

ENDOPHYTE TOXINS IN GRASS AND OTHER FEED SOURCES

Risks to Livestock

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Grazing animals on grass seed fields, or feeding grass seed straw or screenings, can benefit both livestock producers and grass seed growers. However, many varieties of tall fescue and perennial ryegrass contain toxins that are harmful to livestock.

Most turf varieties, and some forage varieties, of tall fescue and perennial ryegrass are infected with a fungal endophyte. These endophytes produce bioactive compounds that enhance plant performance. Some of these compounds are toxic to livestock, and problems may develop when livestock consume these grasses or their by-products.

With the phase-out of field burning in Oregon's Willamette Valley during the 1990s, more grass seed straw is being baled for use as livestock feed, both locally and for international export. Some livestock producers have unknowingly created serious herd health problems by using toxic-endophyte straw or grass seed screening materials (Figure 1) as a direct replacement for hay.

What is an endophyte?

An endophyte is a fungus that lives within a plant (endo = inside + phyte = plant). The fungus grows between plant cells and into the developing seed (Figure 2, page 3). The endophyte does not affect the appearance of the plant, so its presence can be detected only by laboratory analysis.

Endophytic fungi are associated with both forage and turf types of tall fescue (*Schedonorus phoenix* [Scop.] Holub) and perennial ryegrass (*Lolium perenne* L.) These fungi are from the



Figure 1. Pelleted screening materials (top) and straw (bottom) are common by-products of grass seed production. Both are readily available as feed sources in Oregon. Livestock producers should be aware of the risk of endophyte toxicity when livestock consume these products. Photos by Gene Pirelli (top) and Nicole Anderson (bottom), © Oregon State University.

genus *Epichloë*. The most well-known are *Epichloë coenophiala* (formerly *Neotyphodium coenophialum*) and *Epichloë festucae* var. *lolii* (formerly *Neotyphodium lolii*). Tall fescue hosts *E. coenophiala*, while perennial ryegrass hosts *E. festucae* var. *lolii*.

The relationship between a grass plant and its fungal endophyte is symbiotic; that is, they both benefit. The grass host provides nutrients for the endophyte, and the endophyte produces bioactive compounds that help protect the plant from drought stress and pests (insects and diseases). These compounds are especially beneficial in hot, dry regions. Some of them, such as lolines and peramine, are not known to cause livestock toxicity.

Endophyte toxicity

Some of the bioactive compounds produced by endophytes, while beneficial to the plant, can be detrimental to livestock and birds.

Ergot alkaloids (also known as ergopeptides) are associated with fescue toxicosis. Ergovaline is the best known of the ergot alkaloids. It once was thought that ergot alkaloids were present only in tall fescue. However, a survey of Willamette Valley grass seed straw indicated that endophyte-infected perennial ryegrass also contained ergovaline.

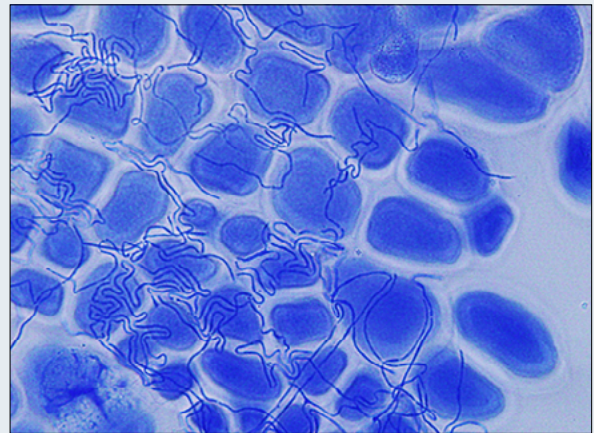
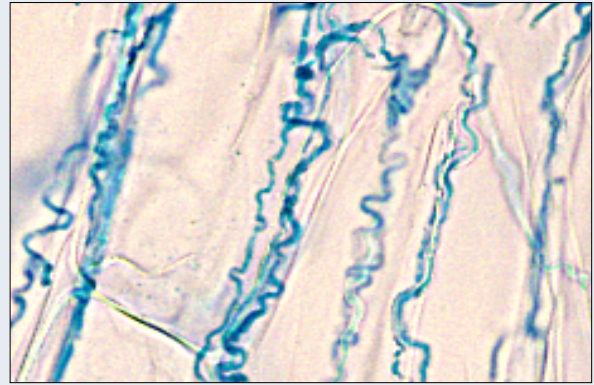


Figure 2. Endophyte within a plant host: tall fescue pseudostem (top) and tall fescue seed (bottom). The endophyte fungus appears as blue-stained convoluted lines. Photos used by permission of The Samuel Roberts Noble Foundation, Inc.

Summary

- Most turf varieties, and some forage varieties, of tall fescue and perennial ryegrass are infected with a fungal endophyte that enhances plant performance.
- Some endophytes produce compounds that are toxic to livestock. These toxins are present in the plant, hay, straw, and seed screenings.
- When livestock consume these toxins in forage or feed, they may develop health problems (fescue toxicosis, ryegrass staggers).
- Production losses can occur well before clinical symptoms appear.
- Some newer forage varieties of tall fescue and perennial ryegrass are infected with novel endophytes, which do not produce toxins that are harmful to livestock.
- In the Pacific Northwest, tall fescue and perennial ryegrass pastures should be planted to endophyte-free or novel endophyte varieties.
- Where toxic-endophyte forage must be grazed, toxin concentrations can be reduced by interplanting endophyte-free or novel endophyte varieties and by avoiding grazing the crowns.
- Before feeding straw or screening materials from unknown or toxic endophyte-infected grass seed fields, have the feed tested for toxin concentrations.
- Toxin concentrations in feed can be diluted by adding good-quality legume or novel endophyte or endophyte-free grass forage to the diet.

Ergovaline is now known to be produced by both *E. coenophiala* (in tall fescue) and *E. festucae* var. *lolii* (in perennial ryegrass).

Indole-diterpenes, such as lolitrem B, are associated with ryegrass staggers. These compounds are produced in perennial ryegrass by *E. festucae* var. *lolii*.

Endophytes in pastures and grass seed fields

In some grass varieties, especially those grown for turf seed, many or most of the plants in a field are endophyte infected. Turfgrass breeders often select for pest resistance and other positive qualities provided by endophytes, without being concerned about potential negative effects on livestock. Because turf varieties represent a large proportion of total grass seed production, the risk

Claviceps purpurea: Another source of toxins

Endophytes are not the only fungal species that produce ergot alkaloids. In some cases, livestock toxicity is caused by the fungus *Claviceps purpurea*, which can produce sclerotia (also known as ergot) in the seed of many grass species (Figure 3). These ergots are full of ergopeptines (toxic alkaloids similar to ergovaline), which are highly toxic to livestock. Seed and seed screenings contaminated with ergots sometimes contain high concentrations of ergopeptines.



Figure 3. Ergot on a tall fescue plant infected by *Claviceps purpurea*. Photo used by permission of The Samuel Roberts Noble Foundation, Inc.

to livestock is high when animals graze on Oregon grass seed fields or consume grass seed straw or screening materials.

Conversely, forage grass breeders consider livestock wellbeing as well as forage persistence. Since the late 1970s, when the association between the endophyte in tall fescue and the livestock disorder fescue toxicosis was discovered, breeders have avoided endophyte-infected varieties. More recently, forage varieties containing nontoxic endophytes have become available (see “Not all endophytes are bad,” page 5).

Choke

Epichloë typhina, an endophyte known to infect orchardgrass, causes choke disease (Figure 4). The emerging inflorescence is overcome, or choked, by the proliferation of fungal hyphae. Because choked stems are unable to produce seeds, yield is reduced. This endophyte is not known to cause livestock toxicity.

Rather than being seed transmitted, this endophyte reproduces sexually. There is no known host resistance, and fungicide treatments have not been effective.



Figure 4. Choke disease, caused by *Epichloë typhina*, on an orchardgrass plant in western Oregon. Fungal hyphae are orange or white and are usually found on stems in the lower part of the plant prior to full seed development. Photos by Nicole Anderson (left) and Sujaya Rao (right), © Oregon State University.

Some Oregon grass seed fields are planted to toxic endophyte-infected forage varieties, such as Kentucky-31. Although Kentucky-31 is widely planted, it is generally not recommended, including in the Pacific Northwest.

The entire life cycle of the endophyte takes place inside the grass host. The fungus is transmitted only through the seed; a plant does not become infected from other plants. Therefore, unless endophyte-infected plants are already present, a grass pasture or grass seed field planted to a noninfected variety will be endophyte-free.

Fungicides have been shown to reduce levels of some fungal pathogens associated with endophytes and ergot in grass seed production fields. However, fungicide applications are not recommended in pastures or hay fields.

Not all endophytes are bad

Over the years, endophytes have been perceived poorly because of their potential toxicity to animals. However, not all endophytes are bad.

Research has identified endophyte strains that benefit the plant without being harmful to livestock. These endophytes are known as novel, selected, or livestock friendly. Grazing grass or feeding products from these endophyte-infected plants does not adversely affect livestock.

In these strains, natural mutations eliminate some or all of the genes required to produce a functional ergot alkaloid. Forage grasses containing these endophytes are commercially available in New Zealand, Australia, and the United States.

Novel endophyte seed must be handled and stored properly to maintain viable endophyte presence. Seed should not be stored in direct sunlight or in hot, humid conditions. Ideally, seed should be refrigerated. Seeds should contain a minimum of 70 percent viable endophyte at the time of purchase. Plant seed soon after purchase; do not carry seed over to subsequent years, as there is risk of losing the beneficial endophyte.

Livestock health problems associated with endophytes

Fescue toxicosis

Fescue toxicosis is associated with ergot alkaloids produced by both *E. coenophiala* (in tall fescue) and *E. festucae* var. *lolii*. (in perennial ryegrass). These toxins are vasoconstrictors; they constrict blood vessels and reduce circulation to body extremities. Symptoms of fescue toxicosis include low average daily gains, reduced fertility, rough hair coats, and a preference for shade or muddy areas, as animals are less able to regulate body temperature (Figure 5).

Eventually, animals may develop fescue foot (in cold weather) or summer slump (in hot weather). However, small production losses can occur well before clinical symptoms appear. For example, there may be slight reductions in average daily gain, with losses becoming more severe as the amount of toxin in the diet increases.

Fescue foot. This disorder is characterized by dry, dead (gangrenous) tissue in the extremities, lameness, and swelling in the legs. After 2 or more weeks, animals develop sloughing of the hooves or lose the tips of their tail or ears. In the winter, frostbite can occur. A 10- to 20-day period of feeding on toxic endophyte-infected tall fescue or its by-products is required before clinical signs appear. In the Pacific Northwest, fescue foot has occurred sporadically in cattle and sheep during the winter.



Figure 5. Signs of fescue toxicosis in cattle that have grazed toxic tall fescue. Animals have low average daily gains and rough hair coats. They are muddy due to a preference for wallowing near the water trough. Photo used by permission of The Samuel Roberts Noble Foundation, Inc.

Summer slump. Hyperthermia (elevated body temperature) is the key problem associated with summer slump. Animals spend less time grazing and more time standing in water or shade in an attempt to stay cool. Other clinical signs include reduced food intake, poor weight gain, lower pregnancy rates, and decreased milk production. Poor livestock performance is more pronounced when temperatures exceed 87°F. In cattle, the reduction in weight gain and hyperthermia may last up to 6 weeks after removal from an endophyte-infected field.

In the southeastern United States, where summers are hot, summer slump is common. With more than 35 million acres of mostly toxic endophyte-infected tall fescue pasture in the southeastern states, fescue toxicosis is the number one large animal toxicity problem in the United States. Livestock losses are estimated by the U.S. Department of Agriculture at nearly \$1 billion per year.

Reproductive problems in horses. Horses that ingest ergot alkaloids can develop serious reproductive abnormalities, including failure to come into heat, early-term abortions, prolonged pregnancies, difficult births, retained placentas, poor udder development, little or no milk production, and poor foal survival. These reproductive problems occur in both the southeastern United States and the Pacific Northwest.

If pregnant mares are removed from toxic endophyte-infected pasture at least 1 month before foaling, they usually recover and have normal foals. However, milk production may be decreased.

Ryegrass staggers

Ryegrass staggers is caused by indole-diterpenes such as lolitrem B, which are produced in perennial ryegrass by *E. festucae* var. *lolii*. These toxins are tremorgens; they cause muscle weakness, tremors, and spasms. Most affected animals show no clinical signs unless they are excited. When they run, they may experience trembling, severe incoordination, and/or

falling. Decreased average daily gains and milk production may occur before these clinical symptoms appear.

In Oregon, this condition is most common in sheep grazing toxic endophyte-infected perennial ryegrass as their only feed. A 7- to 14-day exposure may induce staggers. The signs usually disappear 2 or 3 days after animals are removed from perennial ryegrass, but sometimes can last 2 weeks.

Is there a safe level?

It is always better for an animal not to consume a toxin. Although livestock may tolerate some level of ergot alkaloids and indole-diterpenes, it is important to limit exposure and the concentration of toxins in feed.

Experiments and case studies conducted at the Oregon State University College of Veterinary Medicine and elsewhere have suggested threshold levels of ergovaline and lolitrem B in animal diets (Table 1, page 7). Although clinical disease is not seen at toxin levels below these thresholds, keep in mind that production losses, such as reduced average daily gains, might still occur.

Horse oedema

In Australia, a new clinical syndrome has been reported in horses grazing Mediterranean tall fescue varieties infected with a specific strain of endophyte. Horses develop subcutaneous oedema (fluid retention under the skin) throughout their body, especially in the head, neck, and chest. Other clinical signs include loss of appetite, lethargy, and weakness. Most horses recover in 7 to 21 days, but deaths have been reported.

Most cases were seen when several months of dry weather were followed by a wet period that promoted rapid growth of Mediterranean tall fescue. Cattle and sheep have not been affected. Mediterranean tall fescue varieties are uncommon in North America, and to date there have been no reports of subcutaneous oedema in the United States.

It is important to emphasize that the thresholds in Table 1 refer to the level of toxin in the total diet, not in a single feed component. Threshold levels vary because environment and stress also play a role in the development of clinical disease.

Animals fed endophyte-infected perennial ryegrass almost always exhibit symptoms of ryegrass staggers before symptoms of fescue toxicosis. An OSU study found that, on average, the ratio of lolitrem B to ergovaline in perennial ryegrass straw is about 10:1. Thus, perennial ryegrass with 2,000 ppb lolitrem B would contain about 200 ppb ergovaline. Therefore, ergovaline should not be a problem, except for pregnant mares.

Variation in toxin concentrations

Toxin levels vary across years, seasons, and fields, even within the same grass variety. Variation occurs because of differences in seed storage conditions and environmental conditions during growth.

More ergovaline is produced when plants grow under stress. Research at OSU showed that the ergovaline content in straw from the same plants was 55 percent less in 1992 than in 1991. The

reason was stress from higher than normal spring rainfall in 1991.

Another OSU study showed that ergovaline concentrations were highest in September and October, and lowest in December and January (Table 2).

Nitrogen fertilization can also influence ergovaline levels. More ergovaline is produced when soil nitrogen levels are high.

Managing forage and feed for safety

Pastures

Seed selection. Pastures planted with endophyte-free, low-endophyte, or novel endophyte varieties of tall fescue or perennial ryegrass are safe to graze or cut for hay. In the Pacific Northwest, endophyte-free or novel endophyte varieties are recommended. In the region's cool temperatures, these varieties perform well.

Purchase Oregon-grown certified seed to ensure seed purity and quality. The Oregon Department of Agriculture administers the Endophyte Free Seed Tagging Program, which verifies that seed is free of endophyte fungus.

Table 1. Levels of ergovaline and lolitrem B in the diet that produce disease.

	Ergovaline (ppb) ¹		Clinical disease	Lolitre B (ppb) ²
	No or limited symptoms	May see some symptoms		
Horses ³	0–150	150–300	Above 300	Not determined
Cattle	0–150	150–300	Above 300	Above 1,800
Sheep	0–150	150–500	Above 500	Above 1,800

¹Ergovaline is an ergot alkaloid associated with fescue toxicosis. Some labs test for total ergot alkaloid, rather than for ergovaline alone. In this case, threshold levels are different.

²Lolitre B is an indole-diterpene associated with ryegrass staggers.

³For mares in the final 60 to 90 days of pregnancy, the threshold is zero.

Table 2. Ergovaline levels in a turf-type tall fescue field with high levels of endophyte infection.^{1,2}

	Ergovaline (ppb)		
	Seed	Crowns	Tops and stems
September–October	2,000	900	460
December–January	—	500	75 (tops) 200 (stems)

¹Field was 77 percent infected with endophyte.

²Sheep grazing mostly the tops of grasses showed no signs of fescue toxicosis.

Tagged seed must meet the Oregon forage grass standard of 5 percent endophyte or less.

Forage dilution. Forages with toxin levels above the thresholds in Table 1 (page 7) may be fed safely, as long as they are diluted with other feedstuffs that do not contain endophyte toxins. Dilution can be accomplished by interseeding endophyte-free or novel endophyte forage varieties and species into endophyte-infected grass pastures. Suitable grasses include orchardgrass, annual ryegrass, and some varieties of tall fescue and perennial ryegrass. Legumes such as red clover, white clover, and subclover are another option.

Grazing management. During the vegetative phase of grass plant growth, endophyte fungal hyphae are concentrated in the growing points (the meristematic tissue). The fungus is found first in the leaf sheaths at the base of the plant. For example, the crowns of tall fescue contain about twice as much ergovaline as the tops. Thus, grazing grasses too short increases the risk of toxicity. Grazing crowns in a grass seed field following seed harvest is riskier than grazing the tops of plants in winter or early spring.

Grass seed straw and screening materials

As grass enters its reproductive phase and the stem elongates, endophyte fungal hyphae begin to proliferate in the developing seed heads. As a result, seed heads contain the highest concentrations of toxins (Table 2, page 7).

Toxins persist in straw and screening materials. Toxicity is not reduced by pelleting seed screenings or by making silage. Ammoniating straw reduces the toxin level by approximately 50 percent, but this level might still cause animal toxicity.

Grass seed straw and screening materials are fed to Oregon livestock primarily in the winter. In some areas, extreme cold increases the severity of endophyte-related problems.

Feed selection and testing. Avoid feeding screening materials from unknown grass seed fields. If such feed must be used, testing for toxins is strongly recommended.

Feed sources from fields known to be infected with toxic endophyte should also be tested. Since toxin levels vary greatly, testing is the best way to decide whether, or how, a feedstuff can be used in a livestock diet.

See “Endophyte testing” for detailed information.

Feed dilution. Feeds with higher toxin levels may be fed safely, as long as they are diluted with other feedstuffs that do not contain endophyte toxins. Dilution can be achieved by feeding good-quality legume hay, such as alfalfa, along with grass seed straw or screening materials that do not contain toxic endophyte.

Supplementation. Grass seed straw and screening materials are considered low-quality feed ingredients. A low-quality diet can result in poor body condition, thereby increasing endophyte-related problems. Always supplement low-quality feed to meet nutritional needs.

Stored feed. In both tall fescue and perennial ryegrass, the endophyte fungus begins to lose viability when seed is stored 18 months or more, especially under warm, humid conditions. Novel endophytes may lose viability more quickly. Thus, the infection rate is reduced in old seed and in plants grown from old seed. However, endophyte-produced toxins persist even after several years of storage, so feeding stored grass seed and screening materials is still risky.

Endophyte testing

Sampling procedures

It is important to submit a representative sample for testing. Follow the procedures outlined below.

- **Hay and straw.** Use a core forage sampler that has an exterior diameter of ½ inch and a sample length of at least 12 inches.
 - **For bales,** center the core sampler at the end of the bale and drill horizontally. For each lot, take one core sample each from at least 20 random bales.
 - **For stacks of straw,** core every fourth or fifth stack or take at least five random samples from each side of the stack.

- **Seed screening materials.** Take 15 to 20 small subsamples randomly from the storage containers. Combine into one representative sample by mixing thoroughly. Send at least 1 cup of the mixed material to the laboratory.
- **Fields.** Grass plants can be sampled at any time of the year. However, sampling during a vegetative stage is preferred. Choose at least 20 random locations. Clip grass plants at ground level. Air-dry samples out of direct sunlight. Make sure samples are completely dry before submitting; moist samples are not acceptable.

Testing services

- **Feed sources.** The OSU Endophyte Service Laboratory can measure amounts of specific endophyte alkaloids in hay, straw, and screening pellets. Testing for the ergot alkaloid ergovaline and indole-diterpene lolitrem B is available to the public. Oregon grass seed producers who ship straw internationally also use this service. Many countries require certification that toxin levels are below the thresholds for producing animal disease. Send samples and payment to Endophyte Service Laboratory, 139 Oak Creek Building, Oregon State University, Corvallis, OR 97331. Call for current fees (541-737-2872).
- **Certified seed.** The official test required for Endophyte Fungus Tested seed tags is provided only by the Oregon Department of Agriculture. Many seed growers obtain this certification if they plan to sell their seed out of state. An endophyte seed test result shows the percentage of seeds infected. For example, a 25 percent level means that 25 out of 100 seeds contain an endophyte that will produce infected plants. For more information, see <http://www.oregon.gov/ODA/programs/PlantHealth/Pages/LabServices.aspx>
- **General seed and forage testing.** The OSU Seed Laboratory, as well as private laboratories, can test for the presence of endophyte in grass seeds, seedlings, and vegetative plants. The OSU Endophyte Service Laboratory can also test plants.

Submitting samples

Seal samples in polyethylene freezer bags and label with your name, phone number, sample identification, and the test you want performed (ergovaline, lolitrem B, or both). See the sidebar “Testing services” to choose an appropriate laboratory.

For more information

- Oregon Department of Agriculture Endophyte Testing and Seed Tagging Program. <http://www.oregon.gov/ODA/programs/PlantHealth/Pages/LabServices.aspx>
- Oregon State University Endophyte Service Laboratory. <http://oregonstate.edu/endophyte-lab/>
- Oregon State University Seed Laboratory. <http://seedlab.oregonstate.edu/>
- Oregon Tall Fescue Commission. *Tall Fescue Endophyte Concepts*. <http://www.oregontallfescue.org>

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