Urinary Excretion of the Mycotoxins Lolitrem B and Lysergic Acid in Cattle Consuming Perennial Ryegrass Straw over 56 Days

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Undergraduate Research, Scholarship, and the Arts

Abstract

Perennial ryegrass (*Lolium perenne*) hay is an important source of forage for a variety of livestock species, and is most common in cool, coastal climates such as the Pacific Northwest. An endophyte fungus called *Neotyphodium lolii* frequently colonizes this grass to promote insect resistance, drought tolerance, and growth enhancement without the need to incorperate excessive use of fertilizers, irrigation or pesticides. Unfortunately, encouragement of endophyte infection can also result in the production of mycotoxins including lolitrem B and ergot alkaloids. Lolitrem B is a tremorgenic neurotoxin that effects livestock and other herbivores and is responsible for the condition known as "ryegrass staggers," in which animals develop tremors, ataxia and frequently collapse. The ergot alkaloids are vasoconstrictors and prolactin inhibitors that negatively affect thermoregulation, reproductive efficiency and milk yield. The toxicokinetics of lolitrem B have not yet been fully elucidated; however, that of the ergot alkaloid mycotoxin ergovaline has, and the main urinary metabolic breakdown product was determined to be lysergic acid. A thorough understanding of the metabolism of lolitrem B/ergot alkaloids from perennial ryegrass in cattle must be established before a risk assessment can be extended to humans. Therefore, ***the goal of this project*** was to evaluate one toxicokinetic compartment, the urine, of cattle fed varying doses of lolitrem B- and ergovaline-containing perennial ryegrass hay for 60 days for lolitrem B and the ergot alkaloid breakdown product lysergic acid via LC-MS/MS. Our experiment confirmed that lolitrem B is not secreted in bovine urine; lysergic was detected in a dose-dependent manner. The tendency for lysergic acid to be excreted in aqueous matrices raises concerns for human consumption of milk products from exposed animals.

Methods

Urine extraction of lolitrem B

- Dilute 2 mL urine with 2 mL of 0.1M phosphoric acid
- Wash *Waters Oasis HLB* SPE column with 2 mL dd H_2O
- Load samples onto column under vacuum
- Elute lolitrem B with 6 mL of 1:9 hexane-ethyl acetate
- Dry samples under N_2 gas
- Reconstitute samples with 500 µL acetonitrile, sonicate for 10 seconds, and then vortex
- Syringe samples into HPLC vial using Whatman 1.2 um pore size syringe

Urine extraction of lysergic acid

- Adjust 2.5 ml urine to pH 5.0-5.5 with 10% acetic acid
- Centrifuge samples at 3,500 rpm for 10 minutes
- Condition Phenomenex Discovery DSC- SCX SPE column with 3.0 mL methanol, 3.0 mL 0.1 M HCl, and wash twice with 3.0 mL H_2O
- Load urine samples onto SPE column under vacuum, wash twice with 3.0 mL H_2O, and allow to dry under vacuum
- Elute lysergic acid with 3 mL 95:5 methanol:ammonium hydroxide and dry under vacuum for 2 minutes
- Dry samples under N_2 gas at 43°C
- Reconstitute in 200 µL 1:1 methanol:0.05 M phosphate buffer and sonicate for 30 seconds
- Transfer samples to 1.7 mL centrifuge tubes with filter and spin at 13,200 rpm for 5 minutes
- Transfer filtrate to HPLC vials

Lolitrem B LC-MS/MS settings

Column: Phenomenex Prodigy ODS (30) (150 x 4.6 mm)
Flow Rate: 1.0 mL/min
Mobile Phases: (A)40% acetonitrile with 0.1% acetic acid (B)Acetonitrile with 0.1% acetic acid,

Lysergic Acid LC-MS/MS settings

Column: Phenomenex Luna Phenyl-hexyl (250 x 2 mm) + Phenylpropyl guard
Flow rate: 0.5 mL/min
Mobile Phases: (A)2.5 mM ammonium carbonate (pH 7.0) (B) ACN, over a gradient
MRMs Monitored: 269 → 44, 269 → 182, 269 → 192;
CE: 55V Temp: 250°C, IS: 5000 V, DP: 45, EP: 10

Samples

- Urine samples were collected from four groups of steers, each fed different concentrations of lolitrem B and ergovaline-containing ryegrass at the Oregon State University Beef Barn.
- Feed was quantified using HPLC-fluorescence.

Diet
Endophyte-infected perennial ryegrass

Group	Average LB (ppb)	Average EV (ppb)
1	1997 ± 693	633 ± 89
2	1554 ± 214	373 ± 120
3	1012 ± 199	259 ± 53
4	247 ± 176	< 100

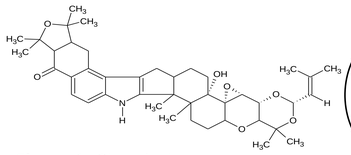

Figure 1: Chemical structure of mycotoxin Lolitrem B

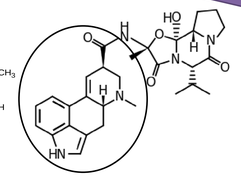

Figure 2: Chemical structure of ergovaline.

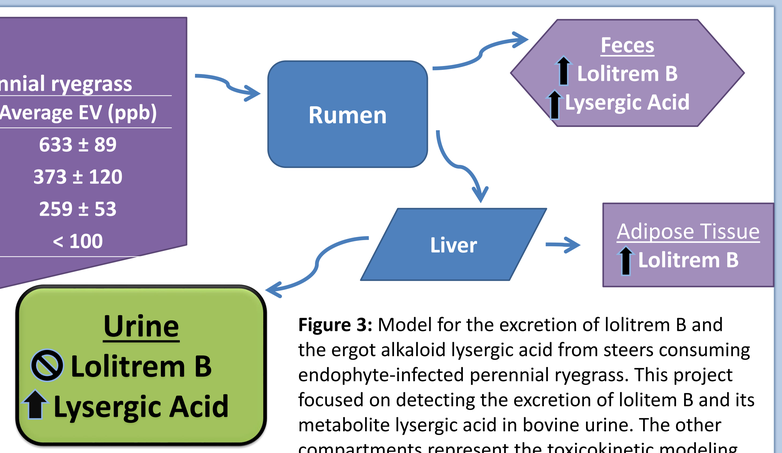

Figure 3: Model for the excretion of lolitrem B and the ergot alkaloid lysergic acid from steers consuming endophyte-infected perennial ryegrass. This project focused on detecting the excretion of lolitem B and its metabolite lysergic acid in bovine urine. The other compartments represent the toxicokinetic modeling of lolitrem B and lysergic acid established outside of this project.

Results

Table 1. Lolitrem B in bovine urine

Group	Concentration (ppb)
1 (~2300 ppb LB)	Not Detectible
2 (~1500 ppb LB)	Not Detectible
3 (~1000 ppb LB)	Not Detectible
4 (~0 ppb LB)	Not Detectible

All urine samples were analyzed for lolitrem B on LC-MS/MS; however no detectable amounts were present.

Lysergic acid in bovine urine

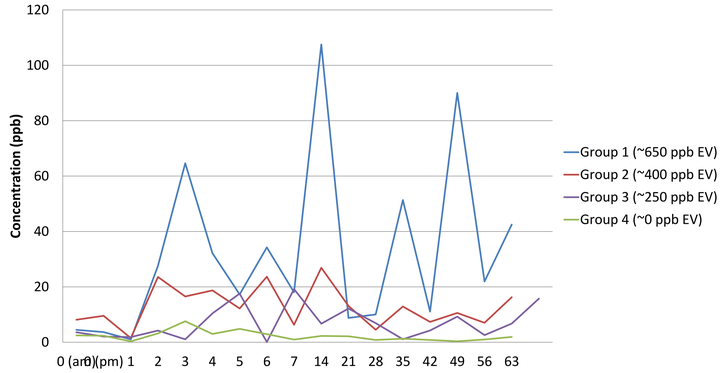

Figure 4: Ergovaline Lysergic acid excreted in bovine urine detected using LC-MS/MS

Lolitrem B in bovine feces

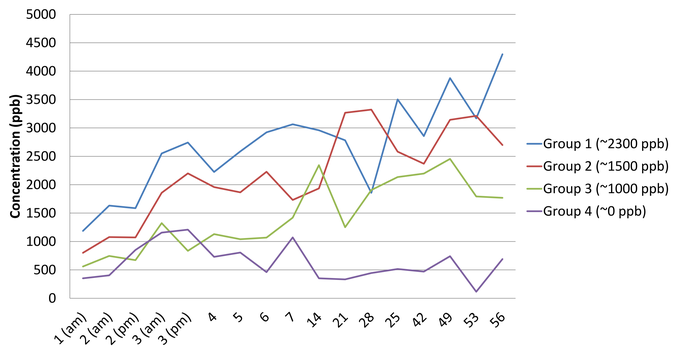

Figure 5: Lolitrem B excreted in bovine urine detected using HPLC –fluorescence

Ergovaline in bovine feces

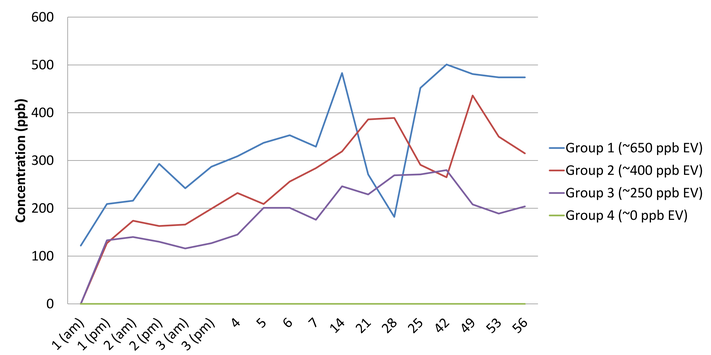

Figure 6: Ergovaline excreted in bovine feces detected using HPLC – fluorescence

- Neurotoxin lolitrem B consumed in concentrations greater than 1800 ppb causes a condition known as "*Ryegrass staggers*"
- Clinical signs include ataxia, tremors and incoordination.

Conclusions

- **The mycotoxin lolitrem B is not excreted in detectible amounts in bovine urine.**
- **Lysergic acid is excreted in urine, and analysis showed a dose-response relationship between the amount of ergovaline fed to the animal and how much was detected in urine.**
- **Residues of the lipophilic mycotoxin compound lolitrem B have been detected in beef fat. This presents a potential food safety hazard to humans consuming this meat, as a risk assessment for humans has not yet been established.**
- **Lysergic acid is a hydrophilic compound, and residues have been detected in bovine milk. This poses a potential food safety hazard to human consumers.**
- **The residue of each compound alone may not produce profound toxicological effects, but when an animal is exposed to both insults concerns for additive toxicity should be taken into consideration when developing risk assessment analyses of these mycotoxins in animal products.**

Acknowledgements

Undergraduate Research, Scholarship, and the Arts (URSA) engage program
Oregon Agricultural Experiment Station