BOARD-INVITED REVIEW: St. Anthony’s Fire in livestock: Causes, mechanisms, and potential solutions\textsuperscript{1,2}

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ABSTRACT: After a brief history of ergot alkaloids and ergotism, this review focuses on the metabolism and mechanisms of action of the ergot alkaloids. The authors provide models of how these alkaloids afflict grazing livestock under complex animal-plant/endophyte-environmental interactions. Alkaloid chemistry is presented to orient the reader to the structure-function relationships that are known to exist. Where appropriate, the medical literature is used to aid interpretation of livestock research and to provide insight into potential modes of action and alkaloid metabolism where these are not known for livestock. In closing the paper, we discuss management of ergot alkaloid intoxication in livestock and future research needs for this field of study.

Key words: ergot alkaloid, ergotism, fescue toxicosis, St. Anthony’s Fire

INTRODUCTION

Ergotism (see Table 1 for signs), named “St. Anthony’s Fire” after the monastic Order of St. Anthony whose members treated sufferers, resulted in tens of thousands of human deaths in Europe during the Middle Ages (Schiff, 2006). At the time of the widespread cases of St. Anthony’s Fire, the cause was not known, only that a pilgrimage to and treatment at the Order’s monasteries appeared to cure the condition miraculously. It is now known that the malady was the result of ingestion of ergot alkaloids from ergot-contaminated rye (\textit{Secale cereale} L.) grains; at the monasteries sufferers were no longer exposed because clean grain was provided. Ergot alkaloids (Figure 1) are naturally occurring mycotoxins (Bush and Fannin, 2009) produced by fungi, including members of the \textit{Claviceps} (external spore-producing fungi) and \textit{Neotyphodium} (endophytic fungi that grow within a plant-without sporulation) genera. The general medicinal and toxic effects of ergot-(\textit{Claviceps purpurea}) contaminated grain have been well recognized for several millennia. In fact, ergot is recorded in written records as early as 1100 B.C.E. for use in obstetrics, as well as in the reports of St. Anthony’s Fire during the Middle Ages (Schiff, 2006). Although the use of ergot persisted as a medicinal as well as a toxicant for several millennia, it was not until 1906 that the first ergot alkaloid (i.e., ergotoxine, a mixture of 4 alkaloids) was isolated by Barger and Dale; ergotamine was isolated and identified shortly thereafter in 1918 by Arthur Stoll (De Costa, 2002).

St. Anthony’s Fire is now largely a malady of history. In fact, cases of ergotism in humans are very rare today and almost always the result of overdosing of an ergot-based drug (e.g., ergotamine tartrate) rather than as a result of consumption of naturally contaminated foodstuffs. This trend is largely due to improvements in crop genetics and management, grain cleaning techniques for removal of sclerotia (Flieger et al., 1997), and toxicant screening protocols and regulation to safe quantities in grain used for foodstuffs. However,
this is not the case for livestock producers. Although ergot itself is not a major problem in livestock production, consumption of some pasture forage is an entirely different story. As early as 50 C.E., the presence of ergot alkaloids (known today to be associated with endophytic fungi) were documented in forage grasses, as evidenced by the mention of the sowing of tares in the Holy Bible (Matthew 13:25–40; as reviewed by Bacon, 1995). More recently, widespread dissemination of tall fescue (*Lolium arundinaceum* [Schreb.] Darbysh. – *Schedonorus arundinaceus* [Schreb.] Dumort., formerly *Festuca arundinacea* Schreb. var. *arundinacea* Schreb.) as a pasture grass in the early 1940s in the United States led to the recognition of the importance and ultimate linkage of endophytic fungi to ergot-alkaloid induced fescue toxicosis (Table 1), a condition similar to St. Anthony’s Fire in grazing livestock (Strickland et al., 1993; Bacon, 1995; Hoveland, 2009).

Fescue toxicosis is a serious problem within the fescue suitability zone (Hannaway et al., 2009) of the United States; it was estimated in 1993 to result in lost cattle production of almost 600 million dollars (Hoveland, 1993). In today’s dollars, when taking into account the impact on the equine and small ruminant industries, the combined losses likely exceed $1 billion annually. Although the losses are great (Porter, 1995; Miles et al., 1996), producers often have not recognized this insidious problem, especially given that the signs (Table 1) usually are not overtly severe and go undetected. This magnitude of losses attributable to fescue toxicosis makes them the largest animal health-related production cost for the grazing livestock industry. Although progress has been made in mitigating the problem (see the Current Management Approaches and Future Research Questions section), a comprehensive solution to the intoxication has not been realized. The lack of ad-

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**Table 1.** General toxicity signs and symptoms that may be present after consumption of ergot (ergotism; *Claviceps* spp.) infested grain or endophyte (*Neotyphodium coenophialum*) infected tall fescue (fescue toxicosis; *Lolium arundinaceum*)

| Sign/Symptom | Cause |
|--------------|-------|
| Dry gangrene (ear, tail tips, or teats) | Increased respiration rate |
| Dystocia/death in horses | Lameness |
| Elevated rectal temperature | Loss of hooves |
| Excessive salivation | Loss of tail switch |
| Extended time in shade, mud holes, ponds, or streams | Poor thrift |
| | Reduced BW gain |
| | Reduced grazing time |
| | Reduced milk production |
| | Reduced serum prolactin |
| | Rough hair coat |
| | Swelling (fetlock/hoof) |
equately selective and sensitive analytical methodologies to measure the alkaloids and metabolites in various animal tissues and fluids; the complex interaction of animal, plant/endophyte, and environmental factors involved in expression of the toxicosis; and an incomplete understanding of the identity, metabolism, clearance, and mechanisms of action associated with the alkaloids that are absorbed by the grazing animal explain the delay in developing comprehensive solutions.

In the last century many natural ergot alkaloids (Table 2) were isolated and identified as contributors to ergotism and fescue toxicosis. These alkaloids have formed the basis for many of the synthetic ergot alkaloids (e.g., cabergoline, lergotrile) of medicinal importance in use today for the treatment of maladies such as migraines and prolactinomas. The body of literature available to the alkaloid researcher is quite large. A National Center for Biotechnology Information PubMed search using “ergot alkaloid” as the search term returns more than 26,000 citations with the first being published in the early 1930s. The intent of this publication is to provide an in-depth review of the literature as it pertains to the state of ergot alkaloid research concerning grazing livestock.

### Natural Sources, Chemistry, and Analysis

#### Natural Sources of Ergot Alkaloids

Fungi within the Hypocreales and Eurotiales orders produce ergot alkaloids. All species of the Claviceps genus within Hypocreales, most notably Claviceps purpurea, parasitize more than 600 plants, including some of the economically important cereal grains such as rye, wheat, barley, millet, and oats (Bové, 1970; Groger, 1972). The fungal mycelia invade the seed and form an ergot-containing body called the sclerotium. Consumption of this sclerotium and attendant ergot alkaloids caused the widespread human epidemics of ergotism throughout history (Schiff, 2006). Most recently, ergot (C. africana) contamination of sorghum [Sorghum bicolor (L.) Moench], an important food and feed crop in Africa, Central America, and South Asia, has been reported (Bandypadhyay et al., 1998) in developing countries. In recent decades, several endophytic fungi of the genera Epichloë, Neotyphodium, and Balansia found in grasses have been shown to produce ergot alkaloids (Bacon, 1988; Clay and Schardl, 2002). These fungi have various host relationships ranging from antagonistic in Epichloë to mutualistic in Neotyphodium species (Flieger et al., 1997). These symbiotic relationships between grasses and fungi often result in improved vigor of the grass host by protecting the plant from environmental stressors (Siegel and Bush, 1994). However, production of ergot alkaloids by these endophytes, especially Neotyphodium coenophialum-infected tall fescue (L. arundinaceum), limits livestock production. Fungi of the Aspergillus and Penicillium (Eurotiales order) genera, known for their production of aflatoxins, also produce ergot alkaloids but in lesser quantity than Claviceps spp. (Reshetilova and Kozlovsikki, 1990; Flieger et al., 1997) and thus are not generally of concern for ergotism. Favorable environmental conditions (e.g., warm temperatures, high rainfall and humidity, high-soil fertility) increase the prevalence of certain fungi (e.g., Claviceps spp.) and the production of ergot alkaloids (Craig and Hignight, 1991). As such, the presence or absence of a fungi itself may not be an adequate indicator of toxic potential within a feedstuff. A better alternative would be to determine the profiles of the ergot alkaloids present.

### Analytical Options

Sensitive and selective methods for ergot alkaloid analysis are needed to measure feeds and foods for ergot alkaloid contamination and to study the toxicokinetics and toxicodynamics in animal systems. Methods currently favored for the analysis of ergot alkaloids in the agricultural/food sciences include a competitive ELISA and HPLC coupled to fluorescent or mass spectrometry (MS) detection systems.

The ELISA method (Shelby and Kelley, 1990, 1992; Hill and Agee, 1994; Schnitzius et al., 2001) excels in sensitive and rapid analysis for total ergot alkaloids. This method has been successfully used to measure total ergot alkaloid concentrations in plant and fungal tissues (Shelby and Kelley, 1990, 1992; Hill and Agee, 1994; Schnitzius et al., 2001), digesta (Hill and Agee, 1994), urinary and biliary excretions (Stuedemann et al., 1998; Hill et al., 2000), and animal fat (Realini et al., 2005). However, the method suffers from a lack of selectivity in that it does not distinguish one ergot alkaloid from another. The method will probably not detect the precursor molecules (e.g., clavine alkaloids) to lysergic acid because the antibody used for the analysis is reportedly (Hill and Agee, 1994) specific for the lysergic acid moiety. This limits the use of ELISA in discovery research aimed at linking specific ergot alkaloids to specific pathological effects. Nevertheless, a commercial assay (Agrinostics Ltd. Co., Watkinsville, GA) based on this technology has been very useful for quick qualitative and quantitative assessment of total ergot alkaloid loads in herbage and feedstuffs, and in the determination of ergot alkaloid exposures of livestock.

High-performance liquid chromatography separation coupled to detection by fluorescence has been used for similar analyses, but is slower than the ELISA method (Yates and Powell, 1988; Rottinghaus et al., 1991, 1993; Jaussaud et al., 1998; Durix et al., 1999; Lodge-Ivey et al., 2006). Although slower, these methods have the advantage of providing a degree of selectivity missing with the ELISA because they can separate and quantify individual analytes. However, HPLC-fluorescence methods are dependent on purified standards to identify retention times and excitation and emission wavelengths for...
Table 2. List of ergot alkaloids identified, general chemical classification, and reference cited

| Alkaloid                  | Source fungi       | Method of detection1 | Chemical classification | Reference |
|---------------------------|--------------------|----------------------|-------------------------|-----------|
| Acid-ergovaline            | *Neotyphodium* coenophialum | LC/MS ESI+         | Ergopeptine             | Shelby et al., 1997 |
| Didehydroergovaline       | *N. coenophialum*  | LC/MS ESI+           | Ergopeptine             | Shelby et al., 1997 |
| Ergocornine               | *N. coenophialum*  | HPLC/Fluorescence   | Ergopeptine             | Yates and Powell, 1988 |
|                           | *Claviceps purpurea* |                      |                         | Rottinghaus et al., 1993 |
| Ergocryptine              | *N. coenophialum*  | HPLC/Fluorescence   | Ergopeptine             | Yates and Powell, 1988 |
| Ergocrystine              | *C. purpurea*      | HPLC/Fluorescence   | Ergopeptine             | Rottinghaus et al., 1993 |
| Ergonine                  | *N. coenophialum*  | LC/MS-MS* (SRM) ESI+| Lysergic acid derivative | Shelby et al., 1997 |
| Ergonovine (ergometrine)  | *N. coenophialum*  | HPLC/Fluorescence   | Ergopeptine             | TePaske et al., 1993 |
|                           | *C. purpurea*      |                      |                         | Leiner et al., 2005 |
|                           | *C. purpurea*      |                      |                         | Burk et al., 2006 |
|                           | *C. purpurea*      |                      |                         | Mohamed et al., 2006 |
|                           | *C. purpurea*      |                      |                         | Krska et al., 2008 |
|                           | *N. coenophialum*  | LC/MS ESI+           | Ergopeptine             | TePaske et al., 1993 |
|                           | *C. purpurea*      |                      |                         | Leiner et al., 2005 |
|                           | *C. purpurea*      |                      |                         | Burk et al., 2006 |
|                           | *C. purpurea*      |                      |                         | Mohamed et al., 2006 |
|                           | *C. purpurea*      |                      |                         | Krska et al., 2008 |
|                           | *N. coenophialum*  | LC/MS-MS* (SRM) ESI+| Lysergic acid derivative | Shelby et al., 1997 |
|                           | *C. purpurea*      |                      |                         | Leiner et al., 2005 |
|                           | *C. purpurea*      |                      |                         | Burk et al., 2006 |
|                           | *C. purpurea*      |                      |                         | Mohamed et al., 2006 |
|                           | *C. purpurea*      |                      |                         | Krska et al., 2008 |
|                           | *N. coenophialum*  | LC/MS ESI+           | Ergopeptine             | TePaske et al., 1993 |
|                           | *C. purpurea*      |                      |                         | Leiner et al., 2005 |
|                           | *C. purpurea*      |                      |                         | Burk et al., 2006 |
|                           | *C. purpurea*      |                      |                         | Mohamed et al., 2006 |
|                           | *C. purpurea*      |                      |                         | Krska et al., 2008 |
|                           | *N. coenophialum*  | LC/MS-MS* (SRM) ESI+| Lysergic acid derivative | Shelby et al., 1997 |
|                           | *C. purpurea*      |                      |                         | Leiner et al., 2005 |
|                           | *C. purpurea*      |                      |                         | Burk et al., 2006 |
|                           | *C. purpurea*      |                      |                         | Mohamed et al., 2006 |
|                           | *C. purpurea*      |                      |                         | Krska et al., 2008 |
|                           | *N. coenophialum*  | LC/MS ESI+           | Ergopeptine             | TePaske et al., 1993 |
|                           | *C. purpurea*      |                      |                         | Leiner et al., 2005 |
|                           | *C. purpurea*      |                      |                         | Burk et al., 2006 |
|                           | *C. purpurea*      |                      |                         | Mohamed et al., 2006 |
|                           | *C. purpurea*      |                      |                         | Krska et al., 2008 |
|                           | *N. coenophialum*  | LC/MS-MS* (SRM) ESI+| Lysergic acid derivative | Shelby et al., 1997 |
|                           | *C. purpurea*      |                      |                         | Leiner et al., 2005 |
|                           | *C. purpurea*      |                      |                         | Burk et al., 2006 |
|                           | *C. purpurea*      |                      |                         | Mohamed et al., 2006 |
|                           | *C. purpurea*      |                      |                         | Krska et al., 2008 |
|                           | *N. coenophialum*  | LC/MS ESI+           | Ergopeptine             | TePaske et al., 1993 |
|                           | *C. purpurea*      |                      |                         | Leiner et al., 2005 |
|                           | *C. purpurea*      |                      |                         | Burk et al., 2006 |
|                           | *C. purpurea*      |                      |                         | Mohamed et al., 2006 |
|                           | *C. purpurea*      |                      |                         | Krska et al., 2008 |
|                           | *N. coenophialum*  | LC/MS-MS* (SRM) ESI+| Lysergic acid derivative | Shelby et al., 1997 |
|                           | *C. purpurea*      |                      |                         | Leiner et al., 2005 |
|                           | *C. purpurea*      |                      |                         | Burk et al., 2006 |
|                           | *C. purpurea*      |                      |                         | Mohamed et al., 2006 |
|                           | *C. purpurea*      |                      |                         | Krska et al., 2008 |
|                           | *N. coenophialum*  | LC/MS ESI+           | Ergopeptine             | TePaske et al., 1993 |
|                           | *C. purpurea*      |                      |                         | Leiner et al., 2005 |
|                           | *C. purpurea*      |                      |                         | Burk et al., 2006 |
|                           | *C. purpurea*      |                      |                         | Mohamed et al., 2006 |
|                           | *C. purpurea*      |                      |                         | Krska et al., 2008 |
|                           | *N. coenophialum*  | LC/MS-MS* (SRM) ESI+| Lysergic acid derivative | Shelby et al., 1997 |
|                           | *C. purpurea*      |                      |                         | Leiner et al., 2005 |
|                           | *C. purpurea*      |                      |                         | Burk et al., 2006 |
|                           | *C. purpurea*      |                      |                         | Mohamed et al., 2006 |
|                           | *C. purpurea*      |                      |                         | Krska et al., 2008 |
|                           | *N. coenophialum*  | LC/MS ESI+           | Ergopeptine             | TePaske et al., 1993 |
|                           | *C. purpurea*      |                      |                         | Leiner et al., 2005 |
|                           | *C. purpurea*      |                      |                         | Burk et al., 2006 |
|                           | *C. purpurea*      |                      |                         | Mohamed et al., 2006 |
|                           | *C. purpurea*      |                      |                         | Krska et al., 2008 |
|                           | *N. coenophialum*  | LC/MS-MS* (SRM) ESI+| Lysergic acid derivative | Shelby et al., 1997 |

Continued
Table 2 (Continued). List of ergot alkaloids identified, general chemical classification, and reference cited

| Alkaloid                  | Source fungi | Method of detection | Chemical classification | Reference                  |
|--------------------------|--------------|---------------------|-------------------------|---------------------------|
| Lysergic acid            | N. coenophialum | LC/MS-MS (MRM) ESI+ | Lysergic acid           | Lehner et al., 2005       |
| Lysergic acid amide (ergine) | N. coenophialum | HPLC/Fluorescence | Lysergic acid derivative | Lodge-Ivey et al., 2006   |
| Lysergol                 | N. coenophialum | LC/MS ESI+          | Lysergic acid derivative | Shelby et al., 1997       |
|                          | N. coenophialum | LC/MS-MS (MRM) ESI+ | Lysergic acid derivative | Lehner et al., 2005       |

1LC/MS = liquid chromatography/mass spectrometry; ESI+ = electrospray ionization in the positive mode; MS-MS = tandem mass spectrometry; MRM = multiple reaction monitoring; SRM = selected reaction monitoring.

analystes of interest. Thus, these methods are limited in discovery and identification of unknown compounds. When HPLC is coupled to MS (TePaske et al., 1993; Lehner et al., 2005; Bürk et al., 2006; Mohamed et al., 2006; Kraska et al., 2008; Smith et al., 2009), the combination offers the selectivity of the HPLC-fluorescence methods and also 3-parameter identification of an analyte within a chromatographic separation via retention time, precursor-ion m/z (single ion monitoring mode), and product ions m/z (specific reaction monitoring mode). Methods utilizing fluorescence typically only have retention time for a specific excitation/emission wavelength combination (2-parameter identification) to select and quantify a given analyte. Additionally, HPLC-MS/MS, because of its ability to identify product ions present in classes of compounds, is a powerful discovery tool for unknown natural compounds and for metabolites generated during biotransformation processes. The advantages of HPLC-MS over traditional HPLC detector combinations and recent declines in the cost of instrumentation led the USDA-ARS, Forage-Animal Production Research (FAPRU) to collaborate on a project with a chemist at Eastern Kentucky University to develop an HPLC-MS method for detection of ergot alkaloids in vascular tissue (Smith et al., 2009). The method demonstrated sensitivity with limits of detection and quantification of 0.05 and 0.1 pmol on column, respectively. The method was used successfully by Klotz et al. (2009) to demonstrate a bioaccumulation of ergovaline, but not lysergic acid, in the bovine lateral saphenous vein. The ergot alkaloids that have been analyzed via HPLC-fluorescence, HPLC-MS, or both are listed in Table 2.

Recently there has been an exciting development within the law enforcement community: ultra-performance liquid chromatography coupled to MS has been used to screen for lysergic acid diethylamide (an ergot alkaloid) in human urine and blood (Chung et al., 2009). Further, the USDA-ARS, FAPRU has been collaborating with chemists at Waters Inc. (Milford, MA) to develop an ultra-performance liquid chromatography-MS/MS method for detecting, identifying, and quantifying ergot alkaloids (lysergic acid, ergonovine, ergovaline, ergocornine, α-ergocryptine, ergotamine, ergocristine) in animal tissues and fluids at sub-picomole concentrations. Current efforts indicate that limits of detection ≤0.005 pmol on column, with chromatographic runs of 10 min, are possible (unpublished data). If this limit of detection holds for different tissue matrices, then we have a major advance in research on the disposition, metabolism and toxicodynamics of ergot alkaloids in animal fluids and tissues. Before these recent attempts, the best limit of detection accomplished for the ergot alkaloids was 0.05 pmol on column using an HPLC-MS in single ion monitoring mode and a 43-min chromatographic separation (Smith et al., 2009).

**Ergot Alkaloid Chemistry**

The chemistry and synthesis of ergot alkaloids has been discussed in several publications over the years (Berde, 1980; Weber, 1980; Garner et al., 1983; Smith and Shappell, 2002; Haefner et al., 2008; Bush and Fannin, 2009), including the 1978 publication of the book titled *Ergot Alkaloids and Related Compounds* (Berde and Stürmer, 1978). Therefore, only a brief synopsis of ergot alkaloid chemistry is provided here for the convenience of the reader.

All ergot alkaloids contain a tetracyclic ergoline ring structure (Figure 1; Berde, 1980). There are 3 major classes of ergot alkaloids: 1) clavine alkaloids, 2) lysergic acid and derivatives, and 3) ergopeptine alkaloids (Figure 1; Lyons et al., 1986; Porter, 1995; Bush and Fannin, 2009). Whereas derivatives of d-lysergic acid (e.g., ergopeptines) are of concern for causing ergotism or fescue toxicosis, those of its epimer, D-Isolysergic acid, have little biological effect reported in animal systems (Berde and Stürmer, 1978; Berde, 1980). However, given the epimerization potential of both epimers (Smith and Shappell, 2002; Haefner et al., 2008) for a given ergot alkaloid, both likely play a role in inducing toxicity in the animal. Therefore, the concentration of both epimers should be determined during sample analysis.

The biological activity of the ergot alkaloids in animal systems is largely due to the structural similarities of the ergoline ring structure to the biogenic amines, serotonin, dopamine, norepinephrine, and epinephrine (Berde, 1980; Weber, 1980). This structural similarity allows many of the ergot alkaloids to bind biogenic...
amine receptors and to elicit such effects as decreased serum prolactin and vasoconstriction. Covalent modifications (e.g., bromination of the number 2 carbon to form bromocriptine, a dopamine agonist used to treat prolactin-producing pituitary gland tumors) to the ergoline ring, confirmation of the flexible D ring, and substitutions at carbon number 8 determine receptor affinity and activity (Weber, 1980). Consequently, ergot alkaloids constitute a very diverse class of chemical compounds with widely different toxicological targets and activities, and potential routes of elimination and rates of clearance.

**ERGOT ALKALOID ABSORPTION, METABOLISM, DISTRIBUTION, AND CLEARANCE**

Currently, very little is known concerning the disposition of the natural ergot alkaloids in livestock and only marginally more for small animal models and humans. The literature appears to support that ruminant livestock handle the metabolism and elimination of ergot alkaloids from their bodies better than nonruminant and hind-gut fiber digesting livestock. This is due to more efficient microbial metabolism before absorption along the gastrointestinal tract. Further, there is evidence that ergopeptines (e.g., ergovaline, ergotamine), but not simpler lysergic acid derivatives (lysergic acid, ergonovine, lysergamide), bioaccumulate in body fat/lipid stores (Realini et al., 2005; Klotz et al., 2009).

**Exposure**

It is well known in toxicology that an animal must be exposed to a sufficient dose of a toxicant, by an effective route, and for a sufficient time, to achieve the concentration at the molecular target site that is effective to elicit a physiological response.

Several potential routes of exposure for toxicants in livestock include injection (intravenous, intraperitoneal, subcutaneous), respiration, ingestion, and cutaneous exposure. Although one might envision that the respiratory route could present as a hazard if ergot sclerotia were aerosolized, this route has not been reported to our knowledge as a concern for ergot alkaloid intoxication in livestock, small animals, or humans. Injection-based routes are of concern primarily in research or in medicine when used for delivery of ergot alkaloid-derived drug treatments; they pose little threat to livestock in production settings. No reports of cutaneous poisoning with ergot alkaloids have been found in the literature. Given the nature of contaminated feeds, forages, and medicinals, ingestion of ergot alkaloids provides the primary route of intoxication for animals (grazing endophyte-infected tall fescue, consumption of contaminated grains) and humans (a drug overdose). For the purposes of this paper, endophyte (*N. coenophialum*)-infected or -infested implies that the tall fescue is toxic due to the presence of ergot alkaloids. Therefore, all reference to ergot alkaloid containing tall fescue will be referred to as endophyte-infected or -infested. Tall fescue not containing ergot alkaloids will be referred to as endophyte free.

For an ergot alkaloid to reach an effective concentration at the target site after exposure, the ergot alkaloid must overcome several protective mechanisms/barriers that are aimed at improving the tolerance of the animal to environmental toxicants. These barriers are 1) mechanisms of influx and efflux, 2) biotransformation reactions, 3) transport/distribution to molecular target sites, including passing the blood-brain and placental barriers, and 4) physical elimination (e.g., via urine) from the body.

**Absorption**

Gastrointestinal absorption of the ergot alkaloids involves the transport either passively or via facilitated/active mechanisms, across the gastrointestinal epithelia. Many toxicants/drugs are absorbed via passive transport across the gastrointestinal epithelia (Eckert et al., 1978). The physicochemical properties of the ergot alkaloids greatly affect the rate and extent of their absorption. Solubility within the digestive tract and the extent of ionization, which determines the partitioning of the alkaloid between water and lipid phases, are important in determining the rate and extent of alkaloid absorption. Most of the ergot alkaloids, as weak bases, are amphipathic (i.e., possessing both polar and nonpolar components). Consequently, the pH of the surrounding environment will affect the water/lipid partitioning of the alkaloids and their subsequent absorption. As the ergopeptide alkaloids are charged at low pH, one may assume that these compounds would not be absorbed in the abomasum (Eckert et al., 1978). Thus, absorption of these compounds would appear to be limited to the small intestine in nonruminants (i.e., duodenum and jejunum; Rothlin, 1933) and the forestomach (Westendorf et al., 1992; Hill et al., 2001) and intestine in ruminants. The rumen is unlike the gastric stomach; the pH is near neutral in forage-fed animals; and a mucosal layer is not necessary to protect the tissue from the digesta (Russell and Rychlik, 2001). This adaptation permits the transport of nutrients across the rumen epithelium. In this way, the rumen has functional features that are more like the intestine than the gastric stomach, thus making it an apparent site of absorption.

Once absorbed across the gastrointestinal epithelium, the ergot alkaloids are transported via the lymphatic system through the thoracic duct and subclavian vein or the mesenteric veins through the portal system and liver to the systemic circulation (Eckert et al., 1978). Although direct in vivo measure of ergot alkaloid absorption across the gastrointestinal epithelia has not been conducted, several lines of indirect evidence supporting absorption after oral exposure do exist, including radiotracer studies (reviewed by Eckert et al., 1978).
across Caco-2 cell monolayers. Shappell and Smith (2005) made using in vitro models. Shappell and Smith (2005) reported the absorption potential for several ergot alkaloids across the forestomach tissues of sheep. When equimolar proportions (30.5 μM) of ergot alkaloids were applied to the mucosal side of the tissues, at least 50% more lysergic acid appeared on the serosal side after 4 h than lysergol, ergonovine, ergotamine, and ergocryptine. Ergot alkaloid absorption occurred across all tissues types tested (ruminal, reticular, and omasal) and appeared to be via active transport as evidenced by reduced alkaloid transport in the presence of sodium azide. Ayers et al. (2009) recently demonstrated transport of lysergic acid across the rumen epithelium in vitro. In addition, they found that ergovoline was not transported across the rumen or the omasum tissues. All these data suggest that, in ruminants as in nonruminant animals, the small intestine may be the most important site for ergopeptine absorption.

Estimates for the flux of ergopeptines have been made using in vitro models. Shappell and Smith (2005) reported that ergovaline (22 μM) was transported across Caco-2 cell monolayers. Shappell and Smith (2005) concluded that the ergovaline transport rate (7.5 ng·min⁻¹·cm⁻²) across the Caco-2 cells compared favorably with the calculated rate (7.0 ng·min⁻¹·cm⁻²) for ergotamine (30.5 μM) across the sheep omasum reported by Hill et al. (2001). Although these data provide theoretical rates of transport for 2 similar ergopeptides (ergovaline and ergotamine) as well as relative absorption potential between lysergic acid and derivatives, it is important to be cautious in drawing direct inferences from data derived from different in vitro models to absorption events within the animal. In addition, physiological differences in species, health, production status, sex, age, nutritional status, or presence or absence of absorption modifiers (e.g., caffeine; Eckert et al., 1978) can affect the rate and extent of alkaloid absorption. Therefore, it is essential that in vivo studies be conducted to confirm in vitro findings. However, measuring ergot alkaloid absorption in vivo has been elusive due to the difficulty in gaining access to the appropriate tissue compartments and the lack of highly sensitive analytical methods. As such, in vivo evidence of ergot alkaloid absorption has relied primarily on analysis of excretory materials coupled to intake estimates to establish the extent of ergot alkaloid absorption in the intact animal.

It has been noted that 96% of the ergopeptine alkaloids consumed by cattle grazing endophyte-infested tall fescue were excreted via the urine (endophyte-infested refers to a population of plants where not all plants are infected, whereas endophyte-infected refers to a single plant or population of plants known to all be infected); very little of that consumed was detected in the bile (Stuedemann et al., 1998). Given that the ELISA (Agrinostics Ltd.) used in this study does not differentiate among ergot alkaloids, it was not possible to determine the structure of the ergopeptines or metabolites excreted via the urine. These findings compared well with those reported earlier by Westendorf et al. (1993), where fecal material of sheep accounted for only 6 to 7% of the total ergot alkaloids consumed. In contrast, a report by Schumann et al. (2009) indicated that ergopeptines analyzed via HPLC were detected in feces of ergot-exposed dairy cows at almost 24% of ergopeptine intake. Breed, physiological status, and dietary differences with attendant differences in rumen pH, digesta flow rates, or differing alkaloid profiles or all 3 may have contributed to the greater fecal recovery of ergopeptine alkaloids than those previously reported by Westendorf et al. (1993) and Stuedemann et al. (1998). Nevertheless, taken together, the findings support extensive absorption of the ergot alkaloids from the gastrointestinal track of the ruminant animal; that does not seem to be the case for nonruminants. Eckert et al. (1978) reviewed the literature and reported that ergotamine tartrate tracked via radiotracers was accounted for in the bile, urine, and feces of monkeys (Macaca mulatta) at 24.1, 7.4, and 68.2% of intake, respectively. The same authors also reported similar values (32.8, 8.6, and 63.9% of intake for bile, urine, and feces, respectively) from the literature in rats. The data indicate that in these 2 nonruminant species, only 31 to 42% of the ergotamine given orally was absorbed vs. the nearly 93 to 96% reported by Westendorf et al. (1993) and Stuedemann et al. (1998) for ruminants.

Total fecal excretion of ergovaline by the horse appears to be intermediate between the amounts reported for laboratory nonruminant species (Eckert et al., 1978) and ruminant species (Westendorf et al., 1993; Stuedemann et al., 1998). Schultz et al. (2006) reported that fecal excretion accounted for only 35 to 40% of the ergovaline consumed, with none accounted for in the urine. In contrast to the data of Stuedemann et al. (1998) and those reviewed by Eckert et al. (1978), the data of Schultz et al. (2006) were obtained from HPLC analysis of feces and urine, thereby providing positive identification of the compounds in the samples. Using only fecal excretion as a marker for absorption, it would appear that at least 60% of the ergovaline consumed by horses was absorbed. Nearly 60 to 65% of the ergovaline consumed apparently was retained or metabolized by the horse. Evidence for biotransformation of ergovaline is evident in the horse when one looks at the excretory lysergic acid concentrations. Schultz et al. (2006) reported that, whereas fecal lysergic acid (a putative breakdown product of ergovaline) concentration was approximately 133% of the total lysergic acid consumed by the horse, the urinary amount was nearly 200% of total intake.

In light of these findings by Schultz et al. (2006), an explanation for the often assumed greater tolerance of...
ruminants to ergot alkaloid exposure may be related to microbial or hepatic metabolism or both. However, the studies reported by Eckert et al. (1978) and Stuedemann et al. (1998) were designed primarily to study the other primary contributor to ergot alkaloid balance in the body, that being metabolism.

**Metabolism**

There is little information available concerning the metabolism of the ergot alkaloids in livestock. For the most part, the biotransformation of the ergot alkaloids in livestock has been assumed to be similar to that reported in laboratory animals and humans. It has been reported that the ergot alkaloids may be biotransformed at several potential sites (Eckert et al., 1978; Maurer and Frick, 1984; Ronca et al., 1996; Mas-Chamberlin et al., 1997) and subject to photolytic and air oxidation, hydration, and epimerization at the C-8 position of the ergoline ring (Garner et al., 1993; Porter, 1994), among other reactions. Biotransformation of the ergot alkaloids has been reported to be mediated primarily by the CYP3A subfamily of cytochrome P450 enzyme systems (Ball et al., 1992). In humans, this subfamily consists of 3 genes: CYP3A4, CYP3A5, and CYP3A7, with the former 2 genes expressed in adults and the latter expressed primarily during fetal life. Whereas CYP3A5 apparently has only a minor role in xenobiotic (e.g., drug, toxicant) metabolism, CYP3A4 is very active in xenobiotic metabolism and is the predominant P450 in human liver and intestine, reportedly comprising up to 60% of total CYP450 (Shimada et al., 1994; McKinnon et al., 1995). It should be noted that CYP3A4 activity varies greatly among individuals and may be driven in part by DNA mutations (Rebbeck et al., 1998), thus potentially accounting for differences in susceptibility to ergot alkaloid intoxication among individuals and species.

Peyronneau et al. (1994) evaluated the interaction between ergopeptines and a series of lysergic acid derivatives on rat and human CYP450. Peyronneau et al. (1994) found that ergopeptines (bromocriptine, ergocryptine, dihydroergotamine) strongly interacted with rat liver microsomes. This was a result of the ergopeptide binding of a protein site close to the heme. In addition, they demonstrated that the tripeptide moiety of ergopeptines was extremely important for their recognition and binding to CYP3A. This was evidenced by the observation that lysergic acid derivatives, without the tripeptide group of ergopeptides, failed to bind with CYP3A. Enzymatic metabolism of ergotamine in tissue microsomes has been shown to be mediated mainly by the CYP3A family and with conversion to more hydrophilic metabolites M1 and M2 (8-hydroxy derivatives; Peyronneau et al., 1994; Moubarak and Rosenkrans, 2000). Further metabolism resulted in a second hydroxylation of M1 and M2 to metabolites M3 and M4 (8, 9-dihydroxy derivatives; Moubarak and Rosenkrans, 2000). Currently, little is known about the biological activities of these metabolites resulting from the biotransformation of ergot alkaloids by CYP3A. However, it has been shown with bromocriptine that metabolites formed through modification of its peptide moiety are able to inhibit prolactin secretion (Valente et al., 1997) and to modulate P-glycoprotein function (Orlowski et al., 1998).

Ruminants present a special case in the study of ergot alkaloid metabolism; unlike other mammals, they have a pregastric fermentation of consumed feed (Hungate, 1975). The fermentation occurs in the rumen, which is colonized by a dense community of phylogenetically diverse microorganisms. The adaptive value of this symbiosis is that the first enzymes that act on the feed are not mammalian, but microbial, and catabolize substrates that are not otherwise catabolized by the host animal (e.g., cellulose). An early hypothesis stated that the rumen was the principal site of alkaloid degradation (Westendorf et al., 1992; Hill et al., 2001). The rumen flora detoxify several mycotoxins, including ochratoxin A, deoxynivalenol, and zearalenone (Kiessling et al., 1984; Fink-Gremmels, 2008). Indeed, the rumen flora degrade exogenous loline alkaloids both in vitro and in vivo (Westendorf et al., 1992), though metabolism of ergot alkaloids has not been shown definitively. However, rumen fermentation does release plant compounds that are not primary metabolites (Sun et al., 2008). Toxicants, such as ergot alkaloids, appear to be among the compounds that are liberated by fermentation (Ayers et al., 2009). When Ayers et al. (2009) incubated endophyte-infested tall fescue in rumen fluid with viable microbes, the concentration of total ergot alkaloids increased over time. These results were similar to those of De Lorme et al. (2007), who performed similar experiments in vivo; in this case, the ruminal concentrations of both ergovaline and lysergic acid increased when sheep were fed endophyte-infested tall fescue, thus increasing the potential for increased absorption and subsequent intoxication.

**Distribution**

Eckert et al. (1978) reported that lysergic acid and its derivatives were found (in descending concentration) in the plasma, lung, liver, kidney, brain, intestines, heart, and fat of cats 90 min after an intravenous administration at 1 mg/kg of BW. For ergopeptines, Eckert et al. (1978) reported that the distribution of ergotamine after intravenous or oral administration (1 mg/kg of BW) in rats was similar to that of lysergic acid in cats, with 1 notable exception. Ergotamine was less in the blood of rats than in all other tissues except for the brain. The limited concentration of ergotamine in the blood may be related to its poor water solubility compared with that of lysergic acid or to the potential for sequestration (bioaccumulation) of ergopeptines in tissues. Evidence for the latter was described in an in
St. Anthony's Fire in livestock

TOXICODYNAMICS

Ergot alkaloid toxicity, whether as a result of consuming ergot-contaminated grains or toxic endophyte-infested tall fescue, results in disruption of several physiological systems (reproduction, growth, cardiovascular) within the body of an animal. The signs (Table 1) of these disruptions vary in severity. For instance, consumption of endophyte-infested tall fescue by mares in their last third of gestation often results in loss of the foal and potentially of the mare if the mare is not treated with domperidone or care is not taken to remove her from the forage for several weeks before the expected due date (as reviewed by Cross et al., 1995). Less dire for the animal, but perhaps more costly to the producer, are the reduced BW gains in grazing livestock (as reviewed by Waller, 2009). Although there is little doubt that the cardiovascular, reproductive, and neural systems of the animal are affected, and there is evidence that the gastrointestinal tract and accessory organs may be affected directly, the mechanisms by which the ergot alkaloids exert the full spectrum of physiological effects in an animal are not fully understood. The following is a discussion (arranged by physiological system) of the current state of knowledge concerning the mechanisms (toxicodynamics) by which the alkaloids may affect the physiological systems of animals, resulting in health or production deficiencies, or both.

Microbial Ecology

Although ruminal fermentation, as discussed earlier, may provide some protection to the ruminant animal through metabolism of the alkaloids by ruminal microbes, it remains that the microbial community itself also may be a target of the ergot alkaloids. For example, it has been established that the loline alkaloids (1-aminopyrrollizidine), which are found in endophyte-infested tall fescue, can inhibit cellulose degradation by ruminal microorganisms (Bush et al., 1976). The impact of the ergot alkaloids on ruminal microbial ecology is less clear. At present, we are unaware of cultivation or molecular ecology studies that have addressed this problem. However, antimicrobial activity may sometimes be inferred by analyzing metabolites of ruminal fermentation. Two well-studied categories of rumen microbial metabolites are gasses and VFA. Pavao-Zuckermann et al. (1999) reported no difference in methane production from steers that were maintained on endophyte-free or -infested tall fescue (i.e., ergot alkaloid-containing). This result is informative because ruminal methanogenesis is dependent on hydrogen production (McAllister et al., 1996). Because methanogenesis was not affected, it can be inferred that there was no net inhibition of either the methanogens or the phylogenetically diverse microorganisms that produce hydrogen. Similarly, when sheep were fed endophyte-infested tall fescue, the concentration of ergovaline did not affect rumen pH (De Lorme et al., 2007).

In contrast to the aforementioned results, Schumann et al. (2008) provides evidence that rumen fermentation was altered by ergotism. When dairy cows were fed a
diet that contained ergot-contaminated rye, the rumen concentrations of propionic acid, isovaleric acid, and ammonia increased. The direct cause of these increases was not clear. However, ammonia and isovalerate are both products of leucine deamination by *Peptostreptococcus anaeorobius* (Chen and Russell, 1988).

The mechanism by which ergot alkaloids affect bacteria is unknown, but the antimicrobial efficacies of dihydroergotamine, ergonovine, and ergotamine on *Escherichia coli* O157:H7 have been demonstrated (Looper et al., 2008). These alkaloids inhibit the growth rate of the pathogen and have an additive effect. *Escherichia coli* is an important foodborne pathogen, but is not considered an ecologically significant species in the rumen. However, there are structural similarities between *E. coli* and predominant rumen bacteria, such as members of genus *Prevotella* (Stevenson and Weimer, 2007). Clearly, the antimicrobial effects of ergot alkaloids cannot be dismissed without further investigation.

**Gastrointestinal System**

Little information exists concerning the toxicodynamics of the ergot alkaloids in the gastrointestinal tracts of foraging livestock. In fact, only 4 reports (Harmon et al., 1991; Rhodes et al., 1991; McLeay and Smith, 2006; Poole et al., 2009) were found concerning ergot alkaloid exposure/intoxication and gastrointestinal function in foraging livestock. Harmon et al. (1991) reported that in steers fed endophyte-infested tall fescue, net portal flux, and portal-arterial concentration differences of acetate were greater than in steers consuming endophyte-free tall fescue. All other metabolites and hormones (other VFA, glucose, lactate, urea N, insulin, glucagon, prolactin) measured were not different. Further, Harmon et al. (1991) reported that blood flow was not affected by treatment. The lack of data concerning ergot alkaloid concentrations in the endophyte-infested tall fescue fed makes it difficult to confirm that these animals were intoxicated. Although prolactin concentrations alone are not conclusive evidence of intoxication, the lack of a prolactin response to the endophyte-infested tall fescue treatment would argue against the animals having been in an intoxicated state. In contrast to Harmon et al. (1991), Rhodes et al. (1991) reported that the blood flows to the duodenum and colon of steers exposed to highly endophyte-infested tall fescue (0.52 mg/kg of ergovaline) were less than those in steers consuming tall fescue of low endophyte status (<0.1 mg/kg). Further, a similar trend was noted in wethers.

McLeay and Smith (2006) reported that intravenous injection of ergotamine (5 to 20 nmol/kg) and ergovaline (0 to 10 nmol/kg) into conscious sheep resulted in inhibition of cyclical contractions in the rumen and reticulum. McLeay and Smith (2006) demonstrated, subsequent to the initial inhibition of cyclical contractions (lasting as long as 14 h), an increase in the basal tone of the reticulum and rumen (lasting as long as 8 h) as a result of alkaloid exposure. Though the effects were delayed in response and somewhat attenuated, ergotamine given intraruminally (400 to 800 nmol/kg) induced similar responses to those reported after intravenous injection of the alkaloids. McLeay and Smith (2006) speculated that the responses to these alkaloids might be the result of interactions with several biogenic amine receptors (dopaminergic, alpha1-adrenergic, serotonergic, acetylcholinergic) via centrally and peripherally located tissues. In a later study, Poole et al. (2009) demonstrated that the activity of ergotamine or ergovaline or both on the reticulum could be antagonized partially in vivo using atropine and in vitro using either atropine or tetrodotoxin. The in vivo and in vitro data taken together were interpreted to indicate that effects of these ergopeptines on reticular motility were at least partially mediated via muscarinic receptors within the mesenteric neurons. Poole et al. (2009) concluded that there were direct effects of these alkaloids on the reticular muscle as well. The data provided clear evidence that the motility of the reticulo-ruminal compartment in forage-livestock is affected. However, the effects on the remaining length of the gastrointestinal tract have not been addressed.

Though not directly shown in forage-livestock, there is evidence that abomasal motility is affected by ergot alkaloid exposure. Frankhuijzen (1975) demonstrated that ergotamine was a partial agonist of serotonergic receptors when tested using contractile response measured in an in vitro preparation of the rat abomasum as a bioassay. Further, the identification of the 5-HT2 (serotonergic) receptor (Komada and Yano, 2007), a known target of the ergot alkaloids in vascular tissue (Dyer, 1993) within the neurons and smooth muscle of the abomasum in rats, provided additional evidence that the ergot alkaloids are likely effectors of gastric motility and function. Studies on the effects of ergot alkaloids on other gastrointestinal tissue functions and motilities do not appear to have been conducted. However, given the similarity in the smooth muscle and neurons of these tissues to those of the abomasum, and the effects of anti-emetics such as domperidone (Cross et al., 1995), it is likely that motility and function of the lower digestive tract would be affected.

Information on the effects of ergot alkaloids on gastrointestinal and accessory organ secretions and transport is essentially nonexistent. However, reduced digestibilities of several nutrients have been reported in livestock consuming endophyte-infested tall fescue, providing clear evidence of ergot alkaloid effects on digestion of forage in livestock. For a review of the literature associated with effects of ergot alkaloids on nutrient digestibility, the reader is referred to Strickland et al. (2009b).

**Pancreas**

Several lines of evidence suggest that the ergot alkaloids may affect pancreatic function directly. Sirek et al. (1977) demonstrated that dihydroergotamine and other...
similar hydrogenated AA alkaloids [dihydroergokryptine (hydergine), dihydroergocornine, dihydroergocristine], were potent amplifiers of sulfonylurea-stimulated insulin secretion. In contrast, alkaloids such as ergotamine, ergonovine, dihydroergonovine, and dihydromoethylergonovine had no amplifying potency. These results suggest that saturation of the double bond at C9 and C10 of the lysergic acid moiety and the presence of an AA chain are essential structural requirements for an ergot alkaloid to amplify the action of sulfonylureas. These results suggest also that ergot alkaloids may affect mechanisms other than α-adrenergic, dopaminergic, and serotonergic receptor interactions. Perhaps they function as regulatory molecules inducing cooperative changes in integral proteins of the plasma membrane of β cells. This postulation is supported by reports of direct interaction between ergot alkaloids and enzyme extracts from various tissues independent of membrane-bound receptor activity (Maurer et al., 1982, 1983; Maurer and Frick, 1984; Mas-Chamberlin et al., 1997; Moubarak and Rosenkrans, 2000; Moubarak et al., 2003). Browning et al. (2000) demonstrated that ergotamine administered (bolus injection; 19 or 20 μg/kg) to cattle resulted in decreased plasma insulin concentrations within 1 h after intravenous administration. Further, Browning et al. (2000) showed that glucagon was increased within 1 h after intravenous ergotamine administration. The exact mechanism by which these effects were elicited is unknown. Several ergot alkaloids are known to interact with dopamine-2 receptors. Further, it has been shown that dopamine-2 receptors may be involved in the effects on insulin regulation (Liang et al., 1998; García-Tornadú et al., 2010), thereby providing one potential mechanism for the alkaloids to affect insulin secretion. In addition, ergot alkaloids are reported to interact as partial agonists/antagonists (Berde, 1980; Weber, 1980) at several serotonin receptor subtypes (Strickland et al., 2009b). Recent evidence has shown that serotonin receptor subtypes are potentially involved in the reversal of insulin secretion inhibition by ginger extracts (Heimes et al., 2009). This finding provides evidence of serotonin receptor involvement in insulin secretion and, thus, another potential mechanism by which the alkaloids may affect insulin secretion.

Although data concerning the effects of ergot alkaloids on digestive secretions by the pancreas are limited, 2 studies of endophyte-infested tall fescue effects on pancreatic function provide evidence that the alkaloids can affect digestive secretions and potentially digestive efficiency. Dew et al. (1989) examined male, weanling Sprague-Dawley rats for the effects of an endophyte-infected seed diet (ergot alkaloid-containing) on the activity of pancreatic hydrolytic enzymes. They found that the activity of protease, trypsin, amylase, and lipase in the pancreas was greater in rats fed endophyte-infected fescue seed than in those fed the control diet. Similar results for greater amylase concentrations in cattle grazing endophyte-infested fescue pastures have been reported by Nutting et al. (1992). The mechanism by which these effects were elicited is not known.

**Neural and Neuroendocrine System**

The autonomic nervous system is affected by lysergic acid that occurs naturally in endophyte-infected tall fescue. Further, cattle consuming endophyte-infested tall fescue have shown large variations in blood concentrations of epinephrine and norepinephrine, which lead to nervous and highly excitable animals (Schmidt et al., 1982). Likewise, Youngblood et al. (2004) found that mares consuming endophyte-infested tall fescue had increased concentrations of urinary ergot alkaloids and depressed endogenous catecholamine activity, suggesting an endocrine disruptive effect of hypothalamic origin. These reported variations in blood catecholamines may be explained partially by the marked affinity that ergot alkaloid derivatives demonstrate for α-adrenoceptors (McPherson and Beart, 1983). However, there is evidence that neuronal dopamine concentrations may also be involved in altered endogenous catecholamine activity. Rowell and Larson (1999) found that whereas ergocryptine, ergocristine, and bromocriptine stimulated the release of dopamine from rat striatal synaptosomes, ergotamine, ergonovine, ergovaline, and ergocornine did not. Similarly, Porter et al. (1990) reported that steers grazing endophyte-infested tall fescue pastures had an impaired balance of dopamine and serotonin in the pituitary and pineal glands, suggesting that adverse effects of ergot alkaloids in endophyte-infested tall fescue on growth, reproduction, and metabolism may be attributed to neurotransmitter disturbances in various regions of the brain.

Although ergotamine has been shown in rats to act as an agonist of both presynaptic dopamine receptors and α₂-adrenoceptors, it functions as an antagonist of the postsynaptic α₁-adrenoceptors (Badia et al., 1988). Evidence of ergot alkaloid interaction with neural serotonin receptors has been provided by Haddjeri et al. (1998). These researchers studied the effects of ergotamine on serotonin-mediated responses in the rodent and human brains. In the rat brain, ergotamine acted as a 5-HT1A receptor agonist and as an agonist of terminal 5-HT autoreceptor. In humans, ergotamine also had some effect on 5-HT1A receptor, but it did not display the same profiles as other 5-HT1A receptor agonists, probably because of lack of receptor selectivity.

Selected ergot alkaloids (ergovaline, α-ergocryptine) from endophyte-infected tall fescue have been shown to function as agonists of rat pituitary D2 dopamine receptors (Strickland et al., 1992, 1994). It has been demonstrated also that administration of domperidone, a dopamine receptor antagonist, was effective in preventing signs of fescue toxicosis in horses without neuroleptic side effects (Redmond et al., 1994). This latter research provides evidence that the ergot alkaloids function similarly at the level of the pituitary gland in...
rats and grazing livestock. Larson et al. (1994) demonstrated that, whereas rat whole brain D2 dopamine receptor density and mRNA were reduced by consumption of endophyte-infected fescue seed, receptor density and mRNA were increased by an injection of a D2 dopamine receptor antagonist. Later, Larson et al. (1995) demonstrated that ergot compounds, especially ergovaline, bind to D2 dopamine receptors and elicit second messenger responses similar to that of dopamine. Larson et al. (1999) also reported that commercially available ergot alkaloids, such as ergotamine tartrate and ergonovine, can simulate effects of extracted ergovaline and ergine, and may be used to examine the responses in receptor binding and the inhibition of cyclic AMP when they are used in the D2 dopamine receptor system. It should be noted that the broad array of targets for the ergot alkaloids and differential effects at the target sites seen between classes of ergot alkaloids are reasons why developing a fully effective treatment for ergotism or fescue toxicosis has been largely unobtainable.

Reproductive System

Evidence of reduced reproductive performance of animals consuming endophyte-infested tall fescue has been measured and reported (Porter and Thompson, 1992; Looper et al., 2009, 2010). However, as with most other systems in the body, the exact mode of action has not been fully determined, but likely involves numerous molecular targets and organs. Further, the extent of this reduction in reproductive performance in ruminants consuming ergot alkaloid-contaminated diets has not been consistent. Modest to severe reductions (7.6 to 39.2% decreases) in pregnancy rates of cows consuming endophyte-infested tall fescue have been reported (Gay et al., 1988; Brown et al., 1992), whereas others failed to observe diminished conception and pregnancy rates in cows (Burke et al., 2001a,b) and heifers (Mahmood et al., 1994; Rorie et al., 1998). Obviously, an interactive relationship exists among several factors (e.g., ambient temperature, genetics, animal age, amount and length of exposure time, and nutritional management) with consumption of ergot alkaloids and reproductive dysfunction in ruminants. Further, there may be direct effects of ergot alkaloids on reproductive tissues, including the ovary, corpus luteum, and embryo at the molecular level, as well as indirect effects on the reproductive system with reduced nutrient intake and subsequent losses in body adipose stores.

The influence of ergot alkaloids on the reproductive system may be due, in part, to altered function of the hypothalamus, and pituitary and pineal glands (Sibley and Creese, 1983; Schillo et al., 1988; Porter et al., 1990). For example, most animals consuming tall fescue-contaminated with ergot alkaloids have reduced prolactin concentrations in sera (Hurley et al., 1980) and pituitary glands (Schillo et al., 1988). Extracts of the tall fescue endophytic fungi inhibit the release of prolactin from pituitary cells in vitro (Sheeler et al., 1985). Normally prolactin secretion is regulated by dopamine acting on a D2-dopamine receptor to inhibit secretion (Lamberts and Macleod, 1990). The ergoline ring structure of the ergot alkaloids contains structural similarities to dopamine, which allow many members of this alkaloid class to bind D2-dopamine receptors (Berde and Stürmer, 1978; Goldstein et al., 1980; Sibley and Creese, 1983) within the anterior pituitary gland, thereby reducing prolactin concentrations (Schillo et al., 1988; Porter and Thompson, 1992). Not only do the alkaloids reduce prolactin secretion via dopaminergic receptor activity, they may also alter prolactin gene transcription (Maurer, 1981). However, the extent to which this mechanism plays in ergot alkaloid-induced reductions in reproductive efficiency has not been fully determined. Regardless of the exact control mechanism for prolactin production, prolactin has been suggested to influence gonadotropin release in sheep (Tortonese et al., 1998) and mares (Gregory et al., 2000). Although the relevance of a direct effect of prolactin on cattle reproduction has not been demonstrated, prolactin receptors have been found in bovine corpora lutea (Pointdexter et al., 1979) and granulosa cells (Lebedeva et al., 2001, 2004). Flores et al. (2008) reported that the diameter of the largest follicle after estrous synchronization was correlated to serum prolactin. These studies suggest that decreased prolactin concentration is involved in reduced reproduction in seasonal breeding animals and possibly to a lesser extent in nonseasonal breeders such as cattle.

Unlike prolactin, progesterone is a hormone necessary for establishment and maintenance of pregnancy in most ungulates. Serum progesterone concentrations have been reported reduced in horses (Monroe et al., 1988), ewes (Burke et al., 2006a), and heifers fed endophyte-infested tall fescue or ergotamine tartrate (Jones et al., 2003; Seals et al., 2005). Mahmood et al. (1994) suggested age of the animal may exacerbate ergot alkaloid effects on progesterone. Weaned heifers had reduced concentrations of progesterone when grazing endophyte-infested tall fescue, whereas yearling heifers were not as sensitive to the effects of ergot alkaloid-exposure (Mahmood et al., 1994). There are several possible mechanisms that may be involved in reduced concentrations of progesterone. Serum cholesterol, a precursor to steroid hormones such as progesterone, was reduced in steers after prolonged consumption of endophyte-infested tall fescue (Oliver et al., 2000; Nihsen et al., 2004). Conversely, cows had increased cholesterol concentrations immediately after an intravenous administration of ergotamine tartrate (Browning, 2003), suggesting an effect of exposure time of ergot alkaloids on certain blood metabolites. Ergot alkaloids stimulate uterine smooth muscle (Saameli, 1978); they could serve as a mechanism responsible for release of the luteolysin PGF<sub>2α</sub>, compromising corpus luteum function (Browning et al., 1999b) and ultimately decreasing progesterone. However, Jones et al. (2003) reported that progesterone concentrations from
luteal tissue extracts were similar in heifers consuming ergot alkaloid-contaminated or ergot alkaloid-free diets. Recognizing that many ergot alkaloids cause vasoconstriction of blood vessels, these authors suggested local vasoconstriction of blood flow to the ovary or corpus luteum, which was likely responsible for inhibiting release of progesterone into the systemic circulation. Doppler ultrasonography (Aiken et al., 2007) lends support to the hypothesis that vascular constriction plays a role in systemic progesterone concentrations. Aiken et al. (2007) demonstrated vasoconstriction of caudal arteries, decreased blood flow, and reduced heart rate in beef heifers and that these physiological responses were rapid, within 4 h of exposure to endophyte-infested tall fescue. Regardless of the mechanism responsible for altered concentrations of progesterone in animals consuming ergot alkaloid-containing diets, decreased progesterone after recognition of pregnancy could lead to abortion.

As with progesterone, limited research exists about the influence of ergot alkaloids on gonadotropin synthesis and release in animals. Preliminary work suggested both LH and FSH were diminished in 3-mo-old heifers when fed ergot alkaloid-contaminated hay (McKenzie and Erickson, 1991). However, changes in LH were not observed in ewes administered ergocornine hydrochloride maleate subcutaneously (Louw et al., 1974) or in heifers fed endophyte-infected tall fescue seed diets for 75 d (Mizinga et al., 1992). In a series of intravenous ergot alkaloid-challenge studies, Browning et al. (1997, 1998a) reported reduced LH release in cattle after ergonovine maleate and ergotamine tartrate exposure. Jones et al. (2003) speculated that ergot alkaloids might bind to dopamine or norepinephrine neurons, or both, that stimulate FSH receptors on the follicle in cattle, as is the case in rodents (Mayerhofer et al., 1997).

Insulin-like growth factor-I along with gonadotropins is important to the growth and differentiation of follicles (Spicer and Echternkamp, 1995). Serum IGF-I was reduced in cows immediately after intravenous administration of ergotamine tartrate (Browning, 2003). Similarly, heifers fed endophyte-infected tall fescue seed diets for 100 d had decreased serum IGF-I, but serum IGF-II and uterine concentrations of IGF-I and IGF-II were not influenced by ergot alkaloid exposure (Rorie et al., 1998). Nutrient restriction uncouples the positive relationship of the GH-IGF axis with increased GH and reduced IGF-I (Armstrong et al., 1993; Bossis et al., 1999). Heifers consuming endophyte-infected tall fescue seed diets in the Rorie et al. (1998) study lost BW, possibly confounding the direct influence of ergot alkaloids with the indirect effects of nutrient restriction. As with gonadotropin-ergot alkaloid interactions, direct effects of prolonged exposure of ergot alkaloids on serum or uterine concentrations (or both) of IGF-I have yet to be elucidated fully and should be included in future studies.

Additionally, exposure to diets containing ergot alkaloids has been shown to decrease the number of large follicles (ovarian) in beef heifers (McKenzie and Erickson, 1991; Burke and Rorie, 2002). However, the diameter of the dominant follicle in beef cattle was decreased only when ambient temperatures were high (Burke et al., 2001b; Burke and Rorie, 2002). Total numbers of follicles (>5 mm), diameters of follicles during specific waves, and sizes of the ovulatory follicles of heifers fed diets containing ergotamine tartrate for 30 d were similar to follicle measurements of heifers fed diets containing no alkaloid (Seals et al., 2005). Similarly, mean number of medium (5 to 7 mm) and large (>8 mm) follicles present on the ovaries at embryo collection of heifers fed endophyte-infected tall fescue seed diets containing ergot alkaloids for 100 d were similar to heifers fed control supplements (Rorie et al., 1998). Consumption of endophyte-infected tall fescue seed diets containing ergot alkaloids did not alter follicle size at ovulation when compared with heifers fed diets containing no alkaloid (Jones et al., 2003). Thus, the available data support a minimal direct effect of ergot alkaloids on follicular development, which may be further exacerbated with increases in ambient temperatures.

Diminished luteal function also has been investigated as a possible mechanism for reduced reproductive performance in ruminants consuming ergot alkaloids. Erratic estrous cycles reported in animals consuming diets with endophyte-infested tall fescue or ergotamine tartrate (Jones et al., 2003; Seals et al., 2005; Burke et al., 2006a) could be a result of altered luteal function. In preliminary research, large luteal cell number and the number of mitochondria within these cells were increased in heifers grazing (56 d) endophyte-infested tall fescue when compared with control heifers (Ahmed et al., 1990) and corpora lutea weights were similar between diets with or without ergot alkaloids. Diameters of corpora lutea at embryo recovery were similar between heifers fed seed diets containing ergot alkaloids for 100 d and heifers fed control supplements (Rorie et al., 1998). Likewise, size and number of corpora lutea were similar between ewes fed tall fescue diets with or without ergot alkaloids (Burke et al., 2006a). Genes associated with apoptosis and cell cycle regulation are reported to be expressed differentially in luteal tissue from heifers consuming endophyte-infected tall fescue seed (Jones et al., 2004). Collectively, there is some evidence for ergot alkaloid influence on gene transcription and damage, but how these may influence the physiology of the corpora lutea remains to be understood. Certainly the potential combined effects of ergot alkaloids on gonadotropin concentrations, IGF, follicular function and morphology, as well as the corpora lutea, provide clues to early pregnancy loss (before 30 d gestation; Rahe et al., 1991; Waller et al., 2001).

Perhaps one of the most important outcomes of ergot alkaloid-induced decreased reproductive efficiency is the profound effect that alkaloids have on DMI and subsequent nutrition (Strickland et al., 2009b). Dry matter intake is usually reduced in ruminants consuming endophyte-infected tall fescue diets (Parish et al., 2007). Additionally, exposure to diets containing ergot alkaloids has been shown to decrease the number of large follicles (ovarian) in beef heifers (McKenzie and Erickson, 1991; Burke and Rorie, 2002). However, the diameter of the dominant follicle in beef cattle was decreased only when ambient temperatures were high (Burke et al., 2001b; Burke and Rorie, 2002). Total numbers of follicles (>5 mm), diameters of follicles during specific waves, and sizes of the ovulatory follicles of heifers fed diets containing ergotamine tartrate for 30 d were similar to follicle measurements of heifers fed diets containing no alkaloid (Seals et al., 2005). Similarly, mean number of medium (5 to 7 mm) and large (>8 mm) follicles present on the ovaries at embryo collection of heifers fed endophyte-infected tall fescue seed diets containing ergot alkaloids for 100 d were similar to heifers fed control supplements (Rorie et al., 1998). Consumption of endophyte-infected tall fescue seed diets containing ergot alkaloids did not alter follicle size at ovulation when compared with heifers fed diets containing no alkaloid (Jones et al., 2003). Thus, the available data support a minimal direct effect of ergot alkaloids on follicular development, which may be further exacerbated with increases in ambient temperatures.

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phytate-infected tall fescue seed diets tended to have reduced motility when compared with bulls consuming a diet free of alkaid during the last 2 wk of a 60-d study. Using an integrated visual optical system, Looper et al. (2009) reported that the sperm of Brahman-influenced bulls consuming endophyte-infested tall fescue had less motility and slower velocities than the sperm of bulls consuming alkaid-free forages, but only during months of high ambient temperature. Collectively, evidence from these studies suggests that exposure to alkaid may compromise sperm cell membranes, thus ultimately reducing fertilization capability.

Cardiovascular System

Two recent reviews (Strickland et al., 2009a,b) are available concerning the effects of endophyte-infested tall fescue and associated alkaid on cardiovascular function. It is not our intent to repeat those reviews. Nevertheless, given the broad implications (e.g., temperature regulation, nutrient transport, and waste elimination) of impaired cardiovascular function by the alkaid, it is important to at least provide the reader a brief overview. There are 2 primary cardiovascular effects for the alkaid in livestock. First, heart rate appears to be reflexively decreased temporarily, after alkaid exposure, with a gradual return to preexposure rates after approximately 172 h (McLean et al., 2002; Aiken et al., 2007). Second, vascular tone and possible alterations in morphology contribute to restricted blood flow to many tissues within the animal (Rhodes et al., 1991; Aiken et al., 2007; Strickland et al., 2009a,b). Vasoconstriction no doubt plays an important role in thermotolerance and heart rates of livestock (Strickland et al., 2009b), as well as in many of the issues with reproduction and other physiological systems addressed above. However, rather than focus on the gross effects resulting from vasoconstriction, this section provides a brief overview of the mechanisms currently thought to account for the vasoconstrictive effects of the alkaid in livestock. This section also highlights research concerning the relative potency and efficacy of alkaid in the livestock vascular system. Understanding the mechanism(s) and the alkaid that are most vasotoxic will allow researchers a better opportunity to develop targeted treatments or prophylactic measures aimed at improving animal tolerance to the alkaid.

Mechanisms. As previously discussed, the alkaid contain structural components in common with endogenous biogenic amines. This structural similarity allows members of the alkaid class to affect cellular activity via biogenic amine receptors (Weber, 1980). Activation of several biogenic amine receptors by alkaid has been shown to induce vasoconstriction in several in vitro livestock vascular models. Dyer (1993) provided some of the first information concerning the interaction of ergovaline (important alkaid in endophyte-infested tall fescue) with serotoninergic receptors. Dyer (1993) reported that ergovaline
induced contraction of bovine uterine and umbilical cord arteries in vitro via 5HT2A serotoninergic receptors but not via α1-adrenergic receptors. Similarly, Schoning et al. (2001) showed that ergotamine and ergovaline stimulated vasoconstriction in a rat tail artery bioassay via the 5HT2A serotoninergic receptors. In contrast, however, they reported that the α2-adrenergic receptor also was involved in the vasoconstriction induced by these 2 alkaloids. Oliver et al. (1993), using both a bovine lateral saphenous vein and dorsal metatarsal artery, demonstrated that ergine induced vasoconstriction, but not via α1-adrenergic receptors. Similarly, Schoning et al. (2001) as discussed above. In fact, Schoning et al. (2001) reported that ergine had both agonist and antagonist effects through the 5HT1B/1D serotoninergic receptor in their bioassay. Although no direct effect on vasoconstriction of ergine was noted to be mediated by α2-adrenergic receptors with bioassays (Oliver et al., 1993), Oliver et al. (1998) later found that α2-adrenergic receptor-induced contractile activity was enhanced in cattle grazing endophyte-infested tall fescue but not in those consuming endophyte-free tall fescue. In complete contrast to these models, ergotamine-induced vasodilatation via 5HT2B serotoninergic receptors has been reported in porcine pulmonary arteries (Ghula and Roos, 1996).

Given numerous reports in the medical literature (not reviewed here) of ergot alkaloid interaction with biogenic amine receptors as predicted by Weber (1980), it is no surprise that these alkaloids also have cardiovascular effects in grazing livestock. However, it remains unclear as to which biogenic amine receptors are important in mediating the response to the ergot alkaloids in differing vascular beds. The interpretation of ergot alkaloid-induced cardiovascular dysfunction is complicated further by vascular congestion and long-term morphological changes in blood vessels resulting from ergot alkaloid exposure (Julien et al., 1974; Williams et al., 1975; Garner and Cornell, 1978) in livestock. All of these researchers reported thickening of the intimal layer of small peripheral blood vessels after exposure to ergot alkaloids as a result of consuming endophyte-infested tall fescue. The congestion is likely from vasoconstriction and thrombosis within the compromised blood vessels. The thickening of the intimal layer would further reduce blood flow as a result of a constant reduction in vascular internal diameter in addition to the vasospasm induced by alkaloid exposure. Several possibilities might explain the thickening of the intimal layer. It may be due to a prolonged vasoconstriction by the alkaloids leading to a cessation of blood flow, similar to that discussed by Lewis and Gelfand (1935) when reviewing ligation effects on blood vessel morphology. Whether this mechanism would result in hypertrophy or hyperplasia is unclear; Strickland et al. (1996) provided support for hyperplasia over hypertrophy as a possible mechanism: quiescent bovine smooth muscle cells (primary culture) were stimulated to grow and replicate as a consequence of exposure to ergonovine, α-ergocryptine, or ergovaline. In contrast, Zhang and Cincotta (1997) reported that bromocriptine (a synthetic ergot alkaloid drug) inhibited the proliferation of growing vascular smooth muscles cells in both rats and humans. Strickland et al. (1996) observed similar responses in growing bovine smooth muscle cells (primary culture) when the cells were incubated with ergovaline. However, both ergonovine and α-ergocryptine appeared to stimulate growing cells. Finally, endothelial cell death (Shappell, 2003) induced by ergot alkaloids may contribute to the vascular pathology and resultant decrease in blood flow. Interpretation of the physiological implications in the animal in these of studies is difficult given the limited understanding of the potency and efficacy of the impact of different ergot alkaloids on various vascular beds. It is clear from the literature that individual ergot alkaloids elicit differing effects because of chemical structure and nutritional level, both of which would influence the tissues and molecular sites of action targeted by the alkaloids.

Vasoconstrictive Potency and Efficacy of Ergot Alkaloids in Livestock. Although potency and efficacy studies are routinely conducted on drugs, the study of ergot alkaloid potency and efficacy in the vasculature system of livestock has not received a great deal of attention. However, a series of studies have been published recently that examined the potency and efficacy of selected ergot alkaloids to induce constriction of the bovine lateral saphenous vein in vitro (Klotz et al., 2006, 2007, 2008, 2010). Ergovaline was shown to be approximately 1,000-fold more potent and 5-fold more efficacious in causing vasoconstriction than lysergic acid (Klotz et al., 2006, 2007). Ergotamine and ergovaline reportedly have very similar contractile dose response curves (Klotz et al., 2007). Other alkaloids such as α-ergocryptine, ergocristine, ergocornine, and ergonovine were intermediate in contractile response to ergovaline and lysergic acid (Klotz et al., 2010). We conclude that individual alkaloids elicit varying responses; thus, broad statements about the ergot alkaloid class in general are not prudent. In fact, as we continue our studies, we find that these alkaloids have differing contractile responses in other tissue beds within the animal.

Known and Potential Regulation of Cellular Transport Mechanisms by Ergopeptide Alkaloids

Compared with the known negative effects of consumed ergot alkaloids on the systemic physiology of cattle, little is known about specific cellular mechanisms affected directly that result in impaired production of animals displaying signs of fescue toxicosis. Mechanistically, the best-characterized ergot alkaloid-cell interactions are the binding of ergot peptides (α-ergocryptine, bromocriptine, ergovaline), lysergic acid amides (ergine and ergonovine), and pyrrolizidines (N-formylyloline and N-acetylloline) to dopamine receptors. These ligand-receptor interactions are thought to be the principal...
mechanism by which ergot alkaloids affect animal performance to mediate their negative effects (Larson et al., 1999). For example, activation of the type-2 dopamine receptor (D2) is thought to mediate many of the whole-animal physiological symptoms observed in tall fescue toxicosis, including intracellular alteration of cardiovascular (Hieble et al., 1990), thermoregulatory (Nuñes et al., 1991; Sanchez and Arn, 1992) and nervous systems (Tomé et al., 2004). Activation of D2 stimulates the inhibitory G protein protein cascade, which inhibits adenylate cyclase activity and, consequently, reduces intracellular cAMP concentrations. Bromocriptine and α-ergocryptine are 2 well-characterized ergopeptine alkaloids (synthetic/model and endogenous, respectively) and are D2 agonists (Sibley and Creese, 1983). Bromocriptine binds D2 with equal affinity to that of α-ergocryptine, 3-fold that of ergovaline, and with 100-fold greater binding affinity for D2 than do the lysergic amides ergine and ergonovine (Larson et al., 1999).

However, recent reports (Settivari et al., 2006, 2008; Brown et al., 2009) indicate other molecular mechanisms that may contribute to the syndrome of ergot alkaloid intoxication such as fescue toxicosis. A survey of the literature suggests that transport proteins, as a class, may be particularly susceptible to the presence of ergot alkaloids. Therefore, to aid in the ongoing quest to identify targets of ergot alkaloids as agents of fescue toxicosis, membrane transport proteins and their activities that are known or hypothesized to be targets of ergot alkaloids are discussed below.

**Vesicular Glutamate Transporters.** Whereas L-glutamate is a well-known primary neurotransmitter of the mammalian nervous system, its role as a metabotropic regulator of many nonneuronal cell types is a developing area of research. Besides the central nervous system, glutamatergic neurons innervate the enteric nervous system, gastrointestinal tract, gastrointestinal smooth muscle and glands (Li et al., 2005), pinealocytes, endocrine cells, and spermatids (Moriyama and Yamamoto, 2004).

Vesicular glutamate transporters (VGLUT) are members of the solute carrier 17 family (SLC17; Reimer and Edwards, 2004). The VGLUT actively concentrate (about 50 mM) neuronal cytosolic glutamate into synaptic vesicles of the presynaptic terminal end of neurons. The driving force for VGLUT activity is the H+ gradient generated by vesicular ATPase. These “loaded” vesicles are then “trafficked” to the synapse and stimulated to release glutamate into the synaptic cleft (Moriyama and Yamamoto, 2004). The released glutamate then binds to glutamate receptors of postsynaptic neurons, thus propagating glutamatergic neurotransmission. When present on the plasma membrane of postsynaptic neurons, VGLUT act to cotransport inorganic phosphate and Na+ into the cytosol in exchange for glutamate, thus stimulating phosphate-dependent glutaminase to generate more cytosolic glutamate for uptake into synaptic vesicles by VGLUT (Takamori, 2006).

The potential effect of selected ergot alkaloids on VGLUT activity in rat synaptic vesicles has been evaluated (Carlson et al., 1989). Vesicular glutamate transporters-mediated glutamate uptake was inhibited (IC50; concentration producing 50% of maximal inhibition) substantially by naturally occurring ergot alkaloids containing a peptide bond (ergotamine, 30 μM; α-ergocryptine, 57 μM; dihydroergocryptine, 73 μM; ergocristine 83 μM). In contrast, alkaloids lacking a peptide moiety (lergotrile, >500 μM; ergonovine, 1 mM) were poor inhibitors of VGLUT activity. The greatest ability to inhibit VGLUT activity was exhibited by bromocriptine (IC50 = 22 μM), a synthetic and model ergopeptine. Moreover, kinetic analyses suggested that bromocriptine competitively inhibited VGLUT glutamate uptake. More recent research suggests that bromocriptine may act to increase transmembrane H+ permeability, thus dissipating the high out-to-in transmembrane H+ gradient required for VGLUT function in synaptic vesicles (Shigeti et al., 2004).

Collectively, these data suggest that ergopeptines may inhibit VGLUT activity, causing a deleterious effect on the normal physiological function of tissues that express VGLUT. Currently, it is known that all 3 VGLUT isoforms (VGLUT1, 2, 3) are expressed by brain tissues (cerebral cortex, cerebral white matter, cerebellum, and pituitary and pineal glands). In the brain-gut axis, VGLUT2 is expressed in the enteric neurons (submucosal and myenteric plexus), nodose, dorsal root ganglia neurons to support afferent signaling from the mucosa to the enteric plexuses and brain (Tong et al., 2001). Outside of the central nervous system tissues, all or at least 1 of the 3 VGLUT isoforms are known to be expressed by bone, skeletal muscle, liver, kidney, pancreas (α-cell, β-cell), or L-cells of small intestinal epithelia (Boulland et al., 2004) whereas nonneuronal gonadotrophs and thryrotrhops of the anterior pituitary gland express only VGLUT2 (Hrabovszky and Liposits, 2008). The expression of nonneuronal VGLUT is thought to support either maintenance of glutamine/glutamate cycles (liver or kidney; Welbourne and Matthews, 1999; Boulland et al., 2004), autocrine/paracrine regulation of Ca2+-dependent signaling of GnRH (Hrabovszky and Liposits, 2008), metabolic regulation of pancreatic glucagon secretion by α-cell glutamate release (Moriyama and Omote, 2008), and osteoclast regulation of bone formation and resorption by VGLUT-mediated release of glutamate and subsequent initiation of metabotropic glutamate receptor type 8-mediated inhibitory cAMP cascades (Morimoto et al., 2006; Moriyama and Omote, 2008). Collectively, these data support the concept that the toxicity from consuming endophyte-infested tall fescue includes alteration of neuronal and nonneuronal glutamatergic signaling by inhibition of VGLUT transport activity.

**Na+/K+ ATPase.** In a manner similar to the peptide bond moiety-dependence for inhibition of synaptic vesicle VGLUT activity, ergovaline, but not α-ergocryptine, lysergic acid, or lysergol-noncompeti-
tively inhibited Na\(^+/K^+\) ATPase activity in homogenates of rat forebrain (Moubarak et al., 1990, 1993a,b). Unlike VGLUT sensitivity, ergonovine also noncompetitively inhibited Na\(^+/K^+\) ATPase activity (Moubarak et al., 1993a,b). These reports suggest that ergonovine and ergovaline have the ability to affect tissue-wide inhibitory effects through their ability to reduce resting membrane potential in brain tissue by inhibiting Na\(^+/K^+\) ATPase activity. Obviously, if this effect is widespread, then all cells may be potentially affected by a generalized reduction of \(\Delta\psi\) membrane potential.

**Anionic AA Transporters.** System X\(_{\text{AG}}\) activity is responsible for the high-affinity, concentrative uptake of glutamate and of D- and L-aspartate, and is mediated by 5 known mammalian isoforms that are all members of the SLC1 transport family (GLAST1, EAAC1, GLT-1, EAAT4, EAAT5; Kanai and Hediger, 2004). Functional expression studies of human GLAST1 indicate that GLAST1-mediated uptake of glutamate is upregulated 41% by 100 \(\mu\)M extracellular bromocriptine (Yamashita et al., 1998) and is not dependent on D1- or D2-mediated events. This dopamine receptor-independent result was surprising because bromocriptine and \(\alpha\)-ergocryptine are known to reduce intracellular cAMP concentrations by binding to D2 (Sibley and Creese, 1983). In contrast, neuronal cell EAAC1 activity is thought to be stimulated by increased cAMP concentrations, although not in C6 glioma cells (Dowd and Robinson, 1996), whereas activation of the cAMP pathway induces a 20-fold increase in GLT-1 mRNA abundance of astrocytes (Schlag et al., 1998). Although GLAST1 has been detected only in the pancreas, EAAC1 and GLT-1 expression by sheep and cattle tissues is widespread (Howell et al., 2001). These data are consistent with the concept that expression and function of system X\(_{\text{AG}}\) activities in many ruminant tissues may be sensitive to regulation by ergot alkaloids (Miles et al., 2005) through dopamine receptor-dependent or -independent mechanisms in a cAMP-dependent or -independent manner.

**CURRENT MANAGEMENT APPROACHES AND FUTURE RESEARCH QUESTIONS**

Animal profitability and performance when consuming ergot alkaloid-contaminated forage or grain can be maximized by managing 2 primary factors, alkaloid intake and nutritional value of the feedstuff. During the last few decades, plant scientists have validated several methods of managing tall fescue for plant persistence and alkaloid load. Those methods include clipping seedheads before grazing, stockpiling forage for winter grazing, increasing the biodiversity of plants in a pasture, and developing new cultivars or identification of unique endophyte strains or both that synthesize alkaloids that are less toxic to livestock (for review see Roberts and Andrae, 2004).

Animal scientists have developed animal management regimens that prevent or alleviate part or all of the signs associated with ergot alkaloid intoxication. An approach that which may be satisfactory is to feed a protein or energy supplement to livestock grazing endophyte-infested tall fescue, which dilutes alkaloid consumption and may maintain a more desirable animal body condition (Forster et al., 1993; Richards et al., 2006). This approach is similar to diluting ergot alkaloid-contaminated grain with clean grain to dilute the ergot alkaloids below a given threshold. Identification of animals with less susceptibility to stress from environmental toxins such as ergot alkaloids has proven useful in some regions. In particular, Brahman-cross cattle have performed better than British cattle breeds when grazing endophyte-infested tall fescue (Brown et al., 2000; Burke et al., 2010). Nonetheless, there are lines of cattle within the British and continental breeds that appear to have tolerance to ergot alkaloids. A variety of nutritional supplements, including minerals and yeast products, have been evaluated for efficacy in animals, with mixed results both within and across species (Brazle and Coffey, 1991; Aiken et al., 2001; Coffey et al., 2001; Norman et al., 2010). Pharmaceutical evaluations have included steroid implants (Aiken et al., 2006), GH injections (Flores et al., 2008), anthelmintics such as ivermectin (Rosenkrans et al., 2001; Looper et al., 2006), and dopamine antagonists like domperidone (Redmond et al., 1994; Jones et al., 2003) and metoclopramide (Lipham et al., 1989). As indicated in this review, the bioaccumulation of ergot alkaloids has multifaceted impacts on animal physiology; therefore, it is unlikely that a single approach will resolve all toxicity issues.

Future research undoubtedly will use an array of technologies to answer the multitude of questions that remain unanswered regarding ergotism, animal well-being, and animal productivity: 1) what gene products are involved with animal metabolism and detoxification of ergot alkaloids, and are those gene families useful for selecting breeding stock, 2) which of the many ergot alkaloid molecules are toxic, and 3) what is the best predictor of animal toxicity: total alkaloids, ergopeptines, lysergic acid derivatives, ergoline alkaloids, or metabolites of these alkaloids? Considerable effort continues to be devoted to analytical methods that extend the detection limits for alkaloids in body fluids and tissues (blood, fat, milk, seminal fluids, and follicular and uterine fluid). As those detection methods develop, investigations will further elucidate the mechanisms involved with animal immunity vs. adaptation to ergot alkaloid poisoning. Further, investigators will be able to more precisely describe recovery. Which are the indicators that best identify animals that have recovered from tall fescue toxicosis: normal serum concentrations of prolactin, no alkaloids in the urine or fat depots, or normal blood vessel function?

These questions are not meant to be all inclusive, but are representative of possible future research directions.
As we couple the continually developing “-omics” with observations of animal behavior and physiology, we are confident that new management practices will be developed that will lead eventually to greater animal health and profitability.

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