Endophyte Toxicoses in Beef Cattle

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Introduction

Tall fescue (*Festuca arundinacea*) and perennial ryegrass (*Lolium perenne*) are perennial cool-season grasses that are infected with the endophytic fungi, *Epichloë coenophiala* and *E. lolii*, respectively (Joost, 1995; Porter, 1995). These endophytes have been increasingly selected for in grass seed by plant breeders, as they confer benefits such as pest resistance and drought tolerance to the plant. However, livestock consuming endophyte-infected grasses, either directly in pastures or from hay/straw produced from grass seed fields, can be negatively impacted by fungal ergot and lolitrem alkaloids which are responsible for a variety of mammalian diseases including fescue toxicosis (summer syndrome, fescue foot and fat necrosis), ergot and ryegrass staggers (Aldrich-Markham et al., 1995; Duringer et al., 2012; Oliver, 2005; Thompson and Stuedemann, 1993). These livestock maladies are estimated to cost >$1 Billion in losses annually in the United States, when taking the impacts to the cattle, equine and small ruminant industries into consideration (Strickland et al., 2011).

Strategic testing of feed material in a service/diagnostic laboratory for ergot and lolitrem mycotoxins is the best preventative measure that can be taken to ensure a safe food supply in beef cattle; samples can also be submitted to diagnose symptoms in animals that are congruent with endophyte toxicoses (Craig et al., 2015, 2014). Factors including proper collection and treatment of a sample are vital to ensuring that the results obtained are reflective of the batch or lot of feed material that is intended to be fed.

Tall fescue and fescue toxicosis

Tall fescue is planted extensively throughout the world, valued for its wide tolerance of soil and climatic factors and high yield potential (Hannaway, D et al., 1999a). It is grown on approximately 40 million acres in the United States, making up ~10% of all pastureland. In addition to its use as livestock feed, tall fescue is also grown for turf grass and erosion control. Varieties developed for beef cattle consumption are usually classified as “forage varieties” and may include the use of a novel endophyte, or one in which the endophytic fungus produces compounds toxic to insect pests but not mammalian herbivores. Tall fescue has a high nutritive value, as seen in Table 1, which can vary based upon the maturity stage and fertility of the plant at harvest (Hannaway, D et al., 1999a).

Table 1. Nutritional composition of tall fescue.

Feed description	TDN (%)	DE (Mcal/kg)	ME (Mcal/kg)	NEm (Mcal/kg)	NEg (Mcal/kg)	CP (%)	Ca (%)	P (%)
Fresh, early vegetative	73	3.22	2.64	1.73	1.11	22.1	0.51	0.37
Fresh, early bloom	67	2.95	2.42	1.54	0.94	16.7	—	—
Hay, late vegetative	76	3.35	2.75	1.82	1.19	21.3	—	—
Hay, early bloom	62	2.73	2.24	1.38	0.80	20.2	—	—
Hay, mid-bloom	60	2.65	2.17	1.31	0.74	16.4	—	—
Hay, full bloom	58	2.56	2.10	1.24	0.68	12.1	0.41	0.30

All values expressed on a dry matter basis. TDN=Total Digestible Nutrients; DE=Digestible Energy; ME=Metabolizable Energy; NEm=Net Energy for Maintenance; NEg=Net Energy for Gain; CP=Crude Protein; Ca=Calcium; P=Phosphorus.
TDN values are listed for ruminants. Values for horses generally are lower.

(Adapted from (Hannaway, D et al., 1999a)).

Epichloë coenophiala infection can be deleterious to beef cattle when concentrations of the ergot alkaloid mycotoxin known as ergovaline (Figure 1) rise above 400 ppb (Table 2) (Aldrich-Markham et al., 1995; Oliver, 2005; Thompson and Stuedemann, 1993). (Many forage grasses can also be infected with the parasitic fungus *Claviceps purpurea*, producing a syndrome known

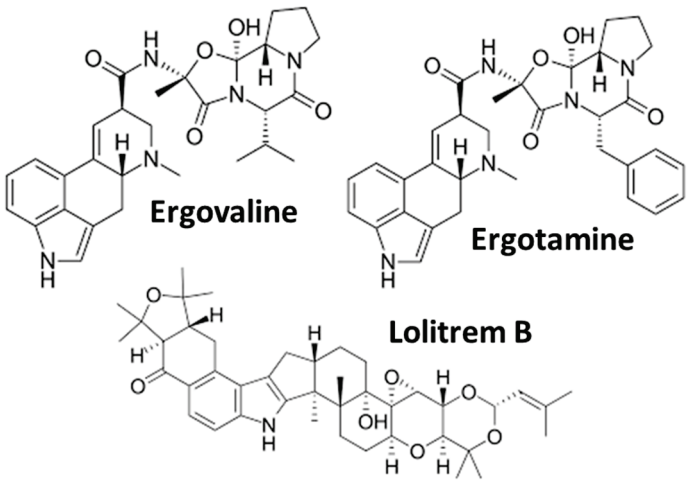

Figure 1. Endophyte and ergot mycotoxins.

Table 2. Threshold concentrations for endophyte toxicoses in livestock species.^

Species	*Ergovaline (ppb or ng/g)#*	*Lolitrem B (ppb or ng/g)*
Cattle	400-750	1800-2000
Sheep	500-800	1800-2000
Horses*	300-500	N.D.
Camels	N.D.	500

Table 2 can be used as a guide for concentrations of endophyte mycotoxins above which you will begin to see clinical signs of endophyte toxicoses in livestock consuming contaminated feed.
^Concentrations vary depending on health of the animal and environmental conditions
#Equivalent values can be used to estimate ergotamine toxicity
*Except for mares in the last 60 to 90 days of pregnancy, when the threshold is zero.
N.D. = Not Determined

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as “ergot.” The main ergot alkaloid produced from this fungus is ergotamine (Figure 1) which has equivalent toxicity to ergovaline in mammals (Craig et al., 2015). Ergot can be a particular issue in pellets, as grass seed screenings and other portions of the plant containing high concentrations of ergot alkaloids can be concentrated in production of this feed). Ergot alkaloids cause vasoconstriction of blood vessels via interaction with α-adrenergic receptors in smooth muscle cells which contract (Oliver, 2005; Thompson and Stuedemann, 1993), leading to reduced circulation in extremities (hooves, ears and tails). In extreme cases, *fescue foot* develops whereby tissue in the foot becomes gangrenous below a bloody line of demarcation and typically occurs in cold weather which is additionally stressful to the animal’s circulation. Earlier signs are demonstrated in animals that appear to be exhibiting lameness or tenderness in their feet and swelling in the legs. *Summer slump*, on the other hand, is characterized by hyperthermia where animals spend more time seeking shade or water to cool themselves which is exacerbated by hot, humid environmental conditions. This results in less time grazing, translating to financial losses in the form of reduced average daily gain values.

Ergot alkaloids also cause reproductive difficulties by acting as agonists through the dopamine D2 receptor, including abortion, premature births, low litter sizes and reduced conception rates, as well as agalactia due to suppression of prolactin (Hurley et al., 1980; Schillo et al., 1988). Lastly, abdominal *fat necrosis* can cause hard masses of fat to accumulate which compress the gastrointestinal tract and other internal organs, leading to digestive upset or calving problems. This appears to be more of a minor clinical symptom however and thus has not been studied as in depth as fescue foot and summer slump.

Perennial ryegrass and ryegrass staggers

Established in temperate pastures throughout the globe, perennial ryegrass is grown on ~0.25 million acres in the United states as pasture due to its high palatability and digestibility (Fisher et al., 2004; Hannaway, D et al., 1999b) (Table 3). Like tall fescue, nutritional values will vary depending upon maturity stage at harvest and fertility. Perennial ryegrass tends to be used more prevalently in managing dairy and sheep herds but can also be a component of beef cattle operations. Perennial ryegrass contains a lower proportion of carotenoids and other colorful

Table 3. Nutritional composition of perennial ryegrass.

Feed description	TDN (%)	DE (Mcal/kg)	ME (Mcal/kg)	NEm (Mcal/kg)	NEg (Mcal/kg)	CP (%)	Ca (%)	P (%)
Fresh, early vegetative	80	3.50	2.87	—	—	19.0	0.65	0.40
Fresh, late vegetative	72	3.15	2.58	—	—	16.0	—	—
Fresh, heading	60	3.00	2.46	1.57	0.97	10.4	0.55	0.27
Hay, sun-cured, early vegetative	64	2.82	2.40	1.41	0.78	8.6	0.65	0.32

* All values expressed on a dry matter basis. TDN=Total Digestible Nutrients; DE=Digestible Energy; ME=Metabolizable Energy; NEm=Net Energy for Maintenance; NEg=Net Energy for Gain; CP=Crude Protein; Ca=Calcium; P=Phosphorus.

TDN values are listed for ruminants. Values for horses generally are lower.

(Adapted from (Hannaway, D et al., 1999b)

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components which make it a chosen food source for Japanese Waygu cattle owners, as it results in a much brighter, whiter fat in the beef that is produced from animals that consume it. Perennial ryegrass is also prized for its high yield and fast establishment, making it ideal for golf courses and sports fields.

Epichloë lolii infection can cause issues in livestock when concentrations of the mycotoxin lolitrem B (Figure 1) are present at concentrations in excess of 1800 ppb (Table 2). Unlike ergot alkaloids, lolitrem B is a neurotoxicant, manifesting in a staggering syndrome known as *ryegrass staggers* in animals consuming contaminated feed material (Gallagher et al., 1981). Acting as a tremorgen, lolitrem B inhibits large conductance calcium-activated potassium (BK) channels, having a hyperpolarizing influence on the membrane potential which impairs motor function, resulting in a shaking or tremoring in the animal (Dalziel et al., 2005; Imlach et al., 2008). More progressive clinical signs include stiff and uncoordinated running, inability to flex limbs and struggling to rise/falling easily (Fisher et al., 2004). Fortunately, to our knowledge, there are no lasting effects from ryegrass staggers; once removed from contaminated feed, animals should return to a normal physiological state within 1-2 weeks.

In addition to lolitrem B, *E. lolii* is also capable of producing ergovaline, warranting additional caution when using this feed material. Fortunately, ergovaline is generally produced in much smaller concentrations (one tenth to one half of the lolitrem B concentration) (Hovermale and Craig, 2001; Repussard et al., 2014); livestock owners will generally observe ryegrass staggers signs well before any of the vasoconstrictive or reproductive effects from ergot alkaloids occur. However, subclinical effects from exposure to lower concentrations of both mycotoxins is something to bear in mind if impacts to production are observed.

Sampling and testing of feed material

Concern about endophyte-infected feed/forage materials has generated repeated questions about submitting samples for "endophyte testing" through the Endophyte Service Laboratory at Oregon State University. Feed/forage samples are analyzed for toxin content, not for endophyte presence. These chemical analyses are only as good as the sample provided. In other words, a toxin test will accurately represent a lot of straw or feed/forage material ***only if the sample is truly representative***.

The following guidelines will ensure that the sample(s) submitted are as representative as possible and would be applicable to other laboratories that you may submit samples to:

- **Random sampling:** Bales within a lot of straw, subsamples from an entire pasture, handfuls from a lot of pellets or other methods of defining a given collection of feed/forage material should be sampled at random. Random means that you have no precise reason for selecting or rejecting a specific portion of the feed/forage material to sample. Here are two ways to guard against biasing: 1) sample every fourth or fifth bale going around the stack (or truck) or driving down the row in the field/pasture, or 2) take a least 5 random samples from each of the four sides of the stack/batch of pellets.

- A lot of straw should represent straw from one variety, harvested from the same field. If two lots of straw are in a stack, sample them separately. A truck load of straw may be considered a lot if there is no information to the contrary.
- **Sample weight:** A minimum weight of 50 grams is needed for adequate testing of replicates in the assays we perform. Feed/forage samples have widely varying density, so if you are unable to ensure at least a 50-gram sample with a kitchen scale, we suggest (at a minimum) fully stuffing a gallon-sized plastic bag with the dried material.
- **Sampling methods and techniques:**
    - **Core sampler:** Commercial forage samplers are available through farm supply sources. You can attach a core sampler to a brace or 2-inch drill for easy sampling. Your county Extension agent can help you locate a supplier. Ideally, the sampler should have an exterior diameter of ½ inch and a sample length of at least 12 inches. Minor deviations from these measurements are acceptable. Sample a bale by centering the core sampler in the end of the bale and drilling horizontally. Take at least 20 cores (1 per bale) for each lot of straw or other feed material.
    - **Pasture sampling:** To take a pasture sample, randomly select 20 separate sites within the pasture. At each site clip a handful of grass just above the ground. If your animal typically pulls the plant up by the roots and eats the entire plant, you may wish to pull up a handful of plants at each site. Otherwise, clipping plants at ground level will be sufficient.
    - **Pellet sampling:** Obtain 20 random samples (handfuls) from the batch of pellet material to be tested.
- **Sample consistency:** Once you have obtained your sample using one of the sampling methods above, it is recommended that you air dry your sample out of direct sunlight before shipping. If your sample is not dry, it is not acceptable for testing and should not be submitted.
- When you are ready to submit your sample, place the entire sample in a polyethylene freezer bag. Commercial clients should use the commercial client website (https://endophyte.emt.oregonstate.edu) to submit their samples, placing the automatically generated sample submission form into the bag and sealing it tightly. Non-commercial clients should follow this link to fill out an electronic form, then read the instructions for shipping detailed therein: https://emt.oregonstate.edu/endophyte-lab then select “Non-commercial clients” from the Sample Submission drop-down menu.

Once submitted, the sample will undergo processing in a commercial grinder to produce particles of uniform size to be extracted using organic solvents and solid phase extraction columns to clean-up the eluent for analysis via HPLC-fluorescence following a rigorous quality assurance/quality control management plan (Craig et al., 2014). Samples will be evaluated against reference materials to determine their concentration. Results will then be communicated either directly in the website (commercial clients) or via email (non-commercial clients) as soon as they are available.

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