

Cattle vs. Sheep in Their Responses to Fescue Toxins

A. Moubarak, S.M. Cannon, and C.F. Rosenkrans, Jr.¹

Story in Brief

Does grazing endophyte-infected fescue have the same effects on sheep as it does on cattle? This study was designed to examine whether metabolism of ergot alkaloid (ergotamine) and the process of detoxification in sheep and cattle are the same when using the liver enzyme cytochrome P450 3A as a marker. Data were collected from livers of 6 steers and 2 sheep to produce preliminary information to design a larger study. Steers used in this study consisted of 3 steers grazing endophyte-infected fescue and 3 steers grazing endophyte-free fescue. The 2 lambs were both fed a grain diet. Livers of both steers and sheep were prepared and examined for the ability to metabolize ergotamine. There were no apparent differences in the metabolism of ergotamine when comparing steers that grazed endophyte-infected fescue to steers that grazed endophyte-free fescue. Therefore, diet of the steers had no effect on the metabolism rate of ergotamine. Liver microsomes from cattle appeared to metabolize a greater amount of ergotamine than did sheep after 30 min (63% vs 20%). This work provides preliminary information on the possible genetic differences between cattle and sheep or between individual animals within the same species which is very helpful in the design of the second phase of this research.

Introduction

Cattle and sheep in the Southern United States routinely graze tall fescue (*Festuca arundinacea*) infected with the endophytic fungus *Neotyphodium coenophialum*. The effects of fescue toxins on cattle have been heavily investigated and debated for a long time. Although, it has been generally accepted that sheep are more tolerant to fescue toxins than cattle, very little information evaluating sheep vs. cattle is available. Fiorito et al. (1991) showed that ewes grazing endophyte-infected fescue had decreased prolactin and lengthened intervals from introduction of the ram until conception. However, mean daily respiration rates, heart rates, rectal temperature, and hematocrit were not affected by the endophyte-infected fescue. Rankins (1996) reported that sheep on diets of endophyte-free and endophyte-infected fescue exhibited no differences and concluded that sheep fed the endophyte-infected fescue did not portray typical fescue toxicosis. Effects of endophyte-infected fescue on cattle have been shown to reduce milk production (Hemken et al., 1979), pregnancy rate of heifers (Schmidt, et al., 1986), and calf weaning weights (Hoveland, 1993). Moubarak and Rosenkrans (2000) reported that the liver enzyme, cytochrome P450 3A, was present and has been shown to metabolize fescue toxins in cattle and is perhaps the major means of detoxification. Cytochrome P450 enzymes catalyze the primary oxidation of a wide variety of natural endogenous substrates. These enzymes also play an important role in the metabolism of exogenous compounds like drugs, procarcinogens, solvents, anesthetics, and ergot alkaloids (Peyronneau et al., 1994). This study was designed to examine whether metabolism of ergot alkaloids (ergotamine, ET) and the process of detoxification in sheep and cattle are different using liver cytochrome P450 3A.

Experimental Procedures

Liver tissues were obtained from steers (900 to 1,300 lb BW) from the University of Arkansas abattoir. Three steers used in this study had grazed endophyte-infected fescue, and 3 steers had grazed endophyte-free fescue. The 2 lambs were both fed a grain diet. Liver tissues (50 to 100 g) were collected and microsomes were prepared according to Kremers et al. (1981). The livers were diced with scissors and then washed with 150 mM sodium chloride buffer. Diced tissue was then ground at 1 g/10 ml in an ice-cold medium (250 mM sucrose, 100 mM Tris-HCl, 1 mM EDTA (ethylenediaminetetraacetic acid), pH 7.4) using a precooled blender for 10 to 20 sec followed by homogenization with Potter-Elvehjem (5X). The homogenate was then centrifuged at 800 x g for 10 min, at 13,500 x g for 20 min collecting the supernate, then at 105,000 x g for 60 min collecting the pellet containing the microsomal fraction. The pellet (microsomal fraction) was washed with 100 mM sodium pyrophosphate (pH 7.5) and resuspended in buffer containing 100 mM sodium phosphate and 20% V/V glycerol to give 50 mg protein/ml concentration. Microsome suspensions were aliquoted and stored at -90°C and were used within 20 to 30 days. Protein concentration was determined using bovine serum albumin (BSA) as a standard.

The cytochrome P450 3A activity in all animals was measured using ergotamine as a substrate. The metabolism of ergotamine was assayed (Peyronneau et al., 1994) in medium containing 100 mM Tris-HCl, 10 mM potassium phosphate, 0.1 mM EDTA, pH 7.5, cofactor generating system (nicotinamide adenine dinucleotide; NADPH), 20% glycerol, 1.0 µg/ml ergotamine that had been fully isomerized, and 0.1 mg/ml microsomal protein in a total volume of 500 µL. The cofactor generating system was: 26 mM NADP⁺, 66 mM D-glucose-6-phosphate, 66 mM magnesium chloride, and 1 U Glucose-6-Phosphate dehydrogenase in sodium citrate. Bovine liver microsomes were diluted in assay buffer to a working concen-

¹ Department of Animal Science, Fayetteville

tration of 2.5 mg protein/ml and kept on ice. The reactions were started by adding the NADPH generating system and were terminated after 30 min by adding 100 μ l of 94% acetonitrile and 6% glacial acetic acid and centrifuged at 12,000 x g for 5 min at pH 7.5. Twenty μ l of the supernatant from each enzyme assay were examined for the disappearance of ergotamine and its isomer and also for the appearance of metabolites by using a modification of the high pressure liquid chromatography (HPLC) method described by Moubarak et al. (1993) and Moubarak and Rosenkrans (2000).

Data were analyzed using one-way ANOVA with type of animal as the independent variable and concentration of remaining ET or the appearance of metabolites as the dependent variable. Data were reported as percentage of total ET or total metabolites remaining.

Results and Discussion

Even though this experiment was exploratory in nature and further work to investigate such differences is in progress, the data indicate that 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 (data not shown). However, when comparing livers of cattle with those of sheep, the metabolism of ergot alkaloids shows differences. Figure 1 is an illustration of an HPLC chromatogram of the products of metabolism of ergotamine by liver microsomes enzyme (cytochrome P450 3A) from cattle and sheep. The enzyme preparation of cattle liver appeared to metabolize ET and its isomer to its respective metabolites. On the other hand, enzyme preparations from sheep liver did not metabolize ET or its isomer to any significant level regardless of the enzyme concentration or the length of the incubation with ET.

The cytochrome P450 3A metabolism of ET and its isomer was time and enzyme concentration dependent. The percentage of ET remaining after incubation with sheep and cattle cytochrome P450 3A after 15, 30 and 45 min is shown in Figure 2. After 30 min of incubation, 63% of ET (100% - 37%) was metabolized by cattle;

whereas, only 20% of ET (100% - 80%) was metabolized by sheep during the same time of incubation. The appearance of metabolites is presented in Figure 3. These data demonstrate the ability of enzyme preparation from steers to convert ET to its metabolites in 15 min and leveling off thereafter; whereas, the sheep enzyme preparation produced very little conversion of ET to its metabolites up to 45 min of incubation under our assay conditions.

Implications

Ergot alkaloids (ergotamine) and fescue toxins are generally detoxified in the liver. We have demonstrated in previous work at the University of Arkansas that cytochrome P450 3A is found in cattle and is capable of metabolizing ergotamine. The low level of activity of cytochrome P450 3A in sheep raises several questions. First, is there a different enzyme system in sheep than cytochrome P450 3A that is involved in the detoxification of ergotamine? Second, is it possible that the toxic form of ergot alkaloids is not the parent compound but rather the metabolite itself? Perhaps sheep can tolerate a higher level of toxins because they produce fewer metabolites.

Literature Cited

- Fiorito, I.M., et al. 1991. *J. Anim. Sci.* 69:2108-2114.
Hemken, R.W., et al. 1979. *J. Anim. Sci.* 49:641.
Hoveland, C.S. 1993. *Agri. Ecosystems Env.* 44:3-12.
Kremers, P., et al. 1981. *Eur. J. Biochem.* 118:599-606.
Moubarak, A.S., et al. 1993. *Agric. Food Chem.* 41:407-409.
Moubarak, A.S. and C.F. Rosenkrans, Jr. 2000. *Biochem. and Biophysical Res. Comm.* 274:246-249.
Peyronneau, M.A., et al. 1994. *Eur. J. Biochem. (EMZ).* 223:947-956.
Rankins, D.L., Jr. 1996. *Vet. Hum. Toxicol.* 38:173-176.
Schmidt, S.P., et al. 1986. *Alabama Agric. Exp. Stn. Highlights Agri. Res.* 33:15.

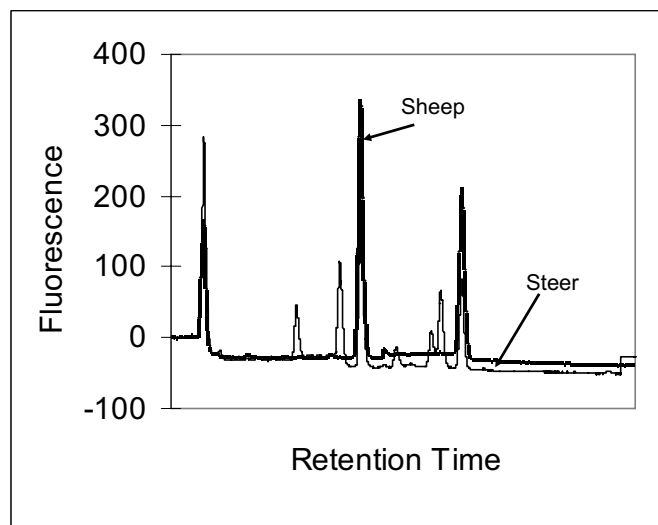


Fig. 1. High pressure liquid (HPLC) chromatogram of products from ergotamine incubation with liver microsomes from steers and sheep.

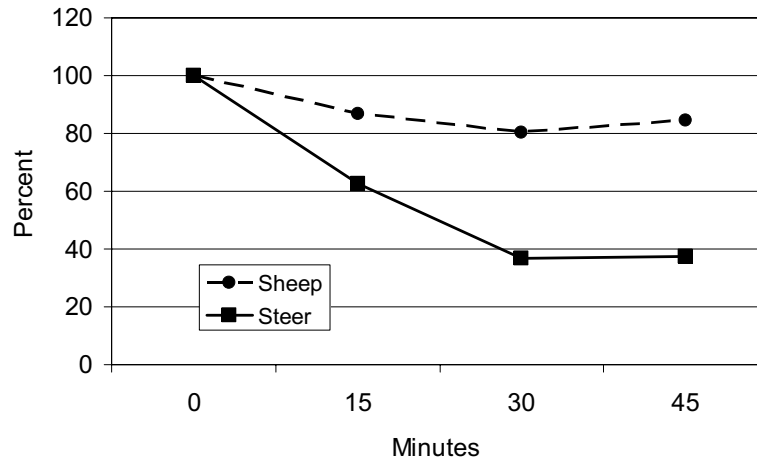


Fig. 2. Time dependent disappearance of ergotamine after incubation with liver microsomes from steers or sheep. Shown is the percentage of ergotamine remaining after incubation. Values differ ($P < 0.05$) between sheep and steers at 15, 30 and 45 min.

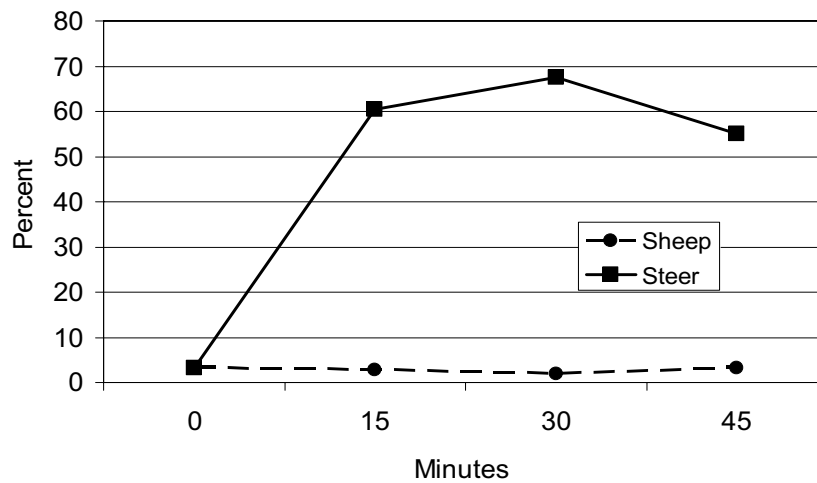


Fig. 3. Time dependent formation of total metabolites when ergotamine was incubated with liver microsomes from steers or sheep. Values differ ($P < 0.05$) between sheep and steers at 15, 30 and 45 min.