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ADAPTATION OF LAMBS TO AN ENDOPHYTE INFECTED TALL FESCUE SEED DIET

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ADAPTATION OF LAMBS TO AN ENDOPHYTE INFECTED TALL FESCUE SEED DIET

THESIS

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science in the
College of Agriculture
at the University of Kentucky

By

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Lexington, Kentucky

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ABSTRACT OF THESIS

ADAPTATION OF LAMBS TO AN ENDOPHYTE INFECTED TALL FESCUE SEED DIET

Ten wether lambs were used to determine the effects of ergovaline consumption from endophyte infected tall fescue, on nutrient utilization and metabolism. Lambs were fed a diet of 23% endophyte free tall fescue seed (E-) and 77% concentrate from d -14 to -1 (adaptation phase). On d 0, five lambs were switched to an endophyte infected seed diet (E+) where they remained through d 14. Nutrient digestibilities tended to increase from adaptation through the acute (d 1 to 4) to subacute (d 10 to 14) phases when E- was fed. E+ digestibilities were highest ($P < 0.05$) in the acute phase. Lambs fed E+ had higher rectal temperatures in the acute ($P < 0.01$) and subacute phases ($P < 0.05$). Fecal recovery of ergovaline increased as day of collection increased in the acute and subacute phases. Lysergic acid fecal recovery increased with day of collection in the acute phase, but no effect was found in the subacute phase. Serum enzyme analyses did not indicate tissue damage from alkaloid consumption. These results demonstrate lambs try to adapt to endophyte infected fescue seed consumption through increased nutrient digestibilities and increased ergovaline and lysergic acid excretion.

KEYWORDS: Lambs, endophyte, fescue, ergovaline, lysergic acid

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December 8, 2011

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Table of Contents

List of Tables

List of Figures

Chapter 1. **Introduction**

Tall fescue is the primary grass species used for forage of grazing livestock in the transition zone of the southern United States. However, the presence of an endophytic fungus in tall fescue causes a syndrome known as “fescue toxicosis” with symptoms such as increased respiration rates, higher body temperatures, lowered feed intakes, and reduced weight gains. There has been considerable debate as to the cause of this toxicosis. Currently, the alkaloids ergovaline and lysergic acid are the primary suspects. Several researchers have attempted to delineate routes of excretion of these alkaloids so, in turn, any successful alleviation of toxicosis could be indicated by increased excretion. Most of the researchers believe the primary excretion route of ergovaline is the feces. Other research has studied blood serum enzyme concentrations, including alkaline phosphatase, creatine kinase, aspartate aminotransferase, and gamma glutamyltransferase, as indicators of toxicity. Therefore, increased recovery of ergovaline and lysergic acid in the feces and lower levels of serum enzymes may indicate some degree of toxicity alleviation.

The primary objective of this study was to determine the extent of ergovaline and lysergic acid excretion by lambs fed endophyte infected tall fescue seed. A secondary objective was to evaluate the potential of lambs to adapt to the consumption of endophyte infected tall fescue seed over a 14 d period.

Chapter 2. **Literature Review**

Tall fescue (*Lolium arundinaceum* [Schreb.] Darbysh., formerly *Festuca arundinacea* Schreb.) is a perennial, cool-season grass commonly used for forage and turf purposes. Approximately 33 million acres of tall fescue are grown in the United States, with close to 50% of that existing east of the Mississippi river. States such as Kentucky, Tennessee, West Virginia, and North Carolina use tall fescue as their primary grass species for forage and turf production (Sleper and Buckner, 1995). Approximately 5.5 million acres are grown in Kentucky alone. However, much of the fescue in the U.S. is infected with an endophytic fungus *Neotyphodium coenophialum* found between cells of the plant (Bacon et al., 1977). Unfortunately, grazing animals consuming common (wild-type) endophyte-infected tall fescue suffer from several disorders collectively known as tall fescue toxicosis as a result of alkaloids produced by the fungus.

Tall fescue is a native of Europe and was most likely introduced to North America as a contaminant in meadow fescue seed imported from England (Hoveland, 2009). It was widely planted in the 1940s and 1950s across the southern United States where ready establishment of a cool season perennial grass had been unsuccessful in the past. Tall fescue was recognized for its dependability and adaptability to a wide range of soils and was planted for forage, roadside cover, and turf (Hoveland, 2009). In 1973, researchers found plant samples, taken from a pasture where grazing cattle were exhibiting signs of toxicosis, were infected with the fungal endophyte *Epichloe typhina* [renamed *Acremonium coenophialum* Morgan-Jones and Gams and later *Neotyphodium coenophialum* (Morgan-Jones and Gams) Glenn, Bacon, and Hanlin] (Hoveland, 2009). These findings were supported with grazing studies conducted in Alabama over multiple years. Steers grazing paddocks with 18% infestation had 51% higher average daily gains than steers grazing paddocks with 80% infestation (Hoveland et al., 1980). Another study by Hoveland et al. (1983) found steers grazing 5% endophyte infected fescue had 66% greater average daily gains and 28% greater gain per acre than those grazing paddocks with 94% infestation. Steers grazing the paddocks with higher infestation also exhibited typical toxicosis signs such as elevated body temperatures, rough haircoats, excess salivation, and nervousness.

Tall fescue and the endophytic fungus have a symbiotic relationship where the plant provides a means of nourishment and growth for the fungus and the fungus protects the plant from environmental stresses (Joost, 1995). Hill et al. (1990) determined that plants infected with the endophyte were larger and generally more competitive in mixtures than non-infected plants. Endophyte infection has also been shown to increase germination rates (Pinkerton et al., 1990) and improve tiller density and whole plant survival during drought stress (West et al., 1991). Furthermore, endophyte infected plants are more resistant to root feeding nematodes and many plant diseases (Pedersen et al., 1988; Welty et al, 1991).

Effects of Fescue Toxicosis

Cattle grazing tall fescue have traditionally been afflicted with three disorders: fescue foot, fat necrosis, and/or summer syndrome (Waller, 2009). Fescue foot traditionally occurs in cattle grazing endophyte infected pastures during the winter and is evidenced by lameness, swelling/tenderness around the fetlock and hoof region, dry gangrene of tips of ears and tails, and loss of tail switch. Severe cases may result in the sloughing of hooves. These symptoms are a result of the effect of the ergot alkaloids on the blood vessels causing vasoconstriction and loss of blood flow (Strickland et al., 1993). Fat necrosis occurs when hard fat accumulates along the intestinal tract of cattle, constricting internal organs, and causes difficulty with digestion and reproduction. Signs of summer syndrome include elevated respiration rate and body temperature, rough haircoat, intolerance to heat, lowered feed intake, poor weight gain, reduced serum prolactin, reduced milk production, and low conception rate and is most severe during warmer weather, hence the name (Waller, 2009; Strickland et al., 1993). Summer syndrome is very costly and the effects of this syndrome are those typically referred to as tall fescue toxicosis.

Intake, Growth, and Nutrition

Hoveland (1993) estimated that slow weight gains and reproductive inefficiency of cattle grazing fescue costs the US beef industry over $600 million every year in 1993. Strickland et al. (2011) estimate current losses greater than $1 billion annually when accounting for the impact on equine and small ruminant industries. The presence of the endophyte in tall fescue generally has a negative effect on intake of grazing animals. Cattle grazing endophyte infected tall fescue had dry matter intakes 24 to 44% lower than those grazing endophyte free fescue (Stuedemann et al., 1989). The decrease in grazing times is attributed to the lowered heat tolerance of affected animals. Animals grazing endophyte infected fescue experience higher internal body temperatures that contribute to vasoconstriction and subsequently reduced blood flow to peripheral tissues. As a consequence, animals spend more time in the shade during the heat of the day, with a subsequent reduction in grazing time, than animals grazing endophyte free fescue (Seman et al., 1990). Stuedemann et al. (1985) found steers consuming endophyte infected tall fescue spent only 4 to 6% of their time grazing from 1200 to 1800 hours compared with 60% of that time for steers grazing endophyte-free fescue. Consumption of endophyte infected tall fescue hay and seed decreases feed intakes, increases rectal temperatures and respiration rates, and increases weight losses of steers (Schmidt et al., 1982; Jackson et al., 1984). In a review by Paterson et al. (1995) summarizing 11 trials conducted across 10 states, gains were 30 to 100% lower for bovines consuming endophyte infected fescue versus endophyte free tall fescue. Aiken et al. (1993) reported that yearling geldings grazing endophyte infected fescue had lower average daily gains than those grazing forage with low endophyte levels. Parish et al. (2003) and Emile et al. (2000) reported lambs grazing endophyte infected tall fescue had lower average daily gains and reduced prolactin levels compared with lambs on endophyte free tall fescue or fescue with a novel (non-alkaloid producing) endophyte.

Many of the growth effects of tall fescue toxicosis may be related to the reduced feed intake and/or digestibility that occurs when endophyte infected tall fescue is consumed. These factors would affect the nutrients available for growth and maintenance (Strickland et al., 2009). A study conducted by Panaccione et al. (2006) evaluated feed preferences of rabbits using genetically modified endophytes (*Neotyphodium* spp. related to wild-type in tall fescue) in perennial ryegrass. Rabbits preferred novel endophytes (endophytes that do not produce toxic alkaloids) over endophyte free perennial ryegrass, both of which are negative for ergot alkaloids. Also, the rabbits' preference for endophyte perennial ryegrass was equivalent to the endophyte free perennial ryegrass with clavine alkaloids (precursors to ergovaline). However, endophyte infected ryegrass with ergovaline had a negative effect on intake levels. These results suggest that ergopeptines, such as ergovaline, may be linked to depressed feed intakes of animals grazing endophyte infected tall fescue.

Several studies have controlled intake to eliminate the effects of reduced feed intakes on digestibility in animals consuming endophyte infected tall fescue. Hannah et al. (1990) compared 0, 1.5, and 3.0 ppm of ergovaline fed to lambs by substituting 0, 25, or 50% ergovaline infected tall fescue seed for noninfected tall fescue seed. Ruminal and total tract organic matter (OM), neutral detergent fiber (NDF), and cellulose digestibilities were decreased with increased ergovaline in the diet. Fiorito et al. (1991) used rumen cannulated lambs to balance intake between treatment groups. They found lambs consuming highly endophyte infected tall fescue hay (>95% infected) had lower total tract digestibilities of dry matter (DM), neutral detergent fiber (NDF), and acid detergent fiber (ADF) than lambs consuming low endophyte infected (<1% infected) hay when nutrient intake was equivalent for both treatment groups. Lambs on the low infected hay also had lower fecal N excretion and thus greater N retention and higher serum prolactin levels. Similarly, Westendorf et al. (1993) reported lambs fed a diet containing endophyte infected tall fescue had depressed total tract DM digestibility, ruminal and total tract ADF and crude protein (CP) digestibilities, and ruminal NDF digestibility.

Thermal Response

Fescue toxicosis is most often observed during the summer months when higher environmental temperatures exacerbate the symptoms of the toxicosis (Hemken et al., 1984; Bacon et al., 1986). Peters et al. (1992) reported similar organic matter intakes (2.6% body weight) for cows grazing endophyte infected tall fescue and those cows grazing endophyte free tall fescue or orchardgrass during June. However, during August, when environmental temperatures were higher, the cows on endophyte infected pastures consumed less (1.6% body weight) than those consuming either endophyte free or orchardgrass pastures (2.0% body weight). Even when intakes were similar in June, cows grazing endophyte infected tall fescue lost weight while those grazing endophyte free tall fescue gained weight. Hemken et al. (1981) reported calves fed a more toxic strain of tall fescue had consistently lower DM intakes, higher respiration rates, higher rectal temperatures, and lower weight gains when the ambient temperature was above 31°C. Hannah et al. (1990) reported lambs fed 1.5 ppm ergovaline had higher rectal temperatures (vs 0 ppm) at both 27 and 34°C. Aldrich et al. (1993a) fed ruminally cannulated wethers an endophyte free diet or an endophyte infected diet containing 1.17 ppm ergovaline when the environment was held at 32°C and 60% humidity. Voluntary DM intake, DM digestibility, and intake of digestible DM was less for the endophyte infected diet.

Several studies have examined the effect of specific ergot alkaloids in relation to changes in thermal status. When Holsteins were injected with ergotamine tartrate at an air temperature of 18.5°C, tail skin temperature dropped 8° indicating reduced blood flow (Carr and Jacobson, 1969). Reduced skin temperature and increased rectal temperatures were also noted when an 80% ethanol extract of endophyte infected fescue was administered either orally or intraperitoneally. These data suggest reduced skin blood flow can decrease heat loss and lead to hyperthermia, a common sign of fescue toxicosis, during heat stress. Likewise, during cold weather, the reduced blood flow to appendages can result in less nutrient flow and, thus, tissue necrosis. Al-Haidary et al. (1995) found cattle maintained in a 31°C environment and injected with ergovaline for 3 days had increased core body temperatures and respiration rates and lowered skin temperatures in the back and hip. McCollough et al. (1994) intravenously injected calves with ergotamine, ergine, and ergovaline. Those receiving ergovaline had the greatest rate and magnitude of reduction in tail skin temperature. In a study with adult rats (Spiers et al., 1995), those maintained at 31 to 33°C and injected with ergovaline experienced hyperthermia, preceded by a reduction in tail skin temperature with no shift in heat production. However, rats held in a cold environment of 7 to 9°C experienced hypothermia, a result of reduced heat production.

Animals fed endophyte infected tall fescue under controlled conditions have also demonstrated shifts in thermal status. Rhodes et al. (1991) reported steers fed a diet containing endophyte infected fescue (0.52 ppm ergovaline) had reduced vascular flow rates to the skin over the ribs (measured using radio labeled microspheres). Gadberry et al. (2003) fed lambs a diet of 10% endophyte infected tall fescue seed (0.640 ppm ergovaline) for 14 days in a heat stressed environment and showed evidence for reduced peripheral heat loss, reduced feed intake, and lower average daily gains. Steers fed endophyte infected tall fescue (0.285 ppm ergovaline) for 20 days at 32°C experienced hyperthermia thought to be the result of a reduction in skin vaporization (Aldrich et al., 1993). Hyperthermia, due to a reduction in heat loss, is considered to be a result of increased peripheral vasoconstriction from alkaloids in tall fescue.

Reproduction

In studies of cow-calf operations, cows grazing endophyte infected tall fescue had lower calf birth weights and both cows and calves on endophyte infected pastures had reduced serum prolactin levels (Watson et al., 2004). Waller et al. (2001) had previously reported first-calf heifers on endophyte infected pastures had lower calf birth weights than heifers grazing endophyte free pastures. The lower calf weights were attributed to reduced dry matter intake commonly associated with fescue toxicosis or reduced uterine blood flow in animals consuming ergot alkaloids present in endophyte infected tall fescue (Porter and Thompson, 1992). Lactating dairy cows fed endophyte infected tall fescue soilage had lower milk yields (Strahan et al., 1987) and Peters et al. (1992) reported a 25% decrease in milk production for cows grazing endophyte infected tall fescue as compared with those grazing endophyte free tall fescue. Decreased calf weaning weights have also been implicated as a result of decreased rates of milk production in cows grazing endophyte infected tall fescue (Ashley et al., 1987; Keltner et al., 1989). Additionally, cows grazing endophyte infected tall fescue typically have lower conception rates than those grazing endophyte free fescue, perhaps indirectly due to increased loss of body weight and condition. Schmidt et al. (1986) reported pregnancy rates of 55% for cows grazing endophyte infected fescue compared with 96% for those on endophyte free tall fescue.

Alkaloids in Tall Fescue

A general summary of definitions for alkaloids defines all alkaloids as naturally occurring, organic, N-containing bases, most often produced by plants (Bush and Fannin, 2009). Numerous alkaloids have been identified in tall fescue, including perloline, peramine, ergot, and loline alkaloids. Perloline is part of a group of alkaloids that were first identified in tall fescue. Perloline is part of the diazaphenanthrene alkaloids produced by the plant. Early studies focused on in vitro effects of perloline and how it pertained to fescue toxicosis. However, no physiological links were established in accordance with the signs of fescue toxicosis (Bush and Fannin, 2009). Peramine, ergot, and loline alkaloids are all associated with the symbiotic relationship between the plant and the endophyte (Bush and Fannin, 2009).

Peramine

Peramine is an insect feeding deterrent and is the only known pyrrolopyrazine alkaloid in endophyte infected tall fescue (Bush and Fannin, 2009). However, a significant role of peramine in tall fescue has not been demonstrated due to the insignificant concentrations typically present plus its inactivity in mammalian bioassays (Bush and Fannin, 2009).

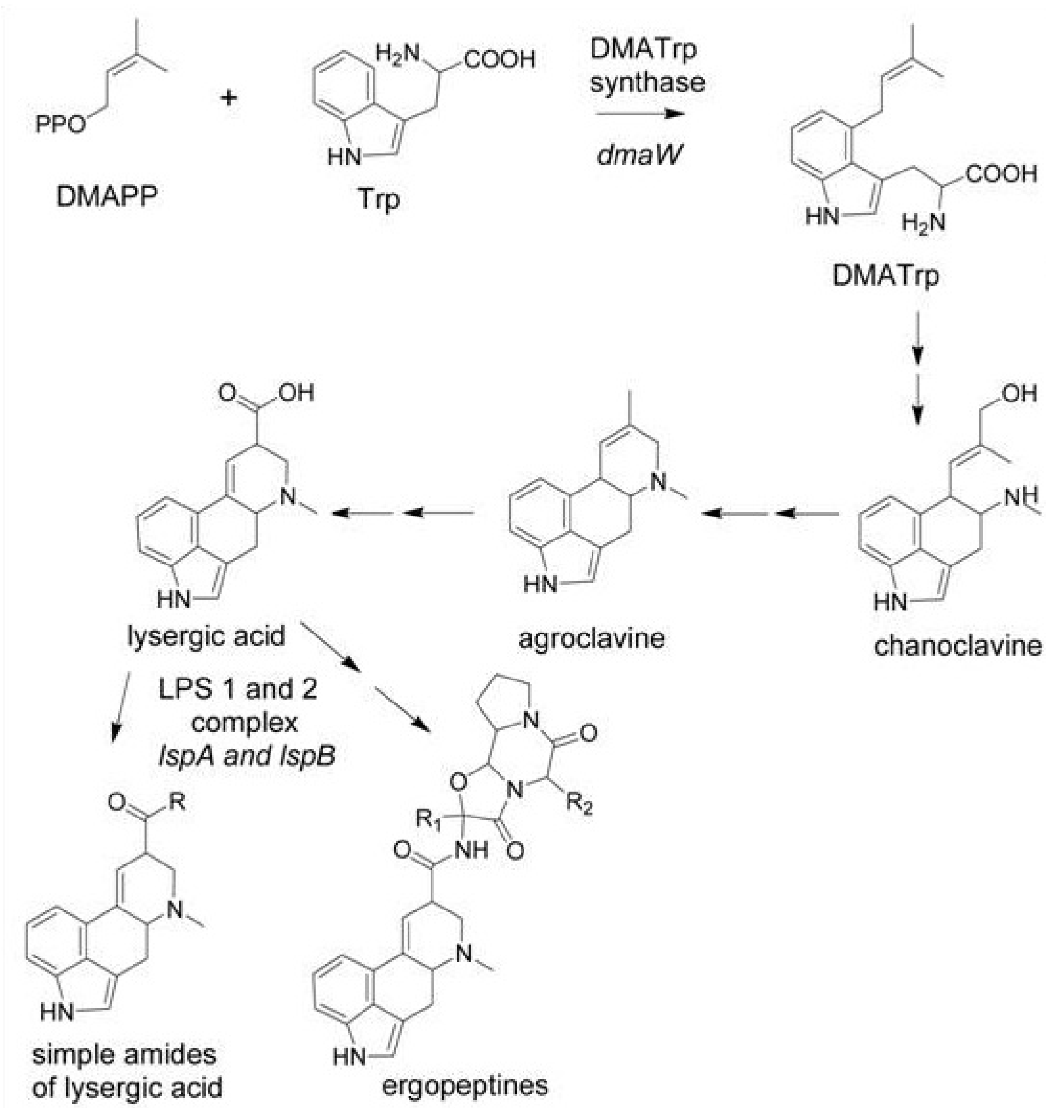
Pyrrolizidine Alkaloids

The pyrrolizidine (loline) alkaloids, also known as lolines, associated with the tall fescue-endophyte relationship are saturated in the pyrrolizidine rings (Bush and Fannin, 2009). Many pyrrolizidine alkaloids of plant origin are significant toxins, being hepatotoxic and carcinogenic, and are unsaturated between carbons 1 and 2. In contrast, the pyrrolizidine alkaloids found in endophyte infected tall fescue are saturated 1-amino alkaloids and contain an oxygen bridge between carbons 2 and 7 of the A and B rings (Cheeke, 1998; Bush et al., 1993; Bush and Fannin, 2009). These loline alkaloids occur in much greater quantities than others found in endophyte infected tall fescue with levels exceeding 10 g/kg (Bush and Fannin, 2009). *N*-acetylloline (NAL) and *N*-formylloline (NFL) are the primary lolines produced by the common endophyte strain found in Kentucky (KY) 31 tall fescue. Also found are loline, *N*-formylnorloline, norloline, *N*-acetylnorloline, and *N*-methylloline (Blankenship et al., 2005). Lolines are broad-spectrum insect toxins, but with much less biological activity in vertebrates (Siegel and Bush, 1996). Concentrations of loline alkaloids in the flowering plant are found from greatest to least in the spikelet, rachis, stem, leaf sheath, and leaf blade with mature seed containing greater amounts of NAL and NFL (Burhan, 1984; Bush et al., 1982). Loline accumulation is greatest in the late summer, typically July and August, for both the northern and southern portions of the tall fescue belt. Loline accumulation is correlated with the heat and drought stress generally occurring in the late summer, which is also when ergot alkaloid accumulation is lowest (Bush and Fannin, 2009).

Ergot Alkaloids

The ergot alkaloids produced by *N. coenophialum* are strongly suspected as the underlying cause of fescue toxicosis due to the similarity of symptoms to classic ergotism in humans. The ergot alkaloids in tall fescue are related by an ergoline ring system or a biosynthetic precursor thereof and include clavines, lysergic acid and derivatives, and the ergopeptide and ergopeptine alkaloids (Bush and Fannin, 2009) (Figures 2.1 and 2.2). Five ergopeptine alkaloids – ergovaline, ergosine, ergonine, ergoptine, and ergocornine – were found in pasture samples of E+ fescue with ergovaline accounting for 84 to 97% of the total (Lyons et al., 1986). Ergovaline has been the focus of much research related to fescue toxicosis due to its vasoconstrictive properties. Ergotamine has also been found in endophyte infected tall fescue, but in smaller amounts (Yates et al., 1985; Porter, 1995). However, ergotamine presence may be a result of contamination from Claviceps purpurea, the cause of ergotism from cereal grain (L. P. Bush, 2011, personal communication). Rottinghaus et al. (1991) reported the concentration from greatest to least for ergovaline is generally seed, crown, stems, leaves, and roots with levels almost 5.0 ppm ergovaline in seedheads compared to less than 0.5 ppm in the stems and leaves. In northern portions of the tall fescue belt, including Kentucky, ergovaline concentrations are low during the winter, then start to increase during the spring growing season until peak levels are reached in mid-May near the time of seed fill (Bush and Fannin, 2009). Ergovaline concentrations then drop off during the summer followed by a second peak in the fall. In the southern portions of the fescue belt, due to the high summer temperatures, fescue mainly grows from March to June with peak concentrations in May (Bush and Fannin, 2009). Ergovaline content in the plant tissue may be affected by several factors including fertilization, water stress, and growth temperature. Lyons et al. (1986) found nitrogen fertilization increased ergovaline in the leaf sheath and blade of greenhouse grown plants by more than 500% compared with unfertilized plants. Rottinghaus et al. (1991) reported increased ergovaline concentrations of 88, 103, and 66% in leaf blades, stems, and seedheads, respectively, when nitrogen fertilization was increased from 0 to 135 kg/ha. Other environmental factors may be confounded with each other. For example, the combined effects of drought stress and high summer temperatures on pastures with low levels of nitrogen resulting in lowered ergovaline concentrations during the hottest summer months, which follows the seasonal distribution pattern of ergovaline (Bush and Fannin, 2009).

Figure 2.1 A generalized scheme for ergot alkaloid biosynthesis in tall fescue/*Neotyphodium coenophialum* symbiotum[1]

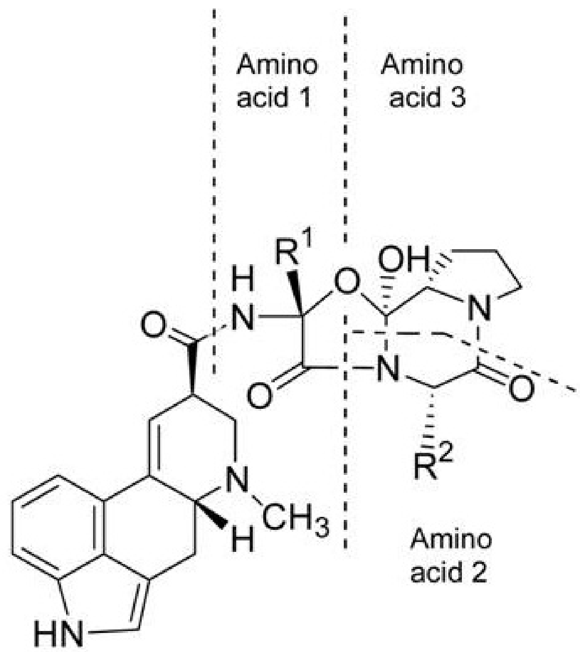


F

[1]Bush and Fannin (2009)

DMAPP: dimethylallyl-diphosphate, Try: tryptophan, DMATrp: dimethylallyl-tryptophan, draW: gene encoding for DMATrp synthase, LPS1 and LPS2: two subunits of D-lysergyl peptide synthetase, IspA and IspB: encode for the LPS enzymes

Figure 2.2 Structures and amino acids in the common ergopeptine alkaloids of the tall fescue/*Neotyphodium coenophialum* symbiotum. Proline is always the amino acid in position 3[1]



	Amino acid 1	R^1	Amino acid 2	R^2
Ergocornine	L-valine	*i*-Pr	L-valine	*i*-Pr
Ergonine	L-2-aminobutyric acid	Et	L-valine	*i*-Pr
Ergoptine	L-2-aminobutyric acid	Et	L-leucine	*i*-Bu
Ergosine	L-alanine	CH_3	L-leucine	*i*-Bu
Ergovaline	L-alanine	CH_3	L-valine	*i*-Pr
Ergotamine	L-alanine	CH_3	L-phenylalanine	CH_2Ph

[1]Bush and Fannin (2009)

Analysis of Alkaloids

Methods for determining ergot alkaloid concentrations are continuing to evolve in order to more accurately identify individual alkaloids and their metabolites. Current methods for the analysis of ergot alkaloids include HPLC (Craig et al., 1994; Jaussaud et al., 1998), competitive ELISA (Hill and Agee, 1994), and HPLC coupled to mass spectrometry (Yates et al., 1985). The ELISA method is an excellent choice for rapid analysis and has been used to measure total ergot alkaloid concentrations in plant and fungal tissues, digesta, urinary and biliary excretions, and animal fat (Strickland et al., 2011). However, its use is limited since it cannot distinguish specific alkaloids and can only determine total ergot alkaloid levels. Research by Hill and Agee (1994) also suggests ELISA is biased towards simpler ergot alkaloids, such as lysergic acid, versus more complex alkaloids, like ergovaline, because the antibody used is specific for the lysergic acid moiety.

Traditional high performance liquid chromatography using fluorescence detection is slower than ELISA, but is excellent for identification and quantification of individual alkaloids. HPLC is good for determining profiles of known alkaloids, but is dependent on pure standards to determine retention time of known alkaloids. Thus, it is not useful for research and discovery of unknown alkaloid metabolites (Strickland et al., 2011). Alternatively, HPLC coupled with mass spectrometry (HPLC-MS) provides the benefits of specific alkaloid determination with a third angle by providing retention times, molecular mass, and empirical formulas from isotopic distribution information (Strickland et al., 2011). Tandem MS-MS allows for identification of product ions present in classes of compounds and is therefore a valuable tool for discovery of unknown ergot alkaloids and metabolites. Furthermore, the USDA-ARS, Forage-Animal Production Research Unit in Lexington, KY has pursued development of an ultra-performance liquid chromatography-MS/MS method, in collaboration with Waters Inc., for detecting, identifying, and quantifying the ergot alkaloids in animal tissues and fluids (Strickland et al., 2011). This new method allows limits of detection ≤0.005 pmol on column and chromatographic runs of 10 min compared with previous best limit of detection of .05 pmol and a 43-min run (Smith et al., 2009).

Toxicokinetics

Naturally occurring ergot alkaloids have been detected in serum (Savary et al., 1990; Bony et al., 2001), urine and bile (Stuedemann et al., 1998; Schultz et al., 2006), ruminal and abomasal fluids (Westendorf et al., 1993; Craig et al., 1994), milk (Durix et al., 1999) and feces (Westendorf et al., 1993; Schultz et al., 2006) of sheep, cattle, and/or horses.

Knowledge of kinetic rates of clearing of ergot alkaloids is limited due to lack of sufficient and/or affordably priced quantities of pure ergot alkaloids for use in large animal studies. Jaussaud et al. (1998) reported a plasma clearance rate of 0.02 L/min/kg body weight in sheep when injected with ergovaline via intravenous injection at 17 ug/kg BW. Bony et al. (2001) recorded a plasma clearance rate of 0.02 L/min/kg BW results when geldings were injected with 15 ug ergovaline/kg BW. Because only ergovaline plasma concentrations were evaluated in these studies, it is unclear if ergovaline was sequestered in tissues, metabolized (e.g. to lysergic acid), or actually eliminated from the body.

Westendorf et al. (1993) fed increased amounts of endophyte infected seed in the diet to abomasally cannulated sheep for 6 days. They reported 50 to 60% of the ergot alkaloids administered in the diet were recovered in the abomasal contents, but only 6 to 7% in the feces, indicating extensive absorption from the gastrointestinal tract. Stuedemann et al. (1998) found 96% of consumed ergot alkaloids were excreted in the urine of steers grazing endophyte infected tall fescue with very little alkaloids found in the bile. On the contrary, Schultz et al. (2006) fed geldings a diet containing endophyte infected tall fescue seed for 21 days and reported 35 to 40% of ergovaline was excreted in the feces. Ergovaline was not detected in the urine. This would indicate that 60 to 65% of the ergovaline is retained or metabolized to another form. De Lorme et al. (2007) also could not detect any ergovaline in the urine of lambs fed endophyte infected tall fescue straw, but approximately 35% of the consumed ergovaline was excreted in the feces.

Work by Hill et al. (2003) suggests lysergic acid may play a larger role in fescue toxicosis than originally thought. These researchers evaluated the transport of ergot alkaloids across ruminal and omasal tissues using parabiotic chambers. Lysergic acid was the only ergot alkaloid reported to be transported across these tissues as measured by ELISA (Hill and Agee, 1994), which led them to conclude that lysergic acid was the primary toxin causing fescue toxicosis. However, an earlier experiment by Hill et al. (2001) showed other ergot alkaloids (ergonovine, ergotamine, ergocryptine) were also transported across ruminal and omasal tissues, although not to the same degree as lysergic acid and lysergol. Schultz et al. (2006) found fecal lysergic acid concentrations averaged 133% of total lysergic acid intake and lysergic acid recovered in the urine was more than 200% of that consumed. Similarly, De Lorme et al. (2007) reported 248% of dietary lysergic acid was recovered in the feces and urine. A significant note is that Schultz et al. (2006) and De Lorme et al. (2007) used HPLC to quantify the individual alkaloids, which allows identification of the specific alkaloids rather than total ergot alkaloids. The site of transport of ergot alkaloids in hind-gut fermenters has yet to be determined, thus, Schultz et al. (2006) speculated the excess levels of lysergic acid excreted may be due to metabolism of ergovaline (or other ergot alkaloids) to lysergic acid in the stomach, small intestine, hindgut, or the hepatic tissues. De Lorme et al. (2007) also theorized the higher levels of excreted lysergic acid may be due to degradation of the ergopeptines by ruminal microbial degradation or by degradation in the lower gastrointestinal tract. Differences in metabolism and/or elimination of ergot alkaloids among ungulates may be due to a number of potential physiological differences including diet selection and intake, rate of digesta flow, hindgut versus foregut fermentation, affinity and capacity of absorption, excretory mechanisms, and hepatic and gastrointestinal tract epithelial metabolism (Strickland et al., 2009).

Vascular Effects

Effects of fescue toxicosis on the cardiovascular system include vasoconstriction, thickened medial layer of blood vessels, endothelial cell damage, vascular stasis and thrombosis, ischemia, and gangrene (Strickland et al., 2009). Strickland et al. (1996) used isolated vascular smooth muscle cells in vitro to demonstrate a link between the thickened medial layer of blood vessels and hyperplasia of the smooth muscle layer. Several other studies have produced evidence that the ergot alkaloids present in endophyte infected tall fescue are associated with hyperthermia of tall fescue toxicosis using in vitro vascular bioassays (Oliver et al., 1992, 1993, and 1998; Klotz et al., 2006, 2007). The alkaloids may be able to decrease blood flow to the peripheral tissues through vasoconstriction, vascular smooth muscle cell hyperplasia, and endothelial cell damage, thus decreasing the efficiency of heat transfer from core body tissue to the surface for dissipation (Strickland et al., 2009). Evidence supporting these effects includes studies by Rhodes et al. (1991) and Oliver et al. (1998) that have shown peripheral vasoconstriction is a measurable response in animals consuming endophyte infected tall fescue. Also, Aiken et al. (2007) used Doppler ultrasonography to show heifers consuming endophyte infected tall fescue seed had reduced caudal artery area and blood flow rates when compared to baseline measures of the same animals and to heifers consuming endophyte free seed.

Ergovaline is presumed to be the primary toxicant of tall fescue toxicosis because it is the most abundant of the ergopeptine alkaloids produced in endophyte infected tall fescue (Yates et al., 1985; Lyons et al., 1986). However, much of the research has been conducted with ergotamine, which is chemically similar to ergovaline, but more readily available due to its use in migraine treatment. Ergotamine has been demonstrated to elicit a contractile response in the bovine dorsal pedal vein (Solomons et al., 1989), cranial branch of the bovine lateral saphenous vein (Klotz et al., 2007), equine lateral saphenous vein and dorsal metatarsal artery (Abney et al., 1993), and rat tail artery (Schoning et al., 2001). On the other hand, ergovaline is also a potent vasoconstrictor of bovine uterine and umbilical arteries (Dyer, 1993), rat tail and guinea pig iliac arteries (Schoning et al., 2001), and the cranial branch of the bovine lateral saphenous vein (Klotz et al., 2006, 2007). Additionally, Klotz et al. (2006, 2007) found the ergopeptines, ergovaline and ergotamine, are more potent and efficacious vasoconstrictors than lysergic acid, with lysergic acid being at least 1000-fold less potent than ergovaline. In comparison to the norepinephrine reference dose (10^{-4} M), the maximum contractile response of lysergic acid was 15 to 20%, whereas ergovaline was 70 to 105%. These studies suggest lysergic acid is a weak vascular toxicant at best. Other alkaloids, such as α-ergocryptine, ergocristine, ergocornine, and ergonovine, were intermediate in contractile response to ergovaline and lysergic acid (Klotz et al., 2010).

Klotz et al. (2007, 2008, 2009) found prior exposure to ergot alkaloids (e.g., tissue from abattoir animals) does attenuate the effect of the alkaloids on vasoconstriction in vitro when compared with tissue from animals with no prior exposure. Specifically, Klotz et al. (2009) found ergovaline, but not lysergic acid, bioaccumulates with repetitive exposure in vitro. Similarly, Oliver et al. (1998) found prior exposure to endophyte infected tall fescue resulted in a shift in α-2 adrenergic receptor activity. Also, Klotz et al. (2008) found there were no synergistic, subtractive, or additive effects of ergovaline, lysergic acid, and N-acetylloline in mixtures of these alkaloids on vasoactive potential in vitro and N-acetylloline alone showed no contractile effect. These results suggest the saturated pyrrolizidine alkaloids, such as N-acetylloline, may play little or no role in fescue toxicosis.

The exact mechanism of alkaloid-induced vascular toxicity is not known, but research implicates the adrenergic and serotonergic receptor systems (Oliver, 1997; 2005). The use of α-adrenergic receptor antagonists have been most successful in treating severe vaso-spastic disease, thus the α-adrenergic receptors may be good targets for alkaloid interaction at the blood vessel level (Strickland et al., 2009). Oliver et al. (1998) demonstrated segments of the cranial branch of the lateral saphenous vein taken from cattle grazing endophyte infected tall fescue had a greater contractile response to BHT-920, a selective α-2 adrenergic receptor agonist, than those from animals grazing endophyte free tall fescue. Also, Oliver et al. (1993) found serotonin 5-HT_2 and α-1 adrenergic receptors were sites of interaction for the ergot alkaloid lysergic acid.

Other biogenic amine receptors are known to be affected by the ergot alkaloids found in endophyte infected tall fescue. Stimulation of α-2 adrenergic receptors by the alkaloids resulted in enhancement of blood platelet aggregation (Oliver et al., 1998), which is likely involved in the coagulopathies and tissue necrosis commonly associated with fescue toxicosis (Oliver, 1997, 2005). Larson et al. (1994, 1995) found stimulation of the dopamine-2 receptor by the ergot alkaloids causes a decrease in prolactin secretion, which is associated with lowered milk production and agalactia found in animals grazing endophyte infected tall fescue (Strickland et al., 1993). Other studies have shown the ergot alkaloids appear to interact with serotonin-2 receptors and activity of these receptors is linked to serotonin-induced contraction of vascular smooth muscle (Dyer, 1993; 2000; Oliver et al., 1993). Serotonin is known to affect the hypothalamic satiety center and increasing levels of serotonin result in suppression of appetite, which may be how the ergot alkaloids decrease feed intake (Strickland et al., 2009). This theory is supported by data from Porter (1995) that showed cattle grazing endophyte infected tall fescue had increased serotonin metabolites in central nervous system tissues. Additionally, Oliver et al. (2000b) found increased levels of tryptophan in sera of cattle grazing endophyte infected tall fescue, which is also associated with decreased feed intake.

Blood Parameters

Many blood cellular parameters are unaffected in animals consuming endophyte infected tall fescue. However, data collected over several years by Oliver et al. (2000a) did find consumption of endophyte infected tall fescue increased red blood cells and decreased mean corpuscular volume and hemoglobin. Also, serum globulin levels were consistently lowered. Additionally, several studies have found consistently lower serum cholesterol (Oliver et al., 2000a; Nihsen et al., 2004; Brown et al., 2009) and consistently higher serum creatinine in steers grazing endophyte infected tall fescue (Oliver et al., 2000a; Nihsen et al., 2004). Most minerals are unaffected by endophyte infected tall fescue intake. However, serum Cu levels are decreased in cattle grazing endophyte infected tall fescue (Saker et al., 1998; Oliver et al., 2000a), possibly due to lower Cu levels found in endophyte infected tall fescue (Dennis et al., 1998). Cu deficiency has been linked to lowered immune function in steers (Saker et al., 1998). Decreased prolactin levels have been well established as a clinical sign of fescue toxicosis in cattle and sheep (Oliver et al., 2000a; Schultze et al., 1999; Fiorito et al., 1991). Furthermore, enzymatic activity is generally lowered by intake of endophyte infected tall fescue, which may be due to decreased feed intake resulting in reduced growth and tissue metabolism and mass. Oliver et al. (2000a) found alanine aminotransferase was consistently lowered in cattle grazing endophyte infected tall fescue over a 3-year grazing period, which correlates with data from Brown et al. (2009). Lower levels of lactate dehydrogenase (Nihsen et al., 2004; Brown et al., 2009) and lower levels of aspartate aminotransferase and creatine kinase (Brown et al., 2009) have also been reported in steers grazing endophyte infected tall fescue. Moreover, several other studies have found that alkaline phosphatase activity is suppressed in cattle affected by tall fescue toxicosis (Brown et al., 2009; Nihsen et al., 2004; Oliver, 1997; Schultze et al., 1999).

Chapter 3. **Materials and Methods**

All research protocols were approved by the University of Kentucky Animal Care and Use Committee. Ten wether lambs weighing approximately 45 kg and 6 months of age were randomly assigned to one of two treatments: 1) control diet with endophyte free tall fescue seed (E-) or 2) a diet containing endophyte infected tall fescue seed (E+). Lambs were fescue naïve, indicating they were not previously exposed to tall fescue prior to the start of this experiment. All lambs were adapted to a diet of 23% endophyte-free tall fescue seed and 77% concentrate during the adaptation period. Lambs assigned to the E+ diet were switched to endophyte infected tall fescue seed on day 0 of the experiment and remained on their respective diets for 2 weeks. Lambs were housed in individual pens during diet adaptation and in metabolism crates during all collection phases in temperature controlled rooms at 21°C. Tall fescue seed was ground (0.5 to 1 mm) and mixed with a concentrate feed consisting primarily of ground shelled corn, soybean meal, distillers dried grains with solubles, and cottonseed hulls (Table 3.1; Table 3.2). Lambs had ad libitum access to fresh water at all times. Diets were fed at 1.8% of individual lamb BW and offered in two equal feedings at 0800 and 2000 h.

The experiment was divided into three phases to evaluate the effects of exposure time to alkaloid containing tall fescue seed. Concentration of ergovaline in the endophyte infected tall fescue seed was 3.6 ppm and concentration of lysergic acid was 4.6 ppm. The first phase (Adaptation Phase; d -14 to d 0) served as a dietary adaptation period in which all lambs (n=10) were adjusted to a diet consisting of 23% endophyte free tall fescue seed and 77% concentrate. During this time basal concentrations of ergovaline and lysergic acid in feces, as well as baseline levels of serum profiles and rectal temperatures were determined. The second phase (Acute Phase; d 1 to 4) included initial exposure to endophyte infected seed (n=5) when the endophyte free seed was replaced with endophyte infected seed followed by the third and final phase (Subacute Phase; d 5 to 14).

Table 3.1 Ingredient and nutrient composition of concentrate mix

Ingredient	%
Ground shelled corn	66.83
Soybean meal	15.00
Cottonseed hulls	10.00
Distillers dried grains with solubles	5.00
Trace mineral salt	1.00
Ground limestone	1.00
Ammonium chloride	0.50
Vit ADE	0.07
Corn oil	0.60
Nutrient Composition	% of DM
NDF	15.3
ADF	6.5
Crude protein	19.7

Table 3.2 Nutritional composition of non-endophyte infected (E-) and endophyte infected (E+) tall fescue seed

Non-endophyte infected (E-)	% of DM
NDF	39.9
ADF	16.3
Crude protein	14.2
Endophyte infected (E+)	% of DM
NDF	40.1
ADF	16.4
Crude protein	15.9

Blood, via jugular venipuncture, was collected twice daily during each phase (Adaptation Phase: d -4 to 0; Acute Phase: d 1 to 4; and Subacute Phase: d 10 to 14). Blood samples were centrifuged at 1500 x g for 20 min. Serum samples from individual lambs were decanted and stored at -20°C for later analysis of alkaline phosphatase (AP), creatine kinase (CK), aspartate aminotransferase (AST), and gamma glutamyltransferase (GGT). Rectal temperatures were taken every 2 h within a 24-h period for each phase (Adaptation Phase: d -4; Acute Phase: d 4; and Subacute Phase: d 10). Total fecal collections were taken using metabolism crates fitted to each lamb. Total feces were collected and weighed once daily at 0800 h during each phase (Adaptation Phase: d -4 to 0; Acute Phase: d 1 to 4; and Subacute Phase: d 10 to 14). A 20% aliquot (by weight) was taken from each day's collection. Samples were stored at -20°C until later freeze-dried and analyzed for alkaloid content and nutrient digestibility.

All diet and fecal samples were ground in a Wiley mill to pass through a 1-mm screen and stored in sealed plastic bags at room temperature until chemically analyzed. Dry matter and ash contents were determined by AOAC (1999) procedures. Neutral detergent fiber (NDF) (Robertson and Van Soest, 1981) and acid detergent fiber (ADF) (Goering and Van Soest, 1970) analyses followed the procedures modified for use in an Ankom 200 Fiber Analyzer (Ankom Co., Fairport, N.Y.). Heat-stable alpha-amylase was added for the neutral detergent fiber analyses of all feed and fecal samples to degrade starch, which could inhibit filtration. Feed and fecal samples were analyzed for N using the automated Kjeldahl method described in AOAC (1999). Serum AP, CK, AST, and GGT were determined using kits obtained from Pointe Scientific, Inc. (Lincoln Park, MI). Samples were processed through the kits using a Konelab 20Xti Clinical Chemistry Analyzer (Thermo Electron Corp., Vantaa, Finland).

Feed and fecal samples were analyzed for ergovaline as described by Craig et al. (1994). A 0.5-g sample size was mixed with 10 ml of 80% methanol. Tubes were sealed and incubated on a shaker at low to medium speed for 2 h. The 2 h incubation is critical because a shorter incubation will not result in full extraction of ergovaline. After the 2 h shaking, samples were vortexed to loosen particulate. Liquid from each sample was decanted into a cotton plugged, 9" borosilicate glass Pasteur pipet sitting in a 13 x 100 mm disposable glass tube to filter out any residual particulate. Ergovaline was extracted and purified using PrepSep columns (SPE, C18 disposable columns, Fisher Sci. #13-678-20A). The columns were pre-conditioned with 2 ml of 80% methanol and covered with foil to keep from drying out. Two ml of supernatant from each sample was pipetted into a labeled PrepSep column and each ml pushed through separately with a syringe and discarded. A third ml was run through each column and collected in a labeled HPLC vial, capped, and run for analysis.

For lysergic acid extraction and analysis, feed and fecal samples were prepared using a 1-g sample size mixed with 10 ml water:acetonitrile (1:1). Tubes were sealed and rotated on a hematology/chemistry mixer (Fisher, Pittsburgh, PA) for 16 h under darkness at room temperature. The sample-water:acetonitrile mixture was separated by centrifugation for 10 min at 2000 rpm. A 5-ml aliquot of the supernatant was transferred to a disposable glass tube and adjusted to pH of 5.0 to 5.5 with 10% acetic acid. Lysergic acid was extracted and purified from the resulting supernatant using strong cation exchange solid phase extraction (SPE) cartridges (Discovery DSC-SCX SPE, Supelco catalog number 52686-U Bellfonte, PA) on a vacuum manifold (Alltech, Deerfield, IL). The SPE cartridge was preconditioned with 3 ml methanol followed by 3 ml of 0.1 M HCl and two, 3-ml portions of pure water. The preconditioning eluents were discarded. The acidified supernatant was applied to the SPE cartridge followed by two, 3-ml portions of pure water. Lysergic acid was eluted from the SPE cartridge with a 3-ml portion of methanol:ammonium hydroxide (95:5). This fraction was collected in a 12 x 75 mm glass test tube and concentrated to dryness using a Savant ISS-100 centrifugal evaporator. The residue was reconstituted in 200 µL of methanol:0.05 M phosphate buffer at pH 8.5 (50:50). Reconstituted samples were placed in an ultrasonic bath then transferred to 1.7-ml microcentrifuge tubes. Samples were centrifuged at 10,000 rpm and supernatant transferred to HPLC vials.

Analyses of ergovaline and lysergic acid with HPLC were carried out using a guard column hand packed with Pellicular C18 material (Alltech Inc., Lexington, KY) and a Luna C18 analytical column (150 x 3.0 mm id, 5 µm particle size, Phenomenex, Torrance, CA) eluted at 1 ml/min under isocratic conditions with a 94:6 ratio of 0.05 M phosphate buffer (pH 8.5):acetonitrile.

Statistical Analysis

Nutrient digestibilities were analyzed as repeated measures using PROC MIXED of SAS (Windows version 5.1/2600, SAS Inst., Inc., Cary, NC). Treatment, phase, and treatment x phase were included in the model. Lamb was assumed to be a random effect and phase was the repeated effect. A compound symmetry (CS) variance-covariance structure was assumed based on AIC values. Enzymes were analyzed, by phase, as repeated measures using PROC MIXED of SAS (Windows version 5.1/2600, SAS Inst., Inc., Cary, NC). Treatment, day, and treatment x day were included in the model. Lamb was assumed to be a random effect and day was the repeated effect. A compound symmetry (CS) variance-covariance structure was assumed based on AIC values. Temperatures were analyzed, by phase, as repeated measures using PROC MIXED of SAS (Windows version 5.1/2600, SAS Inst., Inc., Cary, NC). Treatment, hour, and treatment x hour were included in the model for each phase. Lamb was assumed to be a random effect and hour was the repeated effect. A compound symmetry (CS) variance-covariance structure was assumed based on AIC values. Ergovaline and lysergic acid recovery data were analyzed within the E+ treatment, by phase, using PROC MIXED of SAS (Windows version 5.1/2600, SAS Inst., Inc., Cary, NC). The model included only the repeated effect of day. Lamb was assumed to be a random effect.

Chapter 4. **Results and Discussion**

Nutrient Digestibility

Least squares means for DM digestibility are shown in Table 4.1. Although there was no effect of treatment (E- vs E+), differences among phases were apparent. Digestibility tended to be higher ($P < 0.10$) during the subacute phase within the E- treatment. In contrast, DM digestibility within the E+ treatment was higher ($P < 0.05$) during the acute phase than in either the adaptation or subacute phases. Reasons for these differences are unclear.

Differences in N digestibility (Table 4.2) between E- and E+ were not significant, but phase differences were significant ($P < 0.01$). Digestibility within E- increased from the adaptation (87.8%) through the acute (88.6%) to the subacute (89.1%) phase, whereas the highest value was found during the acute phase of the E+ treatment (89.3%). Based on the highest DM digestibility (Table 4.1), finding the highest N digestibility in the acute phase of E+ might be expected because N is a component of the diet DM. However, finding the high digestibilities for both DM and N during the acute phase, when lambs were consuming the endophyte infected seed, is somewhat surprising. Perhaps the digestive tract is stimulated to compensate for the metabolic stress imposed through consumption of endophyte infected fescue seed by providing more essential entities at the cellular level of the affected animal.

Neutral and acid detergent fiber followed suit with DM and N in that digestibilities within the E+ treatment were highest in the acute phase. However, this occurrence was followed by the lowest ($P < 0.05$) digestibility of both nutrients in the subacute phase of E+. The only treatment differences for NDF ($P < 0.094$) and ADF ($P < 0.055$) were found in the acute phase. Digestibility determinations were made for only 4 days of the acute phase and 5 days of the subacute phase. If determinations had been made continuously from adaptation to acute

Table 4.1 Least squares means of DM digestibility (%) in lambs consuming non-endophyte infected tall fescue seed (E-) or endophyte infected tall fescue seed (E+) by phase

	Treatment[a]		
Phase	E-	E+	P-value[f]
Adaptation	85.3[b]	85.8[d]	0.785
Acute	85.2[b]	87.5[e]	0.169
Subacute	86.3[c]	85.5[d]	0.605

[a]SEM: E- = 1.06 (n = 5) and E+ = 1.18 (n = 4).
[b,c] Values in a column followed by different superscripts are different ($P < 0.10$).
[d,e] Values in a column followed by different superscripts are different ($P < 0.05$).
[f]E- vs E+.

Table 4.2 Least squares means of N digestibility (%) in lambs consuming non-endophyte infected tall fescue seed (E-) or endophyte infected tall fescue seed (E+) by phase

	Treatment[a]		
Phase	E-	E+	P-value[d]
Adaptation	87.8[b]	87.7[b]	0.936
Acute	88.6[b,c]	89.3[c]	0.662
Subacute	89.1[c]	88.3[b,c]	0.579

[a]SEM: E- = 1.01 (n = 5) and E+ = 1.12 (n = 4).
[b,c] Values in a column followed by different superscripts are different ($P < 0.01$).
[d]E- vs E+.

Table 4.3 Least squares means of NDF digestibility (%) in lambs consuming non-endophyte infected tall fescue seed (E-) or endophyte infected tall fescue seed (E+) by phase

	Treatment[a]		
Phase	E-	E+	P-value[d]
Adaptation	66.4	70.5[b,c]	0.308
Acute	66.8	73.8[c]	0.094
Subacute	69.5	68.1[b]	0.720

[a]SEM: E- = 2.59 (n = 5) and E+ = 2.89 (n = 4).
[b,c] Values in a column followed by different superscripts are different ($P < 0.05$).
[d]E- vs E+.

Table 4.4 Least squares means of ADF digestibility (%) in lambs consuming non-endophyte infected tall fescue seed (E-) or endophyte infected tall fescue seed (E+) by phase

	Treatment[a]		
Phase	E-	E+	P-value[d]
Adaptation	59.2	62.5[b,c]	0.405
Acute	58.5	66.6[c]	0.055
Subacute	61.3	59.8[b]	0.704

[a]SEM: E- = 2.58 (n = 5) and E+ = 2.88 (n = 4).
[b,c] Values in a column followed by different superscripts are different ($P < 0.05$).
[d]E- vs E+.

through subacute phases, the effect of E+ seed consumption on nutrient digestibility may have been clearer. Then, a more definitive conclusion could have been made.

Overall, there was a tendency for nutrient digestibility to increase throughout the trial within the E- treatment group. This could possibly be due to adaptation of the lambs to the experimental diet of fescue seed and concentrate diet. However, nutrient digestibility tended to be highest during the acute phase for lambs within the E+ treatment. Differences in nutrient digestibilities, both within and across treatments, may be attributed to variation associated with sampling and analysis. In this study the main effect of treatment was not statistically significant for any of the nutrients analyzed. These results are comparable to those of De Lorme et al. (2007) who found no difference in DM, ADF, or CP digestibilities between lambs consuming E- and E+ tall fescue hay and seed. Matthews et al. (2005) also did not find a difference in NDF digestibility in steers consuming endophyte free or endophyte infected tall fescue hay. In contrast, Hannah et al. (1990) found ruminal and total tract NDF and cellulose digestibilities decreased with increasing ergovaline in the diet of wether lambs and Fiorito et al. (1991) reported lower total tract digestion of DM, NDF, and ADF in lambs consuming endophyte infected tall fescue hay. Other studies have reported lower DM (Westendorf et al., 1993; Aldrich et al., 1993b; Matthews et al., 2005), CP (Westendorf et al., 1993; Matthews et al., 2005), and ADF digestibility (Westendorf et al., 1993; Matthews et al., 2005) in ruminants fed E+ diets compared with those consuming E- diets. To the contrary, Goetsch et al. (1987) reported increased NDF digestibilities when steers were fed endophyte infected tall fescue hay, vs. non-infected hay. Similarly, Neal and Schmidt (1985) found increased DM, crude fiber, and nitrogen-free extract digestibilities when endophyte infected seed was fed to weanling rats. However, in the studies by Goetsch et al. (1987) and Neal and Schmidt (1985), feed intakes were depressed even though animals were given ad libitum access to the ration, which would be expected to increase digestibility. In the present study, as well as those by Westendorf et al. (1993), Fiorito et al.

(1991), and Hannah et al. (1990), intake was equalized across treatments to avoid this confounding influence.

Rectal Temperatures

Rectal temperatures taken every 2 h during day -4 of the adaptation phase are illustrated in Figure 4.1 and least squares means are shown in Appendix Table A.1. Mean values for the E- and E+ treatment groups were 38.75 and 38.71°C, respectively. The main effect of treatment was not statistically significant, which was expected because all lambs consumed E- fescue seed during this phase. Also, there was no significant interaction between treatment and hour. However, there was a statistically significant effect ($P < 0.01$) of time. The peak temperature was recorded at 2000 h for both treatment groups. This peak coincided with the evening feeding. Following this peak, temperatures for both treatment groups dropped over the next two collection times, then experienced another smaller peak at 0200 h. Temperatures leveled out for the remaining collection times.

Rectal temperatures taken at 2-h intervals for 24 h on day 4 of the acute phase are depicted in Figure 4.2. Least squares means used to construct this figure can be found in Appendix Table A.2. There was a significant treatment effect ($P < 0.01$). Temperature tended to increase in E+ fed lambs, but remained constant or decreased in lambs fed E- fescue. Overall differences among hours tended to be significant ($P < 0.10$) resulting in a trend towards a treatment x hour interaction ($P < 0.10$). The mean 24-h temperatures for E- and E+ were 38.69 and 39.20°C, respectively. Significant treatment differences were found at every collection hour except 1400 and 1800 h (Appendix Table A.2).

Subacute phase rectal temperatures are illustrated in Figure 4.3 and least squares means are presented in Appendix Table A.3. The effect of treatment was statistically significant ($P < 0.05$). However, the effect of hour was not significant. Therefore, there was no significant interaction between treatment and hour. Mean temperatures for the subacute phase were 38.67 and 38.98°C for the E- and E+ treatments, respectively. Similar to the acute phase, temperatures for lambs within the E+ treatment remained consistently higher throughout the collection period than for lambs in the E- treatment. However, the mean temperature of the E+ treatment group was numerically lower compared with the acute phase (39.20 vs. 38.98°C). There was a statistically significant difference ($P < 0.10$) between treatments at collection times 1000, 1200, 2000, 2200, 2400, 0200, and 0600 h. Although the overall trend was similar to the acute phase, the magnitude of the differences between treatment groups tended to be less during the subacute phase. This may indicate some adaptation of the lambs to the endophyte infected diet.

The lambs in this study were held in a 21°C environment for the duration of the experiment. In similar studies conducted in a non-heat stressed environment, Fiorito et al. (1991) and De Lorme et al. (2007) did not find an effect of feeding an endophyte infected diet on rectal temperature in lambs for 7 and 28 days, respectively. Matthews et al. (2005) also did not find a difference in rectal temperature when steers consuming endophyte infected or endophyte free tall fescue hay were maintained in a 22 to 27°C controlled environment. Hannah et al. (1990) and Aldrich et al. (1993a) conducted studies with sheep housed in a heat stressed environment (32 to 34°C) and found animals consuming endophyte infected tall fescue did have higher rectal temperatures compared with those consuming endophyte free tall fescue. Nihsen et al. (2004) also reported steers grazing endophyte infected KY 31 and exhibiting signs of heat stress had higher rectal temperatures compared with steers grazing endophyte free tall fescue.

Figure 4.1 Rectal temperatures in lambs consuming non-endophyte infected tall fescue seed (E-) or endophyte infected tall fescue seed (E+) on d -4 of adaptation phase

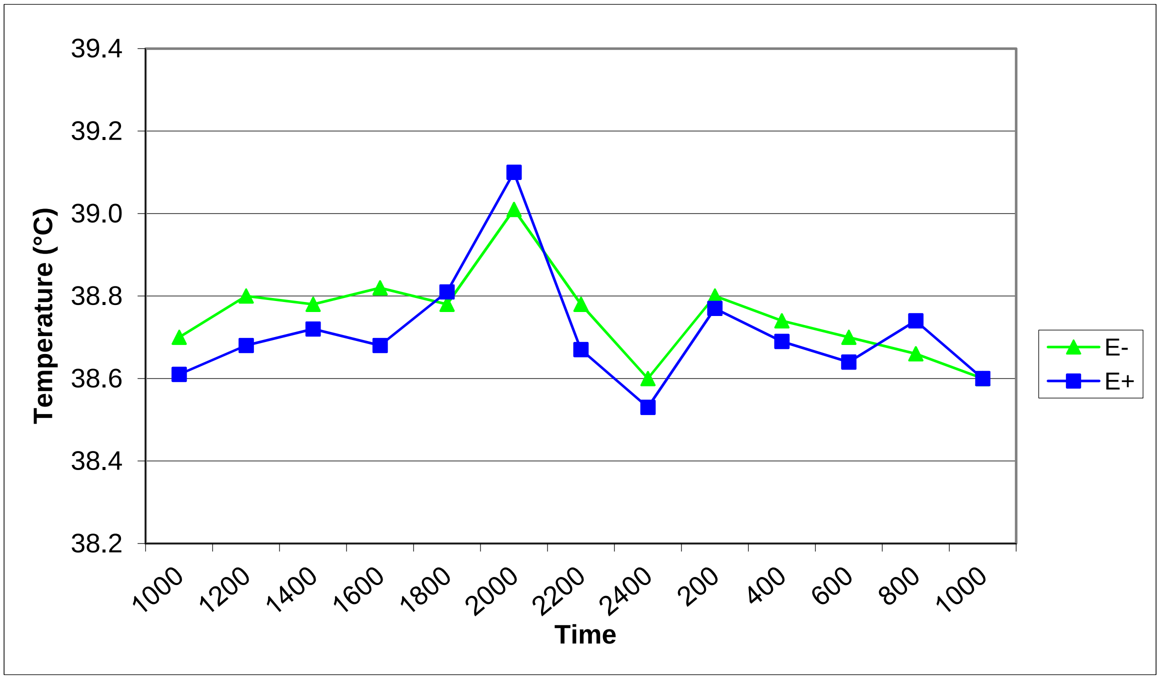


Figure 4.2 Rectal temperatures in lambs consuming non-endophyte infected tall fescue seed (E-) or endophyte infected tall fescue seed (E+) on d 4 of acute phase

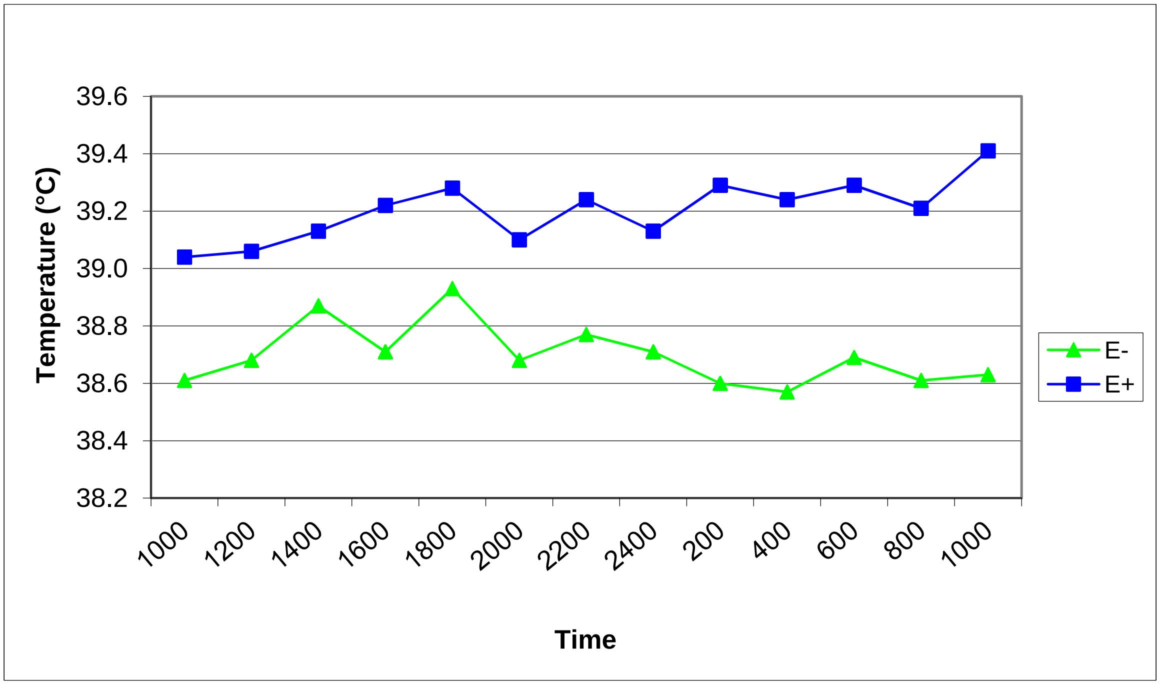
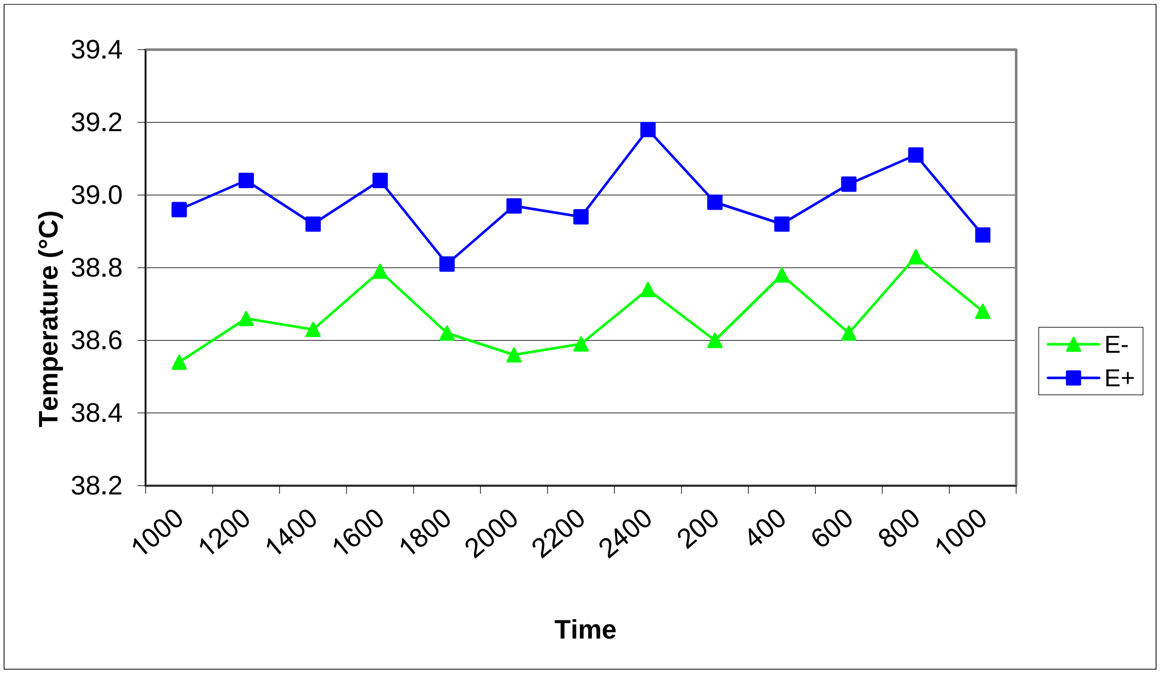


Figure 4.3 Rectal temperatures in lambs consuming non-endophyte infected tall fescue seed (E-) or endophyte infected tall fescue seed (E+) on d 10 of subacute phase



Ergovaline and Lysergic acid

Recovery of dietary ergovaline in the feces of lambs in the E+ treatment is presented in Table 4.5 for the acute phase and in Table 4.6 for the subacute phase. There was a significant ($P < 0.01$) effect of day of the acute phase with recovery increasing from 2.0% on d 1 up to 9.6% on d 4. Although recoveries were numerically higher in the subacute phase (16.6 to 23.3%), the only difference ($P < 0.10$) that could be attributed to day of collection was between d 10 and d 14.

Westendorf et al. (1993) reported 6 to 7% of the ergot alkaloids consumed from an endophyte infected seed diet for 6 days were excreted in the feces of lambs. These results are similar to the average ergovaline recovery found during the acute phase of this study (5.6%), which consisted of 4 days of collection. In comparison, Schultz et al. (2006) found 34.3% of consumed ergovaline was excreted in the feces during an initial phase (d 0 to 3) and another 41.7% during the subacute phase (d 4 to 21) when geldings were fed an endophyte infected seed diet for 21 days. De Lorme et al. (2007) also reported approximately 35% of consumed ergovaline was excreted in the feces of lambs fed endophyte infected tall fescue straw for 28 days. Differences in ergovaline recovery between the acute phase of this study and the initial phase of Schultz et al. (2006) may be due to the slower passage rate of ruminants (foregut fermenters) compared with horses (hindgut fermenters). However, Schultz et al. (2006) did observe increased ergovaline recovery from the initial to the subacute phase which coincides with the numerically higher recovery in the subacute phase of this study compared with the acute. The mean ergovaline recovery of this study was lower during the subacute phase (20.0%) than values reported by Schultz et al. (2006) and De Lorme et al. (2007), but the current study was only 14 days long. If carried out longer, higher recoveries may have been found.

Table 4.5 Least squares means of fecal ergovaline recovery (% of intake) in lambs consuming endophyte infected tall fescue seed (E+) during the acute phase[a]

Day	Intake (mg/d)	Fecal (mg/d)	% Recovery
1	0.61	0.01	2.0[b]
2	0.76	0.02	3.2[b]
3	0.76	0.06	7.8[c]
4	0.74	0.07	9.6[c]
Mean	0.72	0.04	5.6

[a]SEM for all days = 1.44.
[b,c] Values in a column followed by different superscripts are different ($P < 0.01$).

Table 4.6 Least squares means of fecal ergovaline recovery (% of intake) in lambs consuming endophyte infected tall fescue seed (E+) during the subacute phase[a]

Day	Intake (mg/d)	Fecal (mg/d)	% Recovery
10	0.77	0.13	16.6[b]
11	0.77	0.15	19.7[b,c]
12	0.77	0.17	21.7[b,c]
13	0.77	0.15	18.8[b,c]
14	0.77	0.18	23.3[c]
Mean	0.77	0.15	20.0

[a]SEM for all days = 3.46.
[b,c] Values in a column followed by different superscripts are different ($P < 0.10$).

Acute and subacute phase recoveries of dietary lysergic acid in the feces of lambs fed E+ seed are shown in Tables 4.7 and 4.8, respectively. There was a significant effect of day during the acute phase ($P < 0.05$). Days 1 and 2 recoveries were not different, but both were lower than 3 and 4 ($P < 0.10$). Days 2 and 3 did not differ, but both were lower ($P < 0.10$) than day 4. There was no significant effect of day during the subacute phase when lysergic acid recovery ranged from 35.4% to 39.9% over the collection period.

Similar to ergovaline, lysergic acid recovery increased daily during the acute phase with a mean recovery of 15.0%. Mean recovery of lysergic acid was numerically higher during the subacute (37.3%) phase. Schultz et al. (2006) reported fecal lysergic acid recovery averaged 134% in geldings consuming E+ tall fescue throughout the 21-d study. Unlike this study, Schultz et al. (2006) did not observe an effect of day on lysergic acid recovery, which may again be attributed to differences in passage rates of horses compared with ruminants. De Lorme et al. (2007) also found an average lysergic acid recovery of 113% in the feces of lambs consuming endophyte infected tall fescue straw and seed. De Lorme et al. (2007) theorized ergopeptines in the feed were degraded to lysergic acid by ruminal microbial degradation or by degradation in the lower gastrointestinal tract. Schultz et al. (2006) came to a similar conclusion that ergovaline, or other ergot alkaloids, were metabolized to lysergic acid in the stomach, small intestine, hindgut, or hepatic tissues. The diet in this study was more digestible due to a primarily concentrate based ration compared with the forage based diets of those fed by Schultz et al. (2006) and De Lorme et al. (2007). The higher digestibility of the diet in this study would result in a smaller indigestible fraction excreted compared with a larger indigestible fraction found in a forage based diet. Possibly, the smaller lysergic acid recovery in this study may be the result of less lysergic acid excreted with the indigestible fraction in the feces.

Table 4.7 Least squares means of fecal lysergic acid recovery (% of intake) in lambs consuming endophyte infected tall fescue seed (E+) during the acute phase[a]

Day	Intake (mg/d)	Fecal (mg/d)	% Recovery
1	0.91	0.07	7.9[b]
2	0.96	0.12	12.2[b]
3	0.96	0.19	19.3[c]
4	0.95	0.20	20.8[c]
Mean	0.95	0.14	15.0

[a]SEM for all days = 3.90.
[b,c] Values in a column followed by different superscripts are different ($P < 0.10$).

Table 4.8 Least squares means of fecal lysergic acid recovery (% of intake) in lambs consuming endophyte infected tall fescue seed (E+) during the subacute phase[a]

Day	Intake (mg/d)	Fecal (mg/d)	% Recovery
10	0.97	0.34	35.4
11	0.97	0.37	37.8
13	0.97	0.35	35.9
14	0.97	0.39	39.9
Mean	0.97	0.36	37.3

[a]SEM for all days = 4.56.

Enzymes

Serum alkaline phosphatase (AP), creatine kinase (CK), aspartate aminotransferase (AST), and gamma glutamyltransferase (GGT) concentrations were monitored during the adaptation, acute, and subacute phases. The AP least squares means are presented in Tables 4.9, 4.10, and 4.11 and graphically illustrated in Figures 4.4, 4.5, and 4.6. Neither treatment nor day affected the AP concentrations of any phase. This was expected in the adaptation phase (Table 4.9) because all lambs consumed E- tall fescue seed during this phase. Differences due to treatment and day were also nonsignificant in the acute (Table 4.10) and subacute (Table 4.11) phases. Additionally, there was no interaction between treatment and day during the adaptation and acute phases. However, the interaction of treatment and day in the subacute phase was significant ($P < 0.10$) as shown in Figure 4.6. On d 11 of the subacute phase the E+ treatment was significantly lower at 45.2 U/L ($P < 0.021$) compared to the E- treatment at 80.0 U/L. Mean values of AP for lambs on the E+ treatment tended to decrease after d 11. The difference in mean values between the E- and E+ treatments was 4.8 in the adaptation phase, but increased to 9.8 and 17.3 in the acute and subacute phases, respectively. AP is an indicator of bone or liver damage and all the values reported in this study fell within the normal range of 27 to 156 U/L as reported by Boyd (1984) . The numerically lower AP found in this study for lambs consuming E+ tall fescue seed agrees with results by Oliver et al. (2000a), who found steers grazing endophyte infected tall fescue had numerically lower AP levels, but the differences were not statistically significant. Also, Fiorito (1991) reported lambs consuming endophyte infected tall fescue hay had numerically lower AP levels. Other studies (Nihsen et al., 2004; Brown et al., 2009) reported significantly lower AP levels in steers grazing endophyte infected than in those grazing endophyte free tall fescue. Boling et al. (1989) also found AP levels were lower in calves fed endophyte infected tall fescue hay. Greater differences may have been found in the current study if the collection period had

Table 4.9 Least squares means of serum alkaline phosphatase (U/L) in lambs consuming non-endophyte infected tall fescue seed (E-) or endophyte infected tall fescue seed (E+) during the adaptation phase

	Treatment[a]		
Day	E-	E+	P-value[b]
-4	74.4	73.6	0.917
-3	74.1	73.2	0.898
-2	70.8	64.8	0.426
-1	73.0	69.2	0.610
0	82.7	70.4	0.106
Mean	75.0	70.2	0.232

[a]SEM: E- = 4.93 (n = 5) and E+ = 5.52 (n = 4).

[b]E- vs E+.

Figure 4.4 Blood serum alkaline phosphatase (U/L) in lambs consuming non-endophyte infected tall fescue seed (E-) or endophyte infected tall fescue seed (E+) during the adaptation phase

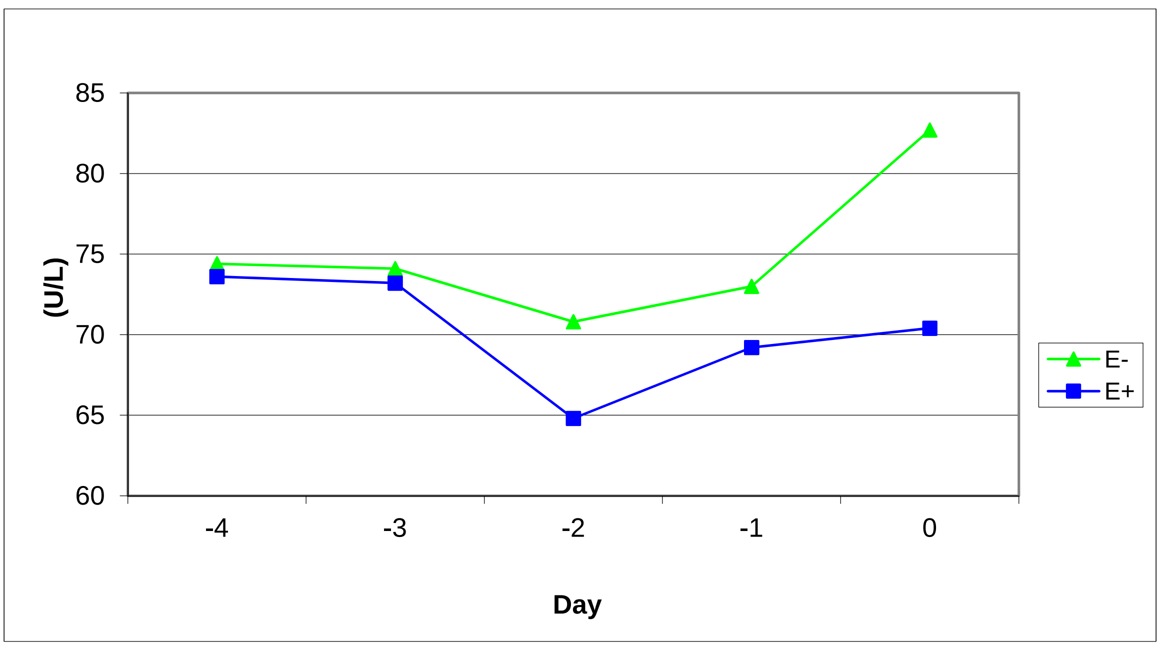


Table 4.10 Least squares means of serum alkaline phosphatase (U/L) in lambs consuming non-endophyte infected tall fescue seed (E-) or endophyte infected tall fescue seed (E+) during the acute phase

	Treatment[a]		
Day	E-	E+	P-value[b]
1	79.3	70.3	0.514
2	81.3	66.5	0.284
3	78.5	61.9	0.230
4	65.9	67.1	0.935
Mean	76.2	66.4	0.402

[a]SEM: E- = 8.95 (n = 5) and E+ = 10.00 (n = 4).

[b]E- vs E+.

Figure 4.5 Blood serum alkaline phosphatase (U/L) in lambs consuming non-endophyte infected tall fescue seed (E-) or endophyte infected tall fescue seed (E+) during the acute phase

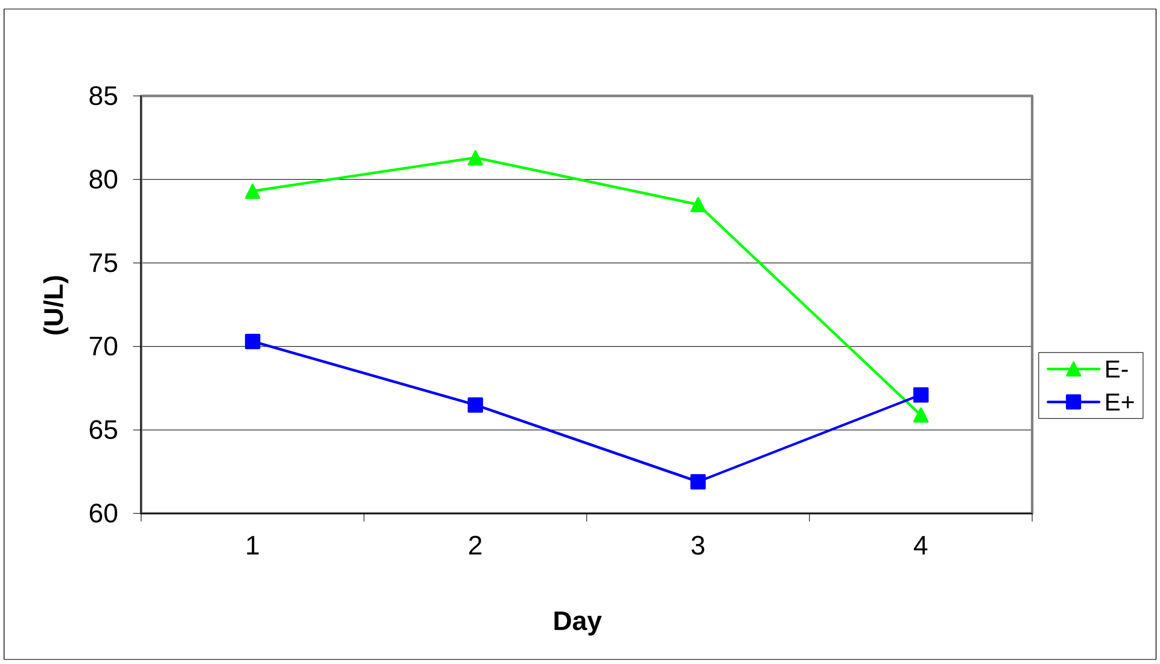


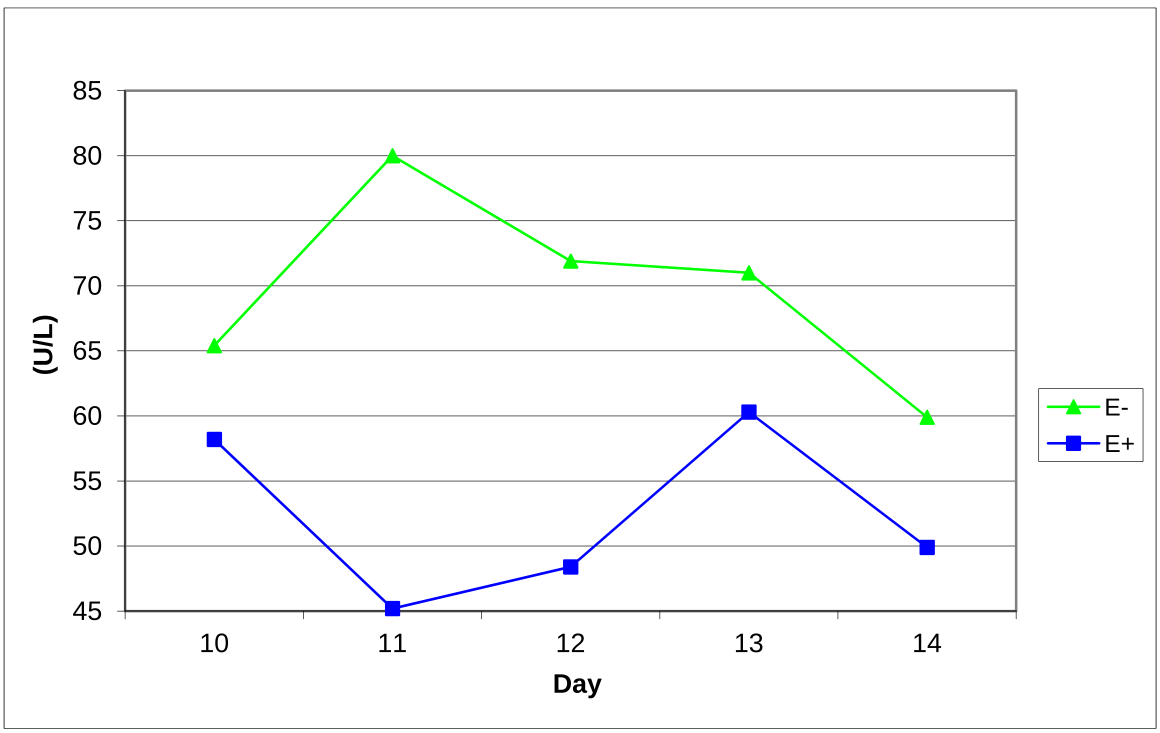
Table 4.11 Least squares means of serum alkaline phosphatase (U/L) in lambs consuming non-endophyte infected tall fescue seed (E-) or endophyte infected tall fescue seed (E+) during the subacute phase

	Treatment[a]		
Day	E-	E+	P-value[b]
10	65.4	58.2	0.620
11	80.0	45.2	0.021
12	71.9	48.4	0.109
13	71.0	60.3	0.459
14	59.9	49.9	0.487
Mean	69.7	52.4	0.178

[a]SEM: E- = 9.51 (n = 5) and E+ = 10.63 (n = 4).

[b]E- vs E+.

Figure 4.6 Blood serum alkaline phosphatase (U/L) in lambs consuming non-endophyte infected tall fescue seed (E-) or endophyte infected tall fescue seed (E+) during the subacute phase



been extended, but more likely the wide range of normal values would still make it difficult to detect significant differences.

The least squares means for CK were not affected by treatment or day within the adaptation and acute phases (Tables 4.12 and 4.13; Figures 4.7 and 4.8). Least squares means for CK in the subacute phase are presented in Table 4.14 and illustrated in Figure 4.9. Again, differences during the adaptation were not anticipated because all lambs consumed E- fescue seed. Differences due to treatment and/or day, or uncovering an interaction, might be expected during the acute phase if striated or skeletal muscle damage occurred as a result of E+ seed consumption. However, this did not appear to be the case. In contrast to AP, CK concentrations increased from adaptation through the acute to highest in the subacute phase in lambs consuming E+ fescue seed. Brown et al. (2009) found lower CK levels in steers grazing endophyte infected tall fescue for 89 d than for those grazing endophyte free fescue. This contrasts with the results of this study where no significant effect of treatment was found during the first two phases and CK levels tended to be higher in lambs in the E+ treatment. The normal range of CK in sheep is 7.7 to 101 U/L (Boyd, 1984). Values from this study fell within this range, but had a large range of variability.

Aspartate aminotransferase (AST), an indicator of hepatic and striated muscle damage, can exhibit a concentration range from 49 to 123 U/L in sheep blood serum (Boyd, 1984). Concentrations in the three phases of the present study mostly fell within this range, with a few slightly lower values (Tables 4.15, 4.16, and 4.17; Figures 4.10, 4.11, and 4.12). Although E+ lambs had a higher level ($P < 0.066$) than E- on d -3 (61.4 vs 39.4) of the adaptation phase, differences on other days were not significant. Overall, however, E+ lamb concentrations averaged 58.4 U/L compared to an average of 48.5 U/L for E- lambs in the adaptation phase. There was no effect of treatment in the acute phase (means = 47.3 U/L for E- and 49.2 U/L for

Table 4.12 Least squares means of serum creatine kinase (U/L) in lambs consuming non-endophyte infected tall fescue seed (E-) or endophyte infected tall fescue seed (E+) during the adaptation phase

	Treatment[a]		
Day	E-	E+	P-value[b]
-4	51.4	54.7	0.869
-3	71.0	66.3	0.813
-2	75.0	49.2	0.207
-1	67.6	64.3	0.870
0	47.8	56.6	0.661
Mean	62.6	58.2	0.674

[a]SEM: E- = 13.31 (n = 5) and E+ = 14.88 (n = 4).

[b]E- vs E+.

Figure 4.7 Blood serum creatine kinase (U/L) in lambs consuming non-endophyte infected tall fescue seed (E-) or endophyte infected tall fescue seed (E+) during the adaptation phase

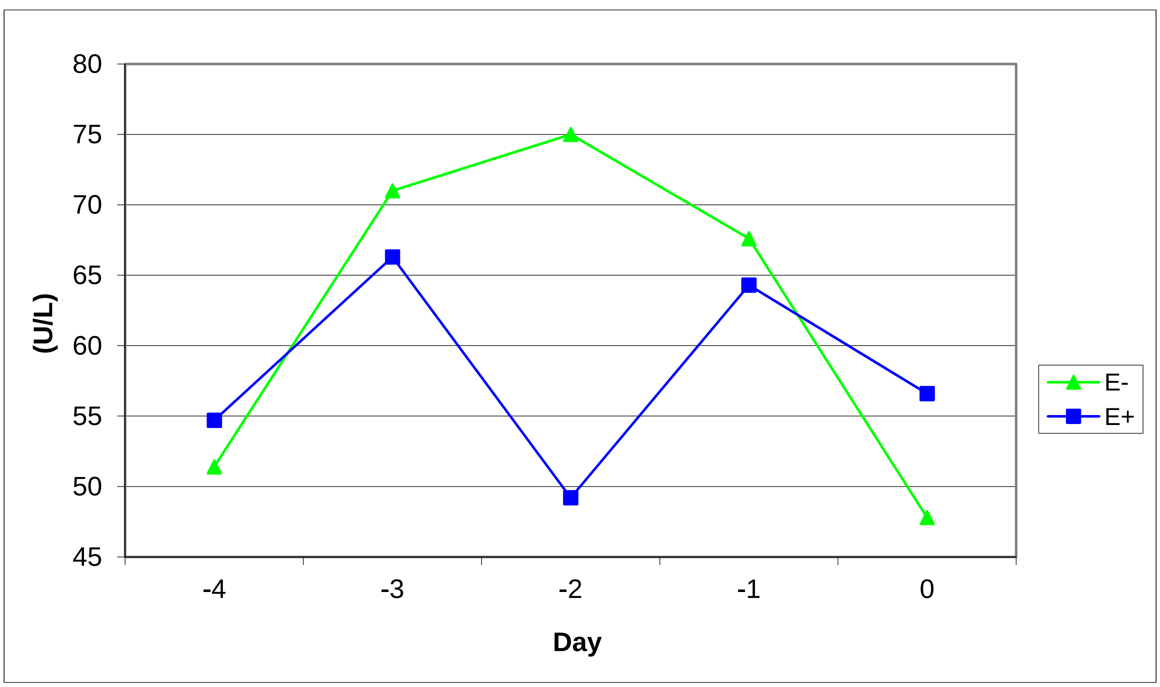


Table 4.13 Least squares means of serum creatine kinase (U/L) in lambs consuming non-endophyte infected tall fescue seed (E-) or endophyte infected tall fescue seed (E+) during the acute phase

	Treatment[a]		
Day	E-	E+	P-value[b]
1	54.1	80.8	0.137
2	68.2	49.7	0.298
3	45.6	63.8	0.305
4	70.6	64.1	0.713
Mean	59.6	64.6	0.570

[a]SEM: E- = 11.54 (n = 5) and E+ = 12.90 (n = 4).

[b]E- vs E+.

Figure 4.8 Blood serum creatine kinase (U/L) in lambs consuming non-endophyte infected tall fescue seed (E-) or endophyte infected tall fescue seed (E+) during the acute phase

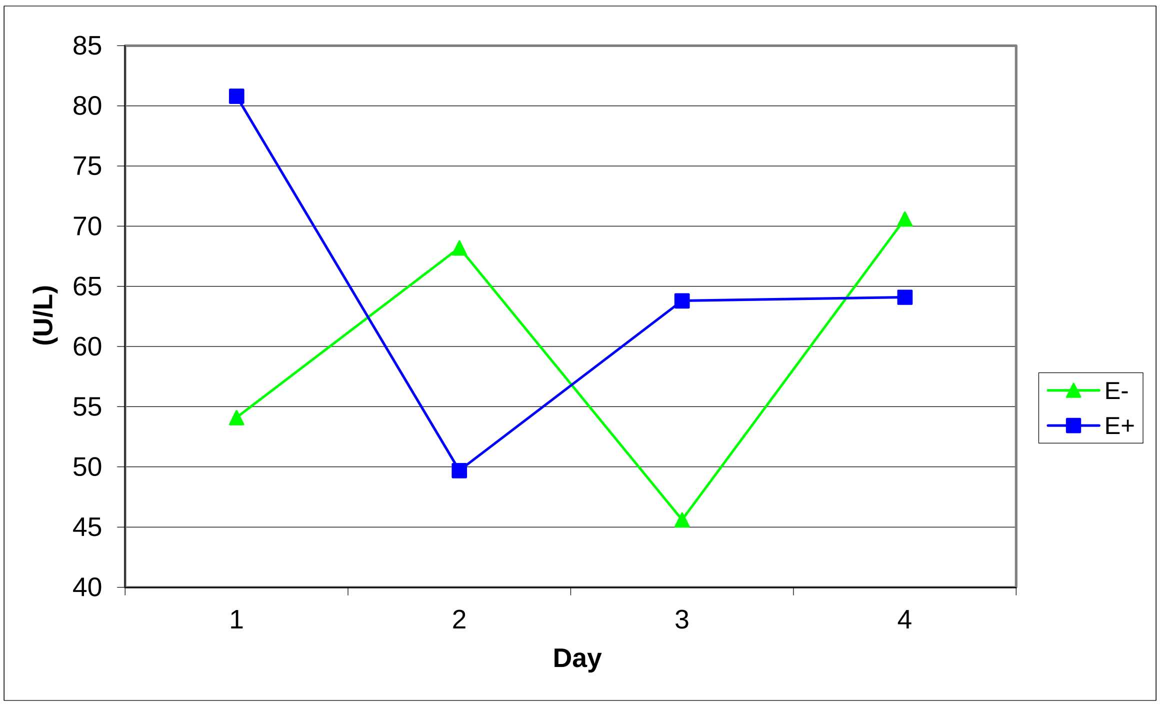


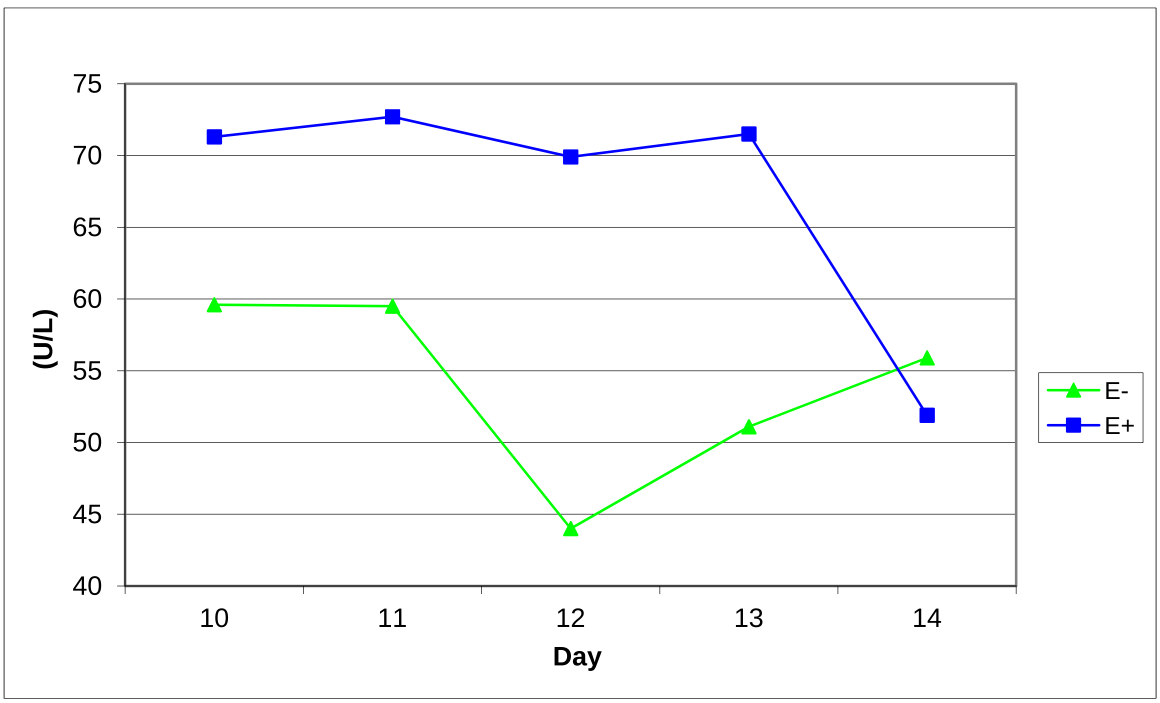
Table 4.14 Least squares means of serum creatine kinase (U/L) in lambs consuming non-endophyte infected tall fescue seed (E-) or endophyte infected tall fescue seed (E+) during the subacute phase

	Treatment[a]		
Day	E-	E+	P-value[b]
10	59.6	71.3	0.353
11	59.5	72.7	0.290
12	44.0	69.9	0.045
13	51.1	71.5	0.108
14	55.9	51.9	0.745
Mean	54.0	67.5	0.100

[a]SEM: E- = 8.20 (n = 5) and E+ = 9.17 (n = 4).

[b]E- vs E+.

Figure 4.9 Blood serum creatine kinase (U/L) in lambs consuming non-endophyte infected tall fescue seed (E-) or endophyte infected tall fescue seed (E+) during the subacute phase



E+), but day was significant ($P < 0.05$). Variation among collection days prevented establishment of any trends during the acute phase other than mirrored concentrations between E- and E+. There were no effects of treatment or day in the subacute phase. Interaction analyses revealed no differences in AST concentrations between day and treatment in the phase. Similar to their AP results, Oliver et al. (2000a) found steers grazing endophyte infected tall fescue had numerically lower AST levels then those grazing endophyte free fescue, but the difference was not statistically significant. Nihsen et al. (2004) also found steers grazing KY 31 (E+) tall fescue had lower levels of AST then E- steers, but the difference was not significant. The results of this study agree with these researchers in terms of not finding a significant effect of treatment on AST levels in ruminants consuming E+ tall fescue. However, in this study animals in the E+ treatment tended to have higher AST levels than those in the E- treatment. In contrast, Brown et al. (2009) reported significantly lower ($P < 0.03$) AST levels in steers grazing endophyte infected tall fescue for 89 d. However, the previous research did not divide their experimental periods into phases as was done in the present study. Differences in experimental protocol make it difficult to extrapolate long term grazing data to short term intensive phases. Nevertheless, it appears that serum AST concentrations are not consistently altered in animals consuming endophyte infected forage or seed.

A fourth serum enzyme that has been evaluated as an indicator of tissue damage from endophyte infected fescue consumption is gamma glutamyltransferase (GGT). The normal range of concentration in sheep is 20 to 44 U/L (Boyd, 1984). Levels measured in this experiment are shown in Tables 4.18, 4.19, and 4.20 and graphically illustrated in Figures 4.13, 4.14, and 4.15. The effect of day was significant ($P < 0.05$) in all three phases, but treatment differences were nonsignificant. Concentrations in E- followed the same general pattern as those of the E+ treatment in acute and subacute phases, leading to the conclusion that GGT was not a significant indicator of tissue damage in lambs consuming endophyte infected tall fescue seed in this

Table 4.15 Least squares means of serum aspartate aminotransferase (U/L) in lambs consuming non-endophyte infected tall fescue seed (E-) or endophyte infected tall fescue seed (E+) during the adaptation phase

	Treatment[a]		
Day	E-	E+	P-value[b]
-4	49.4	60.0	0.366
-3	39.3	61.4	0.066
-2	43.7	52.7	0.442
-1	65.6	66.4	0.939
0	44.4	51.5	0.544
Mean	48.5	58.4	0.242

[a]SEM: E- = 7.68 (n = 5) and E+ = 8.59 (n = 4).

[b]E- vs E+.

Figure 4.10 Blood serum aspartate aminotransferase (U/L) in lambs consuming non-endophyte infected tall fescue seed (E-) or endophyte infected tall fescue seed (E+) during the adaptation phase

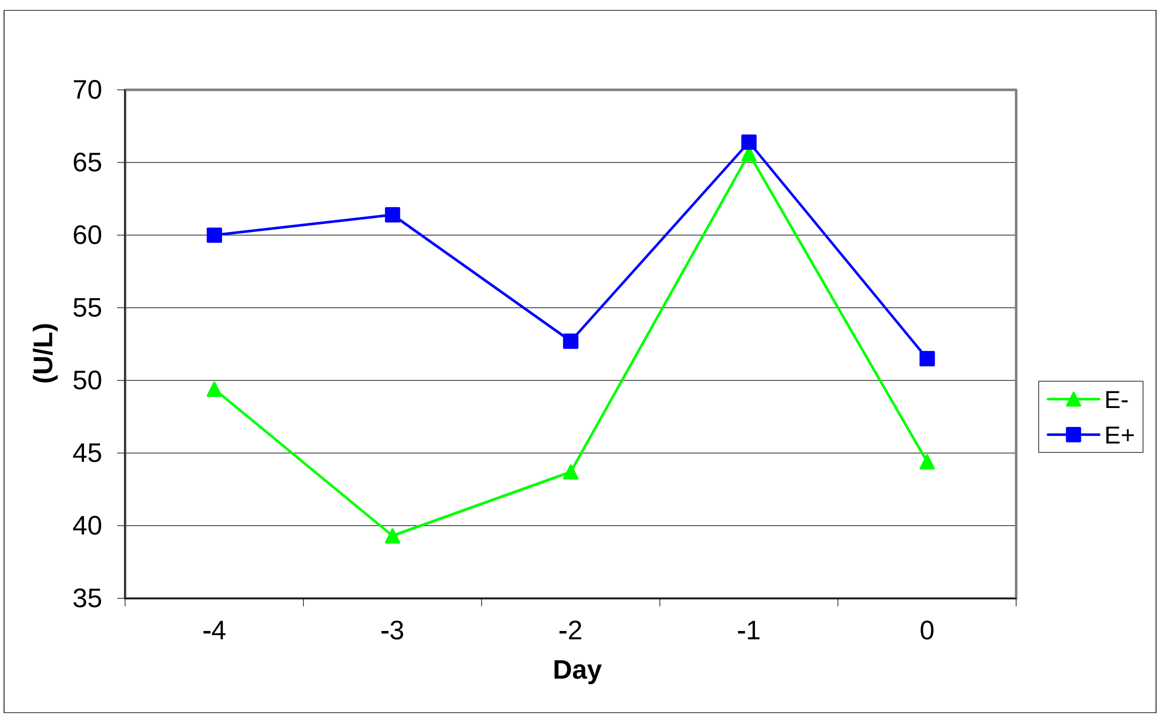


Table 4.16 Least squares means of serum aspartate aminotransferase (U/L) in lambs consuming non-endophyte infected tall fescue seed (E-) or endophyte infected tall fescue seed (E+) during the acute phase

	Treatment[a]		
Day	E-	E+	P-value[b]
1	53.2	55.0	0.885
2	57.9	59.1	0.926
3	38.4	38.0	0.974
4	39.8	44.5	0.713
Mean	47.3	49.2	0.850

[a]SEM: E- = 8.37 (n = 5) and E+ = 9.36 (n = 4).

[b]E- vs E+.

Figure 4.11 Blood serum aspartate aminotransferase (U/L) in lambs consuming non-endophyte infected tall fescue seed (E-) or endophyte infected tall fescue seed (E+) during the acute phase

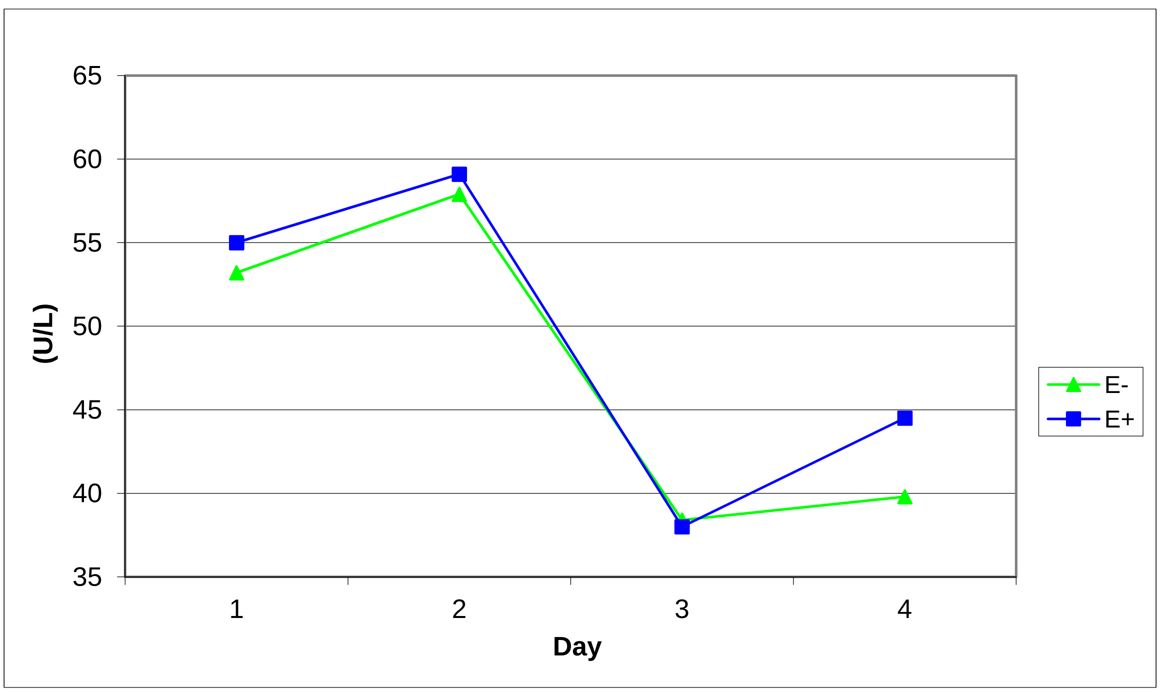


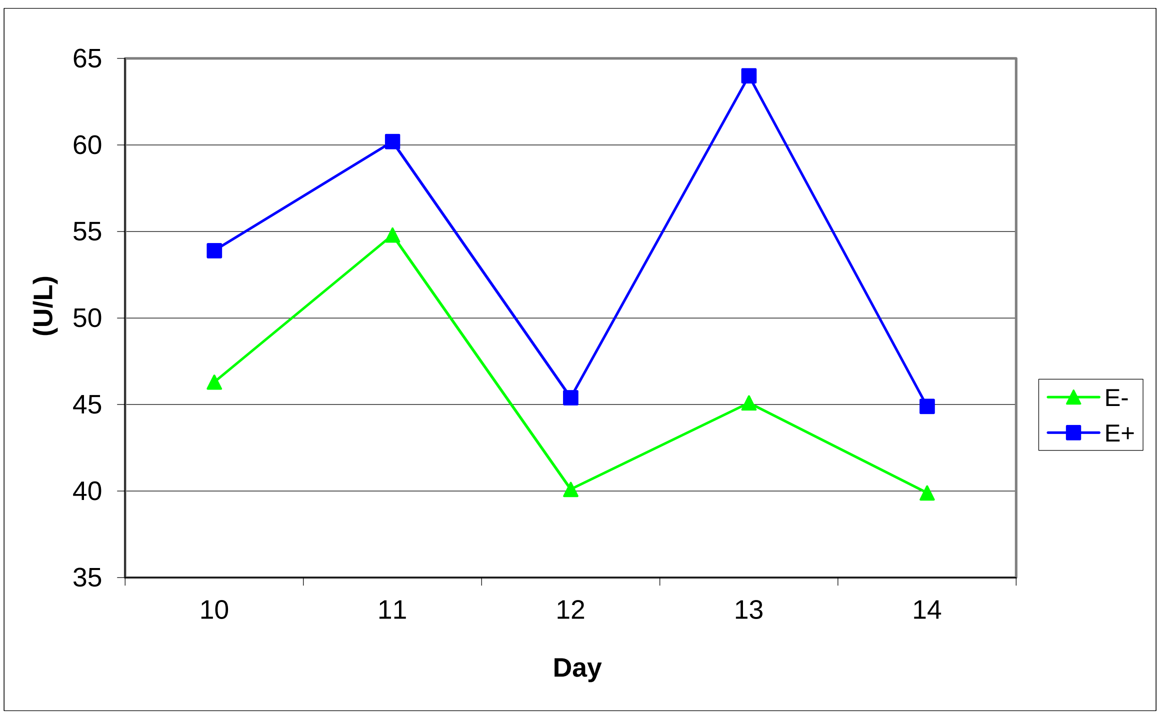
Table 4.17 Least squares means of serum aspartate aminotransferase (U/L) in lambs consuming non-endophyte infected tall fescue seed (E-) or endophyte infected tall fescue seed (E+) during the subacute phase

	Treatment[a]		
Day	E-	E+	P-value[b]
10	46.3	53.9	0.546
11	54.8	60.2	0.662
12	40.1	45.4	0.669
13	45.1	64.0	0.137
14	39.9	44.9	0.687
Mean	45.2	53.7	0.360

[a]SEM: E- = 8.22 (n = 5) and E+ = 9.19 (n = 4).

[b]E- vs E+.

Figure 4.12 Blood serum aspartate aminotransferase (U/L) in lambs consuming non-endophyte infected tall fescue seed (E-) or endophyte infected tall fescue seed (E+) during the subacute phase



experiment. These results agree with those of Oliver et al. (2000a) and Brown et al. (2009) who found levels of GGT were not different between steers grazing endophyte infected tall fescue compared with steers on endophyte free tall fescue.

Overall, there was little effect of treatment on enzyme levels across all phases, indicating consumption of E+ tall fescue seed has little or no effect on the blood serum AP, CK, AST, or GGT or the level consumed in this study was not high enough to elicit a significant response. Although day within a phase was significant in several places, a lack of consistency prevented making any definite conclusions.

Table 4.18 Least squares means of serum gamma glutamyltransferase (U/L) in lambs consuming non-endophyte infected tall fescue seed (E-) or endophyte infected tall fescue seed (E+) during the adaptation phase

	Treatment[a]		
Day	E-	E+	P-value[b]
-4	57.0	58.9	0.821
-3	41.6	58.8	0.050
-2	57.9	64.6	0.426
-1	79.9	80.3	0.963
0	54.9	46.3	0.309
Mean	58.3	61.8	0.379

[a]SEM: E- = 5.59 (n = 5) and E+ = 6.25 (n = 4).

[b]E- vs E+.

Figure 4.13 Blood serum gamma glutamyltransferase (U/L) in lambs consuming non-endophyte infected tall fescue seed (E-) or endophyte infected tall fescue seed (E+) during the adaptation phase

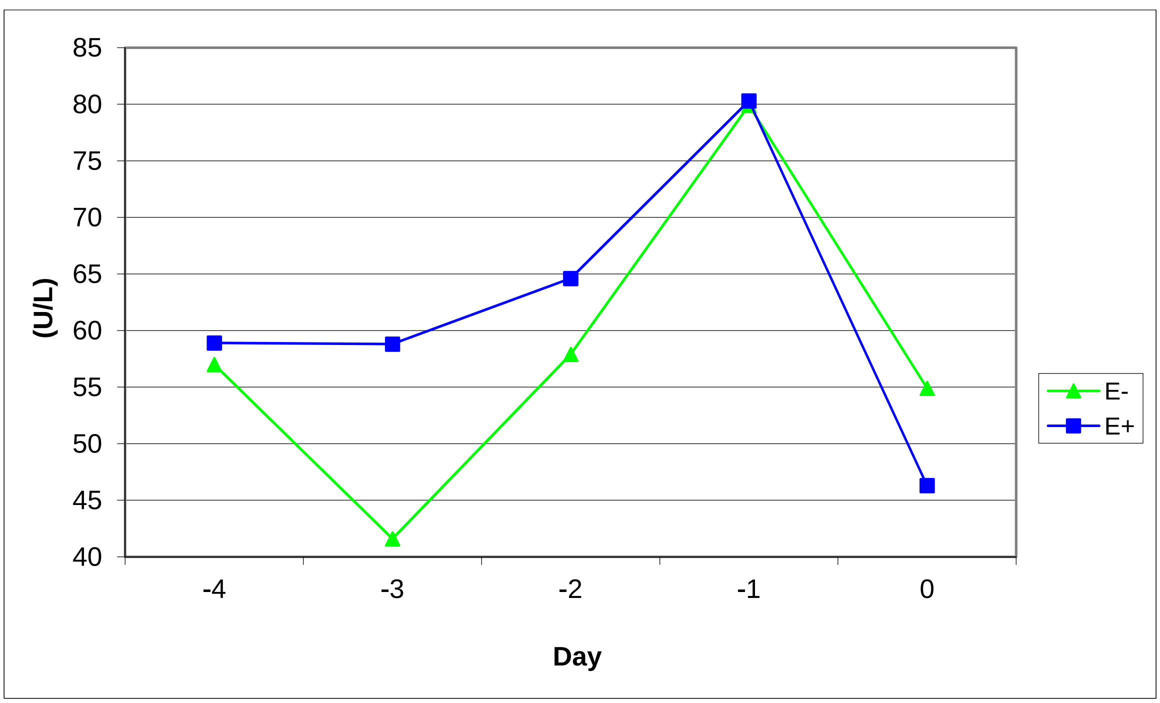


Table 4.19 Least squares means of serum gamma glutamyltransferase (U/L) in lambs consuming non-endophyte infected tall fescue seed (E-) or endophyte infected tall fescue seed (E+) during the acute phase

	Treatment[a]		
Day	E-	E+	P-value[b]
1	64.7	64.3	0.964
2	61.5	69.1	0.474
3	43.8	47.6	0.715
4	44.7	49.5	0.652
Mean	53.7	57.6	0.466

[a]SEM: E- = 6.91 (n = 5) and E+ = 7.73 (n = 4).

[b]E- vs E+.

Figure 4.14 Blood serum gamma glutamyltransferase (U/L) in lambs consuming non-endophyte infected tall fescue seed (E-) or endophyte infected tall fescue seed (E+) during the acute phase

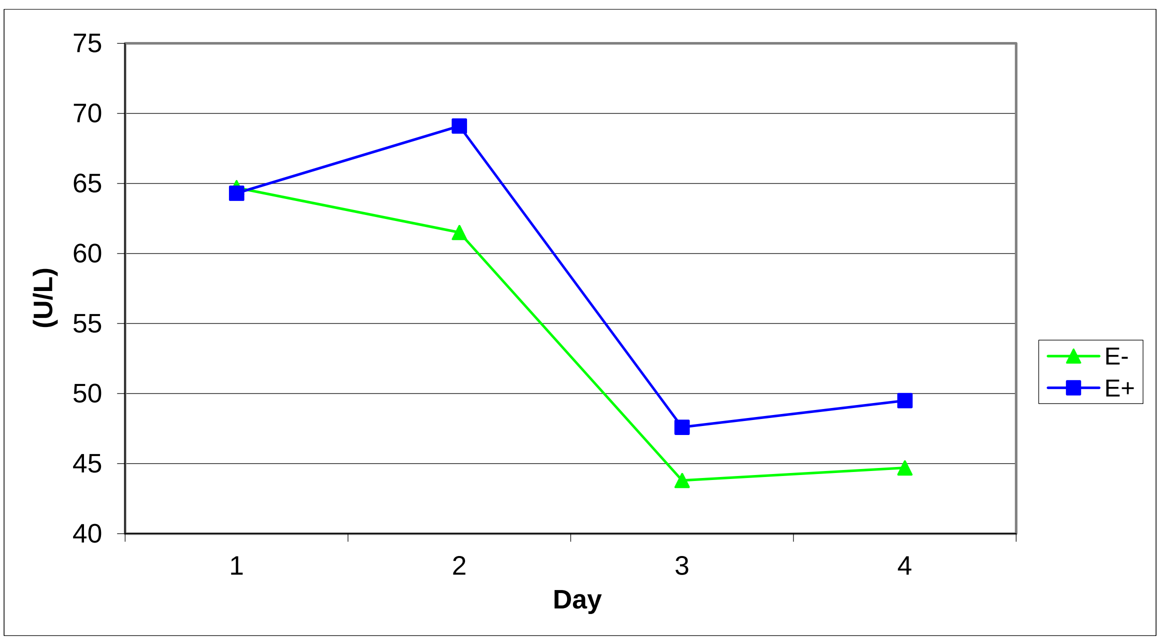


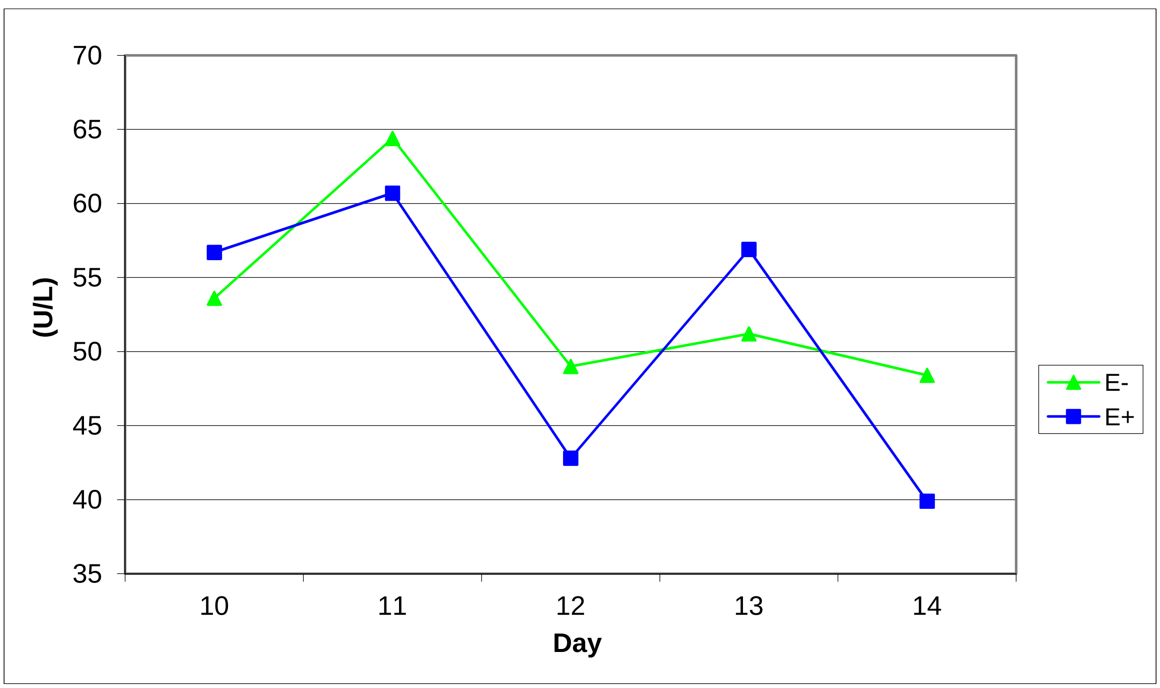
Table 4.20 Least squares means of serum gamma glutamyltransferase (U/L) in lambs consuming non-endophyte infected tall fescue seed (E-) or endophyte infected tall fescue seed (E+) during the subacute phase

	Treatment[a]		
Day	E-	E+	P-value[b]
10	53.6	56.7	0.755
11	64.4	60.7	0.715
12	49.0	42.8	0.538
13	51.2	56.9	0.577
14	48.4	39.9	0.408
Mean	53.3	51.4	0.743

[a]SEM: E- = 6.71 (n = 5) and E+ = 7.50 (n = 4).

[b]E- vs E+.

Figure 4.15 Blood serum gamma glutamyltransferase (U/L) in lambs consuming non-endophyte infected tall fescue seed (E-) or endophyte infected tall fescue seed (E+) during the subacute phase



Chapter 5. **Summary**

Ten wether lambs were used to determine the effects of consuming endophyte infected tall fescue seed on rectal temperature, nutrient digestibility, ergovaline and lysergic acid recovery, and select serum enzyme profiles. Lambs were adapted to a diet of 23% endophyte free tall fescue seed (E-) and 77% concentrate fed at 1.8% BW in equal feedings (12-h intervals). Lambs remained on this diet during the first phase of the experiment from d -14 to d 0. Jugular blood serum and total feces were collected daily from d -4 to 0 (adaptation phase) and rectal temperatures were recorded every 2 h on d -4. On d 0, the E- seed was replaced in the diet of five lambs with endophyte infected seed (E+). Lambs remained on their respective diets (E- or E+) through d 14. Blood serum and total feces were collected from d 1 to 4 (acute phase) and from d 10 to 14 (subacute). Rectal temperatures were recorded on d 4 and 10. Digestibilities of DM, N, NDF, and ADF were calculated and recovery of dietary ergovaline and lysergic acid was determined from fecal analyses. Serum AP, CK, AST, and GGT were measured as indicators of liver and/or muscle tissue damage. The overall effect of treatment was nonsignificant for all nutrient digestibilities. However, there was a trend for lambs within the E+ treatment to have higher digestibilities during the acute phase. Lambs fed the E+ diet had higher rectal temperatures during both the acute and subacute phases. Fecal recovery of dietary EV was 5.6 and 20.0% and recovery of dietary LA was 15.0 and 37.3% during the acute and subacute phases for E+ lambs. Serum concentrations of AP, AST, and GGT were not affected by treatment and CK tended to be higher ($P < 0.10$) during the subacute phase. Increasing recovery of EV and LA in the feces indicates some adaptation of metabolic processes for EV and LA elimination.

Appendix

Table A.1 Least squares means of rectal temperatures (°C) in lambs consuming non-endophyte infected tall fescue seed (E-) or endophyte infected tall fescue seed (E+) on day -4 of the adaptation phase

	Treatment[a]		
Hour	E-	E+	P-value[b]
1000	38.70	38.61	0.598
1200	38.80	38.68	0.478
1400	38.78	38.72	0.741
1600	38.82	38.68	0.401
1800	38.78	38.81	0.869
2000	39.01	39.10	0.609
2200	38.78	38.67	0.509
2400	38.60	38.53	0.668
0200	38.80	38.77	0.830
0400	38.74	38.69	0.766
0600	38.70	38.64	0.717
0800	38.66	38.74	0.632
1000	38.60	38.60	0.987
Mean	38.75	38.71	0.773

[a]SEM: E- = 0.08 (n = 5) and E+ = 0.09 (n = 4).
[b]E- vs E+.

Table A.2 Least squares means of rectal temperatures (°C)in lambs consuming non-endophyte infected tall fescue seed (E-) or endophyte infected tall fescue seed (E+) on day 4 of the acute phase

	Treatment[a]		
Hour	E-	E+	P-value[b]
1000	38.61	39.04	0.049
1200	38.68	39.06	0.083
1400	38.87	39.13	0.234
1600	38.71	39.22	0.020
1800	38.93	39.28	0.113
2000	38.68	39.10	0.055
2200	38.77	39.24	0.032
2400	38.71	39.13	0.058
0200	38.60	39.29	0.002
0400	38.57	39.24	0.003
0600	38.69	39.29	0.006
0800	38.61	39.21	0.007
1000	38.63	39.41	0.001
Mean	38.69	39.20	0.008

[a]SEM: E- = 0.10 (n = 5) and E+ = 0.11 (n = 4).
[b]E- vs E+.

Table A.3 Least squares means of rectal temperatures (°C) in lambs consuming non-endophyte infected tall fescue seed (E-) or endophyte infected tall fescue seed (E+) on day 10 of the subacute phase

	Treatment[a]		
Hour	E-	E+	P-value[b]
1000	38.54	38.96	0.052
1200	38.66	39.04	0.069
1400	38.63	38.92	0.180
1600	38.79	39.04	0.231
1800	38.62	38.81	0.384
2000	38.56	38.97	0.050
2200	38.59	38.94	0.094
2400	38.74	39.18	0.041
0200	38.60	38.98	0.069
0400	38.78	38.92	0.510
0600	38.62	39.03	0.056
0800	38.83	39.11	0.189
1000	38.68	38.89	0.317
Mean	38.67	38.98	0.046

[a]SEM: E- = 0.10 (n = 5) and E+ = 0.11 (n = 4).
[b]E- vs E+.

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