# Improved Method of Analysis for Ergovaline in Tall Fescue by High-Performance Liquid Chromatography

Richard A. Shelby*

Department of Plant Pathology, Auburn University, Alabama 36849

Miroslav Flieger†

Institute of Microbiology, Czech Academy of Sciences, Prague 4 14220, Czech Republic

A rapid HPLC method of analysis for ergovaline in tall fescue plants is presented; it offers the advantages of utilizing readily available materials that are safer to use than those in previous methods. In addition, the sample preparation and chromatographic method allow for the detection and quantification of both C-8 epimers of the target alkaloid. Inclusion of the epimer in the analysis procedure provides a more accurate method of determining total ergovaline in plant materials.

**Keywords:** *Festuca arundinacea; Neotyphodium coenophialum; Acremonium coenophialum*

The ergot alkaloids are produced by several species of phytopathogenic and endophytic fungi, most notably members of the genus *Claviceps* common in grain crops. Yates et al. (1985) first conclusively identified this class of alkaloids in tall fescue seed infested by the endophytic fungus *Acremonium coenophialum* (since renamed *Neotyphodium coenophialum*). They found ergovaline (EV) to be the predominant ergopeptine alkaloid, with lesser amounts of ergosine (ES), ergotamine (EA), and several others. As is the case with ergot infestation of grain crops, these alkaloids in tall fescue have been shown to produce a spectrum of detrimental pharmacological effects in animals consuming endophyte-infested forage, known commonly as fescue toxicosis. These effects include, but are not limited to, poor weight gain (Neal and Schmidt, 1985; Read and Camp, 1986), low milk production (Henson et al., 1984), poor reproduction (Brendemuehl et al., 1994; Zavos et al., 1987), depressed immune response (Dew et al., 1990), and various direct endocrine effects (Porter et al., 1985, 1993; Thompson et al., 1987). Rapid, accurate detection and quantification of these compounds allow laboratories to predict animal performance on pastures and can aid in the diagnosis of fescue toxicosis.

High-performance liquid chromatography (HPLC) is the preferred method of analysis to detect and quantify these alkaloids, due to the sensitivity and quantitative accuracy of the method. Since the ergot compounds with a double bond at position 9,10 fluoresce strongly in UV light, fluorescence detection offers both enhanced sensitivity and decreased background interference in the chromatogram, when compared to other methods of detection. HPLC has been used to detect ergot compounds in wheat or rye (Fajardo et al., 1994; Scott and Lawrence, 1980; Ware et al., 1986), pharmaceutical preparations (Cieri, 1987), plasma (Elund, 1981), and cultures (Flieger et al., 1989). The method was also adapted to detect ergovaline in tall fescue seeds and forage (Hill et al., 1993; Rottinghaus et al., 1991; Craig et al., 1994; Welty et al., 1994; Yates and Powell, 1988). Typically, most of these methods employ reversed phase chromatography, with a $C_{18}$ stationary phase and an alkaline acetonitrile−water mobile phase. Sample preparation is the step most crucial to successful analysis of these compounds and usually involves extraction of the ergot compounds with organic solvent such as alkaline chloroform (Hill et al., 1993; Rottinghaus et al., 1991), methanol (Cieri, 1987; Elund, 1981; TePaske et al., 1993; Yates and Powell, 1988), or ethyl acetate (Fajardo et al., 1994; Ware et al., 1986) and alkaline mixtures of methylene chloride, ethyl acetate, and methanol (Scott and Lawrence, 1980). After extraction, samples are concentrated and separated from other compounds by combinations of solvent partitioning, rotary evaporation, and small silica or $C_{18}$ cleanup columns. Samples are then injected into the HPLC for chromatographic analysis and integration of peaks. A recent method for analysis of ergovaline in tall fescue (Hill et al., 1993) reduces sample preparation time by purifying the extract on small hand-made HL silica columns. This simplified method increases laboratory productivity without a sacrifice in accuracy.

The naturally occurring ergot alkaloids with an intact ergoline moiety can be divided into two groups, based on the position of the double bond at 8−9 or 9−10. The double bond in the 9−10 position of the ergoline ring system has a twofold effect. Those compounds are fluorescent, while those with the 8−9 double bond are not, and those with the 9−10 double bond also exhibit isomerism, which is favored in polar solvents. The degree of isomerization depends upon the particular solvent, and also upon the nature of the group attached at C-8, but typically proceeds to a stable equilibrium mixture of stereoisomers (epimers) over a period of time. Several published methods (Yates and Powell, 1988; Porter et al., 1981) address the problem of epimerization in the extraction and identification of ergot alkaloids, but most do not. Our method is also a simplified extraction and purification procedure that involves fewer steps than classical procedures while maintaining adequate sensitivity for detection of ergovaline at levels lower than would be expected to cause intoxication in grazing animals.

* Author to whom correspondence should be addressed [e-mail rshelby@acesag.auburn.edu; fax (334) 844-1947].

† E-mail flieger@biomed.cas.cz

S0021-8561(96)00575-4 CCC: $14.00 

## MATERIALS AND METHODS

**Sample Collection.** Pastures were sampled in March 1996 by cutting young tillers while walking transects within pastures of the Black Belt Substation of the Alabama Agricultural Experiment Station, Marion Junction, AL. Tillers were packaged and immediately transported to the laboratory, where they were frozen and subjected to microscopic analysis for fungal infection (Shelby and Dalrymple, 1987). All tillers were then dried in a 60 °C oven for 24 h and ground to pass a 1 mm screen.

**Sample Extraction and Purification.** Five grams of each dried, ground plant sample was weighed into a disposable plastic container and spiked with an internal standard of ergotamine tartrate (Sigma Chemical Co., St. Louis, MO; E-4768, fw = 1314) in methanol solution at 1.2 $\mu$g/mL calculated as free base (fw = 581). This spiking solution was stored at −10 °C for several weeks, without apparent loss of detectable ergotamine, other than the expected epimerization. It was added at 1 mL/g dry weight of plant material to give a final concentration of 1.2 $\mu$g/g and allowed to air-dry at room temperature for 1 h. Fifty milliliters of acetic acid solution (20% in distilled water) was then added to the plant material, shaken briefly, and allowed to stand at room temperature in subdued light for 1 h (HAc method). Samples were then gravity filtered (Whatman No. 4 filter paper), and 10 mL of each filtrate was added to $C_{18}$ SepPak Vac columns (Waters Chromatography, Milford, MA; No. 023590) in a vacuum manifold. To prepare columns for use, they were first preconditioned with 1 mL of methanol, followed by 3 mL of water. Columns were washed with 4 mL of 20% acetic acid solution, followed by 4 mL of water, and the samples were eluted with 1 mL of alkaline methanol (+ 0.04% ammonium hydroxide). For comparison, the extraction and purification method of Hill et al. (1993) was conducted on the same spiked samples ($CHCl_3$ method).

The HAc/$C_{18}$ and $CHCl_3$/silica methods were compared using solutions of ergotamine standard to measure relative recovery from the two columns. The methanol stock solution was diluted in the appropriate solvent ($CHCL_3$ or HAc solution) to 1.11 $\mu$g/mL. Ten milliliters of each solution was applied to the appropriate column in five replications and extracted as described above. Ergotamine and ergotaminine recoveries were tested by HPLC.

**HPLC Analysis.** Chromatography of sample extracts was conducted on a Waters HPLC system with a $C_{18}$ 8 mm × 100 mm Nova-Pak radial compression module column (Waters Chromatography; No. 086432) with a guard column (Waters No. 015220) of the same material. Detection was with a Waters No. 470 fluorometer (excitation 310 nm, emission 415 nm, attn = 1, gain = 1000). Mobile phase was isocratic methanol + water + ammonium hydroxide (70:30:0.04), at a flow rate of 1 mL/min. Mobile phase was filtered and degassed by passing through a 4 mm × 0.45 mm PTFE filter (Gelman No. 4472) prior to use. Injection volume was 5 $\mu$L by means of a Waters U6K injector with a 2000 $\mu$L loop. Quantitative analysis was by means of Waters Baseline software. Areas of integrated peaks were compared by linear regression to standard curves generated previously from chromatographic analyses of ergotamine tartrate and ergovaline analytical standards. Quantitative analysis of ergovaline was expressed as nanograms per gram of dry weight on the basis of recovery of ergotamine internal standard and calculated from the equation

$$\text{ng/g EV in plant sample} = (\text{ng of EA in HPLC}/1200) \times \text{ng of EV in HPLC}$$

## RESULTS AND DISCUSSION

**HPLC Chromatographic Conditions.** Our chromatographic conditions differ from those of previous methods mainly in the use of methanol−water as a mobile phase, rather than acetonitrile−water. Although we do not present data here, we have used both mobile phases for the chromatographic separation of ergot alkaloids and prefer methanol, which has the advantages of being less toxic and less expensive with no appreciable loss of sensitivity. We also use a different detector wavelength combination (excitation, 310 nm; emission, 415 nm) than most published ergovaline detection methods, which typically use an excitation wavelength of 230−250 nm and an emission of 389−430 nm. Excitation and emission wavelength scans (Figure 1) indicate two absorbance maxima. Although signal strength is greater at the lower excitation wavelength, the higher excitation wavelength (310 nm) is more specific for ergot alkaloids with the 9−10 double bond.

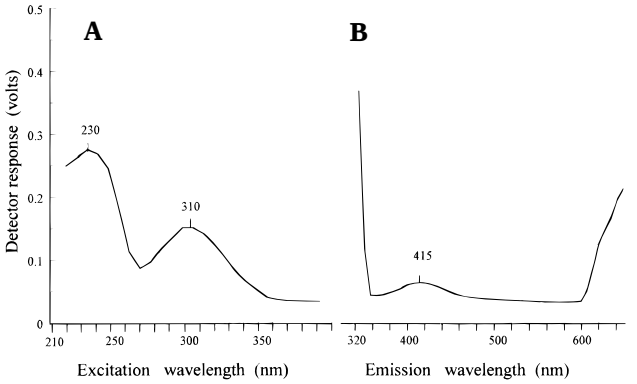


**Figure 1.** Fluorescence scans of ergotamine tartrate 1 $\mu$g/mL in methanol: (A) excitation wavelength scan 210−400 nm, em = 415 nm; (B) emission wavelength scan 320−650 nm, ex = 310 nm.

**Table 1. Comparison of Column Recovery by Both Methods**

| | Hac method | | | | $CHCl_3$ method | | | |
|---|---|---|---|---|---|---|---|---|
| | ng/5 mL injected | | | | ng/5 mL injected | | | |
| | EA[a] | EA-ine | total[b] | %[c] | EA | EA-ine | total | % |
| 1 | 8.78 | 4.26 | 13.04 | 117.3 | 9.17 | 0.11 | 9.28 | 83.4 |
| 2 | 7.49 | 0.60 | 11.09 | 99.0 | 8.29 | 0.09 | 8.38 | 75.3 |
| 3 | 8.13 | 3.97 | 12.10 | 108.8 | 9.04 | 0.15 | 9.19 | 82.6 |
| 4 | 8.62 | 4.22 | 12.84 | 115.5 | 10.12 | 0.18 | 10.30 | 92.6 |
| 5 | 9.82 | 4.82 | 14.65 | 131.7 | 9.44 | 0.24 | 9.68 | 87.0 |
| mean | 8.57 | 4.18 | 12.74 | 115.0 | 9.22 | 0.15 | 9.37 | 84.2 |

[a] EA, ergotamine; EA-ine, ergotaminine. [b] Total = EA + EA-ine. [c] Percent recovery based on total load of 11.1 $\mu$g per column.

**Extraction Columns.** Table 1 demonstrates the difference between extraction strategies in these two methods. The ergotamine stock solution was purposely chosen to have formed ergotaminine epimer to measure relative efficiency of recovery of both epimers by both methods. Both methods compare favorably in ergotamine recovery, but the HL silica columns fail to recover significant amounts of ergotamine.

**Extraction and Purification.** A loss of extractable ergot alkaloids or epimerization may result from the process of air-drying plant material at 60 °C, but we have not tested this hypothesis. Such added precautions as freeze-drying, or drying under a stream of nitrogen, might contribute to increased total extractable ergot alkaloids, if absolute precision of recovery is required. Ergovaline levels are significantly correlated with fungal infection, regardless of the method of analysis ($R^2$ = 0.894 and 0.761 for the HAc and $CHCl_3$ methods, respectively). The two methods of extraction and purification produce significantly different chromatographic profiles (Figure 2). The $CHCl_3$ method does not recover significant quantities of the C-8 epimers of either ergovaline or the internal standard, ergotamine. The -ine epimers form strong hydrogen bonds on silica, while the -inine or "-iso" forms do not, owing to

**Table 2. Comparison of HPLC Analyses of 10 Tall Fescue Forage Samples by Both Methods**

| | | HAc method | | | | | | | $CHCl_3$ method | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | ng/5 mL injected | | | | | | | ng/5 mL injected | | | | | | |
| sample | | -ine | | -inine | | total | | calcd | -ine | | -inine | | total | | calcd |
| ID | INF[a] | EV[b] | EA | EV | EA | EV[c] | EA | EV[d] | EV | EA | EV | EA | EV | EA | EV |
| 7600 | 3 | 0.06 | 5.55 | 0.01 | 1.28 | 0.07 | 6.82 | 12.24 | 0.01 | 0.19 | 0.00 | 0.00 | 0.01 | 0.19 | 61.34 |
| 7585 | 10 | 0.09 | 4.92 | 0.02 | 2.00 | 0.11 | 6.92 | 18.63 | 0.08 | 1.74 | 0.03 | 0.18 | 0.10 | 1.92 | 62.99 |
| 7593 | 10 | 0.05 | 4.57 | 0.04 | 1.83 | 0.09 | 6.40 | 16.00 | 0.01 | 1.33 | 0.00 | 0.00 | 0.01 | 1.33 | 11.01 |
| 7594 | 23 | 0.14 | 4.34 | 0.05 | 1.94 | 0.18 | 6.28 | 34.47 | 0.04 | 1.35 | 0.00 | 0.02 | 0.04 | 1.37 | 37.77 |
| 7589 | 30 | 0.36 | 5.31 | 0.15 | 2.17 | 0.51 | 7.48 | 81.37 | 0.11 | 0.88 | 0.00 | 0.00 | 0.11 | 0.88 | 147.93 |
| 7586 | 50 | 0.81 | 3.72 | 0.38 | 1.82 | 1.19 | 5.54 | 258.93 | 0.06 | 0.27 | 0.00 | 0.00 | 0.06 | 0.27 | 250.44 |
| 7598 | 77 | 1.02 | 4.34 | 0.49 | 2.50 | 1.51 | 6.84 | 265.06 | 0.25 | 1.00 | 0.00 | 0.00 | 0.25 | 1.00 | 301.08 |
| 7582 | 90 | 1.46 | 3.40 | 0.44 | 0.87 | 1.89 | 4.27 | 532.18 | 0.19 | 0.85 | 0.00 | 0.00 | 0.19 | 0.85 | 261.13 |
| 7568 | 93 | 2.33 | 4.71 | 0.74 | 0.98 | 3.07 | 5.69 | 647.71 | 0.22 | 1.07 | 0.00 | 0.00 | 0.22 | 1.07 | 249.40 |
| 7607 | 100 | 1.91 | 2.54 | 0.80 | 0.48 | 2.70 | 3.01 | 1075.29 | 0.59 | 1.24 | 0.00 | 0.00 | 0.59 | 1.24 | 569.47 |
| regression[e] | | $EV = 74 - 5.8\,(INF) + 0.14\,(INF)^2$ $R^2 = 0.894$ | | | | | | | $EV = 32 + 2\,(INF) + 0.017\,(INF)^2$ $R^2 = 0.761$ | | | | | | |

[a] Endophyte infection percentage determined by microscopy. [b] EV, ergovaline; EA, ergotamine. [c] Total = EV + EV-ine, EA + EA-ine. [d] Total ergovaline in sample expressed as ng/g dry weight based on recovery of internal standard. [e] Regression of percent endophyte on ng/g ergovaline in sample.

intramolecular hydrogen bonds in this configuration (Berde and Schild, 1978). This phenomenon is the basis for both normal and reversed phase chromatographic separation of epimers of the ergot alkaloids with the 9−10 double bond. Epimerization of ergopeptine alkaloids occurs in methanol solutions common to both methods described here. In HAc they form an equilibrium mixture quickly, and in MeOH remain so. We have, therefore, chosen to include them in the analysis and report the total to clients concerned about toxicosis. An additional advantage of including C-8 epimers in the chromatogram is that they aid in the positive identification of the target alkaloids. Any ergot compound with a 9−10 double bond which normally forms an epimer should do so in this system designed to encourage epimerization. Epimerization of the ergotamine internal standard as well as the target alkaloids may take place after extraction and should therefore be taken into account by measuring both ergotamine and ergotaminine of the spiking solution. The solvent used for the spiking solution may also affect the relative recoveries of internal standard and the target alkaloids (G. Rottinghaus, personal communication). The $CHCl_3$ method does have the advantage of producing chromatographic profiles with less nontarget background. In particular, the large initial peak in chromatograms produced by the HAc method could obscure extremely small ergovaline peaks that appear early in the chromatograms. We did not observe this problem, however, even at the lowest level of infection.

The ratio of ergovaline to ergotamine internal standard was greater in the $CHCl_3$ method, resulting in a calculated ergovaline level that is greater in those samples having a lower fungal infection and less ergovaline (Table 2). Conversely, at higher fungal infection levels, the HAc method recovers a greater proportion of ergovaline relative to ergotamine internal standard, which results in a higher calculated ergovaline level. Since recovery of the ergot alkaloids varies, and is certainly never 100%, the addition of the ergotamine quantitative internal standard is crucial to generating accurate data. Several previous methods to measure ergovaline have utilized ergotamine as an internal standard (Rottinghaus et al., 1991; Hill et al., 1993; Craig et al., 1994; Welty et al., 1994). Ergotamine is a good choice for an internal standard, because it is readily available due to the fact that it has widespread pharmaceutical applications. Certain precautions should be considered before it can be used accurately as an internal standard to measure ergovaline in tall fescue.

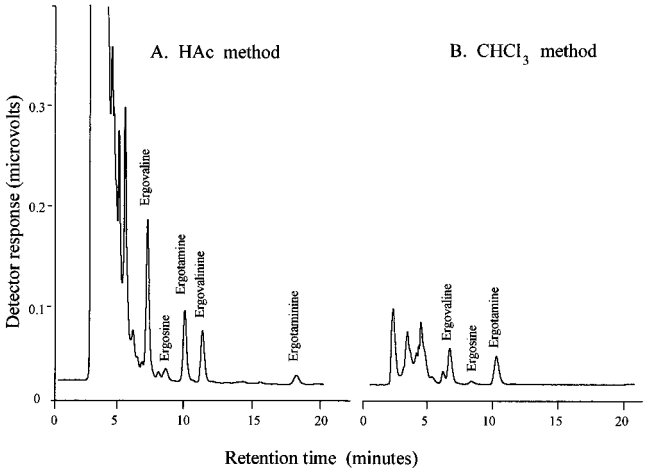


**Figure 2.** Typical chromatograms of infected fescue (sample 7607) comparing the two sample extraction and purification methods. Chromatographic conditions were identical (see text).

Calculations should include epimerization, which will proceed to equilibrium as previously described, so both C-8 epimers should be measured and added together and the sum used for the computation. Some quantitative inaccuracy may also result from the fact that ergotamine may be present in heavily infected forage samples. Yates et al. (1985) reported 0.036 $\mu$g/g in 93−98% infected fescue forage and 0.53 $\mu$g/g in similarly infected seeds (Yates and Powell, 1988), but they suggested *Claviceps sclerotia* may have been responsible for these alkaloids, rather than the endophyte. TePaske et al. (1993) also reported low concentrations of ergotamine and ergosine in endophyte-infected fescue forage. Our observations (data not shown) bear out the fact that occasional *Claviceps* infection may increase levels of ergotamine, ergosine, ergocornine, ergokryptine, and ergocristine, as well, but that ergine, ergosine, ergonine, and some other ergot alkaloids are also typically produced by the endophyte. The typical chromatogram (Figure 2A) shows ergosine in this plant sample, where *Claviceps* infection is not possible, owing to the absence of inflorescence tissues in the sample. Prior to analysis for ergovaline by this method, or any other using ergotamine as an internal standard, the possibility of having *Claviceps* in the sample material should be considered and eliminated if possible.

Other ergot alkaloids may be present in endophyte-infected fescue, particularly seeds, which characteristically have 10-fold higher concentrations of these com-

pounds when compared to stems of the same infected plants. Particularly in the earlier eluting peaks of these chromatograms, infected plants show a number of peaks not found in uninfected controls. These have been variously suggested to be ergonovine (Yates and Powell, 1988) or lysergic acid amide (TePaske et al., 1993).

As Scott and Lawrence (1980) observed, moisture or solvent of crystallization can greatly influence the weight of the ergot alkaloids, when working dilutions of the standards are made. We observed a significant increase in detector response of quantitative standards that had been stored at 60 °C for 2 days. For example, in two different lots of ergotamine, we observed increases in total detector response (-ine + -inine C-8 epimers) of 10 ng injections expressed as peak areas in microvolts of $8.48^6$ to $1.12^7$ (133%) and $8.31^6$ to $3.51^7$ (163%).

Both methods are more rapid than previous methods using rotary evaporation or solvent partitioning. Total sample processing time is equivalent for the two methods. Although HL silica is now commercially available without scraping ("Ergosil", Analtech No. 46050), the columns must still be packed as opposed to using commercial $C_{18}$ preparative columns. These columns can be purchased from many different manufacturers, and in different configurations such as Luer fitting for syringes, vacuum manifold columns, and robotic compatible columns. These should be equivalent, if attention is paid to column volume/sample/solvent ratios. An additional benefit in the HAc method is the absence of the more toxic $CHCl_3$ used in the former method. We use this method on a routine basis for clients of the services provided by the Fescue Diagnostic Lab who wish to have pasture and hay samples analyzed for potential fescue toxicosis.

ACKNOWLEDGMENT

We thank Dr. F. T. Smith, Pharmacal Sciences, Auburn University, AL, for providing ergovaline standards.

LITERATURE CITED

Berde, B.; Schild, H. O. Ergot alkaloids and related compounds. *Handbook of Experimental Pharmacology*; Springer Verlag: Berlin, 1978; Vol. 49, pp 179−178.

Brendemuehl, J. P.; Boosinger, T. R.; Pugh, D. G.; Shelby, R. A. Influence of endophyte-infected tall fescue on cyclicity, pregnancy rate and early embryonic loss in the mare. *Theriogenology* **1994**, *42*, 489−500.

Cieri, U. R. Determination of ergotamine tartrate in tablets by liquid chromatography with fluorescence detection. *J. Assoc. Off. Anal. Chem.* **1987**, *70*, 538−540.

Craig, A. M.; Bilich, D.; Hovermale, J. T.; Welty, R. E. Improved extraction and HPLC methods for ergovaline from plant material and rumen fluid. *J. Vet. Diagn. Invest.* **1994**, *6*, 48−352.

Dew, R. K.; Boissonneault, G. A.; Boling, J. A.; Cross, R. J.; Cohen, D. A. The effect of the endophyte (*Acremonium coenophialum*) and associated toxins of tall fescue on serum titer response to immunization and spleen cell flow cytometry analysis and response to mitogens. *Vet. Immunol. Immunopathol.* **1990**, *26*, 285−295.

Elund, P. O. Determination of ergot alkaloids in plasma by high-performance liquid chromatography and fluorescence detection. *J. Chromatogr.* **1981**, *226*, 107−115.

Fajardo, J. E.; Dexter, J. E.; Roscoe, M. M.; Nowicki, T. W. Retention of ergot alkaloids in wheat during processing. *Cereal Chem.* **1994**, *72*, 291−298.

Flieger, M.; Zelenkova, N. F.; Sedmera, P.; Zima, J.; Sajdl, P.; Stuchlik, J.; Cvak, L. New alkaloids of *Claviceps paspali*. *J. Nat. Prod.* **1989**, *52*, 1003−1008.

Henson, M. C.; Piper, E. L.; Daniels, L. B.; Barnett, M. K. Effect on lactating dairy cows fed high alkaloid fescue vs. bermudagrass. *Arkansas Farm Research*; Arkansas State University: Jonesboro, AR, March 1984; p 9.

Hill, N. S.; Rottinghaus, G. E.; Agee, C. S.; Schultz, L. M. Simplified sample preparation for HPLC analysis of ergovaline in tall fescue. *Crop Sci.* **1993**, *33*, 332−333.

Neal, W. D.; Schmidt, S. P. Effects of feeding Kentucky 31 tall fescue seed infected with *Acremonium coenophialum* to laboratory rats. *J. Anim. Sci.* **1985**, *61*, 603−611.

Porter, J. K.; Robbins, J. D.; Norred, W. D.; Garner, G. B.; Bacon, C. W. Serum prolactin and brain catecholamine metabolite depression in rats administered extracts of endophyte-infected fescue. *J. Agric. Food Chem.* **1985**, *33*, 34−36.

Porter, J. K.; Stuedemann, J. A.; Thompson, F. N.; Buchanan, B. A.; Tucker, H. A. Melatononin and pineal neurochemicals in steers grazed on endophyte-infected tall fescue: effects of metaclopramide. *J. Anim. Sci.* **1993**, *71*, 1526−1531.

Read, J. C.; Camp, B. J. The effect of the fungal endophyte *Acremonium coenophialum* tall fescue on animal performance, toxicity, and stand maintenance. *Agron. J.* **1986**, *78*, 848−850.

Rottinghaus, G. E.; Garner, G. B.; Cornell, C. N.; Ellis, J. L. HPLC method for quantitating ergovaline in endophyte-infected tall fescue: seasonal variation of ergovaline levels in stems with leaf sheaths, leaf blades, and seed heads. *J. Agric. Food Chem.* **1991**, *39*, 112−115.

Scott, P. M.; Lawrence, G. A. Analysis of ergot alkaloids in flour. *J. Agric. Food Chem.* **1980**, *28*, 1258−1261.

Shelby, R. A.; Dalrymple, L. W. Incidence and distribution of the tall fescue endophyte in the United States. *Plant Dis.* **1987**, *71*, 783−786.

TePaske, M. R.; Powell, R. G.; Clemet, S. L. Analyses of selected endophyte-infected grasses for the presence of loline-type and ergot-type alkaloids. *J. Agric. Food Chem.* **1993**, *41*, 2299−2303.

Thompson, F. N.; Stuedemann, J. A.; Sartin, J. L.; Belesky, D. P.; Devine, O. J. Selected hormonal changes with summer fescue toxicosis. *J. Anim. Sci.* **1987**, *65*, 727−733.

Ware, G. M.; Carman, A. S.; Francis, O. J.; Kuan, S. S. Liquid chromatographic determination of ergot alkaloids in wheat. *J. Assoc. Off. Anal. Chem.* **1986**, *69*, 697−699.

Welty, R. E.; Craig, A. M.; Azevedo, M. D. Variability of ergovaline in seeds and straw and endophyte infecton in seeds among endophyte-infected genotypes of tall fescue. *Plant Dis.* **1994**, *78*, 845−849.

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.

Zavos, P. M.; Varney, D. R.; Jackson, J. A.; Siegel, M. R.; Bush, L. P.; Hemken, R. W. Effect of feeding fungal endophyte (*Acremonium coenophialum*)-infected tall fescue seed on reproductive performance in CD-1 mice through continuous breeding. *Theriogenology* **1987**, *27*, 549−559.

Received for review August 5, 1996. Revised manuscript received January 27, 1997. Accepted February 20, 1997.[⊗]

JF9605759

[⊗] Abstract published in *Advance ACS Abstracts*, April 1, 1997.