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# Assessment of vasoconstrictive potential of D-lysergic acid using an isolated bovine lateral saphenous vein bioassay<sup>1</sup>

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**ABSTRACT:** Vasoconstriction has been associated with several symptoms of fescue toxicosis thought to be alkaloid induced. Lysergic acid, an ergot alkaloid, has been proposed as a toxic component of endophyte-infected tall fescue. The objective of this study was to examine the vasoconstrictive potential of D-lysergic acid using a bovine lateral (cranial branch) saphenous vein bioassay. Before testing lysergic acid, validation of the bovine lateral saphenous vein bioassay for use with a multimyograph apparatus was conducted using a dose-response to norepinephrine to evaluate the effects of limb of origin (right vs. left) and overnight storage on vessel contractile response. Segments (2 to 3 cm) of the cranial branch of the lateral saphenous vein were collected from healthy mixed breed cattle (n = 12 and n = 7 for the lysergic acid and norepinephrine experiments, respectively) at local abattoirs. Tissue was placed in modified Krebs-Henseleit, oxygenated buffer and kept on ice or stored at 2 to 8°C until used. Veins were trimmed of excess fat and connective tissue, sliced into 2- to 3-mm sections, and suspended in a myograph chamber containing 5 mL of oxygenated Krebs-Hensel-

eit buffer (95% O<sub>2</sub>, 5% CO<sub>2</sub>; pH = 7.4; 37°C). Tissue was allowed to equilibrate at 1 g of tension for 90 min before initiation of treatment additions. Increasing doses of norepinephrine (1 × 10<sup>-8</sup> to 5 × 10<sup>-4</sup> M) or lysergic acid (1 × 10<sup>-11</sup> to 1 × 10<sup>-4</sup> M) were administered every 15 min after buffer replacement. Data were normalized as a percentage of the contractile response induced by a reference dose of norepinephrine. Veins from both left and right limbs demonstrated contractions in a dose-dependent manner (*P* < 0.01) but did not differ between limbs. There were no differences in dose-response to norepinephrine between tissue tested the day of dissection and tissue tested 24 h later. Exposure of vein segments to increasing concentrations of lysergic acid did not result in an appreciable contractile response until the addition of 1 × 10<sup>-4</sup> M lysergic acid (15.6 ± 2.3% of the 1 × 10<sup>-4</sup> M norepinephrine response). These data indicate that only highly elevated concentrations of lysergic acid result in vasoconstriction. Thus, in relation to the symptoms associated with vasoconstriction, lysergic acid may only play a minor role in the manifestation of fescue toxicosis.

**Key words:** alkaloid, bovine, fescue, lysergic acid, vasoconstriction

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## INTRODUCTION

Lysergic acid (Figure 1, panel A), a common moiety of ergot alkaloids (Hill, 2005), is composed of a carboxylic acid attached to an ergoline ring structure. Lysergic

acid amide (Figure 1, panel B), a simple chemical analog of lysergic acid, has been shown to have vasoconstrictive activity in bovine vessels (Oliver et al., 1993). Hill et al. (2001) reported that lysergic acid, lysergol, and ergonovine had greater absorption potential across ruminant foregut tissues in vitro than the more complex ergopeptines (Figure 1, panel C). Ergot alkaloids are excreted primarily via the urinary system as lysergic acid amide or biotransformed ergopeptine alkaloids in cattle (Stuedemann et al., 1998). In horses, ergovaline is excreted solely via the fecal route, whereas lysergic acid is excreted via both fecal and urinary routes (Schultz et al., 2006). These data implicate lysergic acid

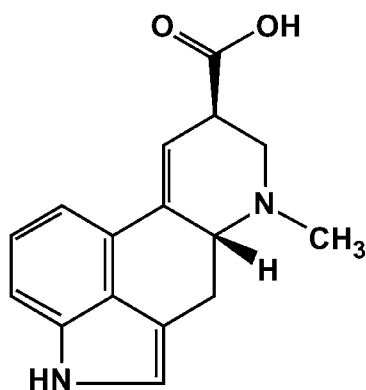
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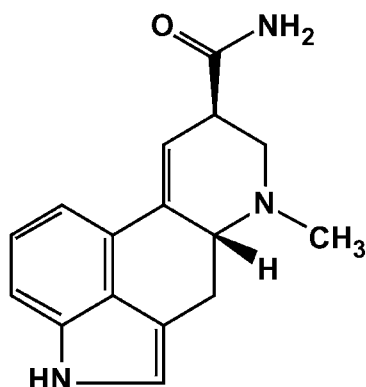
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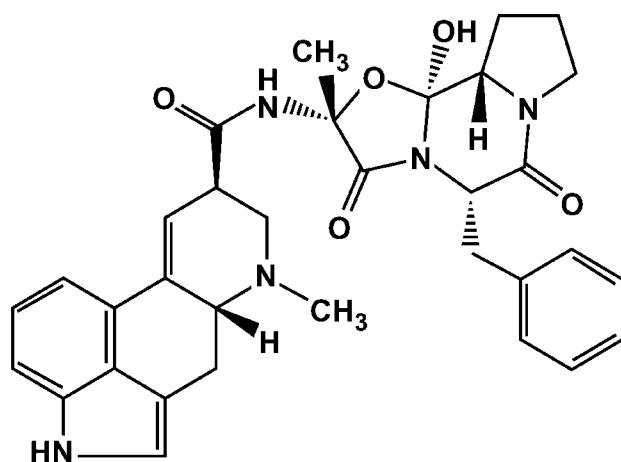
A



B



C



**Figure 1.** A) Chemical structure of lysergic acid; B) chemical structure of lysergic acid amide; C) chemical structure of ergotamine, an example of an ergopeptine.

as a circulating metabolite of ergot alkaloid metabolism in herbivores.

The effects of lysergic acid on bovine vasculature have not been investigated. Given the vasoconstrictive potential of the ergopeptines (e.g., ergotamine; Solomons et al., 1989) and lysergic acid amide on bovine vasculature, it was hypothesized that lysergic acid would cause vaso-

constriction of the bovine lateral saphenous vein at physiologically relevant levels.

Collection, processing, and evaluation of vascular tissue from pedal and saphenous veins using organ baths and a polygraph have been previously described (Solomons et al., 1989; Oliver et al., 1990). Multimyograph systems are available that permit rapid screening of alkaloids and other compounds for vascular activity (Mulvany and Halpern, 1977; Nielsen-Kudsk et al., 1986). Thus, the objective of this study was to evaluate the vasoconstrictive effects of increasing concentrations of D-lysergic acid using an isolated bovine lateral saphenous vein bioassay and a multimyograph.

## MATERIALS AND METHODS

### *Animals and Tissues*

The procedures used in this study were not submitted for approval by the Animal Care and Use Committee because no live animals were used. Tissues were collected from cattle of mixed breeds and sex ( $n = 19$ ; BW = 227 to 545 kg) immediately after slaughter at local abattoirs and used to validate the bioassay and evaluate the vascular effects of lysergic acid. Specifically, segments (2 to 3 cm in length) of the cranial branch of the lateral saphenous vein were removed and placed in a modified Krebs-Henseleit, oxygenated buffer solution (95% O<sub>2</sub>, 5% CO<sub>2</sub>; pH = 7.4; mM composition = D-glucose, 11.1; MgSO<sub>4</sub>, 1.2; KH<sub>2</sub>PO<sub>4</sub>, 1.2; KCl, 4.7; NaCl, 118.1; CaCl<sub>2</sub>, 3.4; and NaHCO<sub>3</sub>, 24.9; K3753, Sigma Chemical Co., St. Louis, MO) for transport and kept on ice until processed.

Excess fat and connective tissue were removed from the segments, which were then sliced into 2- to 3-mm cross-sections. Cross-sections were examined under a dissecting microscope (Stemi 2000-C, Carl Zeiss Inc., Oberkochen, Germany) at 12.5 $\times$  magnification to verify the physical integrity of the tissue and to measure its dimensions (Axiovision, version 20, Carl Zeiss Inc.) to assure a consistent segment size.

Duplicate cross-sections from each animal were horizontally suspended in a tissue bath (DMT610M Multi-chamber myograph, Danish Myo Technologies, Atlanta, GA) containing 5 mL of continuously gassed (95% O<sub>2</sub>, 5% CO<sub>2</sub>), modified Krebs-Henseleit buffer (37°C). The incubation buffer consisted of the transport buffer but with the addition of desipramine ( $3 \times 10^{-5}$  M; D3900, Sigma Chemical Co.) and propranolol ( $1 \times 10^{-6}$  M; P0844, Sigma) to inactivate catecholamine-neuronal uptake and beta-adrenergic receptors, respectively.

The tissue was allowed to equilibrate under a resting tension of 1 g for 90 min. The buffer solution was replaced at 15-min intervals during the equilibration period. To assure tissue responsiveness, the tissue was exposed to norepinephrine ( $1 \times 10^{-4}$  M). Tissues that did not achieve a maximal contraction of 10 g were not considered viable and were discarded. Viable tissues were again washed every 15 min until the original 1 g of resting tension was reestablished.

### Validation Experiment

The bovine lateral saphenous vein bioassay was validated using norepinephrine to compare veins dissected from left and right hind limbs and for analysis of tissue the day of collection vs. analysis after 24-h storage. Norepinephrine, an  $\alpha$ -adrenergic agonist, has been previously shown to induce contractile responses in bovine dorsal pedal and lateral saphenous veins (Solomons et al., 1989; Oliver et al., 1990) as well as equine digital and lateral saphenous veins (Baxter et al., 1989; Abney et al., 1993).

Cranial branch segments of the lateral saphenous vein were dissected from the left and right legs ( $n = 7$ ), and comparisons of norepinephrine dose-response were done in duplicate (2 sections prepared from each leg) between legs the same day as collection. The remaining venous tissue was stored in fresh, modified Krebs-Henseleit transport buffer overnight at 2 to 8°C before conducting a second identical norepinephrine dose-response experiment 24 h later. The final working concentrations of norepinephrine (A0937, Sigma) in the myograph chamber were  $1 \times 10^{-8}$ ,  $5 \times 10^{-8}$ ,  $1 \times 10^{-7}$ ,  $5 \times 10^{-7}$ ,  $1 \times 10^{-6}$ ,  $5 \times 10^{-6}$ ,  $1 \times 10^{-5}$ ,  $5 \times 10^{-5}$ ,  $1 \times 10^{-4}$ , and  $5 \times 10^{-4}$  M. Standards were added in the order listed to minimize concern for carryover from 1 addition to the next.

After an addition of norepinephrine, the tissue was allowed to incubate with the given concentration for 9 min. After the incubation period, the buffer was replaced and allowed to incubate for 2.5 min (2 $\times$ ). The buffer was changed a third time, and after a 1-min recovery interval, the next norepinephrine concentration was added. These buffer changes were done to wash residual norepinephrine from the myograph chamber and to expedite the relaxation of the tissue back to its baseline tension. This process was repeated until all norepinephrine concentrations had been tested.

### Lysergic Acid Experiment

**Dose-Response of Lysergic Acid.** Cross-sections of the cranial branch of the lateral saphenous vein were run in duplicate from each animal ( $n = 12$ ). After recovery from the  $1 \times 10^{-4}$  M norepinephrine addition to assure tissue responsiveness, the tissues were exposed to lysergic acid standards ( $1 \times 10^{-11}$ ,  $1 \times 10^{-10}$ ,  $1 \times 10^{-9}$ ,  $1 \times 10^{-8}$ ,  $1 \times 10^{-7}$ ,  $1 \times 10^{-6}$ ,  $1 \times 10^{-5}$ , and  $1 \times 10^{-4}$  M). Standards were added from the least to the most concentrated every 15 min, as described above for the validation experiment. Stock standards of D-lysergic acid hydrate (95%; Acros Organics, Geel, Belgium) were prepared in 80% (vol/vol) methanol that contained  $1.2 \times 10^{-4}$  M acetic acid. To achieve a  $1 \times 10^{-4}$  M concentration in the incubation buffer, a  $2 \times 10^{-2}$  M stock solution was prepared. Serial dilutions of this stock made up the remaining standards, and 25- $\mu$ L aliquots of the dilutions were added to the incubation buffer to attain the desired treatment concentrations. Using this proto-

col, methanol concentrations in the incubation buffer were kept below 0.5%.

**Liquid Chromatography-Mass Spectrometry of Lysergic Acid.** Because lysergic acid is sparingly soluble in neutral or organic solvents (Merck, 2001), it was necessary to acidify the overall stock solution with acetic acid. To verify that lysergic acid was present in solution with the tissues, for each standard 1-mL aliquots of lysergic acid-containing incubation buffer were removed from the myograph after the incubation period. Relative concentrations of the lysergic acid standards used were confirmed by HPLC-mass spectrometry (MS) using a Varian 1200L quadrupole system equipped with a Varian electrospray source, 2 Varian ProStar solvent delivery modules, and a Varian ProStar 430 autosampler (Varian Inc., Walnut Creek, CA). No clean-up or preconcentration of the samples occurred before injection onto a reverse-phase column.

A 100- $\mu$ L aliquot of lysergic acid-containing incubation buffer with specific concentrations ranging from  $10^{-4}$  to  $10^{-9}$  M was injected onto a 2-mm reverse-phase column (Synergi 4u Hydro-RP C18, Phenomenex, Torrance, CA). Each sample was first eluted with 10:90% methanol:water (both solvents containing 0.1% formic acid) for 6.0 min. A linear gradient then increased the methanol to 95% by 16.3 min, and the system was held at this solvent composition for 3 additional minutes. The solvent composition was then taken back to 10:90% methanol:water for 10 min to equilibrate the column for the next sample run. The flow rate for each sample run was held at 0.1 mL/min. The elution from the column was coupled with the electrospray source (ESI; needle set at 5 kV, shield 600 V) to generate positive ions such that mass spectrometry analysis could then occur (Varian 1200L MS conditions: detector, 1,400 V; N<sub>2</sub> drying gas, 200°C). The 0.1% formic acid in the solvent was present to aid in the ionization process. The retention time of the lysergic acid was on the order of  $14.2 \pm 0.1$  min.

### Data Collection and Statistical Analysis

Isometric contractions were recorded as grams of tension in response to exposure to norepinephrine or lysergic acid. Data were digitally recorded using a Powerlab/8sp (ADInstruments, Colorado Springs, CO) and Chart software (Version 5.3, ADInstruments). The contractile response was recorded as the greatest tension within the first 9 min after a treatment addition and was determined by the difference in tension from the baseline measured immediately before that treatment addition.

The validation experiment data comparing the maximal gram contractile and dose-response of venous segments to norepinephrine isolated from the right and left leg and after 24-h storage were analyzed as a completely randomized design using JMP (version 5, SAS Inst. Inc., Cary, NC). Right vs. left leg responses were analyzed as a separate data set from d 1 vs. 2 responses. Right leg responses had a lower number of experimental units

( $n = 5$  per leg) because tissues from 2 separate limbs did not meet the viability requirements.

Dose response data were normalized as a percentage of the contraction produced by the agonist norepinephrine at  $1 \times 10^{-4}$  M and  $5 \times 10^{-4}$  M for the lysergic acid and validation experiments, respectively. This normalization was performed to compensate for the variation of tissue responsiveness due to differences in tissue size or individual animals. To construct the dose-response curve for the lysergic acid experiment, normalized data (i.e., percentage of the norepinephrine response at  $1 \times 10^{-4}$  M) were averaged within dose, and SE were calculated. Mean response and SE were then plotted to illustrate the dose-response of the bovine lateral saphenous vein to lysergic acid in vitro.

## RESULTS AND DISCUSSION

### *Validation of Isolated Bovine Saphenous Vein Bioassay*

The average length, i.d., and o.d. for tissue segments ( $n = 7$ ) used in the validation experiment were  $2.7 \pm 0.04$ ,  $1.4 \pm 0.2$ , and  $3.6 \pm 0.3$  mm for the left leg and  $2.8 \pm 0.07$ ,  $1.0 \pm 0.1$ , and  $3.5 \pm 0.3$  mm for the right leg. Figure 2 illustrates typical electronic traces of vein segment responses taken from the left and right limbs exposed to increasing concentrations of norepinephrine in duplicate. Vein segments from both left and right limbs demonstrated contractions in a dose-dependent manner ( $P < 0.01$ ) but did not differ between limbs (Figure 3). This outcome was not unexpected because Baxter et al. (1989) did not find any differences when comparing arteries and veins between forelimbs and hind limbs in horses. However, it was necessary to rule out the possibility that tissue taken from different limbs would be a source of variation in preparation for future experiments using this bioassay. There were no differences in dose-response to norepinephrine between tissue tested the day of dissection and tissue exposed to norepinephrine 24 h later (Figure 3). Maximal contractile responses to norepinephrine ( $5 \times 10^{-4}$  M) did not differ between day for the left (d 1 = 20.1; d 2 = 15.8 g; SE = 3.9) or right limbs (d 1 = 18.2; d 2 = 15.1 g; SE = 2.4). The absence of any differences in tissue responsiveness between days tested was important because this permits additional treatment tests to be conducted from a single tissue collection, thereby maximizing response information generated from a single animal.

The contractile responses to  $1 \times 10^{-5}$  and  $1 \times 10^{-4}$  M norepinephrine additions (Figure 3) did appear refractory to the previous addition. This differed slightly from previously reported dose-response curves for norepinephrine (Baxter et al., 1989; Solomons et al., 1989; Abney et al., 1993). This may be a result of the plotted data being corrected for the minimum measured tension during each 9-min sampling period instead of generating a cumulative dose-response where each addition would be corrected by the original baseline. This

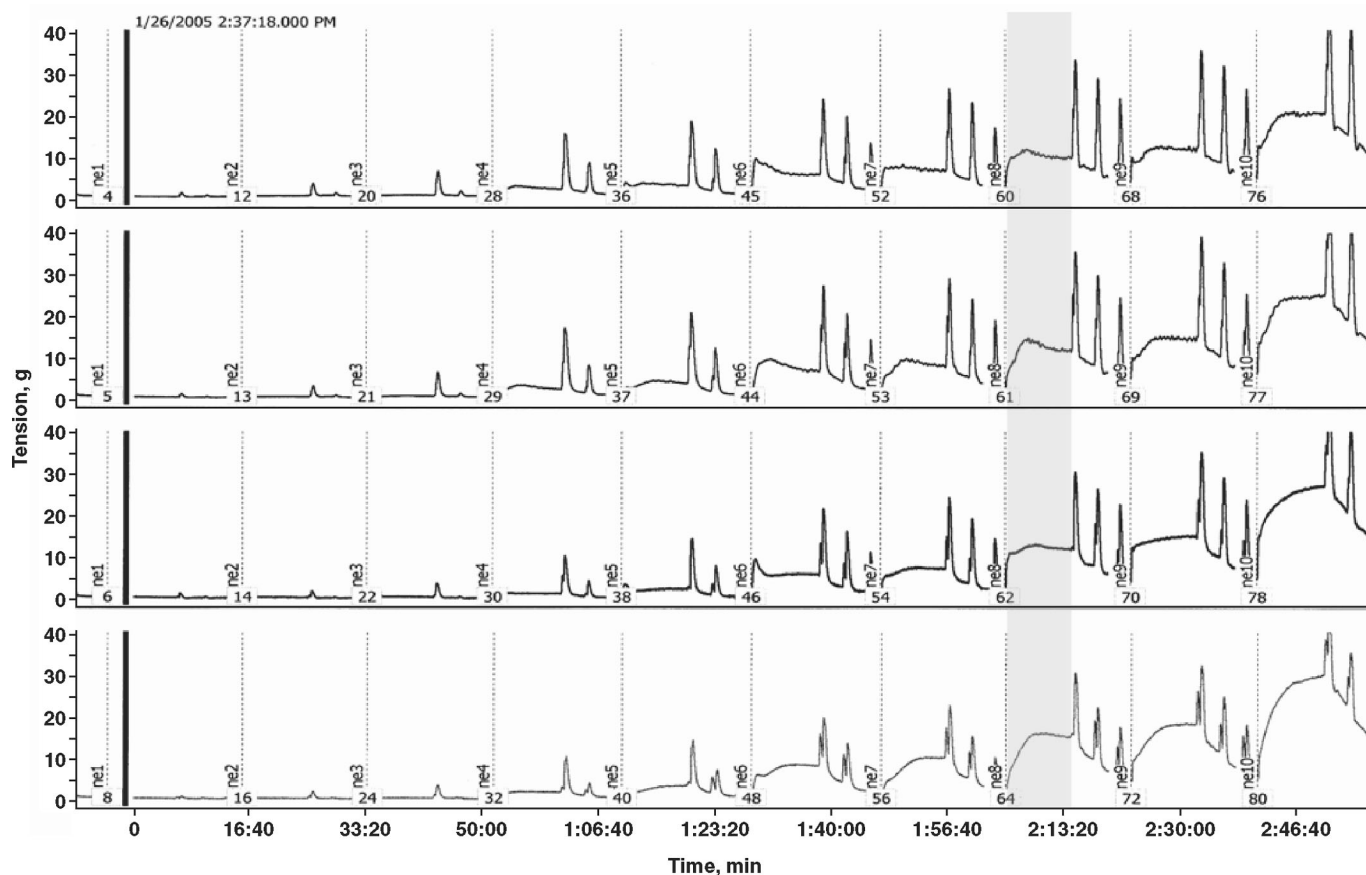
is evident in Figure 2 because the maximum contractile response increased with each addition but might have not had enough time to relax back to the 1-g baseline prior to the subsequent addition.

### *Vasoconstrictive Potential of Lysergic Acid*

Vasoconstriction, an effect noted in livestock afflicted with fescue toxicosis, has been directly linked to ergot alkaloids found in endophyte-infected tall fescue. Solomons et al. (1989) provided the first in vitro evidence that ergotamine, a representative of the ergopeptine class of ergot alkaloids found in endophyte-infected tall fescue, caused vasoconstriction of the dorsal pedal vein of cattle. Further, they reported that agroclavine and ergosine, both alkaloids reported (Porter et al., 1981; Lyons et al., 1986) to be present in endophyte-infected tall fescue, were also vasoconstrictive but much less so than ergotamine. These data provide evidence of differing potentials of various alkaloids in the ergot class for inducing vasoconstriction associated with fescue toxicosis. Rhodes et al. (1991) provided in vivo evidence in sheep and cattle that endophyte-infected tall fescue based diets caused a reduction of blood flow to the skin as well as core body tissues (e.g., duodenum, colon). Other researchers have reported vasoconstriction of cattle or horse vasculature in vitro by ergovaline (Dyer, 1993), ergotamine and ergonovine (Abney et al., 1993), and lysergic acid amide (Oliver et al., 1993). However, no data are available that indicate the potential of lysergic acid as a direct vasoconstrictor. Given the differences in potential vascular activity discussed above and the likelihood of lysergic acid being a major circulating alkaloid in the animal, it was deemed necessary to evaluate the alkaloid for vasoconstrictor activity.

In the current study, the presence and relative concentrations of lysergic acid in the incubation buffer were confirmed via HPLC-MS for the  $1 \times 10^{-4}$ ,  $1 \times 10^{-5}$ ,  $1 \times 10^{-6}$ , and  $1 \times 10^{-7}$  M additions of lysergic acid with a retention time of about 14.2 min (Figure 4). At  $1 \times 10^{-8}$  M, lysergic acid produced a signal that was indistinguishable from baseline noise (Figure 4, panel B). Using a 100- $\mu$ L injection of  $1 \times 10^{-7}$  M lysergic acid, the HPLC-MS (Varian 1200L, Varian Inc., Walnut Creek CA) was capable of detecting ~10 pmol of lysergic acid.

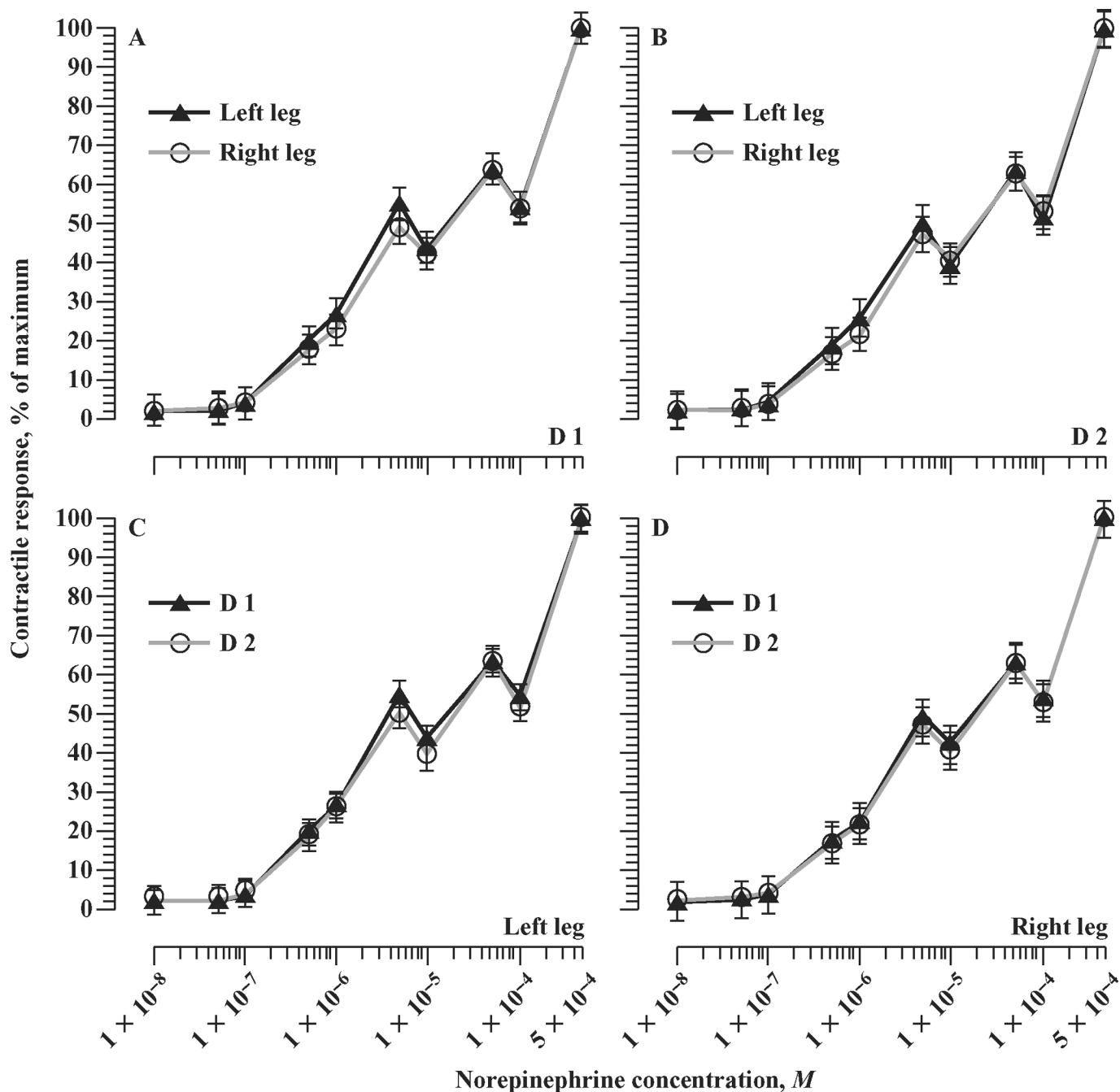
The average length, i.d., and o.d. for tissue segments used in this experiment were  $2.8 \pm 0.1$ ,  $0.8 \pm 0.05$ , and  $3.0 \pm 0.1$  mm, respectively. Figure 5 shows a typical trace of lateral saphenous vein segments exposed to increasing concentrations of lysergic acid. An enlarged portion of the trace was included to affirm the absence of a response to the addition of lysergic acid. The mean maximal contraction induced by  $1 \times 10^{-4}$  M norepinephrine was  $20.70 \pm 2.86$  g ( $n = 12$ ). Treatment of bovine saphenous vein segments with increasing concentrations of lysergic acid showed no contractile response until  $1 \times 10^{-5}$  M was added (Figure 6). However, this



**Figure 2.** Typical dose-response of tissue to norepinephrine. Norepinephrine additions are illustrated by comments (e.g., ne1 =  $1 \times 10^{-8}$  and ne10 =  $5 \times 10^{-4}$  M). The shaded area is an example of a data collection region of the trace. The spikes that follow are artifacts generated by buffer replacement and were not included in the data analysis.

response was slight at only  $2.2\% \pm 0.2$  of maximal norepinephrine response. It was not until the addition of  $1 \times 10^{-4}$  M lysergic acid that a noticeable contractile response of  $15.6\% \pm 2.3$  of maximal norepinephrine response was observed. Interestingly, the contractile response to lysergic acid was similar to that seen for norepinephrine (Figure 2) following subsequent flushing of the incubation chambers with fresh media devoid of lysergic acid. This is in contrast to data published by Solomons et al. (1989) showing that isolated bovine dorsal pedal vein exposed to ergotamine, at concentrations similar to those eliciting a response to lysergic acid in our study, resulted in a prolonged (>60 min) contractile response (even after repeated flushing with ergotamine-free buffer). Likewise, Dyer (1993) found that ergovaline exposure of isolated bovine uterine and umbilical veins resulted in a slow and prolonged vasoconstriction mediated by serotonin receptors. Contractile response the vessel to ergovaline ( $1 \times 10^{-8}$  M) did not abate after more than 3 h of repeated rinses with buffer devoid of ergovaline. Findings of this study and those of Solomons et al. (1989) and Dyer (1993) may indicate that the ergopeptines (Figure 1, panel C) have a much greater potential for bioaccumulation than lysergic acid.

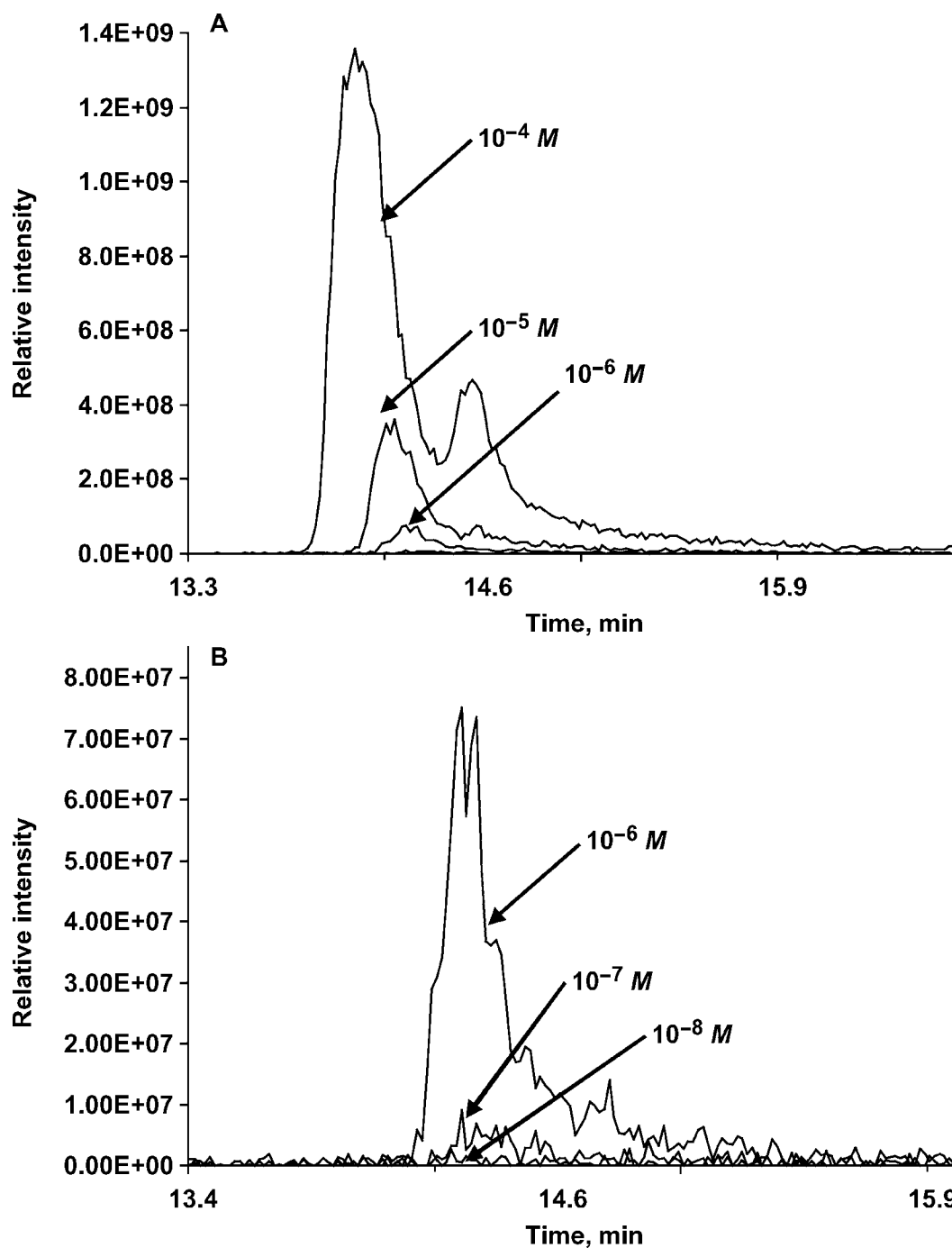
It is difficult to directly compare the maximal contractile response of the bioassay system used in the current study with those previously conducted using cattle or horse vessels because those reports did not normalize their findings to a contractile response of norepinephrine. However, some indirect comparisons can be made. Abney et al. (1993) reported that ergotamine ( $1 \times 10^{-4}$  M) and ergonovine ( $1 \times 10^{-4}$  M) produced >30% of the norepinephrine response at  $3.5 \times 10^{-4}$  M when tested using an isolated equine lateral saphenous vein bioassay. This is approximately twice the 15.6% reported here for lysergic acid based on a response to norepinephrine at the reduced concentration of  $1 \times 10^{-4}$  M. Also, the work of Dyer (1993) indicates that a response in the vessel system to ergovaline at  $1 \times 10^{-8}$  M was somewhat equivalent to a response generated by a  $1 \times 10^{-6}$  M concentration of norepinephrine. Responses to lysergic acid in the bioassay of the current experiment were minimal compared with the response indicated by Dyer (1993) for ergovaline. These comparisons, though indirect, may indicate that lysergic acid is a weak direct acting vasoconstrictor. The data presented here cannot, however, rule out the possibility of indirect effects of lysergic acid on vascular function.



**Figure 3.** A) Comparison of mean contractile dose-responses ( $n = 5$ ) of lateral saphenous veins isolated from the right and left hind limbs to increasing concentrations of norepinephrine; B) Comparison of mean contractile dose-responses ( $n = 5$ ) of lateral saphenous veins isolated from the right and left hind limbs to increasing concentrations of norepinephrine after overnight storage (2 to 8°C); C) Effects of 24-h storage on mean contractile dose-response ( $n = 7$ ) of lateral saphenous vein of the left hind limb to increasing concentrations of norepinephrine. Day 1 refers to analysis the same day as collection, and d 2 refers to analysis after storage overnight (2 to 8°C); D) Effects of 24-h storage on mean contractile dose-response ( $n = 7$ ) of lateral saphenous vein of the right hind limb to increasing concentrations of norepinephrine. Day 1 refers to analysis the same day as collection, and d 2 refers to analysis after storage overnight (2 to 8°C).

Lyons et al. (1986) reported that the highest total ergot alkaloid content in the plant occurs in the sheath, and the highest level reported was 13.8  $\mu\text{g/g}$  of plant tissue. If optimal conditions for intake and absorption are assumed, then a 300-kg steer consuming 2.5% of

BW could only consume a maximum of 103.5 mg of total ergot alkaloid from endophyte-infected tall fescue per day. If 100% of this were absorbed and converted to lysergic acid, assuming negligible bioaccumulation as indirectly evidenced by our data and assuming 10%



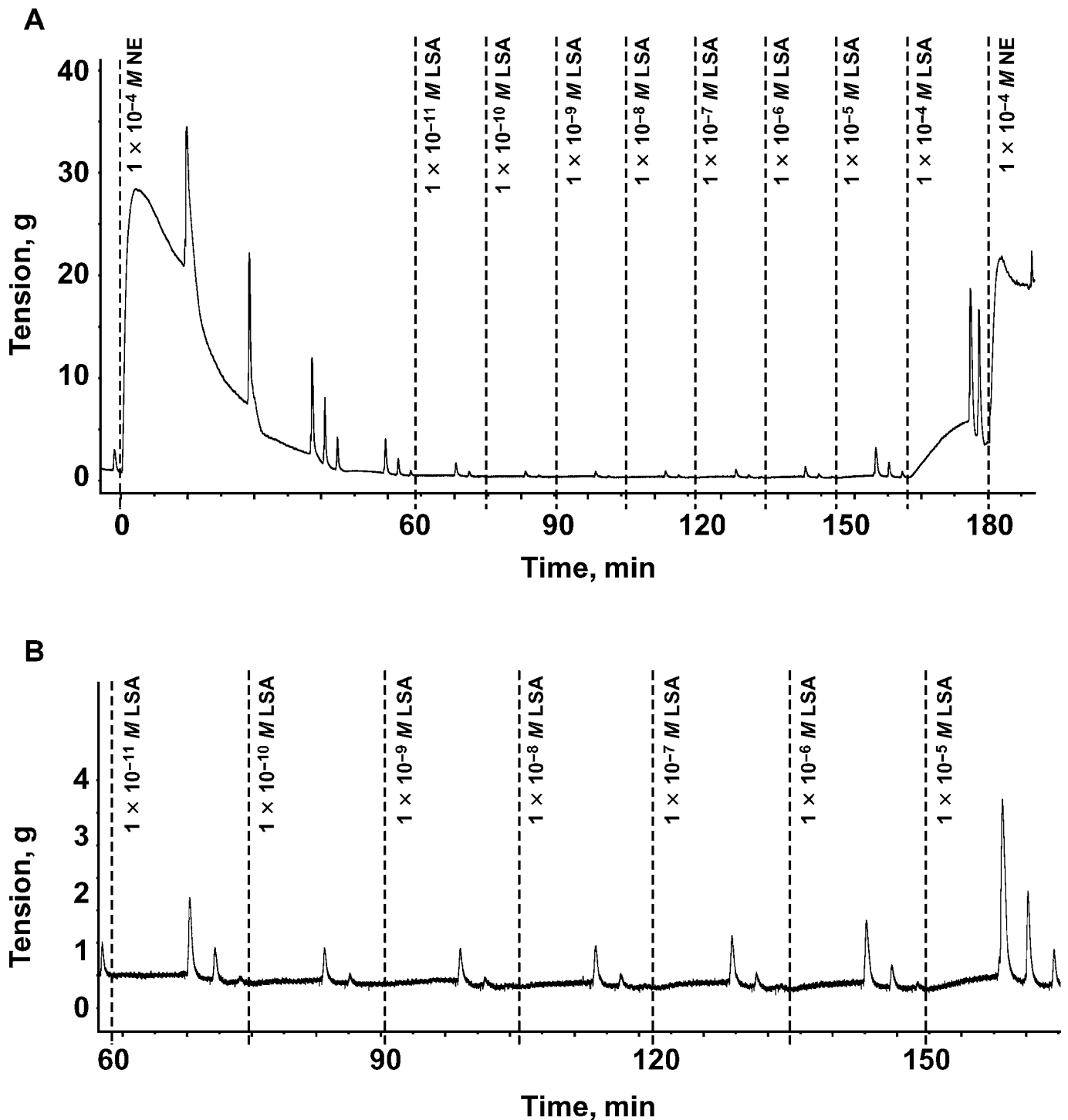
**Figure 4.** A) Total ion chromatograms for various concentrations ( $1 \times 10^{-4}$  to  $1 \times 10^{-8} M$ ) of lysergic acid in a modified Krebs-Henseleit buffer as analyzed using HPLC-mass spectrometry. The singly protonated molecular ion ( $MH^+$ ) for lysergic acid ( $m/z$  269) is plotted. B) A magnified view of the total ion chromatograms for the  $1 \times 10^{-6}$  to  $1 \times 10^{-8} M$  lysergic acid samples illustrates the detection limits for lysergic acid.

of the animal's body weight as blood, the maximum concentration of lysergic acid that could occur in the animals blood would be  $3.45 \mu\text{g/mL}$ . The  $1 \times 10^{-4} M$  concentration used in this study would approximate  $27 \mu\text{g/mL}$  of lysergic acid equivalents in blood. This concentration exceeds (approximately 7-fold) what was calculated above as possible under extremely optimum conditions and was the only concentration to generate meaningful vasoconstriction *in vitro*. Therefore, it is

unlikely that lysergic acid is the primary direct vascular toxicant associated with vasoconstriction caused by endophyte-infected tall fescue.

In summary, the use of venous tissue isolated from the left and right hind limbs exhibited contractile responses to norepinephrine in a dose-dependent manner and did not differ between limbs. The overnight storage of venous tissue in a modified Krebs-Henseleit buffer at 2 to  $8^\circ\text{C}$  did not affect tissue responsiveness to norepi-





**Figure 5.** Example of typical response of isolated bovine lateral saphenous vein cross sections to increasing concentrations of lysergic acid (LSA). The spikes that precede an addition are artifacts generated from buffer replacement and were not included in the data collection and analysis. A) Complete data recording on the myograph that includes initial addition of norepinephrine (NE), the addition of the LSA standards, and the concluding addition of NE. B) A magnified view of  $1 \times 10^{-11}$  to  $1 \times 10^{-5}$  M LSA additions.

nephrine. These findings will permit future experiments to utilize tissue from one leg and then utilize tissue from a second leg following an event such as exposure to endophyte-infected tall fescue. Additionally, data presented here show that lysergic acid is a

weak vasoconstrictor and then only at highly elevated levels as assessed by this blood vessel model. Lysergic acid does not appear to bind tightly to the vascular tissues, thereby reducing the potential for bioaccumulation, and may play a minor role in the expression of

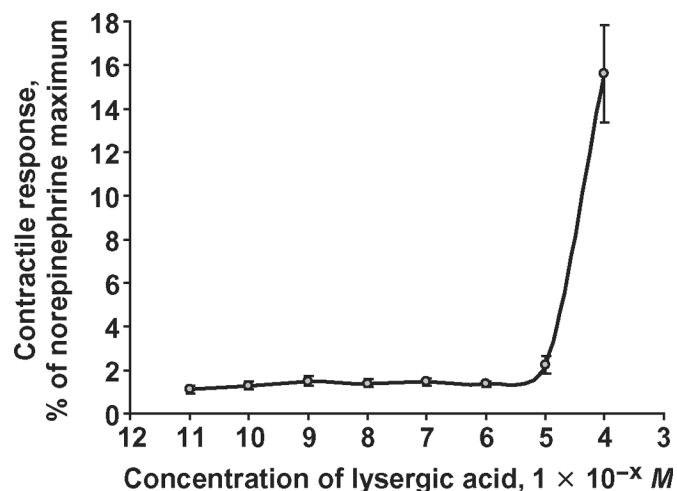


Figure 6. Mean contractile dose-response of bovine lateral saphenous vein to lysergic acid ( $n = 12$ ).

vascular complications associated with fescue toxicosis. However, further research is needed to fully address other direct and indirect effects that lysergic acid may contribute to fescue toxicosis.

#### LITERATURE CITED

- Abney, L. K., J. W. Oliver, and C. R. Reinemeyer. 1993. Vasoconstrictive effects of tall fescue alkaloids on equine vasculature. *J. Equine Vet. Sci.* 13:334-340.
- Baxter, G. M., R. E. Laskey, R. L. Tackett, J. N. Moore, and D. Allen. 1989. In vitro reactivity of digital arteries and veins to vasoconstrictive mediators in healthy horses and in horses with early laminitis. *Am. J. Vet. Res.* 50:508-517.
- Dyer, D. C. 1993. Evidence that ergovaline acts on serotonin receptors. *Life Sci.* 53:223-228.
- Hill, N. S. 2005. Absorption of ergot alkaloids in the ruminant. Pages 271-290 in *Neotyphodium in Cool Season Grasses*. C. A. Roberts, C. P. West, and D. E. Spiers, ed. Blackwell Publishing, Ames, IA.
- Hill, N. S., F. N. Thompson, J. A. Stuedemann, G. W. Rottinghaus, H. J. Ju, D. L. Dawe, and E. E. Hiatt, III. 2001. Ergot alkaloid transport across ruminant gastric tissues. *J. Anim. Sci.* 79:542-549.
- Lyons, P. C., R. D. Plattner, and C. W. Bacon. 1986. Occurrence of peptide and clavine ergot alkaloids in tall fescue grass. *Science* 232:487-489.
- Merck. 2001. Page 1010 (Record 5653) in *The Merck Index*. 13th ed. Merck and Co., Inc., Whitehouse Station, NJ.
- Mulvany, M. J., and W. Halpern. 1977. Contractile properties of small arterial resistance vessels in spontaneously hypertensive and normotensive rats. *Circ. Res.* 41:19-26.
- Nielsen-Kudsk, F., B. Poulsen, C. Ryom, and J. E. Nielsen-Kudsk. 1986. A strain-gauge myograph for isometric measurement of tension in isolated small blood vessels and other muscle preparations. *J. Pharmacol. Methods* 16:215-225.
- Oliver, J. W., L. K. Abney, J. R. Strickland, and R. D. Linnabary. 1993. Vasoconstriction in bovine vasculature induced by the tall fescue alkaloid lysergamide. *J. Anim. Sci.* 71:2708-2713.
- Oliver, J. W., R. G. Powell, L. K. Abney, R. D. Linnabary, and J. Petroski. 1990. N-acetyl loline induced vasoconstriction of the lateral saphenous vein (cranial branch) of cattle. Pages 239-243 in *Proc. Int. Symp. Acremonium/Grass Interactions*. New Orleans, LA. Elsevier Sci. Publ. B.V., Amsterdam, the Netherlands.
- Porter, J. K., C. W. Bacon, J. D. Robbins, and D. Betowski. 1981. Ergot alkaloid identification in Clavicipitaceae systemic fungi of pasture grasses. *J. Agric. Food Chem.* 29:653-657.
- Rhodes, M. T., J. A. Paterson, M. S. Kerley, H. E. Garner, and M. H. Laughlin. 1991. Reduced blood flow to peripheral and core body tissues in sheep and cattle induced by endophyte-infected tall fescue. *J. Anim. Sci.* 69:2033-2043.
- Schultz, C. L., S. L. Lodge-Ivey, L. P. Bush, A. M. Craig, and J. R. Strickland. 2006. Effects of initial and subacute exposure to an endophyte-infected tall fescue seed diet on faecal and urine concentrations of ergovaline and lysergic acid in mature geldings. *N. Z. Vet. J.* In Press.
- Solomons, R. N., J. W. Oliver, and R. D. Linnabary. 1989. Reactivity of the dorsal pedal vein of cattle to selected alkaloids associated with *Acremonium coenophialum*-infected grass. *Am. J. Vet. Res.* 50:235-238.
- Stuedemann, J. A., N. S. Hill, F. N. Thompson, R. A. Fayrer-Hosken, W. P. Hay, D. L. Dawe, S. H. Seman, and S. A. Martin. 1998. Urinary and biliary excretion of ergot alkaloids from steers that grazed endophyte-infected tall fescue. *J. Anim. Sci.* 76:2146-2154.

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