HPLC Method for Quantitating Ergovaline in Endophyte-Infested Tall Fescue: Seasonal Variation of Ergovaline Levels in Stems with Leaf Sheaths, Leaf Blades, and Seed Heads†

George E. Rottinghaus,*,‡ George B. Garner,§ Creighton N. Cornell,∥ and James L. Ellis§

Veterinary Medical Diagnostic Lab, College of Veterinary Medicine, Department of Animal Sciences, and Department of Biochemistry, University of Missouri, Columbia, Missouri 65211

A method for analysis of ergovaline in seed and vegetative tissue of endophyte-infested tall fescue by high-performance liquid chromatography with fluorescence detection was developed. Freeze-dried samples were extracted with alkaline chloroform, filtered, and applied to disposable HL silica gel columns. Following elution of plant pigments with acetone/chloroform, ergopeptine alkaloids were eluted with methanol under vacuum. Column eluant was evaporated to near dryness and the residue redissolved in methanol/water and analyzed by high-performance liquid chromatography; ergotamine was the internal standard. Average recoveries of ergovaline and ergotamine were 85 and 88%, respectively, with a relative standard deviation of 7.5%. The detection limit of ergovaline in fescue seed and vegetative tissue was 50 μg/kg. Replicate samples, collected each week during two growing seasons from experimental plots of KY-31 tall fescue (85–100% endophyte-infested), were separated into stems with leaf sheaths, leaf blades, and developing seed heads. Ergovaline levels in all plant tissues were 300–500 μg/kg from March through mid-June. In late June, ergovaline increased in seed heads to 1000–5000 μg/kg, while stems with leaf sheaths and leaf blade values remained at 300–500 μg/kg. Ergovaline in all plant tissues increased with nitrogen fertilization.

Most tall fescue grown in the United States is derived from Kentucky 31 (KY-31), originally collected from an eastern Kentucky farm in 1931 and released to the public by the University of Kentucky in 1942 (Buckner et al., 1979). Although the agronomic characteristics of KY-31 tall fescue are excellent, animal performance has been inconsistent and signs of toxicity have been observed. Specific disorders in cattle associated with toxic tall fescue include fescue foot (Garner and Cornell, 1978), fescue toxicosis (Hemken et al., 1984), fat necrosis (Bush et al., 1979; Stuedemann et al., 1973), and reduced milk production (Hemken et al., 1984; Wallner et al., 1983). Agalactia has been primarily associated with postparturient horses (Heimann et al., 1981).

In 1977, Bacon et al. reported the presence of an endophytic fungus, later identified as *Acremonium coenophialum*, in toxic tall fescue samples from several states. However, it was not until 1980 that a negative correlation between the level of endophyte and weight gain of steers grazing tall fescue was made (Hoveland et al., 1980). Other states soon published data confirming these observations (Garner et al., 1984; Read et al., 1984; Williams and Backman, 1984; Crawford et al., 1989). Endophyte-infested fescue was found to have its most insidious effects under conditions of high ambient temperature (Hemken et al., 1981). Affected cattle gained weight poorly or lost weight, salivated profusely, had reduced milk production, and exhibited increased body temperature and respiration rate; they sought shade or water in an attempt to dissipate body heat.

The similarity of fescue foot to chronic ergotism led researchers to search for vasoactive compounds similar to the ergopeptine alkaloids. In 1979, Porter et al. reported ergopeptine alkaloids were produced in broth cultures of the endophytic fungus. Yates et al. (1985), with the aid of tandem mass spectrometry (MS/MS), then identified significant levels of ergopeptine alkaloids in crude extracts of tall fescue from pastures producing clinical signs of fescue foot in cattle. Lyons et al. (1986) demonstrated ergot alkaloids to be ubiquitous in endophyte-infested tall fescue but absent when fescue was endophyte free. Ergovaline accounted for 84–97% of total ergopeptine alkaloid content; higher levels were present in leaf sheaths than in blades. In addition, high nitrogen fertilization substantially increased the ergopeptine alkaloid content of fescue plants (Lyons et al., 1986).

Fescue research efforts have been hampered by the lack of laboratories having MS/MS capabilities for ergopeptine analysis. Cost of MS/MS analysis is prohibitive, drastically limiting the number of samples that can economically be tested. Although a high-pressure liquid chromatographic (HPLC) method for ergovaline in fescue seed was reported by Yates et al. in 1988, it was inadequate for fresh plant tissue analysis. We have developed an HPLC method for quantitating ergovaline in both fescue seed and fresh plant material at low micrograms per kilogram levels. The endophytic fungus, *A. coenophialum*, can be identified microscopically in stems, leaf sheaths, and seeds, but has not been detected in leaf blades. We were interested in determining seasonal variation in ergovaline concentration of various parts of the fescue plant (stems with leaf sheaths, leaf blades, and seed heads). In addition, the effect of nitrogen fertilization on ergovaline production was investigated.

EXPERIMENTAL PROCEDURES

KY-31 Tall Fescue Plots (85–100% Endophyte-Infested). Experimental plots were located at University of Missouri South Farm, Columbia, MO. Each plot was treated with a single application of ammonium nitrate at rates of 0, 67.4, or 134.8 kg of N/ha in 1988. Three replicate samples (1 × 1 m) were collected weekly (mid-April through November) from each site in 1987

† Presented at the AAVLD Meeting, Little Rock, AR, Oct 16–18, 1988.

‡ College of Veterinary Medicine.

§ Department of Animal Sciences.

∥ Department of Biochemistry.

0021-8561/91/1439-0112$02.50/0

and 1988. Each sample was manually separated into stems with leaf sheaths, leaf blades, and seed heads. Composite samples of spring growth were collected from June 28 until August 16, 1988. Composite samples of fall regrowth of previously spring-harvested plots were collected from September 7 through November 3, 1988. Samples were freeze-dried, ground to pass a 2-mm screen, and stored at −20 °C to prevent decomposition of ergopeptine alkaloids before analysis.

Endophyte-Free Tall Fescue. Samples were supplied by the University of Georgia from a genotype of KY-31 tall fescue propagated for six generations and free of endophyte on microscopic examination.

Ergot Alkaloid Standards. Ergovaline standard was a gift from Sandoz Ltd., Basel, Switzerland. Ergotamine tartrate, used as an internal standard, was purchased from Sigma Chemical Co.

Extraction and Cleanup. Freeze-dried samples (10 g) were extracted with 100 mL of chloroform/0.001 M sodium hydroxide (9:1) in 250-mL screw-cap bottles containing 1 mL of internal standard (9 μg/mL ergotamine); bottles were placed on a wrist action shaker for 30 min. Extracts were then filtered through Whatman 1PS filter paper to remove any residual water. HL silica gel TLC plates (Analtech, Inc.) were scraped, and the silica gel–organic binder was ground in a Stein mill (Seedburo Equipment Co.). Silica gel cleanup columns were prepared by placing an 0.5-in. biological disk [Schleicher & Schuell (S&S) 740-E] in the bottom of a 6-mL Monoject disposable syringe barrel (Division of Sherwood Medical) followed by (a) 3 g of ground HL silica gel, (b) an S&S biological disk, (c) 1 g of sodium sulfate, and (d) an S&S biological disk. Columns were prewashed with chloroform. Ten milliliters of sample filtrates was applied to the disposable silica gel columns, and pigments were removed by washing the columns with 20 mL of acetone/chloroform (75:25) under vacuum. Ergot alkaloids were then eluted with 8 mL of methanol under vacuum. Methanol eluants were concentrated under reduced pressure at ca. 30 °C, redissolved in 1 mL of methanol/water (1:3) and filtered through a 0.22-μm nylon 66 membrane filter (Micron Separations, Inc.). All glassware was pretreated with Surfasil siliconizing agent (Pierce Chemical Co.) to prevent binding of ergopeptine alkaloids.

Liquid Chromatography. Samples were examined for ergovaline by a modification of the HPLC method described by Scott and Lawrence (1980). Primary standard solutions of ergotamine and ergovaline (0.5–1 mg/mL) in methanol were prepared weekly and stored at 0 °C. Working standard solutions (50–500 ng/mL) in methanol/water (1:2) were prepared daily. Analyses were performed on a Perkin-Elmer Series 2 liquid chromatograph equipped with a Rheodyne Model 7125 loop injector valve (20-μL capacity), a Perkin-Elmer 8.3-cm cartridge C_{18} column (3-μm particle size), and a Perkin-Elmer 650-10S fluorescence spectrophotometer; excitation wavelength was 250 nm (10-nm slit) and emission wavelength 420 nm (10-nm slit). Flow rate of mobile phase, 33% acetonitrile in a 200 mg/L solution of ammonium carbonate in distilled water, was 1 mL/min. As reported by Scott and Lawrence (1982), a saturator silica gel column was required prior to the injector to prevent deterioration of the analytical column at the alkaline pH of the mobile phase.

Statistical Design. The statistical design was a split plot in time with three replications. Main plots were the effects of level of nitrogen fertilization. Split plots were the effects of harvest date. The two-way interaction of fertilization level and harvest date was also included in the model (Snedecor and Cochran, 1980). Ergovaline concentration of each plant part (leaf blades, stems with leaf sheaths, seed heads) was analyzed by the GLM procedure of SAS (1985).

RESULTS AND DISCUSSION

Ergotamine proved to be an acceptable internal standard; it was readily available and had an elution time and a fluorescence response similar to those of ergovaline. Some ergotamine is produced by the endophyte (Yates et al., 1985) but at levels below the detection limit of the method (50 μg/kg). Ergovaline and ergotamine were found to bind tightly to silica gel containing an organic binder (HL silica gel plates); this property was used to rapidly clean up fescue seed and vegetative tissue extracts. The ergopeptine alkaloids and a number of plant pigments were bound to the silica gel column; plant pigments were eluted with chloroform/acetone, allowing the ergopeptine alkaloids to be quantitatively eluted from the cleanup column with methanol. A modification of the HPLC method of Scott and Lawrence (1980) readily separated the major ergopeptine alkaloid produced by the endophyte (ergovaline) and ergotamine, the internal standard. Typical HPLC chromatograms of endophyte-infested fescue tissue extracts and endophyte-free seed extracts are shown in Figure 1, with ergotamine added as the internal standard. Ergovaline was not detected in endophyte-free seed (Figure 1B). There was no interference from sample matrices. Peaks eluting prior to ergovaline were unidentified compounds extracted from tall fescue tissue; the small peak (5.5 min) eluting immediately after ergovaline was due to an impurity in a solvent. The ergovaline peak (5 min) was well separated from the ergotamine internal standard (10 min) by the isocratic mobile phase, and no late eluting peaks were observed, allowing a single analysis to be completed in just 12 min. Average recoveries of ergovaline and ergotamine were 85 and 88%, respectively, with a predetermined detection limit of 50 μg/kg for ergovaline. Lower detection limits were attainable but not applicable to our studies. Ergovaline was linear over the range 50–1000 μg/kg with a relative standard deviation of 7.5%. The response factor for ergovaline relative to ergotamine, 2.1, was determined by repetitive analysis.

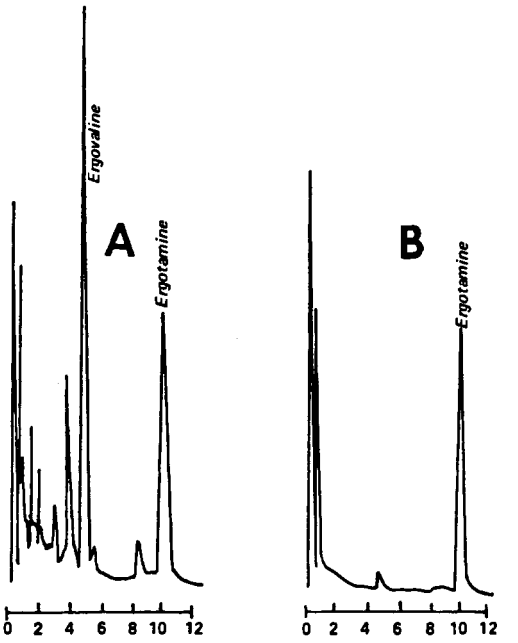

Figure 1. HPLC chromatograms illustrating separation of ergovaline and ergotamine in endophyte-infested fescue extracts (A) and in endophyte-free fescue seed extracts (B); ergotamine was the internal standard.

Previous research (Garner, unpublished data) indicated fescue diets containing 200 μg/kg ergovaline produced clinical signs of toxicity in heat-stressed steers. In 1987 (Figure 2) and 1988 (Figure 3) leaf blade ergovaline was less than 250 μg/kg during late April but increased to 450–500 μg/kg in early May; stems with leaf sheaths increased from 500 to 800–1300 μg/kg. Since endophyte is not present in leaf blades, ergovaline must be translocated from stems or leaf sheaths to leaf blades. These levels are sufficient to induce symptoms of toxicity in heat-stressed cattle. As seed heads began to develop in mid-

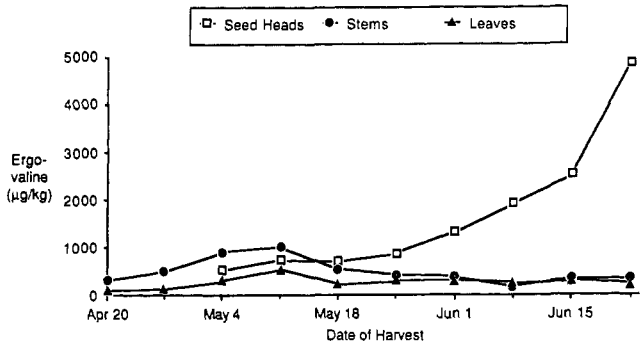

Figure 2. Seasonal variation (1987) of ergovaline in endophyte-infested tall fescue leaf blades, stems with sheaths, and seed heads. Nitrogen application was 67 kg/ha. Values presented are the average of three determinations.

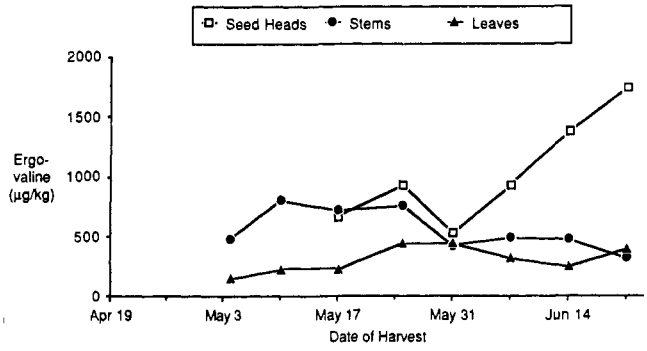

Figure 3. Seasonal variation (1988) of ergovaline in endophyte-infested tall fescue leaf blades, stems with sheaths, and seed heads. Nitrogen application was 67 kg/ha. Values presented are the average of nine determinations.

Table I. Least-Squares Means[a] of the Effect of Sampling Date on Ergovaline Concentration[b]

date	leaf blades	stems with leaf sheath	seed heads
5/4/88	177[c]	498	–[d]
5/10/88	321	1083	–
5/17/88	297	754	806
5/25/88	425	822	998
5/31/88	529	721	763
6/7/88	375	698	1123
6/14/88	296	511	1527
6/21/88	379	399	1648

[a] Each value is the mean of nine samples. [b] $P < 0.01$. [c] Micrograms of ergovaline per kilogram of sample. [d] Prior to seed formation.

May, ergovaline declined in both stems with leaf sheaths and leaf blades; ergovaline then peaked in seed heads by mid-June (5000 μg/kg, 1987, and 1700 μg/kg, 1988). After seed head maturation, ergovaline in stems with leaf sheaths or leaf blades remained at 200–400 μg/kg. Although ergovaline content of the whole plant after seed head maturation was reduced by loss of seeds, it remained at sufficient levels to produce toxicity symptoms in cattle grazing endophyte-infested fescue during hot humid conditions.

Leaf blades containing 177–529 μg/kg ergovaline ($P < 0.01$, Table I) are higher than the 100–300 μg/kg reported by Lyons et al. (1986). However, their date of harvest was not specified. Stems with leaf sheaths contained 399–1083 μg/kg ergovaline ($P < 0.01$, Table I). These values are lower than those (300–2800 μg/kg) reported by Lyons et al. (1986). Since mycelial mass is greater in leaf sheath than stem, our lower values would be expected. Seed head ergovaline was affected by harvest date as well ($P < 0.01$, Table I).

Table II. Least-Squares Means[a] of Effect of Nitrogen Fertilization on Ergovaline Levels

	nitrogen treatment, kg/ha		
plant tissue	0.0	67.4	134.8
leaf blades[b]	258[c]	306	485
stems with sheaths[d]	494	561	1003
seed heads[e]	895	1050	1488

[a] Each value is the mean of 24 samples (leaf blades and stems with leaf sheaths) or 18 samples (seed heads). [b] $P < 0.05$. [c] Micrograms of ergovaline per kilogram of sample. [d] $P < 0.01$. [e] $P = 0.053$.

Table III. Least-Squares Means[a] of Interaction of Nitrogen Level and Sample Date[b] (Seed Heads)

	nitrogen treatment, kg/ha		
sample date	0.0	67.4	134.8
5/17/88	786[c]	686	947
5/25/88	939	924	1013
5/31/88	691	527	1071
6/7/88	901	938	1530
6/14/88	1091	1372	2117
6/21/88	959	1735	2251

[a] Each value is the mean of three samples. [b] $P = 0.075$. [c] Micrograms of ergovaline per kilogram of sample.

Ergovaline in all endophyte-infested tissues increased as nitrogen increased from 0 to 67.4 to 134.8 kg of N/ha (Table II). A large ergovaline increase ($P < 0.01$) in stems with leaf sheaths followed the highest nitrogen application. Similarly, leaf blade values were elevated ($P < 0.05$). The same pattern of ergovaline increase occurred in seed heads ($P = 0.053$).

Leaf blades from fescue receiving 134.8 kg/ha nitrogen contained 485 μg/kg ergovaline (Table II). This is similar to 470 μg/kg ergovaline in leaf blades receiving high levels of ammonium sulfate (Lyons et al., 1986). However, stems with leaf sheaths from the 134.8 kg of N/ha plots contained 1003 μg/kg ergovaline (Table II), considerably less than the 4000 μg/kg in leaf sheaths of fescue receiving high levels of ammonium sulfate (Lyons et al., 1986). Ergovaline in stems with leaf sheaths would be expected to be lower than in leaf sheaths alone. There was an interaction of nitrogen level with sample date in seed heads ($P = 0.075$, Table III). During early seed head development (through the end of May), ergovaline was not affected by nitrogen fertilization. However, as seed heads matured in June, ergovaline increased relative to nitrogen fertilization; 134.8 kg of N/ha stimulated a 2-fold increase when compared with no fertilization.

After seed head maturation, ergovaline slowly declined in weekly composite samples from early July to early August and then increased during mid-August ($P < 0.01$, Table IV). Ergovaline varied from 166 to 340 μg/kg. After August, composite samples of fall regrowth from spring-harvested plots were collected. Ergovaline in fescue regrowth varied from 839 μg/kg in early October to 314 μg/kg in early November ($P < 0.01$). These values are higher than those reported by Yates et al. (1985), even though their samples were fall regrowth taken in October.

These discrepancies in ergovaline levels may be explained by differences in environmental conditions during plant growth, differences in endophyte and/or fescue genotypes (Hill et al., 1990), or differences in crop management (Hill et al., 1990).

Ergovaline in both late-summer composite (July and August) and fall composite regrowth samples was increased by nitrogen fertilization ($P < 0.01$ and $P < 0.05$, respectively, Table V). This trend is in agreement with that reported by Lyons et al. (1986). Only late-summer low and

Table IV. Least-Squares Means[a] of Composite Samples by Harvest Date

date	ergovaline, μg/kg	date	ergovaline, μg/kg
spring growth[b]		fall regrowth[c]	
6/28/88	238	9/7/88	562
7/5/88	340	9/14/88	596
7/12/88	248	9/21/88	510
7/19/88	197	9/28/88	632
7/27/88	176	10/4/88	839
8/3/88	166	10/12/88	590
8/11/88	196	10/19/88	392
8/16/88	291	11/3/88	314

[a] Each value is the mean of nine samples. [b] $P < 0.01$. [c] $P < 0.01$.

Table V. Least-Squares Means[a] of Ergovaline in Composite Samples by Nitrogen Treatment

nitrogen treatment, kg/ha	spring growth[b]	fall regrowth[c]
134.8	377[d]	658
67.4	181	544
0.0	123	453

[a] Each value is the mean of 24 samples. [b] $P < 0.01$. [c] $P < 0.05$. [d] Micrograms of ergovaline per kilogram of sample.

medium nitrogen treatments (0 and 67 kg of N/ha) resulted in ergovaline levels below the 200 μg/kg required to produce toxicity symptoms in heat-stressed steers.

In practice, this method has been a valuable screening tool, allowing as many as 18 samples to be analyzed per day. The simple sample preparation in conjunction with HPLC and fluorescence detection allows rapid detection of low levels of ergovaline. Because 3 million acres of tall fescue in the United States cannot be easily or economically renovated to an endophyte-free status, this rapid analysis should provide a valuable tool for determining the level of ergovaline and/or presence of the endophytic fungus. Continued study of ergovaline content of endophyte-infested tall fescue forage under different environmental and management conditions will lead to the development of agricultural practices enabling producers to better manage their tall fescue pastures.

ACKNOWLEDGMENT

We thank Dr. A. Lindemann, Sandoz Ltd., Basel, Switzerland, for the ergovaline analytical standard; Dr. Nick S. Hill, Department of Agronomy, University of Georgia, for the endophyte-free tall fescue samples; and Frank Ross, National Veterinary Service Laboratory, U.S. Department of Agriculture, Ames, IA, and Ron Plattner, Northern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Peoria, IL, for tandem mass spectrometry confirmation analysis. We also thank Michael Rankin, Marsha Carrol, Cori Sanders, and Linda Schultz for technical assistance. This work was supported in part by USDA Animal Health Research Formula Funding, The Seasonal Variation of Ergovaline Levels in Endophyte Infected Tall Fescue Stems, Leaves and Seed Heads.

LITERATURE CITED

Bacon, C. W.; Porter, J. K.; Robbins, J. D.; Luttrell, E. S. *Epichloe typhina* from Toxic Tall Fescue Grasses. *Appl. Environ. Microbol.* **1977**, *34*, 576–581.

Buckner, R. C.; Powell, J. B.; Frakes, R. V. Historical Development. In *Tall Fescue Monograph*; Buckner, R. C., Bush, L. P., Eds.; American Society of Agronomy: Madison, WI, 1979; pp 1–8.

Bush, L.; Bowling, J.; Yates, S. Animal Disorders. In *Tall Fescue Monograph*; Buckner, R. C., Bush, L. P., Eds.; American Society of Agronomy: Madison, WI, 1979; pp 247–292.

Crawford, R. J., Jr.; Forwood, J. R.; Belyea, R. L.; Garner, G. B. Relationship Between Level of Endophyte Infection and Cattle Gains on Tall Fescue. *J. Prod. Agric.* **1989**, *2*, 147–151.

Garner, G. B.; Cornell, C. N. Fescue Foot in Cattle. In *Mycotoxic Fungi, Mycotoxins, Mycotoxicoses: An Encyclopedic Handbook*; Wyllie, T. D., Morehouse, L. G., Eds.; Dekker: New York, 1978; Vol. 2, pp 45–62.

Garner, G. B.; Morrow, R. E.; Fairbrother, T. E.; Gerrish, J. R.; Nelson, C. J.; Jacobs, V. E.; Hires, W. G. Nursing Cows, Calves and Steers Response to Simultaneously Grazed Tall Fescue Pastures with Varying Levels of Endophyte *E. typhina*. *Proceedings*, Forage and Grassland Conference, Houston, TX; American Forage and Grassland Council: Lexington, KY, 1984; pp 246–250.

Heimann, E. D.; Garrett, L. W.; Loch, W. E.; Morris, J. S.; Pfander, W. H. Selenium and Reproductive Abnormalities in Pregnant Pony Mares Grazing Fescue Pastures. *Proceedings*, 7th Equine Nutrition Physiology Symposium; 1981; p 62.

Hemken, R. W.; Boling, J. A.; Bull, L. S.; Hatton, R. H.; Buckner, R. C.; Bush, L. P. Interaction of Environmental Temperature and Anti-quality Factors on the Severity of Summer Fescue Toxicosis. *J. Anim. Sci.* **1981**, *52*, 710–714.

Hemken, R. W.; Jackson, J. A., Jr.; Boling, J. A. Toxic Factors in Tall Fescue. *J. Anim. Sci.* **1984**, *58*, 1011–1016.

Hill, N. S.; Stringer, W. C.; Rottinghaus, G. E.; Belesky, D. P.; Parrott, W. A.; Pope, D. D. Growth, Morphological, and Chemical Responses of Tall Fescue to *Acremonium coenophialum*. *Crop Sci.* **1990**, *30*, 156–161.

Hoveland, C. S.; Haaland, R. L.; King, C. C., Jr.; Anthony, W. B.; McGuire, J. A.; Smith, L. A.; Grimes, H. W.; Holliman, J. L. Steer Performance on AP-2 Phalaris and Kentucky 31 Tall Fescue Pasture. *Agron. J.* **1980**, *72*, 375–377.

Lyons, P. C.; Plattner, R. D.; Bacon, C. W. Occurrence of Peptide and Clavine Ergot Alkaloids in Tall Fescue Grass. *Science (Washington, D.C.)* **1986**, *232*, 487–489.

Porter, J. K.; Bacon, C. W.; Robbins, J. D. Ergosine, Ergosinine, and Chanoclavine I from *E. typhina*. *J. Agric. Food Chem.* **1979**, *27*, 595–598.

Read, J. C.; Davis, C.; Camp, B. J.; Giroir, E. Effect of an Endophyte in Tall Fescue on Animal Performance. *Proceedings*, Forage and Grasslands Conference, Houston, TX; American Forage and Grassland Council: Lexington, KY, 1984; pp 240–243.

SAS. *SAS Users Guide: Statistics*; SAS Institute: Cary, NC, 1985.

Scott, P. M.; Lawrence, G. A. Analysis of Ergot Alkaloids in Flour. *J. Agric. Food Chem.* **1980**, *28*, 1258–1261.

Scott, P. M.; Lawrence, G. A. Loss of Ergot Alkaloids during Making of Bread and Pancakes. *J. Agric. Food Chem.* **1982**, *30*, 445–450.

Snedecor, G. W.; Cochran, W. G. *Statistical Methods*, 7th ed.; Iowa State University Press: Ames, IA, 1980.

Stuedemann, J. A.; Wilkinson, S. R.; Williams, D. J.; Jackson, W. A.; Jones, J. B., Jr. The Association of Fat Necrosis in Beef Cattle with Heavily Fertilized Fescue Pastures. In *Proceedings*, Fescue Toxicity Conference, Lexington, KY; Garner, G. B., Tookey, H., Jacobson, D. R., Eds.; Extension Publication: University of Missouri, Columbia, MO, 1973; pp 9–23.

Wallner, B. M.; Booth, N. H.; Robbins, J. D.; Bacon, C. W.; Porter, J. K.; Kiser, T. E.; Wilson, R.; Johnson, B. Effect of an Endophytic Fungus Isolated from Toxic Pasture Grass on Serum Prolactin Concentrations in the Lactating Cow. *Am. J. Vet. Res.* **1983**, *44*, 1317–1322.

Williams, M. J.; Backman, P. A. Treatments for Control of the Fungal Endophyte of Tall Fescue. *Proceedings*, Forage and Grasslands Conference, Houston, TX; American Forage and Grassland Council Lexington, KY, 1984; pp 78–82.

Yates, S. G.; Powell, R. G. Analysis of Ergopeptine Alkaloids in Endophyte-Infected Tall Fescue. *J. Agric. Food Chem.* **1988**, *36*, 337–340.

Yates, S. G.; Plattner, R. D.; Garner, G. B. Detection of Ergopeptine Alkaloids in Endophyte-Infected, Toxic Ky-31 Tall Fescue by Mass Spectrometry/Mass Spectrometry. *J. Agric. Food Chem.* **1985**, *33*, 719–722.

Received for review April 24, 1990. Accepted June 25, 1990.