

Discovery, The Student Journal of Dale Bumpers College of Agricultural, Food and Life Sciences

Volume 2

Article 4

Fall 2001

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Recommended Citation

Cannon, S. M., Rosenkrans, C. F., & Moubarak, A. (2001). The possible enzymatic differences between cattle and sheep in their response to ergot alkaloids. *Discovery, The Student Journal of Dale Bumpers College of Agricultural, Food and Life Sciences*, 2(1), 3-7. Retrieved from <https://scholarworks.uark.edu/discoverymag/vol2/iss1/4>

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The possible enzymatic differences between cattle and sheep in their response to ergot alkaloids

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ABSTRACT

Ergotamine is an ergot alkaloid associated with fescue toxicosis of livestock who have grazed endophyte-infected fescue. High performance liquid chromatography (HPLC) was used to detect individual or species-specific differences in the metabolism of ergotamine by liver cytochrome P450 of sheep and cattle. Livers were collected from four steers and two sheep. The diet of the steers used in this study consisted of two being fed a grain diet, one steer grazing endophyte-infected fescue, and the final steer grazing endophyte-free fescue. The two lambs were both fed a grain diet. Livers were prepared and examined for the disappearance of ergotamine and its isomer by HPLC analysis. Liver microsomes from cattle appeared to metabolize ergotamine to a greater degree than those from sheep. There were no apparent differences in the metabolism of ergotamine when comparing cattle that grazed endophyte-infected fescue to cattle that grazed endophyte-free fescue. Therefore, diet had no effect on the metabolism rate of ergotamine. This work provides insight into the possible genetic differences between species-specific and individual animals. Further study of such differences should improve breeding programs and produce animals that can more effectively tolerate fescue toxins.

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INTRODUCTION

Cattle in the Midwest and South routinely graze pastures consisting of tall fescue (*Festuca arundinacea*) infected with the endophytic fungus *Neotyphodium coenophialum*. The fungus secretes ergot alkaloids that enhance stress-tolerance of the plant but result in poor performance by some of the cattle grazing the infected herbage. It is estimated that tall fescue is grown on over 14 million ha in the United States (Stuedemann and Hoveland, 1988). Although fungal endophytes of grasses have been known since the 1930s, their economic importance was not recognized until an association was made in the late 1970s between *Neotyphodium coenophialum* and a toxicity syndrome in livestock consuming tall fescue (Hoveland, 1993). This syndrome is known as fescue toxicosis and is often referred to as 'summer slump' or 'summer syndrome' due to the unthrifty animal appearance and poor performance during summer (Schmidt and Osborn, 1993). This widespread syndrome is characterized by poor animal gains, intolerance to heat, excessive salivation, rough

hair coat, elevated body temperature, poor appetite, nervousness, lower milk production, and reduced conception rate (Hoveland, 1993). Additionally, animal behavior is altered in that animals seek shade, stand in water, and consequently spend less time grazing during the hot part of the day (Bond et al., 1984; Stuedemann et al., 1986).

Trials have been performed to determine how much performance is lost in relation to fescue toxicosis. A study on milk production found that performance was reduced by as much as 45% in beef cows (Schmidt, et al., 1983), 50% in beef heifers, and 60% in dairy cows (Hemken, et al., 1979). A study of the pregnancy rate of heifers on endophyte free (E-), as compared with infected (E+) tall fescue pasture, concluded that pregnancy was reduced from 96% to 55% in those who grazed endophyte infected (E+) pasture (Schmidt, 1986). A third study related to calf weaning weights, concluded that weaning weights were reduced by 23 kg/calf because of fescue toxicosis (Hoveland, 1993). Together, losses from reduced conception rates and weaning weights are estimated at \$600 million annu-

Meet the Student-Author

I am a senior from Ashdown majoring in agricultural education, with a minor in communication. I plan to graduate in December of 2001. As a student at the University of Arkansas I have received several scholarships and have had the opportunity to participate in many extracurricular and scholastic activities. I am a member of the Collegiate Livestock Judging Team, Agricultural Communicators of Tomorrow, Associate Student Government, University Programs, Alpha Zeta Fraternity, and the Gamma Sigma Delta Agricultural Honorary Society. Additionally, I am currently serving as a Dale Bumpers College of Agricultural, Food and Life Sciences Ambassador.

I chose this research project because of the significant effects of fescue toxicosis on cattle and sheep. This experience has allowed me to enhance my research techniques as well as write a paper for publication. These skills will not only be beneficial to me as I further my education but also as I advance into my professional career.

I would like to express my thanks to Dr. Ali Moubarak and Dr. Charles F. Rosenkrans for their support and guidance throughout this research project.



Susan Cannon

ally in the United States (Paterson, et al., 1995).

Unlike beef, the characteristics of fescue toxicosis exhibited in sheep that have grazed endophyte-infected fescue are relatively small. Studies have shown that ewes grazing endophyte-infected fescue have decreased prolactin and lengthened intervals from introduction of the ram until conception. However, trials evaluating sheep that have grazed endophyte-free (less than 1% infected) and endophyte-infected fescue (greater than 95% infected) have shown that mean daily respiration rates and heart rates, rectal temperature, and hematocrit were not affected by the endophyte-infected fescue (Fiorito, et al., 1991). In addition, a study performed by Rankins (1996) showed that 15 crossbred sheep with a diet of endophyte-free fescue (0% infected, 50% hay, 40% seed, 10% molasses) and endophyte-infected fescue (95% infected, 50% hay, 40% seed, 10% molasses) exhibited no differences. This study concluded that sheep fed the endophyte-infected fescue did not portray typical fescue toxicosis (Rankins, 1996).

Several methods of pasture management have been researched to ameliorate the effects of fescue toxicosis. These methods include interseeding with clovers, preventing the formation of seed heads either by overstocking or clipping, pasture renovation, moving cattle to non-fescue pasture during hot weather, use of plant growth regulators, supplementing with grain or 50:50 broiler litter/shelled corn mix, and use of creep feed or creep grazing with cow-calf pairs (Schmidt and Osborn, 1993). Studies have shown that interseeding endophyte-infected fescue pastures with clover improved pregnancy rates of cows, improved gains of grazing steers, and increased calf weaning weights. However, the pregnancy rates were not improved to levels considered economical for the beef industry (Schmidt and Osborn, 1993). In addition, calves continuously grazing endophyte-infected fescue at lower stocking rates appeared to have more severe toxicosis characteristics because of the tendency to selectively graze seed heads, even though the endophyte is concentrated there (Schmidt and Osborn, 1993).

Many possible methods have been proposed to reduce fescue toxicosis; however, none of the proposed solutions have alleviated the syndrome, and fescue remains a widely used forage crop due to its wide range of adaptation, ease of establishment, tolerance of poor soil and climatic conditions, and long grazing season with good winter growth.

In 2000, Moubarak and Rosenkrans reported that the liver enzyme, cytochrome P450 3A, was present and has been shown to metabolize fescue toxins in cattle. Cytochrome P450 enzymes constitute a superfamily of heme-thiolate proteins that catalyze the primary oxidation of a wide variety of natural endogenous substrates like steroids, fatty acids, prostaglandins, leukotrienes, and lipid hydroperoxides. They also play an important role in the metabolism of exogenous compounds like drugs, procarcinogens, solvents, anesthetics, and environmental pollutants (Peyronneau, et al., 1994).

Our objective was to determine if HPLC analysis could be used to detect individual or species-specific differences in the metabolism of ergotamine by liver microsomes of sheep and cattle. Ergotamine is one of many ergot-alkaloids and is our test compound.

MATERIALS AND METHODS

Livers were obtained from four steers and two lambs. The animals were taken from experiments that had been approved by the University of Arkansas' Institutional Animal Care and Use Committee. The nutritional diet of the four steers (450 to 650 kg body weight) used in this study consisted of two steers being fed a grain diet, one steer grazing endophyte-infected fescue, and the final steer grazing endophyte-free fescue. The two lambs (80 to 100 kg body weight) used in this study were both fed a grain diet.

Liver microsomes were prepared according to Kremers, et al. (1981). Liver tissues (50 to 100 g) were collected and frozen in Collins buffer. Frozen samples were thawed, washed in sodium chloride (150 mM), and homogenized in a sucrose Tris-buffered medium. Microsomes were prepared according to a procedure consisting of a three-step centrifugation process of the tissue homogenate. The first centrifugation was at 800 xg for 10 minutes. The supernatant was collected and the second centrifugation was performed at 13,500 xg for 20 minutes. Centrifugation was done a third time at 105,600 xg for 60 minutes. Supernatant from the final centrifugation procedure was discarded, the pellet resuspended in a sodium phosphate/glycerol solution, and protein content determined. Microsome suspensions were stored in a freezer at -20°C until used within 20 to 30 days.

Ergotamine reactions were prepared according to Peyronneau, et al. (1994) and Moubarak and

Rosenkrans (2000). Microsomes (50 µl of protein) were incubated in microcentrifuge tubes containing ergotamine (20 µl) and a NADPH generating system for 30 minutes at 37°C. Immediately following the incubation, the enzymatic reaction was stopped by adding a deproteinizing agent (94% acetonitrile, 6% glacial acetic acid). Reaction tubes were centrifuged at 12,000 xg for 4 minutes and the supernatant was collected. Tubes had a total volume of 500 µl. Twenty µl of each supernatant from the enzyme assays were examined for the disappearance of ergotamine and its isomer by HPLC analysis (Moubarak, et al., 1993).

RESULTS AND DISCUSSION

Metabolites M-1, M-2, and M-3, as well as M1-Iso and M2-Iso are exhibited in cattle; however, there were no signs of these metabolites displayed in sheep (Fig. 1). Ergotamine metabolism in cattle and the appear-

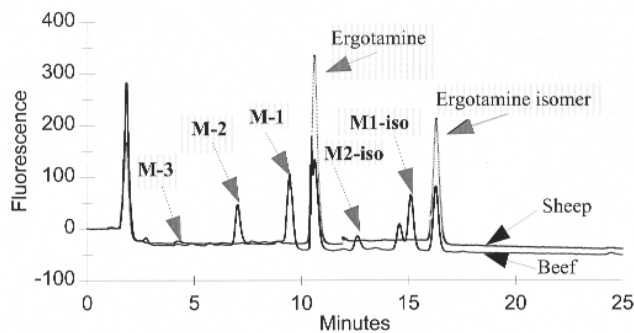


Fig. 1. HPLC chromatogram of products from ergotamine incubation with liver microsomes from cattle and sheep grazing endophyte-infected tall fescue.

ance of metabolites (M-1, M-2, M-3) in Fig. 2 show that ergotamine is gradually reduced by 50% during the first 30 minutes of incubation, while metabolites are increased as a function of incubation time. The time course for ergotamine metabolism by sheep and the unvarying rate of metabolites indicate that ergotamine is gradually reduced by only 10% during the first 30 minutes of incubation, while metabolites remain constant at 0% (Fig. 3).

Based on these data, cattle liver microsomes appear to metabolize ergotamine differently than do those of sheep. On the other hand, there were no apparent differences in the metabolism of ergotamine when comparing cattle that grazed endophyte-infected fescue to

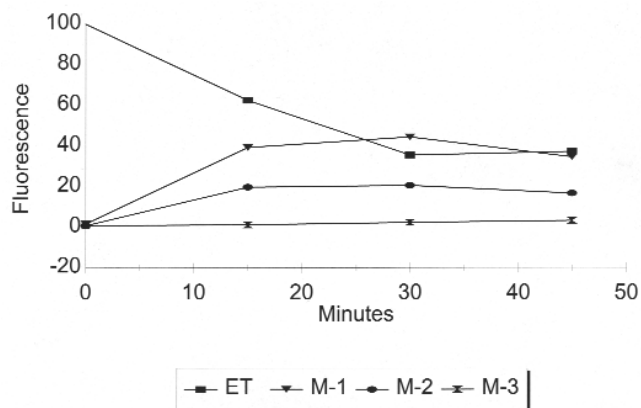


Fig. 2. Time dependent formation of metabolites M-1, M-2, and M-3 when ergotamine was incubated with liver microsomes from cattle.

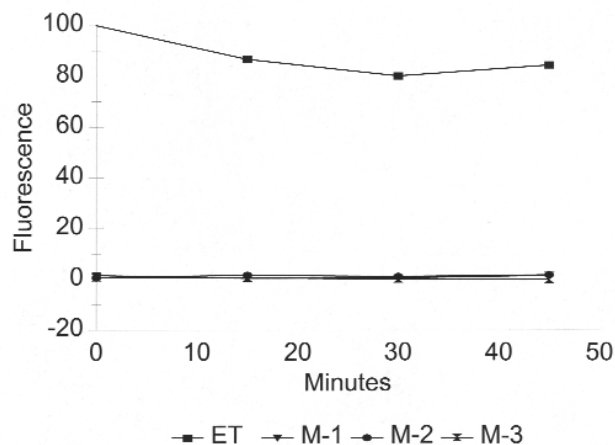


Fig. 3. Time dependent formation of metabolites M-1, M-2, and M-3 when ergotamine was incubated with liver microsomes from sheep.

cattle that grazed endophyte-free fescue. However, the lack of differences could be due to the small number of animals studied in this experiment. This experiment was exploratory in nature and further work to investigate such differences is in progress.

This work provides insight into the possible genetic differences between species and individual animals that were fed dissimilar diets. When the genetic marker for animals with a high tolerance for fescue toxins is identified, such information will be useful in selecting animals for breeding programs to produce livestock that can more effectively tolerate high levels of fescue toxins.

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