Effects of grazing different ergovaline concentrations on vasoactivity of bovine lateral saphenous vein[1]

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ABSTRACT: Previous research has demonstrated that exposure to ergot alkaloids reduces vasoactivity of serotonin (5HT) receptors. Chemical suppression of tall fescue seedhead production is a tool to reduce the level of exposure to ergot alkaloids by a grazing animal. Therefore, the objective was to evaluate contractility of lateral saphenous veins biopsied from mixed breed steers following a 87- to 101-d grazing period on 3-ha pastures of bermudagrass (*Cynodon dactylon*; $n = 5$ steers; BW = 340 ± 9 kg), or toxic endophyte-infected tall fescue (*Lolium arundinaceum*) that was not treated ($n = 5$ steers; BW = 300 ± 6; 0.56 ppm ergovaline) or was treated ($n = 5$ steers; BW = 294 ± 9 kg; 0.24 ppm ergovaline) with herbicide containing aminopyralid and metsulfuron-methyl. To evaluate contractility, biopsied veins were mounted in a multimyograph and exposed to increasing concentrations of a tall fescue seed extract (EXT; ergovaline source) and $5HT_{1B}$ (CP 93129), $5HT_{1D}$ (L-694,247), and $5HT_{2A}$ (TCB2) agonists. All contractility data were normalized to a maximal response of 1×10^{-4} M norepinephrine and were analyzed as a split plot treatment design using SAS for effects of pasture treatment, agonist concentration, and the interaction. There was no contractile response to any concentration of $5HT_{1B}$ agonist in any of the pasture treatments. There were pasture × concentration interactions for contractile responses to $5HT_{2A}$ agonist ($P < 0.01$) and EXT ($P < 0.01$). For both EXT and TCB2, veins from bermudagrass steers were more vasoactive to the higher concentrations of these compounds ($P < 0.05$), and there were no differences between veins collected from the unsuppressed or seedhead-suppressed treatments ($P = 0.66$). There was also a pasture × concentration interaction for the contractile responses to $5HT_{1D}$ agonist ($P < 0.01$). However, these responses were not sigmoidal and reached a zenith at 5×10^{-7} and 1×10^{-6} M. At these concentrations, the response was greatest for veins from the unsuppressed treatment ($P < 0.05$) and did not differ between veins from suppressed and bermudagrass treatments ($P = 0.41$). Although reduced levels of ergovaline in seedhead-suppressed pastures did not alter vasoactivity of $5HT_{2A}$ or $5HT_{1B}$ receptors in the lateral saphenous vein, elevated vasoactivity of $5HT_{1D}$ in veins from unsuppressed tall fescue pasture treatment suggests that lower ergovaline levels in seedhead-suppressed pastures can influence the vascular effects of ergot alkaloids.

Key words: cattle, ergot alkaloids, serotonin receptors, tall fescue, vasoconstriction

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INTRODUCTION

Fungal production of ergot alkaloids is a global problem for pasture- and grain-based livestock feeding operations (Klotz and Smith, 2015). Consumption of ergot alkaloids by livestock disrupts numerous biological processes resulting in numerous clinical signs and productive losses in livestock (Klotz, 2015). This is exemplified in the United States with the endophytic infestation of tall fescue (*Lolium arundinaceum*) by *Epichloë coenophiala* and results in the syndrome known as fescue toxicosis. Many signs of fescue toxicosis have been related to the negative effects ergot alkaloids have on vascular circulation (Oliver, 1997).

Vascular effects of ergot alkaloids are mediated by the toxin's ability to interact with various biogenic amine receptors (Berde and Stürmer, 1978; Pertz and Eich, 1999). Specifically, serotonin receptors have been directly implicated in ergot alkaloid–induced vasoconstriction across multiple species and vessel types (Dyer, 1993; Schöning et al., 2001; Klotz et al., 2013). There are 14 subtypes of serotonin receptors, and several are expressed in vascular smooth muscle and endothelial layers (Raymond et al., 2001). Previous work has demonstrated a decreased vasoactivity of serotonin receptors in blood vessels associated with cattle that had consumed ergot alkaloids (Klotz et al., 2012; Egert et al., 2014; Klotz et al., 2016). A strategy has been developed to mitigate the effects of fescue toxicosis by chemical suppression of tall fescue seedhead production (Aiken et al., 2012; Goff et al., 2012) and thereby reduce pasture ergovaline concentrations. It was hypothesized that a reduced concentration of ergovaline in tall fescue pastures will diminish the associated vascular effects. Thus, the objective was to evaluate the vasoactivity of serotonin receptors in lateral saphenous veins biopsied from steers grazing nontoxic bermudagrass (*Cynodon dactylon*), toxic endophyte–infected tall fescue untreated, and treated to suppress seedhead production.

MATERIALS AND METHODS

All experimental procedures involving live steers were approved by the University of Kentucky Institutional Animal Care and Use Committee and conducted at the University of Kentucky C. Oran Little Research Center in Woodford County (38°5′N, 84°44′W).

Animals and Pastures

Fifteen, *Bos taurus*, predominantly Angus crossbred steers were used at the conclusion of a larger grazing study that evaluated grazing management of toxic endophyte–infected tall fescue pastures chemically treated to suppress production (Williamson et al., 2016; Williamson and Aiken, 2017). This study used 3 pastures of either bermudagrass (n = 5 steers; BW = 340.3 ± 9.3 kg), toxic endophyte–infected Kentucky-31 tall fescue untreated (n = 5 steers; BW = 300.5 ± 6.5 kg), or toxic endophyte–infected Kentucky-31 tall fescue treated (n = 5 steers; BW = 294.1 ± 9.1 kg) with Chapparal herbicide mixture (Dow AgroSciences, Indianapolis, IN) to provide 87.1 g/ha of an aminopyralid (2-pyridine carboxylic acid, 4-amino-3,6-dichloro-) and 13.2 g/ha of metsulfuran-methyl (methyl 2-[[[[(4-methoxy-6-methyl-1,3,5- triazin-2-yl)- amino]carbonyl] amino]sulfonyl]benzoate) that were divided into six 3.0-ha paddocks. For the tall fescue pastures, there was a 90% endophyte infection level as determined using immunoblot test kits (Agrinostics Ltd. Co., Watkinsville, GA). The 3.0-ha paddocks were rotationally stocked with 2 steers/ha as described by Williamson and Aiken (2017). Steers grazed these pastures 87 to 101 d prior to lateral saphenous vein biopsies. Five steers were sampled from each pasture treatment (steers were randomly chosen from different 3.0-ha paddocks within treatment) and subjected to a lateral saphenous vein biopsy from the right hind leg over the course of a 2-wk period. Steers had ad libitum access to water and mineral supplement (Burkmann Mills, Danville, KY) during the study.

Lateral Saphenous Vein Biopsy and Myograph Experiments

Cranial branches of lateral saphenous veins were biopsied and processed as described in detail by Klotz et al. (2008). Briefly, a steer was restrained in a left lateral recumbency using a tilt table (Spring-O-Matic Inc., Marion, KS). Hair was clipped and biopsy site on the right hind limb was cleaned with povidone-iodine soap and disinfected with 70% ethanol. The surgical site was locally anesthetized with a line block using 2% lidocaine (2% injectable; Lidoject, Henry Schein Animal Health, Dublin, OH) that was applied proximal to the incision line. Once the cranial branch of the lateral saphenous vein was identified, ligatures were placed, the isolated section (~ 3 cm) of vein was removed, and the skin was sutured. Isolated venous tissue was placed in a modified Krebs–Henseleit oxygenated buffer solution (95% O_2/5% CO_2; pH = 7.4; mM composition = D-glucose, 11.1; $MgSO_4$, 1.2; KH_2PO_4, 1.2; KCl, 4.7; NaCl, 118.1; $CaCl_2$, 3.4; and $NaHCO_3$,

24.9; Sigma Chemical Co., St. Louis, MO) for transport back to the laboratory, and kept on ice until processed (approximately 4 °C). Immediately after the biopsy, steers received penicillin (Procaine G, 6,600 U/kg of BW; Butler Schein Animal Health, Dublin, OH) and flunixin meglumine (Flunixiject, 1.1 mg/kg of BW; IVX Animal Health Inc., St. Joseph, MO). Administration of flunixin meglumine was continued for 3 d postoperatively. One steer from each pasture treatment was biopsied on each given biopsy day.

Biopsied blood vessels were used in vitro to evaluate changes in contractile response to increasing concentrations of serotonergic agonists and ergovaline. Postbiopsy processing for all vein segments followed methods validated by Klotz et al. (2006). Preparation of vein samples for experiments consisted of removal of excess fat and connective tissue. Cleaned segments were sliced into 2- to 3-mm cross sections using an adjustable acrylic channel tissue matrice (Braintree Scientific, Braintree, MA). Venous cross sections were examined using a dissecting microscope (Stemi 2000-C, Carl Zeiss Inc., Oberkochen, Germany) at 12.5× magnification to measure dimensions for assurance of consistent segment size and to verify physical integrity of tissue. Cross sections were suspended horizontally in a 5-mL tissue bath (DMT610M multichamber myographs, Danish Myo Technologies, Atlanta, GA) containing continuously oxygenated modified-Krebs Henseleit buffer (95% O_2/5% CO_2; pH = 7.4; 37 °C), with 3×10^{-5} M desipramine and 1×10^{-6} M propranolol (Sigma Chemical Co.) to inactivate catecholamine-neuronal uptake and β-adrenergic receptors, respectively. After equilibration to 1 g of tension (~1.5 h), veins were exposed to the α-adrenergic agonist norepinephrine (1×10^{-4} M; Sigma Chemical Co.) to verify tissue viability and for subsequent use as a reference for normalization of corresponding contractile response data.

Cross sections of lateral saphenous veins were run in duplicate from each steer for each contractile response treatment. Following recovery from the 1×10^{-4} M norepinephrine addition (45 to 60 min) and reestablishment of the 1-g baseline tension, agonist or alkaloid additions occurred in 15-min intervals. Each 15-min interval consisted of a 9-min treatment incubation period, followed by a washout period during which 5-mL aliquots of buffer without treatment were incubated with the vein segment for 2 consecutive 2.5-min periods, followed by a final buffer replacement and 1-min incubation prior to the next addition.

Treatment additions consisted of an ergot alkaloid, ergovaline that was provided in the form of a toxic endophyte-infected tall fescue seed extract produced as described by Ji et al. (2014) and validated against pure ergovaline in bovine lateral saphenous veins by Foote et al. (2012), and serotonin (5HT) receptor agonists selective for $5HT_{1B}$ (CP 93129; 1,4-dihydro-3-(1,2,3,6-tetrahydro-4-pyridinyl)-5*H*-pyrrol[3,2-*b*]pyridin-5-one dihydrochloride; Tocris-Biotechne, Minneapolis, MN), $5HT_{1D}$ (L-694,247; 2-[5-[3-(4-methylsulfonylamino)benzyl-1,2,4-oxadiazol-5-yl]-1*H*-indol-3-yl]ethanamine), and $5HT_{2A}$ ((4-bromo-3,6-dimethoxybenzocyclobuten-1-yl) methylamine HCl, TCB2; Tocris-Biotechne). The contractile response curves for the 5HT receptor agonists were constructed with 10 consecutive additions every 15 min at fixed concentrations ranging from 5×10^{-9} to 1×10^{-4} M in the myograph tissue bath. The response curve for ergovaline consisted of 8 additions that ranged from 5×10^{-9} to 1×10^{-5} M because of limiting ergovaline concentrations in the tall fescue seed extract stock.

Isometric contractions of biopsied lateral saphenous vein were recorded as grams of tension in response to exposure to norepinephrine and the 5HT receptor agonists and alkaloid treatments. Data were digitally recorded using a Powerlab 16/35 (ADInstruments, Colorado Springs, CO) and Chart software (Version 7.2, ADInstruments). Contractile response was recorded as the greatest response, in grams, during the 9-min incubation following treatment addition and adjusted by baseline tension recorded just prior to the addition of 1×10^{-4} M norepinephrine. Response data were normalized as a percentage of the maximal contraction produced by norepinephrine (1×10^{-4} M). Normalization of contractile response data proportionally adjusted for any variation of tissue responsiveness resulting from differences in tissue size across individual cattle. Concentration-response curves were constructed by plotting the normalized data using GraphPad Prism (version 5.0f; San Diego, CA). This graphical presentation employed a nonlinear regression (sigmoidal concentration-response curve) to fit a line to contractile response data points for a treatment using the following 3-parameter equation:

$$y = bottom + \frac{(top - bottom)}{\left\{1 + 10^{\left[(logEC_{50} - x)\right]}\right\}}$$

where the top and bottom are plateaus in the units of the y-axis. This calculation permitted the

calculation of a compound's potency in this bioassay expressed as the concentration the compound required to produce 50% of the observed contractile response (EC_{50}).

Sample Collection and Analyses

Fifteen of the steers (5 steers per pasture treatment randomly selected across paddocks) from the larger grazing study described by Williamson et al. (2016) and Williamson and Aiken (2017) were held on their respective treatment pastures until day of biopsy. Tall fescue tiller samples were collected on day 1 relative to the initiation of the first biopsy day. Single tillers were clipped at the crown from 25 randomly selected tall fescue or bermudagrass plants. These samples were dried in a Botanique freeze drier (Model 18DX485A; Botanique Preservation Co., Peoria, AZ) and ground to pass through a 1-mm screen (Cyclotec 1093 Sample Mill, Foss North America, Eden Prairie, MN). Dried and ground tiller samples were composited and analyzed for ergovaline and ergovalinine content with high performance liquid chromatography and fluorescence detection using a slightly modified procedure of Carter et al. (2010). Separation was achieved using a Kinetex (Phenomenex Inc., Torrance, CA) 100 mm × 4.6 mm C18 column with 2.6-µm particle size. Elution solutions were 97-mM ammonium acetate (A) and water:acetonitrile (97:3%, vol/vol) and acetonitrile (B). The linear binary gradient was 78:22% (A:B) at the initial time for 0.5 min with a linear change to 35% (B) during the next 20 min, increased to 58% (B) during following 8 min, stepped up to 100% (B) in 0.5 min and maintained for 5 min to wash the column, and decreased to 22% (B) in 0.5 min and maintained for 5 min prior to the subsequent injection.

Jugular blood samples (approximately 8 mL) were collected from each steer during the lateral saphenous vein biopsy using serum blood collection tubes without an anticoagulant (Becton Dickinson, Franklin Lakes, NJ). Samples were stored on ice (approximately 4 °C) until processed. Serum was collected following centrifugation at 3,000 × *g* for 10 min at 20 °C. Serum samples were stored at −20 °C until analysis. Using the procedures of Bernard et al. (1993), serum prolactin analysis was conducted by the laboratory of F. N. Schrick (University of Tennessee, Knoxville). The intra- and inter-assay CV for serum prolactin were 3.84% and 4.24%, respectively.

Statistical Analyses

All statistics were conducted using the Mixed models of SAS (SAS 9.3; SAS Inst. Inc., Cary, NC), and for tests of fixed effects, the Satterthwaite approximation of denominator degrees of freedom was used. Contractile response data to receptor agonist and ergovaline treatments were analyzed as a completely randomized design with a split-plot treatment design. Although there were pasture replicates used by Williamson and Aiken (2017) in the study that led up to the current study, the current study does not report pasture or steer performance outcomes and is interested in the effect of pasture ergovaline on receptor vasoactivity within steer. Thus, whole plot experimental unit was steer with pasture as the treatment factor. The biopsied blood vessel was the subplot experimental unit and the concentration of 5HT receptor agonist or ergovaline used as the subplot treatment factor. The model included fixed effects of pasture treatment, agonist concentration, and the interaction. For the variables prolactin and vessel dimensions (inside and outside diameter), analysis of variance was conducted as a completely randomized design for the main effect of pasture treatment.

For all statistical analyses, pairwise comparisons of least squares means (± SEM) were conducted only if the probability of a greater *F*-statistic in the ANOVA was significant for the tested effect. If significant, means separation was conducted using least significant difference (LSD) feature in SAS and comparisons were considered significant at $P \leq 0.05$, unless reported otherwise.

RESULTS

The total ergovaline (ergovaline + ergovalinine) concentrations of the pastures just prior to initiation of the biopsies were 0.0 mg ergovaline/kg DM for the bermudagrass, 0.56 mg ergovaline/kg DM for the untreated toxic endophyte-infected tall fescue, and 0.24 mg ergovaline/kg DM for the pasture treated with aminopyralid and metsulfuron-methyl to suppress tall fescue seedhead production. Concentrations of serum prolactin collected on the day of saphenous vein biopsy were lower ($P < 0.05$) in steers from both toxic endophyte-infected tall fescue pastures (1.11 ± 6.72 and 3.42 ± 6.72 ng/mL for untreated and seedhead suppressed pastures, respectively) compared with steers from the bermudagrass pasture (47.92 ± 7.52 ng/mL).

The vascular dimensions (inside and outside diameters) were assessed for all blood vessel cross sections used in the study (Table 1). Veins biopsied

Table 1. Vascular dimensions of bovine lateral saphenous veins biopsied from steers that had grazed pastures of bermudagrass (*Cynodon dactylon*) or toxic endophyte-infected tall fescue (*Lolium arundinaceum*) treated or untreated with aminopyralid and metsulfuran-methyl (Chaparral) herbicide[1]

Dimension	Bermudagrass	Tall fescue, treated	Tall fescue, untreated	pSEM	*P*-value
Outside diameter, mm	2.28[b]	2.25[b]	2.48[a]	0.05	0.007
Inside diameter, mm	0.62[a]	0.44[b]	0.67[a]	0.03	< 0.01
Wall thickness, mm	0.83[b]	0.92[a]	0.90[ab]	0.02	0.06

[1]Administration of Chaparral (Dow AgroSciences, Indianapolis, IN) to provide 87.1 g/ha of an aminopyralid (2-pyridine carboxylic acid, 4-amino-3,6-dichloro-) and 13.2 g/ha of metsulfuran-methyl (Methyl 2-[[[[(4-methoxy-6-methyl-1,3,5- triazin-2-yl)- amino]carbonyl] amino]sulfonyl]benzoate) results in suppression of seedhead production by tall fescue.

[ab]Values not sharing like superscripts within a row are different ($P < 0.05$).

from steers that had grazed the untreated toxic endophyte-infected tall fescue pasture had the largest outside diameter ($P < 0.05$), whereas veins biopsied from steers that had grazed the seedhead suppressed toxic endophyte-infected tall fescue pasture had the smallest inside diameter ($P < 0.05$) when compared with the other pasture treatments. However, when looking at the wall thickness, veins biopsied from steers that had grazed bermudagrass pastures were thinner than those from the pasture treated to suppress seedhead formation ($P < 0.05$) and tended to be thinner than those originating from the untreated pasture ($P = 0.07$).

The exposure to increasing concentrations of CP 93129, the $5HT_{1B}$ agonist, did not result in a significant contractile response that differed between pasture treatments ($P = 0.24$; Figure 1A). There was a significant pasture × concentration interaction when biopsied lateral saphenous veins were exposed to increasing concentrations of L-694,247, a $5HT_{1D}$ agonist ($P < 0.01$; Figure 1B). This was due to a complete absence of $5HT_{1D}$ receptor-derived vasoactivity in veins biopsied from steers that had grazed nontoxic bermudagrass pastures. Conversely, the veins collected from steers that had grazed the seedhead-suppressed toxic endophyte-infected tall fescue pasture had a significant response to the $5HT_{1D}$ receptor agonist. Lateral saphenous veins biopsied from steers grazing untreated toxic endophyte-infected tall fescue had the greatest contractile response to the $5HT_{1D}$ agonist ($P < 0.05$). However, the responses from these 2 groups were not sigmoidal and reached maximal contractile intensity by the midpoint of the response curve at 5×10^{-7} to 1×10^{-6} M L-694,247 (Figure 1B).

The contractile response to the $5HT_{2A}$ agonist TCB2 was sigmoidal for all treatments (Figure 1C) and had a significant pasture × concentration interaction to increasing concentrations of the agonist ($P = 0.01$). Veins originating from steers that had grazed nontoxic bermudagrass pasture had the greatest contractile intensity ($P < 0.05$) at the high concentration side of the concentration response curve compared with both toxic endophyte-infected tall fescue pasture treatments. Veins collected from steers on seedhead suppressed and untreated tall fescue pasture treatment did not differ in response to increasing concentrations of $5HT_{2A}$ agonist TCB2 ($P = 0.66$). Although the contractile intensity was the greatest for veins from the bermudagrass nontoxic control steers, the EC_{50} values, a measure of a compound's potency, were not different ($P = 0.91$) across any of the pasture treatments (Table 2).

The concentration response curve to increasing concentrations of the ergot alkaloid ergovaline (administered as a tall fescue seed extract that was diluted based on the concentration of ergovaline) had a significant pasture × concentration interaction (Figure 1D; $P < 0.01$). Unlike the serotonin agonists evaluated, the maximum concentration of ergovaline evaluated was 1×10^{-5} M and was limited by the concentration of ergovaline in the stock seed extract. Similar to the $5HT_{2A}$ agonist response, veins collected from steers that had grazed bermudagrass pastures had a greater contractile intensity to ergovaline at the high end of the response curve ($P < 0.05$) compared with the untreated and seedhead-suppressed toxic endophyte-infected tall fescue pastures which did not differ ($P = 0.46$). Interestingly, the EC_{50} value for ergovaline in bermudagrass veins was greater ($P < 0.05$), or less potent than veins from either tall fescue pasture treatment (Table 2).

DISCUSSION

Treatment of toxic endophyte-infected tall fescue pastures with aminopyralid and metsulfuron-methyl (Chaparral) results in a suppression of seedhead production by tall fescue plants and has been shown to reduce the negative effects of fescue toxicosis (Aiken et al., 2012). Tall fescue seedheads contain the greatest concentration of ergot alkaloids within an endophyte-infected plant

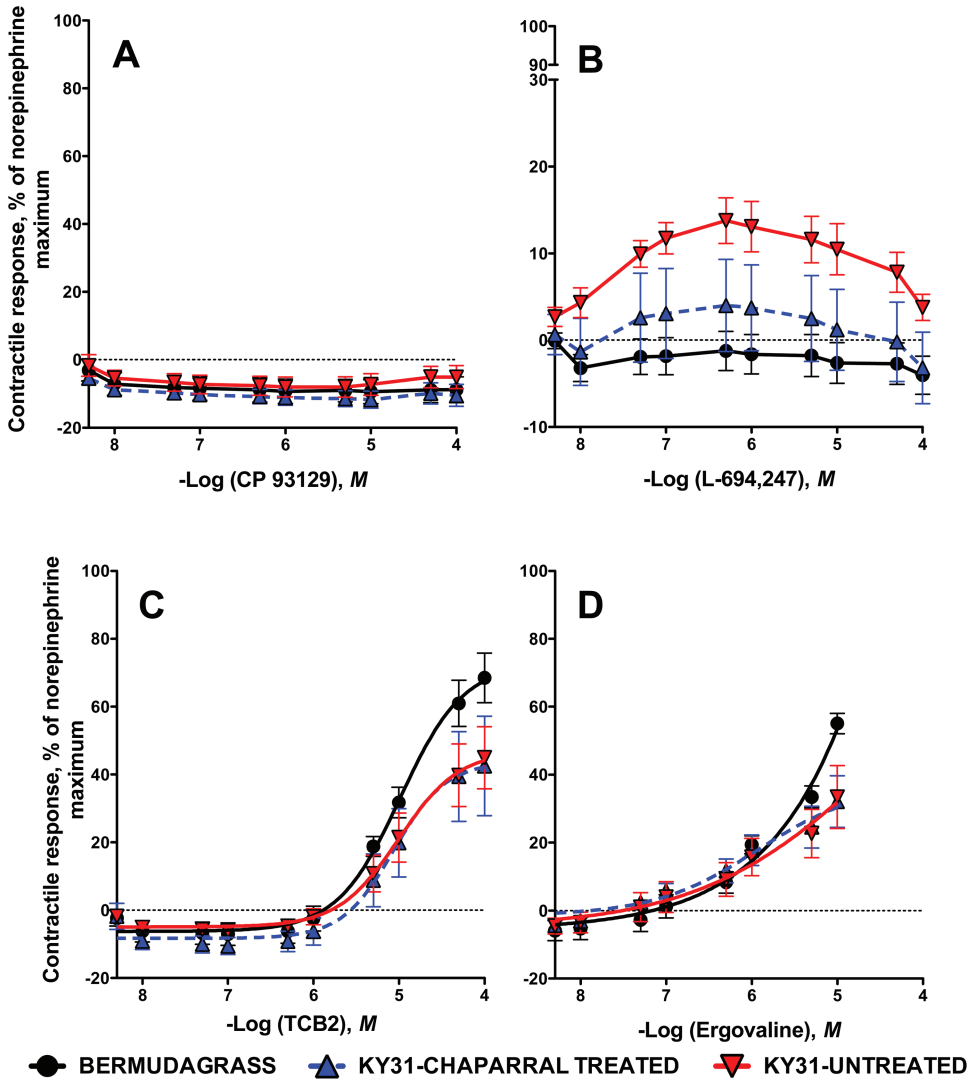

Figure 1. Mean contractile response of lateral saphenous vein to increasing concentrations of 5-hydroxytryptamine (5HT) receptor agonists: (A) CP 93129, a selective agonist for receptor $5HT_{1B}$; (B) L-694,247, a selective agonist for receptor $5HT_{1D}$; (C) TCB2 a selective agonist for receptor $5HT_{2A}$; (D) ergovaline, an ergot alkaloid obtained from a tall fescue seed extract. Veins were biopsied from steers following an 87- to 101-d grazing period on a bermudagrass pasture (n = 5), Kentucky-31 (KY31) toxic endophyte-infected tall fescue pastures not treated (n = 5), or treated with aminopyralid and metsulfuran-methyl (Chaparral; n = 5) to suppress seedhead production.

Table 2. The −log EC_{50} means and SEM of lateral saphenous veins biopsied from steers that had grazed pastures of bermudagrass (*Cynodon dactylon*) or toxic endophyte-infected tall fescue (*Lolium arundinaceum*) not treated or treated with aminopyralid and metsulfuran-methyl (Chaparral) herbicide[1]

	−LogEC$_{50}$, M				
Myograph treatment	Bermudagrass	Tall fescue, treated	Tall fescue, untreated	pSEM	*P*-value
TCB2[2]	4.89	4.93	4.88	0.08	0.91
Ergovaline[3]	5.44[b]	6.10[a]	6.01[a]	0.16	0.01

[1]The effective concentration at which 50% of the contractile response is achieved (EC_{50}). Administration of Chaparral (Dow AgroSciences, Indianapolis, IN) to provide 87.1 g/ha of an aminopyralid (2-pyridine carboxylic acid, 4-amino-3,6-dichloro-) and 13.2 g/ha of metsulfuran-methyl (Methyl 2-[[[[(4-methoxy-6-methyl-1,3,5- triazin-2-yl)- amino]carbonyl] amino]sulfonyl]benzoate) results in suppression of seedhead production by tall fescue.

[2]A selective agonist for serotonin receptor 2A.

[3]Ergovaline was applied in the form of a tall fescue seed extract.

[ab]Values not sharing like superscripts within a row are different ($P < 0.05$).

(Rottinghaus et al., 1991), and tall fescue seedheads have been shown to be selectively grazed by cattle and horses (Aiken et al., 1993). Thus, by suppressing the reproductive development of seedheads in the tall fescue plant, the levels of ergot alkaloids available for consumption by grazing livestock can be substantially reduced. This was the case in the current study, as the ergovaline concentrations (ergovaline + ergovalinine) in the untreated pasture were 2× greater than the measured amounts in the seedhead-suppressed tall fescue pasture at the time that the biopsies were initiated.

Although there were differences in the available pasture ergovaline concentrations for steers to graze at the time of the lateral saphenous vein biopsies, there were no differences in the levels of serum prolactin or vascular wall thickness to indicate a gradation in physiological effects associated with different pasture levels of ergovaline. This could be a consequence of the seedhead-suppressed pastures containing a higher concentration of ergovaline earlier in the grazing period (Williamson et al., 2016), and those residual effects could have carried over into this biopsy portion of the study (Aiken et al., 2013; Klotz et al., 2016).

The serotonin receptors were selected for evaluation in the current study based on functional aspects of each receptor associated with vasoconstriction. The $5HT_{1B}$, $5HT_{1D}$, and $5HT_{2A}$ receptors are involved in vasoconstriction (Hannon and Hoyer, 2008). More specifically, vasoconstriction associated with exposure to ergovaline has been previously shown in rodent models to be mediated by $5HT_{1B/1D}$ and $5HT_{2A}$ receptors (Schöning et al., 2001). Previous work has also demonstrated a decreased vasoactivity of serotonin receptors on bovine blood vessels exposed to ergot alkaloids (Klotz et al., 2012; Klotz et al., 2013; Egert et al., 2014; Jia et al., 2015). This decrease in vasoactivity has been shown to recover over time (Klotz et al., 2016); thus, it was hypothesized that different levels of exposure could produce different levels of effect on vasoactivity. What was observed in the current study was a decrease in vasoactivity to increasing concentrations of the $5HT_{2A}$ agonist and ergovaline in both fescue pastures compared with bermudagrass. The pasture level of ergovaline, however, did not have an effect on the magnitude of decreased vasoactivity to these 2 compounds. Similarly, Williamson and Aiken (2017) reported no difference in luminal area of caudal artery (an assessment of vasoconstriction) in steers taken from seedhead-suppressed compared with untreated toxic endophyte-infected tall fescue pastures.

In contrast to the $5HT_{2A}$ agonist TCB2 and ergovaline, exposure of the lateral saphenous veins biopsied from both fescue pasture treatments and the bermudagrass control to increasing concentrations of the $5HT_{1B}$ agonist CP 93129 did not produce a measureable contractile response. This suggests that this receptor subtype is absent from the bovine lateral saphenous vein. This is different from what has been previously reported in peripheral vasculature in other species such as the rabbit saphenous vein (Bhattacharya et al., 2004) and the rat caudal artery (Craig and Martin, 1993), where $5HT_{1B}$-derived vasoactivity was demonstrated. This difference is probably related to differing species used as models.

An interesting observation in the current study was the overall increase in contractile response to increasing concentrations of the $5HT_{1D}$ agonist L-694,247 as the ergovaline levels in the pasture increased. The majority of data generated with bovine vascular bioassay models exhibit the opposite result, where prior exposure to ergovaline on pasture or in feed causes a decrease in vasoactivity (Klotz et al., 2013; Egert et al., 2014; Jia et al., 2015; Klotz et al., 2016). In the current study, the bermudagrass control had no $5HT_{1D}$-derived contractile response, whereas lateral saphenous veins that were biopsied from steers that had grazed the untreated tall fescue pasture (highest level of ergovaline exposure) had a greater contractile response. This result was observed previously by Klotz et al. (2012) using the same bioassay and a dose response to 5-carboxamidotryptamine hemiethanolate maleate, an agonist at $5HT_{1A}$, $5HT_{1B}$, $5HT_{1D}$, and $5HT_{7}$ receptors. In the Klotz et al. (2012) study, steers that had grazed a high toxic endophyte-infected tall fescue pasture had a significantly greater response to increasing concentrations of this less specific agonist than veins collected from steers that had grazed a low toxic endophyte-infected tall fescue pasture. Presumably the suppressed contractile response seen at the $5HT_{2A}$ receptor with TCB2 and ergovaline is a result of a rate of receptor internalization that is accelerated by agonist occupancy (Tan et al., 2004). It is possible that the results reported by Klotz et al. (2012) and in the current study could be the opposite of the observed suppression of the $5HT_{2A}$ receptor. The $5HT_{1D}$ receptors may be upregulated and be present in greater quantities as a result of pasture ergovaline exposure and may be a mechanism to compensate for suppression of vasoactivity at the $5HT_{2A}$ receptor. This is an aspect that requires

further exploration, as the mechanism by which ergot alkaloids such as ergovaline can cause the diverse number of symptoms and outcomes associated with ergotism, and fescue toxicosis is not fully elucidated at the receptor level.

In conclusion, herbicide suppression of seedhead production in toxic endophyte-infected tall fescue can yield lower concentrations of pasture ergovaline. This difference in ergovaline concentration offered on pasture did not yield differences in the manifestation of toxicosis metrics like serum prolactin concentrations. Nor did the lower ergovaline concentrations produce a different level of vasoactivity at the $5HT_{2A}$ receptor, the primary serotonin receptor associated with vascular smooth muscle contraction. Interestingly, the level of $5HT_{1D}$-derived vasoactivity was elevated in the untreated toxic endophyte-infected tall fescue treatment and lower ergovaline seedhead-suppressed treatment was similar to the nontoxic bermudagrass control, suggesting the possibility of some vascular changes occurring at the $5HT_{1D}$ receptor above 0.24 mg of ergovaline/kg of DM. Different serotonin receptor subtypes may be affected at different ergovaline concentrations and these vascular observations will contribute to furthering the definition of pasture ergovaline thresholds for grazing cattle.

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