heart and respiration rate, associated with heat stress. Whereas the vasoconstriction of caudal, ovarian, and uterine vessels observed in this study, demonstrate the direct effects of ergot alkaloids, i.e., ergovaline, acting through adrenergic receptors as previously described by Klotz (2015b), even in the absence of a prolactin response.

Ergot Alkaloids and Vasoconstriction

Ergot alkaloids in tall fescue have been shown to cause decreased serum prolactin concentrations (Fanning et al., 1992), vasoconstriction, and reduced blood flow to the peripheral arteries (Aiken et al., 2007, reviewed by Klotz, 2015a, 2015b) leading to altered physiological processes. In the current study, area of the caudal vessels was monitored throughout the treatment period to evaluate peripheral vasoconstriction. Heifers consuming the E− diet had greater caudal artery area compared to heifers consuming the E+ diet (35.5 vs. 33.6 mm^2, respectively; P = 0.0216; Table 3). However, no differences were observed in caudal vein area between the 2 treatment groups (43.2 mm^2 average; P = 0.1223; Table 3). Ergot alkaloids bind to biogenic amine receptors in the muscles responsible for contractions (Dyer, 1993; Oliver et al., 1998), potentially causing the reduction in artery, but not venous, area seen in heifers consuming the E+ diet. The capacity of ergot alkaloids to reduce blood flow to peripheral tissues through vasoconstriction and thickening of small peripheral vessels was shown in this trial as well as other studies (Aiken et al., 2007, Klotz et al., 2016); however, literature describing the extent to which vasoconstriction occurs to the internal organs is limited (reviewed by Klotz, 2015a, 2015b). Uterine and ovarian vein and artery vessel areas were measured on day 0, 4, 10, and 17 of the estrous cycle (day 42, 46, 52, and 59 of the treatment period, respectively), representing critical stages within the reproductive cycle. Uterine artery and vein vessel areas were not different between heifers consuming the E− and E+ diets on day 0 and 4 of the estrous cycle (P > 0.05; Fig. 4A). However, heifers consuming the E− diet had greater uterine artery and vein areas compared to heifers consuming the E+ diet (P < 0.05; Fig. 4A) on day 10 and 17 of the estrous cycle when progesterone concentrations are greatest. Interestingly, this reduction in uterine vessel area occurred prior to the timing of maternal recognition of pregnancy (day 14 to 16), which could reduce hormonal communication between the ovary and uterus during this time of embryo development, thereby decreasing pregnancy retention (Roberts et al., 1996). Similar to the uterine artery, the ovarian artery area was not different between heifers consuming the E− and E+ diets on day 0 and 4 of the estrous cycle (P > 0.05; Fig. 4B), whereas heifers consuming the E+ diet had a reduced ovarian artery area compared to heifers consuming the E− diet (P < 0.05; Fig. 4B) on day 10 and 17 of the estrous cycle. Intriguingly, heifers consuming the E− diet had a greater ovarian vein area compared to heifers consuming the E+ diet (P < 0.05; Fig. 4B) on day 0 and 17 of the estrous cycle, when progesterone concentrations are low and estrogen concentrations are increasing. Previous studies have demonstrated estrogen-induced vasodilation in various arteries and veins (reviewed by Miller and Duckles, 2008); however, this effect was not observed in heifers consuming the E+ diet, thus ergot alkaloids may inhibit estrogen-induced regulation of the vascular system.

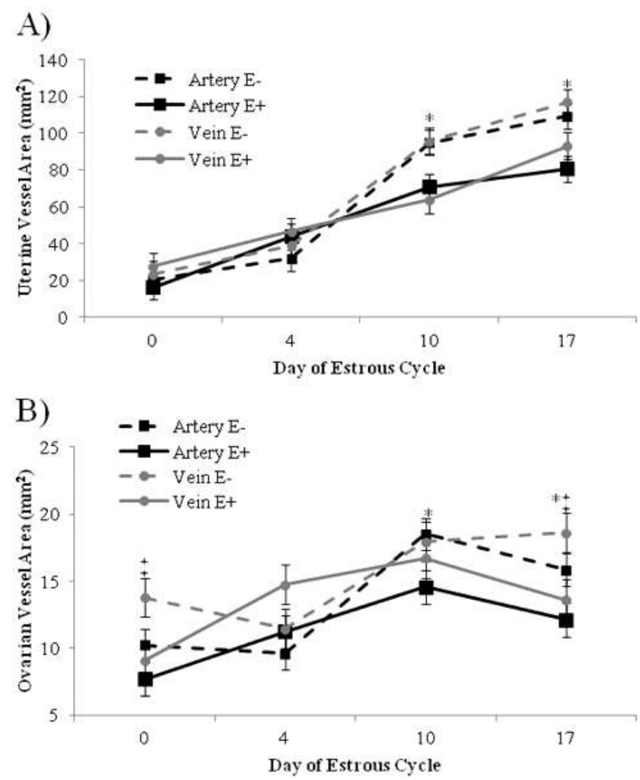

Figure 4. Changes in uterine (A) and ovarian (B) artery (black lines) and vein (gray lines) area (mm^2) in beef heifers chronically exposed to either endophyte-infected (E+) and noninfected fescue (E−). Vessel measurements were taken at critical times (day 0, 4, 10, and 17) based on ovarian activity within the estrous cycle. (A) *Effect of treatment (P < 0.01); treatment × day interaction (P < 0.01). (B) *Effect of treatment (P < 0.01); treatment × day interaction (P < 0.05) for ovarian artery. ‡Effect of treatment (P < 0.01); treatment × day interaction (P < 0.01) for ovarian vein.

Ergot Alkaloids and Reproductive Performance

Ergot alkaloids in tall fescue cause decreased progesterone concentrations in cattle and from extracted luteal tissue from heifers consuming diets with or without ergot alkaloids (Jones et al., 2003). However, others did not observe differences in circulating progesterone concentrations in cattle consuming endophyte-infected tall fescue (Fanning et al., 1992; Burke and Rorie, 2002; Seals et al., 2005. In agreement with other reports, the current study found no differences in progesterone concentrations (P = 0.7408) or luteal area (P = 0.4088) between heifers consuming E+ and E− diets (Table 4).

Decreases in serum prolactin have been associated with fescue toxicity and are often used as an indicator of ergot alkaloid exposure in cattle (Fanning et al., 1992). Increased prolactin concentrations are associated with increasing day lengths and ambient temperatures, and initiate hair

Table 4. Reproductive parameters in beef heifers chronically exposed to either endophyte-infected (E+) and noninfected fescue (E−) seed for 63 d

Item	E−[1]	E+[2]	SEM	Seed *P*-value	Time *P*-value	Interaction *P*-value
Reproductive hormones						
Progesterone, ng/mL	2.58	2.48	0.22	0.7408	<0.0001	0.3662
Prolactin, ng/mL	195.7	167.2	17.4	0.2439	0.0011	0.2427
Follicle characteristics						
Class 0[3]	2.2	2.2	0.30	0.9111	0.7053	0.7282
Class 1[4]	3.5	3.8	0.25	0.4571	0.1950	0.8246
Class 2[5]	1.9[a]	1.3[b]	0.12	0.0018	0.0565	0.0236
Class 3[6]	1.7	1.1	0.31	0.1253	0.0365	0.1006
Ovarian structure sizes						
Ovulatory follicle, mm	12.3	12.8	0.28	0.2541	0.0846	0.0016
Luteal area, mm^2	288.1	273.2	10.6	0.4088	<0.0001	0.7007

[a,b]Means with differing superscripts indicate $P < 0.05$.

[1]E−: noninfected fescue seed (Southern States KY-31).

[2]E+: endophyte-infected fescue seed (Pennington KY-31).

[3]Class 0 = antral follicle size <2.9 mm.

[4]Class 1 = antral follicle size 3.0 to 5.9 mm according to Burke and Rorie (2002).

[5]Class 2 = antral follicle size 6.0 to 9.9 mm according to Burke and Rorie (2002).

[6]Class 3 = antral follicle size ≥10 mm according to Burke and Rorie (2002).

shedding (Smith et al., 1977; Igono et al., 1988). As expected, prolactin concentrations increased in both groups throughout the treatment period in response to environmental changes (increased day length and ambient temperature); however, no statistical differences were observed in heifers consuming E+ diet compared to heifers consuming E− diet (*P* = 0.2439; Table 4). While the heifers consuming the E+ diet displayed many other symptoms of fescue toxicosis, the lack of hypoprolactinemia was unexpected. The interaction of increased THI and exposure to ergot alkaloids impacts the severity of fescue toxicosis (reviewed by Strickland et al., 2011) and the relatively low inclusion rate of the ergot alkaloid into the E+ diet accompanied by the lower THI in the current study may have prevented the depression in serum prolactin concentration.

Previous studies examining changes in follicular number on the ovaries described no difference in the number of class 1 (3 to 5 mm) and class 3 (>10 mm) ovarian follicles in cows grazing E+ and E− fescue pastures (Burke and Rorie, 2002). Conversely, the number of class 2 (6 to 9 mm) follicles were reduced in cows grazing E+ fescue compared to cows grazing E− fescue. In the current study, no differences were observed in the number of class 0 (<2.99 mm), 1, or 3 follicles on the ovaries of heifers consuming the E− and E+ diets; however, number of class 2 (6 to 9 mm) follicles were less (*P* = 0.0018) in heifers consuming the E+ diet compared to heifers consuming the E− diet (Table 4). Burke and Rorie (2002) observed that the diameter of the largest follicle tended to be larger in cows grazing E− fescue between day 7 and 12 of the estrous cycle. In contrast, there was no difference in the size of the ovulatory follicle on the ovary of heifers consuming the E− and E+ diets in the current study (*P* = 0.2541; Table 4). The decrease in 6 to 9 mm follicles on the ovaries suggests that exposure to ergot alkaloids may hinder follicular selection though inadequate delivery of nutrients and steroid precursors due to insufficient blood flow to the reproductive organs. Additionally, a reduced systemic blood flow would negatively affect reproductive cyclicity by hindering ovarian hormonal feedback to the brain (Burke et al., 2001).

Fescue toxicosis is a multifaceted syndrome resulting from chronic exposure to ergot alkaloids that has devastating effects on growth and reproductive performance in beef cattle grazing endophyte-infected tall fescue. Based on these data, chronic exposure to ergot alkaloids from endophyte-infected tall fescue seed reduced ovarian and uterine vessel area which, without a change in BP as was seen in this study, likely restricted blood flow to the reproductive organs. Reduced blood flow to the reproductive organs during critical times in the estrous cycle may contribute to reduced ovarian function and pregnancy rates associated with fescue toxicosis. While temporary solutions have been suggested to mitigate the symptoms of this syndrome, developing a better understanding of the mechanistic action of the ergot alkaloids on the reproductive tract will lead to new and innovative ways to improve reproductive function in cattle consuming endophyte-infected fescue.

SUPPLEMENTARY DATA

Supplementary data are available at *Journal of Animal Science* online.

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