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I am submitting herewith a thesis written by Alison Marie Corrigan entitled "Ruminal responses in cattle grazing tall fescue pastures differing in endophyte level." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

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RUMINAL RESPONSES IN CATTLE GRAZING TALL FESCUE PASTURES DIFFERING IN ENDOPHYTE LEVEL

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Presented for the
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Degree
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Alison Marie Corrigan
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ABSTRACT

Three 1.2-ha tall fescue (*Festuca arundinacea*, Schreb.) pastures at the Blount Livestock Unit of the Knoxville Experiment Station containing 1) Kentucky-31 endophyte (*Neotyphodium coenophialum*) infected tall fescue (KY-31/E+), 2) Jesup endophyte-free tall fescue (Jesup/E-), or 3) Jesup infected with a non-ergot alkaloid producing endophyte tall fescue (Jesup/MaxQ^{TM}) were grazed. The objectives of this experiment were: 1) to validate endophyte status of the experimental pasture through determining ergovaline levels of masticate samples and animal response using serum prolactin concentrations, weight gain, and rectal temperatures, 2) to compare weight and composition of rumen contents of cannulated beef steers grazing tall fescue pastures that differ in endophyte status, 3) to determine the effect of time of grazing on the *in situ* and *in vitro* nutrient disappearance of commonly used supplemental feedstuffs, and 4) to compare nutrient composition of rumen content to masticate sampling of the pastures.

Two ruminally cannulated steers were randomly assigned to each pasture treatment in early May 2003. After an 18-day acclimation period, steers were subjected to four 0700 and four 1900 rumen evacuations, where contents were weighed and sub-sampled for DM, NDF, ADF, and CP analyses. After rumens were evacuated and washed, steers were returned to their respective pastures to graze for about 45 minutes. Masticate samples were collected from the rumen for determination of pasture forage quality. Between evacuation days, *in situ* degradation of dehydrated alfalfa hay, corn grain, corn gluten feed, and soybean hulls was performed by placing five of substrates into 10x20 cm Dacron bags and incubating them in the rumen of steers grazing the forage treatments. Samples were incubated for 12 h, from 0700-1900 and from 1900-0700, to

evaluate differences in day and night ruminal environments. Samples incubated *in situ* were analyzed for DM, NDF, ADF, and CP disappearance. Steers gained less ($P < 0.05$) weight while consuming KY-31/E+ tall fescue than Jesup/E- or Jesup/MaxQ. The ADG for KY-31/E+ was 0.29 kg/d ($P < 0.05$), while Jesup/E- and Jesup/MaxQ were 0.65 kg/d and 0.70 kg/d, respectively. Serum prolactin levels were significantly reduced in steers grazing KY-31/E+ compared to steers grazing Jesup/E- or Jesup/MaxQ. Rumen DM contents for steers grazing Jesup/E- or Jesup/MaxQ were greater ($P < 0.05$) than steers grazing KY-31/E+ and 1900 content weights were greater than 0700 content weights, indicating that steers consume more forage during the day than overnight. Rumen contents were compared to masticate samples to determine if rumen samples could be used to evaluate forage quality. There were significant ($P < 0.05$) differences between the two sampling methods. Therefore, rumen content sampling should not be used in place of masticate sampling to evaluate forage quality. Steer rectal temperature was higher ($P < 0.05$) at 1900 in steers consuming KY-31/E+ compared to Jesup/E- or Jesup/MaxQ. *In situ* DM, NDF, ADF, and CP disappearance of corn grain was greater ($P < 0.05$) in steers grazing Jesup/E- and Jesup/MaxQ than in steers grazing KY-31/E+. *In situ* alfalfa hay and corn gluten feed DM, NDF, and ADF disappearance tended ($P < 0.15$) to be greater in steers grazing Jesup/E- and Jesup/ MaxQ than in steers grazing KY-31/E+. Soybean hull disappearance was not affected by endophyte status of pastures. Degradation of substrates was not affected by time of day of incubation.

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PART I

INTRODUCTION

Tall fescue toxicosis is one of the most costly plant origin problems facing the beef cattle industry in the United States. About 23% of the U.S. beef cattle are grazing tall fescue infested with an endophytic fungus. Signs of fescue toxicosis include reduced dry matter intake, reduced weight gains and reduced reproductive efficiency. Removing the fungus from the plants by use of fungicides and heating of seed prior to planting resulted in an endophytic-free plant. However these endophyte-free (E-) plants do not survive under environmental and grazing conditions commonly found in the mid-south transition zone of the US. In recent years, new endophytic fungi that do not produce ergot alkaloids have been introduced into adapted cultivars of tall fescue. Cattle grazing endophyte-infected (E+) tall fescue have reduced dry matter intake compared to animals consuming E- and cultivars containing non-ergot alkaloid producing endophytes. Data on differences in the rumen fill and composition of animals grazing tall fescue with different endophyte status is limited.

Supplementation strategies to reduce tall fescue toxicosis have not taken into consideration the potential differences in the rumen environment of cattle grazing endophyte-infected tall fescue, endophyte-free and non-ergot producing endophyte-infected tall fescue. The *in situ* technique for determining nutrient disappearance is closely linked to the ruminal environment in which incubations occur. While *in vitro* techniques used to screen feedstuffs for use by ruminants depends heavily on rumen microbes from a known environment.

The objectives of this study were: 1) to validate endophyte status of the experimental pasture through determining ergovaline levels of masticate samples and animal response using serum prolactin concentrations, weight gain, and rectal

temperatures, 2) to compare weight and composition of rumen contents of cannulated beef steers grazing tall fescue pastures that differ in endophyte status, 3) to determine the effect of time of grazing on the *in situ* and *in vitro* nutrient disappearance of commonly used supplemental feedstuffs, and 4) to compare nutrient composition of rumen content to masticate sampling of the pastures.

PART II

LITERATURE REVIEW

TALL FESCUE

Tall fescue (*Festuca arundinacea* Schreb.) is a cool season perennial grass. It is persistent, highly productive most of the year, and the forage base for about 20 million cattle in the United States (Fribourg et al., 2002). Tall fescue is a long-lived bunchgrass with short rhizomes that usually grow two to four feet tall (Ball et al., 1991). The rhizomes develop more abundantly in the winter months, decline in spring and increase again in the summer. Tall fescue is the most important cultivated pasture grass in the USA, occupying over 35 million acres (Hoveland, 2003). It is well adapted to wide array of environments but predominates in the transition zone between the northern and southern regions of the eastern U.S. (Williams et al., 1984). It was originally cultivated in Europe and was first released in the United States in 1942 by the Kentucky Agricultural Experiment Station as "Kentucky 31" (Fergus, 1972). Tall fescue grows best on deep, moist soils that are heavy to medium in texture and high in organic matter. It grows on soils that vary from strongly acidic (pH 4.7) to alkaline (pH 9.5). Best growth occurs when soil pH is maintained between 5.5 and 8.5. It tolerates long periods of flooding (24 to 35 days) when temperatures are below 27°C (Hannaway, 2005).

The reason for the popularity of this grass are ease of establishment, wide range of adaptation, tolerance to abuse, pest resistance, good seed production, excellent appearance when used as a turf grass (Hoveland, 1993), and the ability to withstand drought, poor soil conditions, and intensive defoliation from grazing (Thompson et al., 2001). It is used for livestock feed, turf grass and erosion control (Ensley and Larson, 2001). Tall fescue, when managed correctly has high quality digestible dry matter, crude

protein, amino acid and mineral content (Bush and Buckner, 1973) and should provide for good animal performance; however this was not the case. Tall fescue became associated with three diseases: fescue foot, bovine fat necrosis, and fescue toxicity. Fescue foot symptoms include elevated respiration rate and gangrene resulting in loss of hooves, tails and ears, that occur mostly during cold weather (Yates, 1983) from insufficient blood flow to the extremities (Browning, 2003). Bovine fat necrosis results in hard fat that accumulates along the intestinal tract, which results in poor digestion and difficult birth (Bush et al., 1979). Fescue toxicosis general symptoms include decreased weight gain, decreased conception rates, intolerance to heat, and failure to shed winter coats with these symptoms being more severe in warmer weather (Hoveland, 2003).

The most concerning of the three diseases is tall fescue toxicosis, which is also known as "summer slump" or "summer syndrome" (Robbins et al., 1973). Since the beef cattle industry has such a high economic importance, a great deal of research has been conducted to determine the cause of this toxicosis. In 1977, Bacon et al. reported the presence of an endophytic fungus in tall fescue and its correlation to fescue toxicosis in cattle.

THE ENDOPHYTE

The endophytic fungus associated with tall fescue toxicosis is *Neotyphodium coenophialum*. Surveys suggest that 95% of all tall fescue pastures are infected with an endophytic fungus (Hill and Stringer, 1985; Shelby and Darymple, 1987); however, there is no visible sign of the infection. Annual economic losses of $600 million reported for the US beef cattle industry are probably an underestimate, covering both growth and

reproduction (Allen and Segarra, 2001). In addition, the endophyte adversely affects the $60 million grass seed and hay export industry.

The endophyte lives in intercellular spaces of sheath, stem, leaf, and seed tissues (Thompson et al., 2001). The mycelium is usually present in abundance in the leaf sheath and stems, sparsely in leaf blades and appears absent in roots (White and Cole, 1986; White, 1987). The endophyte is present in the seed when it is planted. When the seed germinates and grows, the endophyte infects the plant at the base of the leaf (Roberts, 2000). When the plant begins its reproductive cycle the endophyte moves to the stem and grows as the stem elongates. Eventually, the stem forms a seed head and the endophyte moves into the seed (Roberts, 2000). The endophytes are restricted to asexual reproduction, and are transmitted vertically from mother plant to its seed (Ahlholm et al., 2002). The endophyte found in the seed will be genetically identical to the mother plant (Bates, 2004). There is no invasion of the plant cells, nor does the endophyte become pathogenic (Thompson et al., 2001).

A true mutualistic symbiotic relationship exists between the endophyte and tall fescue (Hill, 1990, Clay, 1988; Bacon and Siegel, 1988). The endophyte depends on the host plant for energy (Hill et al., 1990). This means the endophyte is never subjected to environmental forces and is only dependent upon plant health for survival (Hill, 1994). This relationship also benefits the endophyte by nutrition, protection, and dissemination through the seed. The host plant benefits from improved drought and pest tolerance, resistance to phytotoxic metal ions in soil (Malinowski and Belesky, 2000), plant tillering and utilization of soil nitrogen (Arechavaleta et al., 1989) seedling vigor, and growth potential (Latch, 1997). These host plant benefits occur through the endophytes

production of secondary metabolites known as alkaloids. These endophytes are the source of several types of secondary compounds that cause fescue toxicosis. These include lolines, peramine, indole acetic acid, ergonovine, ergovaline, lolitrem B, and terpenoids (Lane et al., 2000).

THE ERGOT ALKALOIDS

Ergot alkaloids are isolated from the ergot fungus *Claviceps purpurea*. There are two broad classes: the ergoline alkaloids that contain the lysergic ring structure with hydroxyl, carboxyl, and carboxamide functional groups and the ergopeptine alkaloids that have a tripeptide cyclo moiety attached at the carboamide site (Rutschmann and Stadler, 1978). Chemically the ergot alkaloids are 3, 4 substituted indole derivatives having a tetracyclic ergoline ring structure (Tudzynski et al., 2001). The basic chemical structure of ergot alkaloids is similar to dopamine, noradrenalin and serotonin (Berde and Strurmer, 1978; Muller-Schweinitzer and Weidmann, 1978). These three compounds are neurotransmitters found in the body that regulate appetite, cardiovascular function, endocrine motility, gastrointestinal motility, muscle contraction, and temperature regulation (Browning, 2003).

Ergovaline is the predominant ergopeptine alkaloid found in infected tall fescue and is found at biologically active levels (Garner et al., 1993). Ergopeptines account for as much as 50% of the total ergot alkaloid concentration in endophyte-infected tall fescue and ergovaline accounts for greater than 80% of the total ergopeptine fraction (Lyons et al., 1986). In 1979, Porter and et al. that isolates of *Epichloe typhina* from ‘Kentucky-31’ tall fescue, with demonstrated cattle toxicity, produced ergot alkaloids under laboratory conditions. Ergopeptine alkaloids have also been detected in endophyte infected tall

fescue plants from field (Yates et al., 1985) and controlled environment studies (Lyons et al., 1986).

In 1998, Stuedemann et al. found that approximately 94% of alkaloids from cattle grazing endophyte-infected tall fescue were found in the urine and 6% in the bile. Hill et al. (2001) showed that little ergopeptides cross the gastrointestinal tract and the primary ergot alkaloid that is transported across gastrointestinal tissue are lysergic acid and lysergic acid amide.

FESCUE TOXICOSIS

Tall fescue toxicosis is a condition that alters ruminant grazing behavior (Seman et al., 1999) and adversely affects cattle performance (Stuedemann and Hoveland, 1988). It is not a lethal condition and may be subclinical (Browning, 2003). It affects over 8.5 million beef cattle and 700,000 horses (Ball et al., 1996). Beef cattle grazing endophyte-infected tall fescue ingest ergot alkaloids and develop fescue toxicosis. Some signs of fescue toxicosis include: increased body temperature, increased respiration, excessive salivation, decreased weight gain, reduced reproductive performance, lowered serum prolactin levels, lower milk production, and inability to shed rough hair coats (Stuedemann and Hoveland, 1988; Paterson et al., 1995).

Temperature

Increased body temperature usually occurs during hotter summer months of the grazing season. It has been reported that it can be the result of vasoconstrictive properties of the alkaloids (Schmidt and Osborn, 1993; Porter and Thompson 1992). Diets containing endophyte-infected tall fescue caused a reduction in blood flow to the skin of steers and lambs. Steers with reduced blood flow to the periphery showed an increase in

rectal temperatures (Rhodes et al., 1989). They hypothesized that reduced blood flow to the periphery would reduce the animals' ability to move heat from core tissues to surface for cooling, which would explain the increase in rectal temperatures. Eight days after the removal of endophyte-infected tall fescue, blood flows returned to endophyte-free levels. Rhodes et al. (1989) also showed that administration of a dopamine antagonist, metoclopramide, resulted in increased blood flow indicating that the decrease in blood flow was via dopaminergic activity.

Animal Performance

Decreased animal weight gain is also associated with consumption of endophyte-infected tall fescue toxicosis in cattle (Bond et al., 1986; Schmidt et al., 1982; Hoveland et al., 1983). One explanation for reduced body weight gains may be a reduction in feed intake by animals grazing endophyte-infected tall fescue. Peters et al. (1992) showed that during August, when environmental temperatures were higher, cows on endophyte-infected (E+) tall fescue pastures consumed less (1.6% Body Weight) than cows on endophyte-free (E-) tall fescue or orchard grass (2.0% BW). Steers grazing E- tall fescue had a 43-69% greater dry matter intake compared to steers on E+ tall fescue (Stuedemann et al., 1989). Another explanation for decreased weight gains could be the digestibility effects. A study conducted by Fiorito et al. (1991), using lambs, showed that total tract digestibilities of dry matter, neutral detergent fiber, and acid detergent fiber were lowered by the presence of endophyte-infected tall fescue hay in the diet. Therefore, both, decreased feed intake and decreased digestibility are potential causes of decreased weight gain in steers (Strickland et al., 1993).

Live weight gains in lambs were similar for E- and AR501 in all seasons, but significantly lower in E+, especially in summer and autumn (Fletcher et al., 2000). Mizinga et al. (1992) conducted a pair-fed experiment where the intake of animals receiving E- diet was maintained at the intake level of the E+ diet group. The results showed a depression in body weight gains in the animals consuming the E+ diet. Therefore growth is affected by the endophyte in a matter beyond intake (Thompson et al., 2001). It has been suggested that for each 10% increase in infestation there was a 0.045 kg/d decrease in steer average daily gain (Garner et al., 1984; Crawford et al., 1989).

Reproduction

One of the most economically devastating aspects of fescue toxicosis in cattle is reduced reproductive performance (Schmidt and Osborn, 1993). Only significant effect of the endophyte-infected diet on bulls was lowered serum prolactin (Evans et al., 1988). However, Schuenemann et al. (2005) reported that the ability of oocytes to cleave after fertilization with sperm from bulls exposed to endophyte-infected fescue decreased compared with bulls grazing non-toxic endophyte-infected pasture. The female reproductive efficiency is more severely affected. Reports of reproductive toxicity of endophyte-infected tall fescue in the diet during gestation have resulted in lowered calf birth weights. Calving rates are also decreased by the presence of the endophyte, as observed by Schmidt et al. (1986). They showed that 96% of beef heifers raised on endophyte-free tall fescue calved compared to 55% of those raised on endophyte-infected tall fescue. A second study conducted by Gay et al. (1988) showed similar results. Their

three-year study revealed that calving rates were 94.6% and 55.4% for cows grazing endophyte-free and endophyte-infected tall fescue, respectively.

Milk Production

Another problem of fescue toxicosis is decreased milk. The effect of consuming endophyte-infected tall fescue, range from a decrease in milk production to agalactia (Strickland et al., 1993; Strahan et al., 1987). The most likely cause of decreased milk production is the decrease in serum prolactin levels. Beef cattle fed endophyte-infected fescue at the Black Belt Experiment Station in Alabama showed a 43% reduction in milk yield and weaned calves were 50 kg lighter than cows on endophyte-free fescue (Hill et al., 1985). In 1992, Peters et al. reported that cows grazing endophyte-infected fescue produced 25% less milk than cows grazing either endophyte-free tall fescue or orchard grass pastures. Milk production was estimated to decline by 0.15 kg/d for each 10% increase in pasture infestation level (Danilson et al., 1986). Calf weights associated with nursing cows grazing E+ were significantly reduced compared to those grazing E- (Gay et al., 1988).

ENDOPHYTE FREE TALL FESCUE

Plant breeders developed endophyte free (E-) cultivars by removing the wild-type ergot alkaloid producing endophyte from the seed (Andrae, 2002). Endophyte-free tall fescue does not have any negative affects on growth rate or reproduction of the cattle. However, plant persistence is lower in E- than in E+ tall fescue (Hill et al., 1991), and higher level of management is required to maintain E- stands. Therefore the endophyte-free stands frequently result in failure (Andrae, 2002).

NON-ERGOT ALKALOID PRODUCING ENDOPHYTE-INFECTED TALL FESCUE

This new approach to reducing tall fescue toxicosis was developed by re-infecting E- tall fescue cultivars with a non-ergot alkaloid producing endophyte strain. Research trials were conducted on both lambs (Fletcher et al, 2000; Bouton et al., 2002) and steers (Nihsen et al., 2000) and showed that non-ergot alkaloid producing endophyte-infected tall fescue pastures had similar performance results to those on E- tall fescue, had greater results than those on E+ tall fescue, and did not have signs of toxicosis. One of the first new non-ergot alkaloid producing endophytes is commercially available as MaxQ^{TM} and is marketed in Jesup and Georgia 5 tall fescue cultivars by Pennington Seed of Madison, Georgia (Andrae, 2002). The best combination, Jesup with MaxQ^{TM}, possessed greater yield and better survival than E- and was equal to the fescue with a wild endophyte (Bouton et al., 2002). Also animal average daily gains were equal to those obtained from E- forage and greater than those consuming E+ pastures, and animals did not have the depressed prolactin or elevated body temperature seen in animals grazing E+.

Another non-ergot alkaloid producing endophyte and tall fescue cultivar has been released by researchers in Arkansas as 'Hi Mag". Nihsen et al. (2000) used steers to evaluate three tall fescue cultivars, E-, Hi Mag and E+. The results showed that growing steers grazing Hi Mag perform as well as steers grazing E-. The results of this study are presented in Table 1. All table and figures are located in the Appendices.

The novel endophyte fescues appear to have the potential to provide the livestock producer with forage that has the stand persistence of E+ fescue and the animal production positives provided by E- fescue (Best et al., 2002).

SUPPLEMENTATION

Supplementing cattle, grazing tall fescue, in the form of concentrated feeds or harvested forages can reduce the toxic effects of the endophyte on cattle (Roberts, 2000). When selecting a supplement it is important to know how it will affect forage intake (McCollum, 1997). To achieve acceptable levels of production, many producers supplement cattle that graze or consume harvested forages with feeds that predominantly provide either energy or protein (Bodine et al., 2001). The nutrient composition of four feedstuffs commonly used to supplement beef cattle grazing tall fescue are presented in Table 2.

Dehydrated Alfalfa

Alfalfa is a legume. Legumes are nitrogen-fixing plants that extract nitrogen from the atmosphere and incorporate it into plant proteins (Bruce et al., 2005). Alfalfa is grown throughout the U.S, contains 15-22% crude protein, and is an excellent source of vitamins and minerals. The quality of alfalfa protein is excellent with more than 70 percent of its total protein being digestible.

Corn Grain

Corn is commonly used as a feed grain in beef cattle diets. Compared to other feed grains corn is lower in protein and slightly higher in energy. Corn is also high in starch, which can negatively affect forage utilization, especially in diets based on lower quality forages (Lardy, 2002). Therefore, corn grain should be fed in forage-based diets at low levels. Although feeding corn at a rate of 1% of body weight (BW) can be effective, it may interfere with efficient forage fiber digestion. So, feeding corn at a rate of 0.6% of

BW allows efficient digestion of forage fiber, lowers feed costs, and reduces the effect of toxicity (Roberts, 2000).

Corn Gluten Feed

Corn gluten feed is the part of the commercial shelled corn that remains after the extraction of the larger portion of the starch, gluten, and germ by the processes of the wet milling manufacture of corn starch or syrup. Corn gluten feed is a viable source of protein and energy for cattle that are grazing low and moderate quality forages (Fleck and Lusby, 1986; Fleck et al., 1987; Willms et al., 1992; Cordes et al., 1988). It is a moderately high source of protein (approximately 20-25%), low in starch (approximately 20%), high in digestible fiber and low in oil. Because of these characteristics cattle can be fed in large amounts (Schroeder, 1997).

Soybean Hulls

Soybean hulls are a by-product of soybean processing for oil and meal. Typically, soybean hulls do not require special processing to feed. The nutrients in soybean hulls are highly digestible and are considered an energy feed as opposed to a roughage feed. Soybean hulls are high in NDF and ADF, but they are low in lignin (2% lignin: NRC 1996), resulting in an *in vitro* DM digestibility that may exceed 90% (Quicke et al., 1959). Soybean hulls are frequently used as a supplemental, high-fiber, energy feed supplemented to cattle grazing poor-quality pastures.

IN SITU

In situ techniques allow us to study digestion within the rumen itself and reduce the need for ruminal simulation (Vanzant et al., 1998). It gives the ability to compare ruminal degradation characteristics among different feedstuffs. The technique is

described as placing bags that have a variety of feedstuffs in the rumen of cattle and measuring the amount of nutrients that have disappeared over a certain amount of time (Stern et al., 1997). It is a relatively simple, low cost method compared with methods involving intestinally cannulated animals. The *in situ* method uses fabric bags made of polyester. These bags contain many small pores, which allow for the bacteria and ruminal fluid to pass into the bag where they can begin to breakdown the feed (Cox et al., 2003). Material that disappears from the bag is considered to be digested (Church, 1988).

There are a number of factors associated with the *in situ* method that need to be considered to make this technique efficient. These factors include porosity of bag material, ratio of sample weight to bag surface area, particle size of sample, method of bag placement in the rumen, diet of animal, frequency of animal feeding, and degree of bacterial attachment to feed residues remaining in the bag (Lindberg, 1985; Nocek, 1988; Michalet-Doreau and Ould-Bah, 1992).

Porosity of the bag is important because selection of the appropriate pore size represents a compromise between allowing sufficient influx of digestive agents, permitting adequate efflux of digestion end products, and minimizing influx of ruminal digesta residues and efflux of small sample particles (Vanzant et al. 1998). The suggested pore size is 40 to 60 μm. Another important factor is ratio of sample weight to bag surface area. Research has shown that increased sample size: surface area resulted in decreased *in situ* disappearance by altering the bag size, the sample size or both (Van Keuren and Heinemann, 1962; Figroid et al., 1972; Van Hellen and Ellis, 1977; and Varga and Hoover 1983). The sample size: surface area ratio should be calculated with the following equation: sample size (mg)/(bag width [cm]* bag length [cm] *2) (Vanzant

et al., 1998), which is recommended to be no more than 10 mg of dry matter per cm^2 (Fahey et al., 1994). Particle size is also an important factor because grind size used for samples interacts with pore size in its effects on disappearance of feed. Grinding increases degradation of protein in feeds; however the increase in degradation varies with the type of feedstuff (Michalet-Doreau and Cerneau, 1991). The suggested sample processing size is 2-mm in a Wiley mill (Vanzant et al., 1998).

A few other factors to consider while performing an *in situ* experiment are procedural concerns such as: pre-incubation, timing of insertion and removal of bags, placement of bags in the rumen and rinsing procedures. Pre-incubation is wetting of the Dacron bags before placing them in the rumen. Theoretically, wetting samples before placing them in the rumen could enhance immediate accessibility of the microorganisms to the substrates (Vanzant et al., 1998). There are two methods for timing of bags; the standard method is to place all bags in the rumen at once and take them out at different times. The other method is to place them in at different times and take them all out at once at the end of the experiment. Bags can also be placed in different locations in the rumen. The standard location is in the ventral sac. Another factor to consider is the rinsing technique. Balch and Johnson (1950) described a hand washing procedure for incubated bags that was determined to be complete when the water ran clear. The alternative method is to use a washing machine.

In addition to the *in situ* method, nutrient digestion of forages and supplemental feedstuffs can be determined by using *in vitro* procedure in the laboratory. The *in vitro* technique most commonly used is the Tilley and Terry method (1963). It is used to predict digestibility and as a selection tool for improving the nutritional quality of forages

(Stern et al., 1997). Several factors can affect *in vitro* digestion, such as: dilution of ruminal inoculum, type of buffer, sample particle size, type of mill used for grinding, and the type of diet the donor animal is fed (Stern et al., 1997).

PART III

EFFECT OF ENDOPHYTE STATUS ON RUMINAL CONTENT AND DIGESTION OF FEEDSTUFFS

ABSTRACT

Three 1.2-ha tall fescue (*Festuca arundinacea*, Schreb.) pastures containing 1) Kentucky-31 endophyte (*Neotyphodium coenophialum*) infected tall fescue (KY-31/E+), 2) Jesup endophyte-free tall fescue (Jesup/E-) or 3) Jesup tall fescue infected with a non-ergot alkaloid producing endophyte (Jesup/MaxQ) were grazed. Objectives were to: 1) validate endophyte status of the experimental pastures, 2) compare weight and composition of rumen contents of steers grazing tall fescue pastures that differ in endophyte status, 3) determine effect of time of grazing on *in situ* and *in vitro* nutrient disappearance of feedstuffs, and 4) compare nutrient composition of rumen content to masticate samples. Two ruminally cannulated steers were randomly assigned to each pasture in early May. After an 18-d acclimation period, steers were subjected to four 0700 and four 1900 evacuations; contents were weighed and sub-sampled for DM, NDF, ADF, and CP analyses. After rumens were evacuated, steers were returned to their respective pastures to graze for 45 min. Masticate samples were collected from the rumen to determine pasture forage quality. Between evacuation days, *in situ* degradation of dehydrated alfalfa, corn grain, corn gluten feed, and soybean hulls was performed by incubating them in the rumen of steers grazing the forage treatments. Samples were incubated for 12 h, from 0700 to 1900 and from 1900 to 0700, to evaluate differences in day and night ruminal environments. Samples incubated *in situ* were analyzed for DM, NDF, ADF, and CP disappearance. Steers gained less ($P < 0.05$) weight while consuming KY-31/E+ (0.29 kg/d) than Jesup/E- (0.65kg/d) and Jesup/MaxQ (0.70kg/d). Rectal temperature was higher ($P < 0.05$) at 1900 in steers consuming KY-31/E+ compared to Jesup/E- and Jesup/MaxQ. Rumen DM contents for steers grazing Jesup/E- and

Jesup/MaxQ were greater ($P < 0.05$) than steers grazing KY-31/E+ and 1900 content weights were greater than 0700 content weights, indicating that steers consume more during the day than overnight. Rumens contents and masticate samples were different ($P < 0.05$) indicating that rumen content sampling should not be used to evaluate forage quality of pastures. *In situ* DM, NDF, ADF, and CP disappearance of corn grain was greater ($P < 0.05$) in steers grazing Jesup/E- and Jesup/MaxQ than in steers grazing KY-31/E+, while soybean hull disappearance was not affected by pasture. Degradation of substrates was not affected by time of day of incubation.

INTRODUCTION

Tall fescue toxicosis is one of the most costly plant origin problems facing the beef cattle industry in the United States (Allen and Segarra, 2001). Signs of fescue toxicosis include reduced dry matter intake, reduced weight gains and reduced reproductive efficiency (Paterson et al., 1995). Removing the fungus from the plants by use of fungicides and heating of seed prior to planting resulted in an endophytic-free plant. However these endophyte-free (E-) plants do not survive under environmental and grazing conditions commonly found in the mid-south transition zone of the US. In recent years, new endophytic fungi that do not produce ergot alkaloids have been introduced into adapted cultivars of tall fescue (Fribourg and Waller, 2004). Cattle grazing endophyte-infected (E+) tall fescue have reduced dry matter intake compared to animals consuming E- and cultivars containing non-ergot alkaloid producing endophytes (Parish et al., 2003). Data on differences in the rumen fill and composition of animals grazing tall fescue with different endophyte status is limited.

Supplementation strategies to reduce tall fescue toxicosis have not taken into consideration the potential differences in the rumen environment of steers grazing endophyte-infected, endophyte-free and non-ergot producing endophyte-infected tall fescue. The *in situ* technique for determining nutrient disappearance is closely linked to the ruminal environment in which incubations occur, while *in vitro* techniques used to screen feedstuffs for use by ruminants depends heavily on rumen microbes from a known environment.

The objectives of this study were: 1) to validate endophyte status of the experimental pasture through determining ergovaline levels of masticate samples and animal response using serum prolactin concentrations, weight gain, and rectal temperatures, 2) to compare weight and composition of rumen contents of cannulated beef steers grazing tall fescue pastures differing in endophyte status, 3) to determine the effect of time of grazing on the *in situ* and *in vitro* nutrient disappearance of commonly used supplemental feedstuffs, and 4) to compare nutrient composition of rumen content to masticate sampling of the pastures.

MATERIALS AND METHODS

General Procedures

This study was conducted in the early May to mid July 2003 at the Blount Livestock Unit of the Knoxville Experiment Station, Knoxville, TN. Three 1.2 ha pastures were used with one of three assigned pasture treatments: 1) Kentucky-31 endophyte (*Neotyphodium coenophialum*) infected tall fescue (KY-31/E+). 2) Jesup endophyte-free tall fescue (Jesup/E-), or 3) Jesup infected with a non-ergot alkaloid producing endophyte tall fescue (Jesup/MaxQ^{TM}).

Pastures

The pastures were seeded with tall fescue in September 2001. The endophyte-infected and endophyte-free seed was obtained from Plant Science Farm of the Knoxville Experiment Station. This seed source was developed in 1989 and has supplied seed of known endophyte status for research studies conducted at the University of Tennessee and other research stations in the mid-south region of the United States. The non-ergot alkaloid producing endophyte-infected seed, Jesup/MaxQ^{TM}, was from Pennington Seed Company; Madison GA. Pastures were prepared by taking soil samples to determine soil pH and fertility status of area. Lime was applied when pH was below 6.0. P and K fertilizers were applied as needed to provide medium levels of fertility based on soil tests. Old tall fescue pastures were grazed close in late April to avoid seed production. Pastures were sprayed with 1375 ml/ha of paraquat (Gramoxone Extra7) when tall fescue was 5 to 7 cm tall. After green regrowth occurred (10-15 d) area was sprayed again with 1375 ml/ha of paraquat. Each paraquat application included 1-2 L of non-ionic surfactant per 378 L of spray. Perennial weeds were controlled by applying glyphosate (Roundup7) at 2.75 ml/ha with 2 to 4 L of surfactant per 378 L of spray. In late May, Roundup-Ready soybeans were drilled into tall fescue stubble. In early September the soybeans were chopped with a forage silage harvester and the material was blown back on the field to serve as mulch for drilling in the new seed. In early September the designated tall fescue cultivars were planted using a no-till drill. During late spring through summer of 2002 all pastures were grazed using moderate stocking rates. In early September 2002 cattle were removed and all pastures were fertilized with 56 kg of N/ha and forage was then allowed to grow until early December. Steers were then placed on the pasture at a moderate

grazing pressure until mid-February. They were removed and returned to pasture in late March, again at a moderate grazing pressure, which consisted of four animals per 1.2 ha.

Animal Management

Six Angus steers from the Knoxville Experiment Station Blount Beef Unit Herd, Alcoa, TN. were surgically fitted with rumen cannulas in January 2002. After recovery from surgery, these steers were used in several metabolic feeding studies until assigned to pastures used for this research. Steers were randomly assigned to 1.2-hectare pastures of tall fescue at the Blount Beef Unit. They were assigned, on April 23, 2003 in pairs, to one of three pasture treatments 1) Kentucky-31 endophyte (*Neotyphodium coenophialum*) infected tall fescue (KY-31/E+), 2) Jesup endophyte-free tall fescue (Jesup/E-), or 3) Jesup infected with a non-ergot alkaloid producing endophyte tall fescue (Jesup/MaxQ). Steers continuously grazed the pastures and had free access to water, salt, trace minerals, and shade. Steers were acclimated to the treatments for 18 d prior to sampling. All procedures involving animals were conducted under a protocol approved by the University of Tennessee Animal Care and Use Committee (protocol #1168).

Data Collection

Steers were weighed on two consecutive days at the beginning and end of the trial and single day weights were obtained at 21 d intervals. Every time the steers were handled, for a part of the experiment, rectal temperatures were obtained using an electric thermometer and recorded. Blood was collected on two consecutive weigh dates, June 4 and June 25, 2003, for serum prolactin analysis. Whole blood was obtained from the jugular vein by venipuncture into a 20 ml vacuum tube, placed on ice and allowed to clot. Serum was separated by centrifugation at 20,000 x g. Blood serum was stored in pre-

labeled glass vials and frozen at -20ºC. Serum samples were thawed and samples were analyzed using radioimmunoassay procedures described by Bernard et al. (1993) to determine serum prolactin concentration (ng/ml).

***In Situ* Procedures**

Feed substrates: 1) dehydrated alfalfa, 2) corn grain, 3) corn gluten feed, and 4) soybean hulls, were evaluated for rate of nutrient disappearance in the rumen of steers grazing KY-31/E+, Jesup/E-, and Jesup/MaxQ tall fescue pastures during day grazing (0700 to 1900) and night grazing (1900 to 0700). Nutrient composition of substrates is presented in Table 3.

Substrates were ground to pass a 2-mm screen in a Wiley Mill (Arthur Thomas Company, Philadelphia, PA). Substrate samples were analyzed for DM, OM, CP, NDF and ADF. A 5.0 g sample of each substrate was weighed into a separate labeled pre-weighed #R1020 Dacron bag that was 10 cm x 20 cm, 50 µm to 70 µm pore size (ANKOM Technology, Fairport, NY). Bags were sealed with a heat sealer. A set of four Dacron bags, each containing one of the four substrates, was prepared for each steer and insertion time.

A set of Dacron bags, contained in a nylon mesh bag, was pre-incubated in warm water for five min prior to placement in the rumen. The mesh bags (used to prevent the loss of Dacron bags in the rumen) were placed under the particulate mat of each steer at 1900 of the first sampling period. Bags were allowed to incubate over night for 12 h. At 0700 the next morning, bags were removed and a new set of bags was placed in the rumen and again allowed to incubate for 12 h during the day. The second set of bags was

removed at 1900. This procedure was repeated for three more sampling periods. This resulted in a total of four day grazing incubations and four night grazing incubations.

After bags were removed, they were hand washed in cold water. Bags were rinsed until water ran clear; at that point bags were laid flat in a pan and placed in a 55ºC oven to dry. After 48 h of drying the bags were removed from the oven and allowed to equilibrate to lab environment. Bags were then weighed to determine disappearance of DM of the substrates. Percent dry matter disappearance was calculated in the following manor: [(initial weight - residual weight)/initial weight]*100%.

In situ incubated residue samples were analyzed for N (LECO FP-2000, LECO Corporation, St. Joseph, MI). Resulting N content was multiplied by 6.25 to obtain CP. Neutral detergent fiber and ADF were determined on the substrate residue using ANKOM Technology with F57 filter bags (ANKOM 200 Fiber Analyzer, ANKOM Co., Fairport, NY).

***In Vitro* Procedures**

Feed substrates: 1) dehydrated alfalfa, 2) corn grain, 3) corn gluten feed, and 4) and soybean hulls, were evaluated for extent of dry matter disappearance in rumen fluid obtained from steers grazing KY-31/E+, Jesup/E-, and Jesup/MaxQ tall fescue at 0700 and 1900.

Substrates were ground to pass a 2-mm screen in a Wiley Mill (Arthur Thomas Company, Philadelphia, PA). A 0.5 g sample of each substrate was pre-weighed into a 125-mL bottle. For each pasture (KY-31/E+, Jesup/E-, and Jesup/MaxQ) a set of four bottles (one bottle for each substrate within each pasture) were prepared.

On the day prior to sampling, 400-mL of McDougal's buffer was added to three round bottom flasks (one flask for each pasture treatment). Flasks were placed in a water bath at 39ºC to allow them to reach a steady state temperature comparable to rumen environment.

All steers from KY-31/E+, Jesup/E-, and Jesup/MaxQ pastures were brought to the holding area and placed in a headgate. Rumen fluid samples were collected, via rumen cannula, in a pre-warmed 2-L thermos. Approximately 1-L of fluid was collected from each steer by straining rumen digesta through two layers of cheesecloth. Rumen fluid obtained from steers within the same pasture was combined in a thermos representing each pasture treatment.

After collection was complete rumen fluid was transported to the Ruminant Nutrition Laboratory on campus. At the laboratory Carbon Dioxide (CO_2) was infused into the three round bottom flasks (one for each pasture treatment) containing buffer while rumen fluid preparation occurred. Each thermos was filtered through four layers of cheesecloth and a layer of glass wool into graduated cylinder gassed with CO_2. One hundred mL of rumen fluid from each pasture was added to the 400 mL of pre-warmed buffer in the round bottom flask designated for that pasture source. The final ratio of rumen fluid to buffer was 1:4. Carbon dioxide was bubbled through the mixture of rumen fluid and buffer for 5.0 min.

Fifty mL of the rumen fluid and buffer mixture was transferred from the source designated round bottomed flask to each of the four 125 mL bottle containing the pre-weighed substrate. This process was repeated for each pasture treatment for a total of 12 bottles (three pastures treatments with four different substrates) for each incubation. The

bottles were then capped and placed in a 39ºC shaker water bath for 96 h. The bottles were then filtered and the residue substrate sample was collected in pre-weighed coarse porosity sintered glass crucibles. The crucibles were dried in a100ºC oven and weighed. Percent DM disappearance of the substrates was determined by [(initial pre-digested weight - residue weight)/initial pre-digested weight]*100%. This procedure was repeated for day grazing and night grazing during each of the four sampling periods.

Rumen Evacuations

Rumen evacuations were completed to determine total weight of rumen contents and to evaluate the nutrient profile of the rumen contents. Evacuations were carried out for a day grazing and a night grazing during each of the four sampling periods.

Steers were brought to the holding area and placed in a headgate, where they were haltered. Steers were tied to a fence and total rumen contents were removed via the rumen fistula. Rumen contents were placed in pre-weighed 125-L plastic barrels (Rubbermaid®, Newell Rubbermaid Freeport, IL) and weighed on a digital platform scale. Total weight of each barrel was recorded. The barrels were then hand mixed. Duplicate samples were taken from each steer (~750 g) and placed in pre-weighed aluminum pans. Pans were weighed to determine total sample wet weight, and then placed in 55ºC oven for 48 h or until dry. After samples were dried they were removed from the oven and a dry weight was obtained. Percent dry matter was calculated by subtracting [(initial weight – dry weight)/initial weight]*100%.

Dried rumen samples were then ground to pass a 1-mm screen in a Wiley Mill (Arthur Thomas Company, Philadelphia, PA) and analyzed for DM in 100ºC oven and OM (AOAC, 1990), N (LECO FP-2000, LECO Corporation, St. Joseph, MI) which was

multiplied by 6.25 to determine CP. Neutral detergent fiber and ADF determined using ANKOM Technology F57 Filter Bags (ANKOM 200 Fiber Analyzer, Ankom Co., Fairport, NY).

Masticates

Masticate samples were concurrently collected immediately after rumen evacuations. After complete evacuation, rumens were washed with warm water and a sponge to remove any excess particulate matter and cups were used to remove excess fluid. Steers were then returned to their respective pastures, allowed to graze for about 45 min, and then fresh masticate samples were obtained via rumen cannula for pasture nutrient composition. Duplicate samples were obtained from each pasture and placed in pre-weighed 1-L aluminum pans and masticate contents were weighed for total sample weight. After masticate samples were taken, the original rumen contents were replaced in the steers before being returned to their pasture. Masticate samples were placed on ice until returned to the Ruminant Nutrition Laboratory.

At the lab, masticate samples were placed in a -20ºC freezer until lyophilized in a Vitris: Freezemobile 24 until dry. Sample weights were obtained. Samples were ground to pass a 1-mm screen in a Wiley Mill (Arthur Thomas Company, Philadelphia, PA)

Masticates samples were analyzed for DM, OM, N which was multiplied by 6.25 to obtain CP, NDF and ADF. Ergovaline levels were determined in masticate samples collected in May, June and July from Ky-31/E+, Jesup/E-, and Jesup/MaxQ using the procedure described by Rottinghaus et al. (1992).

Rumen Temperatures

Rumen temperatures were continuously monitored throughout the experiment using Hobo sensors. The sensor microchips were encased in waterproof capsules with the sensor wire running through an opening in the top of the capsule. A small hole was drilled through the outer portion of the steers' cannulas to anchor the capsule. The sensor wire was then placed in the rumen via the cannula and temperatures were continuously recorded every 15 minutes. Two temperature and humidity weather stations were used to record ambient temperature near the research pastures. These recorded temperatures every 15 minutes and the internal clocks of the weather stations and the temperatures sensors on the animals were synchronized.

Statistical Analysis

A randomized block design with a split plot was used for the *in situ* and *in vitro* study. Days were blocked to control for variation between days. Pasture was in the whole plot and time of day was in the sub-plot. Data were analyzed using PROC MIXED, mixed model analysis of variance procedure of SAS (SAS, 2002) with pasture, time of day, and pasture by time of day interactions as fixed effects. Data from *in situ* study were analyzed, for each substrate (alfalfa, corn, corn gluten feed, and soybean hulls), for DM disappearance, NDF, ADF, and CP. Data from the *in vitro* study was analyzed for each substrate for DM disappearance. Significant treatment interactions were separated using least significant differences features in SAS.

A complete randomized design was used for rumen contents, masticate samples, rectal and ruminal temperatures, body weights, serum prolactin, rumen content weights, and rumen content versus masticate samples. Data were analyzed using PROC MIXED,

in SAS (SAS, 2002) with pasture, time of day, and pasture by time of day interactions as fixed effects. The data for rumen samples and masticate samples were analyzed for DM, OM, NDF, ADF, and CP. Linear regression was run to determine if nutrient composition of rumen samples could predict nutrient composition of masticate samples. Significant treatment interactions were separated using least significant differences features in SAS.

RESULTS

All pastures used in this study were vegetative and had adequate forage mass to support *ad libitum* intake. Determination of ergovaline levels in the masticate samples confirmed the endophyte status of the pastures used in this study. The endophyte-infected pasture had 83, 63, and 82 ppb ergovaline on May 14, June 14, and July 14, 2003, respectively. The endophyte-free pasture (Jesup/E-) and non-ergot alkaloid producing endophyte-infected pasture (Jesup/MaxQ) had 0 ppb ergovaline on each of three sampling dates.

Steers were weighed on 21-d intervals to determine overall weight gain while consuming each pasture. Steers that grazed KY-31/E+ gained less ($P < 0.05$) total weight than steers grazing Jesup/E- and Jesup/MaxQ (Figure 1). The ADG was similar for Jesup/E- and Jesup/MaxQ (0.65, and 0.70 kg/d, respectively) and less ($P < 0.05$) for steers consuming KY-31/E+ (0.29 kg/d). Rectal temperatures taken at 1900 were affected by the presence of ergovaline than those taken at 0700. Temperatures taken at 1900 were higher ($P < 0.05$) in steers consuming KY-31/E+ tall fescue than Jesup/E- or Jesup/MaxQ tall fescue (Figure 2). At 0700, there was not a difference ($P > 0.05$) in rectal temperatures (Figure 3). Changes in serum prolactin have been used an indicator of

endophyte status of tall fescue pastures. Serum prolactin concentrations were lower ($P > 0.05$) in steers consuming KY-31/E+ than Jesup/E- or Jesup/MaxQ (Table 4).

Rumen evacuations were conducted to determine total weight of rumen contents. Weight of evacuated contents taken at 0700 was lighter than those taken at 1900 ($P < 0.005$). At 0700, KY-31/E+ had significantly lower ($P < 0.001$) rumen content weight than Jesup/E- and Jesup/MaxQ (Figure 4). At 1900, rumen content weights were different ($P < 0.0001$) for KY-31/E+, Jesup/E-, and Jesup/MaxQ having weights of 37.5, 42.2, and 49.4 kg, respectively (Figure 5).

Rumen content DM was significantly different between 0700 and 1900 evacuations ($P < 0.05$) (Table 5) with DM higher at 1900. However, DM was not ($P > 0.05$) different between pastures. Organic matter of rumen contents tended to be different ($P = 0.09$) between pastures and it was lower ($P < 0.05$) at 0700 compared to 1900. Neutral detergent fiber of rumen contents was not different ($P > 0.05$) between pastures, but was different ($P < 0.05$) between the 0700 and 1900 with NDF lower at 1900 than at 0700. Acid detergent fiber and CP of rumen contents were not different ($P > 0.05$) between pastures or evacuation times. There was no pasture x time interaction ($P > 0.05$).

Masticate DM and nutrient composition was not different ($P > 0.05$) (Table 6) among pastures. However, there were significant differences due to time of sampling. Dry matter was lower ($P < 0.05$) at 0700 than at 1900. Organic matter tended ($P = 0.11$) to be higher at 1900. Neutral detergent fiber was not different ($P > 0.05$) between sampling times. Acid detergent fiber was lower ($P < 0.05$) at 1900 than 0700. Crude protein tended ($P = 0.06$) to be higher at 0700 than at 1900. There was no ($P > 0.05$) pasture x time interaction.

The comparison of rumen content to masticate samples in KY-31/E+ showed that DM was different ($P < 0.05$), however CP and NDF, were not different ($P > 0.05$). Acid detergent fiber tended ($P = 0.06$) to be less for masticate samples than in the rumen content. In Jesup/E-, DM was less ($P < 0.05$) in masticate samples, ADF tended ($P = 0.06$) to be less and there was no difference ($P > 0.05$) for NDF, and CP compared to rumen content. For Jesup/MaxQ, CP was not different ($P > 0.05$) between rumen content and masticate samples. Dry matter, ADF, and NDF were lower ($P < 0.05$) in masticate samples than rumen content. Regressing nutrient composition rumen content on nutrient composition of masticate samples resulted in R^2 values of 0.17, 0.05, 0.09, 0.12, and 0.00 for DM, OM, NDF, ADF, and CP, respectively.

Alfalfa *in situ* DM disappearance tended ($P = 0.12$) to be less for KY-31/E+ than Jesup/E- or Jesup/MaxQ. Alfalfa NDF disappearance ($P = 0.12$) and ADF disappearance ($P = 0.14$) tended to be less for KY-31/E+ than for Jesup/E- and Jesup/MaxQ. Alfalfa CP disappearance was less ($P < 0.05$) for KY-31/E+ compared to Jesup/E- and Jesup/MaxQ (Figure 6). Corn *in situ* DM disappearance was less ($P < 0.05$) for KY-31/E+ compared to Jesup/E- and Jesup/MaxQ. Corn *in situ* NDF disappearance in KY-31/E+ was less ($P < 0.05$) than in Jesup/E- and Jesup/MaxQ. Corn *in situ* ADF disappearance was not different ($P > 0.05$) between pastures. Corn *in situ* CP disappearance was less ($P < 0.05$) for KY-31/E+ compared to Jesup/E- and Jesup/MaxQ (Figure 7). Corn gluten feed *in situ* DM disappearance tended ($P = 0.08$) to be lower for KY-31/E+ compared to Jesup/E- and Jesup/MaxQ. Corn gluten feed *in situ* NDF ($P = 0.12$) and ADF ($P = 0.10$) disappearance tended to be lower for KY-31/E+ compared to Jesup/E- and Jesup/MaxQ.

Corn gluten feed *in situ* CP was not different ($P = 0.20$) (Figure 8). Soybean hull *in situ* DM, NDF, ADF, and CP were not different ($P > 0.05$) between pastures (Figure 9).

Alfalfa *in situ* DM, NDF, ADF, and CP disappearance was not different ($P > 0.05$) due to time of day incubated (Table 7). Corn *in situ* DM disappearance tended ($P = 0.08$) to be less during 0700 to1900 incubation than the 1900 to 0700 incubation in steers consuming KY-31/E+ tall fescue (Table 8). Neutral detergent fiber, ADF, and CP disappearance were not different ($P > 0.05$) between times of day of incubation. In steers consuming Jesup/E- tall fescue pastures, DM, NDF, and CP disappearance were not different ($P > 0.05$) between of time of day of incubation. However, ADF disappearance tended ($P = 0.06$) to be lower during 1900 to 0700 incubation. Dry matter, NDF, ADF, or CP disappearance was not different ($P > 0.05$) between times of day for incubation of corn in steers consuming Jesup/MaxQ. Corn gluten feed *in situ* DM, NDF, ADF, and CP disappearance did not differ ($P > 0.05$) by time of day incubated (Table 9). Soybean hulls *in situ* DM, NDF, and ADF disappearance did not differ ($P > 0.05$) between times of day of incubation in steers consuming KY-31/E+ tall fescue (Table 10). CP disappearance did have a tendency ($P = 0.12$) to be slower during 0700 to 1900 compared to 1900 to 0700. Soybean hulls DM, NDF, ADF, and CP disappearance did not differ ($P > 0.05$) between times of incubation in steers consuming Jesup/E-, or Jesup/MaxQ tall fescue pastures.

Rumen temperatures were also continuously monitored throughout the trial. The results of rumen temperature were highly variable and a comparison of pasture treatments was not feasible. These variations in readings were probably caused by opening and closing of the rumen cannula and placement of the probe into the rumen.

In vitro analysis was conducted to determine if there was a difference in DM disappearance of alfalfa, corn, corn gluten feed, and soybean hulls in rumen fluid collected from steers consuming KY-31/E+, Jesup/E-, and Jesup/MaxQ tall fescue pastures (Table 11). Alfalfa, corn, corn gluten feed, and soybean hull DM disappearance was not significantly affected by the source of rumen fluid. There was no difference in DM disappearance of alfalfa, corn, corn gluten feed, and soybean hulls between times of rumen fluid sampling.

DISCUSSION

Bacon et al. (1977) reported that the presence of an endophytic fungus in tall fescue was correlated to tall fescue toxicosis in cattle. This endophytic fungus produces ergovaline and other ergot alkaloids. In the three-pasture treatments used in our study, we expected to find ergovaline in masticate samples only from the endophyte-infected pasture (KY-31/E+). The absence of ergovaline in masticates from Jesup/E- and Jesup/MaxQ confirmed that these pasture treatments were correctly designated in our research plans. Jesup/MaxQ was produced in US by Bouton and associates (2002) when they re-infected endophyte-free (E-) 'Jesup' tall fescue with different non-ergot alkaloid-producing endophyte strain (AR542) isolated by New Zealand researchers. The AR542 endophyte was selected primarily for its lack of production of ergovaline. We expected to see animal responses commonly associated with tall fescue toxicosis to confirm the presence of ergovaline in the KY-31/E+ tall fescue pasture used in our study.

When steers were first placed on pasture the weights of all steers were similar averaging 259, 260, and 248 kg for KY-31/E+, Jesup/E-, and Jesup/MaxQ respectively. However, after the 18-day adjustment period on pasture, the initial experiment weight

was 243, 268, and 264 kg for KY-31/E+, Jesup/E-, and Jesup/MaxQ respectively. Steers grazing KY-31/E+ lost 16 kg, while steers grazing Jesup/E- and Jesup/MaxQ gained weight. This difference in weight indicates that cattle grazing KY-31/E+ consumed less forage than those grazing Jesup/E- and Jesup/MaxQ and were adversely affected by the ingestion of the ergot alkaloids present in the KY-31/E+ tall fescue. During the remainder of the experiment, steers grazing KY-31/E+ tall fescue had reduced ADG compared to those grazing Jesup/E- and Jesup/MaxQ. These results agree with those reported by Parish et al. (2003) where ADG for steers consuming Jesup/E+ was less ($P < 0.05$) in May, June, and July than Jesup/E- and Jesup/542 (MaxQ).

An explanation for the difference in weight gain became evident when we compared rumen evacuation weights of steers grazing our pasture treatments. Steers grazing KY-31/E+ tall fescue, were consuming less from 0700-1900 ($P < 0.0001$) and from 1900-0700 ($P < 0.001$) than steers grazing Jesup/E- or Jesup/MaxQ tall fescue. Consequently, because they were consuming less fescue, they had reduced nutrient intake, which led to a reduction in weight gain. All steers consumed more forage ($P < 0.005$) during the day (0700-1900) than at night (1900-0700). Higher temperatures during the day and the vasoconstrictive properties of the alkaloids reported by Schmidt and Osborn (1993) would have contributed to the reduction in intake by cattle grazing KY-31/E+ tall fescue. Research reported by Parish et al. (2003) revealed that cattle took less ($P < 0.10$) bites/d while grazing Jesup/E+ than grazing Jesup/E- and Jesup/542 (MaxQ). This verifies that cattle grazing KY-31/E+ consume less forage.

Rectal temperatures at 1900 were higher ($P < 0.005$) in steers consuming KY-31/E+ tall fescue than those consuming Jesup/E- and Jesup/MaxQ tall fescue. Differences

in temperature between pasture treatments were also documented in other research conducted by Parish et al. (2003) and Nihsen et al. (2000). This body temperature difference could hinder steers from grazing because they seek shade to stay cool. Therefore, they are altering their grazing pattern and reducing their nutrient intake and this would result in a reduction in weight gain. The thermoregulation problems seen in steers consuming KY-31/E+ can be caused by the ergot alkaloids produced in KY-31/E+. Oliver et al. (1993) documented that the ergot alkaloids have vasoconstrictive effects on bovine vasculature in animals grazing pastures at the same location as our pastures. Rhodes et al. (1991) stated that reduced peripheral blood flow would reduce the animals' ability to move heat from core tissues to the surface for cooling, which would explain the increase in rectal temperatures. Al-Haidary et al. (2001) reported reduced blood flow over a prolonged period of time could result in tissue death and impair the ability to dissipate heat.

Rumen samples that were collected during rumen evacuations showed there was no difference in nutrient composition between pastures. The difference of DM, OM, CP, NDF and ADF were not significantly different between KY-31/E+, Jesup/E- and Jesup/MaxQ tall fescue. This indicates that there is no difference in nutrient composition of the pastures. However, there was a difference ($P < 0.05$) in time (0700 vs. 1900) of sampling for DM, OM, and NDF.

To determine forage quality, masticate samples could be collected or forage samples could be clipped. Masticate samples are what the steers graze, not just random samples from the field, which offers true pasture forage quality. Olson (1991) indicated that there were no changes in nutritional composition of the masticate to indicate that

being in the rumen affected the samples. Therefore masticate samples would be the best way to evaluate forage nutrient composition. Masticate samples that were collected showed there was no difference between pasture forage quality. The difference for DM, OM, CP, NDF and ADF were not significantly different between KY-31/E+, Jesup/E-, and Jesup/MaxQ. The results indicate there is no difference in growth of the pastures and the presence of the endophyte does not change the composition of the fescue. Time of sampling was not significant enough to be of concern. Samples did not differ between pastures at 0700 and did not differ at 1900; therefore samples can be taken at any time during the day. DM was significantly higher at 1900 than at 0700, which can be explained, again, by the sun drying out the pasture throughout the day, and the morning dew adding moisture to the pasture.

Rumen samples collected during rumen evacuations indicated no significant differences in DM, OM, CP, NDF, and ADF between KY-31/E+, Jesup/E- and Jesup/MaxQ tall fescue pastures. We conclude that pastures were similar in nutrient content regardless of the endophyte status. However, there was a difference ($P < 0.05$) in time of sampling (0700 vs. 1900) for DM, OM, and NDF. This difference may be related to time of forage intake and the moisture content of the forage consumed.

When assessing forage quality of a pasture, obtaining a good representative sample of the forage consumed by grazing animals is important. In our study masticate samples were chosen over clipping of random samples because they represent the plant material selected by the grazing animals. One concern in using masticate samples is the possible contamination of the sample with rumen fluid or particulate matter within the rumen. In our study the rumen contents were removed completely followed by warm

water rinsing to reduce possible contamination. Olson (1991) reported no changes in nutrient composition of the masticate demonstrating that being in the rumen for a short time period did not affect the samples. We concluded that using our protocol for collecting masticate samples would be the best way to evaluate forage nutrient composition. Having found no significant differences in DM, OM, CP, NDF, and ADF of the masticate samples between KY-31/E+, Jesup/E-, and Jesup/MaxQ pastures indicated that these forages were similar in quality regardless of the endophyte status.

There was no difference among pastures when masticate samples were obtained at 0700 and at 1900, leading us to conclude that time of sampling was not of concern when comparing pastures that differ in endophyte status. Therefore, based on our results, samples can be taken either in early morning or early evening.

We compared rumen content with masticate samples based on the hypothesis that there was no difference in DM, NDF, ADF and CP between samples within each pasture. However, this was not true because differences did appear between rumen and masticate samples. In steers grazing KY-31/E+, rumen content samples could be used for CP and NDF composition of pastures, and masticate samples would not need to be obtained. However, masticate samples had significantly less DM and tended to have less ADF than rumen contents.

In steers grazing Jesup/E-, ADF was the only nutrient that tended to be different with masticate samples having less ADF present than rumen contents. Either rumen content samples or masticate samples could be used to determine DM, CP, or NDF content of Jesup/E-. However for Jesup/MaxQ, CP of masticate samples and rumen contents was not significantly different. We conclude that either method would be

sufficient for determining CP regardless of endophyte status of the pastures. Other nutrients reflecting forage quality were different and we do not recommend the use of rumen samples to replace masticate samples. If our proposed hypothesis that no difference existed between rumen and masticate sampling to evaluate pasture quality had been true, then performing complete rumen evacuations would have been unnecessary.

In situ study was conducted using alfalfa because it is a legume similar to clovers, which are often used in tall fescue pastures. It has been shown by Andrae et al. (2003) that the addition of legumes to tall fescue pastures resulted in a positive response in animal performance regardless of the endophyte status of the pasture. They reported steers grazing Jesup/E+ alone had an ADG of 0.76 lbs., while steers grazing Jesup/E+ with clover had an ADG of 1.75 lbs. ($P < 0.05$). The same performance trend was also seen when clover was added to Jesup/E- and Jesup/MaxQ. Legumes have been added to supplement steers consuming endophyte-infected pastures because legumes could possibly reduce the toxic effect of the endophyte. Andrae stated that legumes diluted the ergot alkaloid toxins. Based on animal performance, alfalfa or other legumes would be a good choice when supplementing cattle grazing endophyte-infected fescues. *In situ* results in the current study indicated that alfalfa had a tendency to be degraded less while incubated in steers consuming KY-31/E+, however, reduced degradation was not of sufficient magnitude to warrant concern about using alfalfa as a supplement.

Corn was selected as an *in situ* substrate because the cattle industry commonly supplements with corn grain. However, corn was not degraded as well in KY-31/E+ compared to Jesup/E- and Jesup/MaxQ. The DM, NDF, and CP were degraded less ($P < 0.05$) during incubation in steers consuming KY-31/E+. Therefore corn would not be

utilized efficiently in steers grazing KY-31/E+ tall fescue. The reason why corn is not being efficiently utilized in this study is unknown. It is possible that the microbes may have a problem breaking down the starch, or there may be a microbial shift due to the steers not eating as much tall fescue and consuming the forage at times that alter the rumen environment. As a result the microbes may have to adjust to infrequent substrate addition to the rumen. This study demonstrates supplementing cattle grazing KY-31/E+ tall fescue with corn may result in reduced utilization of the grain consequently reducing expected animal performance.

Corn gluten feed is the supplemental feed of choice for beef cattle producers in east Tennessee because of a large fructose plant located in the region. Corn gluten feed has no starch and a larger amount of fiber compared to corn. Therefore, since we have hypothesized that corn may not be efficiently digested by the microbes due to the high starch, we should expect supplementation with corn gluten feed to have a different response in the rumen than observed with corn. Corn gluten feed incubated in the rumen of cattle grazing KY-31/E+ tall fescue tended to degrade to a lesser extent than when it was incubated in either Jesup/E- or Jesup/MaxQ.

Soybean hulls were evaluated as a supplemental source because they are a good fiber source that provides energy in the form of cellulose rather than starch (i.e. corn grain) or pentose sugars (i.e. corn gluten feed). During incubation of soybean hulls, there was no significant difference in degradation of the substrate among the three different tall fescue pastures. Soybean hulls are highly degradable in the rumen. This may explain why soybean hulls are unaffected by the presence of an endophytic fungus. The alterations in rumen environment when ergot alkaloids are present may only affect substrates requiring

a longer time to degrade. Another reason for selecting soybean hulls was based on other research at Tennessee that reported excellent performance of cattle grazing E+ tall fescue when supplemented with soybean hulls (Pugh, 2003).

To determine whether time of incubation would have an effect on the degradation of the substrates, incubations periods were established to represent supplementation in the evening (1900-0700) and in the morning (0700-1900). However, we concluded that there was no significant difference between periods of incubation of the substrates. Therefore, if any of these feedstuffs were used for supplementing cattle grazing tall fescue the time of feeding would not be critical.

In vitro dry matter disappearance study was conducted to determine if time of rumen fluid collection would alter digestion of these feedstuffs. To accomplish this, rumen fluid was collected at 0700 and at 1900 h. There were no significant pasture differences at either time. This information is important in planning *in vitro* dry matter disappearance studies, in that time of rumen fluid collection does not alter our ability to determine differences in digestion of feedstuffs. Additionally, there was no difference in DM disappearance of substrates in rumen fluid collected from the different tall fescue pastures. This information leads us to conclude that when conducting *in vitro* analysis we should not be concerned about the endophyte status of the pastures grazed by the source animal.

In conclusion, steers grazing KY-31/E+ tall fescue consumed less forage and had lower ADG, higher rectal temperature during the day, and reduced serum prolactin than steers consuming Jesup/E- and Jesup/MaxQ. Rumen content and masticate samples were not different in nutrient composition between pastures. However, the rumen sampling

procedure resulted in different nutrient composition values compared to the masticate sampling procedure indicating a need to continue to collect masticate samples to assess forage quality. The *in situ* study revealed that corn is not utilized well by steers grazing KY-31/E+ tall fescue and would not be a supplement of choice. However, alfalfa, corn gluten feed, and soybean hulls are good supplements for steers consuming endophyte-infected pastures. It was also determined that time of incubation is not of importance for both *in situ* or *in vitro* procedures when comparing tall fescue that differ in endophyte status.

IMPLICATIONS

Cattle grazing endophyte-infected tall fescue had reduced quantity of forages in the rumen. The nutrient composition was similar for all tall fescue pastures regardless of endophyte status of the plants. Therefore, the reduced performance associated with animals consuming endophyte-infected tall fescue is a result of reduced nutrient intake by the grazing animal combined with the adverse affects of the ingestion of the ergot alkaloids. Supplementation strategies need to take into consideration the endophyte status of the forage when selecting feedstuffs. Highly digestible fiber sources such as soybean hulls would be a good choice for supplementing tall fescue pastures because endophyte status did not alter digestion of this feedstuff.

LITERATURE CITED

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Ahlhom, J.U., M. Helander, S. Lehtimaki, P. Wali, and K. Saikkonen. 2002. Vertically transmitted fugal endophytes: Different responses of host-parasite systems to environmental conditions. Oikos 99:177-183.

Al-Haidary, A., D.E. Spiers, G.E. Rottinghaus, G.B. Garner, and M.R. Ellersieck. 2001. Thermoregulatory ability of beef heifers following intake of endophyte-infected tall fescue during controlled heat challenge. J. Anim. Sci. 79:1780-1788.

Allen, V.G., and E. Segarra. 2001. Anti-quality components in forage: Overview, significance, and economic impact. J. Range Manage. 54:409-412.

Andrae, J. 2002. An Update on MaxQ™ Tall Fescue. pp. 7-10 in Proc. 57th Southern Pasture and Forage Crop Improvement Conference Athens, Georgia April 23-25, 2002.

Andrae, J., N. Hill, and J. Bouton. 2003. Effect of tall fescue endophyte status and white clover addition on ergot alkaloids urinary excretion and performance of stocker cattle grazing tall fescue. pp. 18-19 in Tall Fescue Toxicosis Workshop. Southern Extension Research Activity Info. Exchange Group-8. Wildersville, TN.

ANKOM Technology. The ANKOM200 Fiber Analyzer. Fairport, NY.

AOAC, 1990. Official methods of analysis. Association of Official Analytical Chemists, Arlington, VA.

Arechavaleta, M., C.W. Bacon, C.S. Hoveland, and D.E. Radcliffe. 1989. Effect of the tall fescue endophyte on plant response to environmental stress. Agron. J. 81:83-90.

Bacon, C.W., J.K. Porter, J.D. Robbins, and E.S. Luttrell. 1977. Epichloe typhina from toxic tall fescue grasses. Applied and environmental microbiology. 34:576-581.

Bacon, C.W., and M.R. Siegel. 1988. Endophyte parasitism of tall fescue. J. Prod. Agric. 1:45.

Bacon, C.W. 1995. Toxic endophyte-infected tall fescue and range grasses: Historic perspectives. J. Anim. Sci. 73:861-870.

Balch, C.C., and V.W. Johnson. 1950. Factors affecting the utilization of food by dairy cows. 2. Factors influencing the rate of breakdown of cellulose (cotton thread) in the rumen of the cow. Br. J. Nutr. 4:389-395.

Ball, D.M., C.S. Hoveland, and G.D. Lacefield. 1991. Southern Forages. p. 48. Potash and Phosphate Institute. Atlanta, GA.

Ball, D.M., C.S. Hoveland, and G.D. Lacefield. 1996. Southern Forages. pp. 169-174. Potash and Phosphate Institute. Atlanta, GA.

Bates, G. 2004. Novel endophytes in tall fescue. pp. 2-6 in UT Beef and Forage Field Day Proceedings. Knoxville Experiment Station, Knoxville, TN. June 10, 2004.

Belesky, D.P., J.A. Stuedemann, R.D. Plattner, and S.R. Wilkinson. 1988. Ergopeptine Alkaloids in Grazed Tall Fescue. Agron. J. 80:209-212.

Berde, B., and E. Sturmer. 1978. Introduction to the pharmacology of ergot alkaloids and related compounds as a basis of their therapeutic application. In B. Berde and H.O. Schild (ed.) Handbook of Experimental Pharmacology, V. 49 Ergot Alkaloids and Related Compounds. pp. 1-28. Springer-Verlag, Berlin.

Bernard, J.K., A.B. Chestnut, B.H. Erickson, and F.M. Kelly. 1993. Effects of prepartum consumption of endophyte infested tall fescue of serum prolactin in subsequent milk production of Holstein cows. J. Dairy Sci. 76:1928-1933.

Best, T.G., J.L. Howell, J.E. Huston, and R.R. Evans. 2002. Evaluation of fungus free and novel endophyte fescues as roughage sources for developing replacement heifers. pp. 57-59 in Tall Fescue Toxicosis Workshop. Southern Extension Research Activity Info. Exchange Group-8. Wildersville, TN.

Bodine, T.N., H.T. Purvis II, and D.L. Lalman. 2001. Effects of supplement type on animal performance, forage intake, digestion, and ruminal measurements of growing beef cattle. J. Anim. Sci. 79:1041-1051.

Bond, J., and D.J. Bolt. 1986. Growth, plasma prolactin and ovarian activity in heifers grazing fungus infected fescue. Nutr. Rep. Int. 34:93.

Bouton, J.H., G.C.M. Latch, N.S. Hill, C.S. Hoveland, M.A. McCann, R.H. Watson, J.A. Parish, L.L. Hawkins, and F.N. Thompson. 2002. Reinfection of tall fescue cultivars with non-ergot alkaloid-producing endophytes. Agron. J. 94:567-574.

Browning, R. 2003. Tall fescue endophyte toxicosis in beef cattle: Clinical mode of action and potential mitigation through cattle genetics. http://beefimprovement.org/03proceedings/Browning.pdf Accessed on June 1, 2004.

Bruce, B., R. Torell, W. Riggs. 2005. Alfalfa for Beef Cows. In Alfalfa Information Systems, Oregon State University. http://forages.oregonstate.edu/IS/AIS/enfpmain.cfm?PageID=218. Accessed on April 12, 2005.

Bush, L.P., and R.C. Buckner. 1973. Tall fescue toxicity: In A.G. Matches (Ed.) Antiquality components of forages. pp. 99-112. Crop Sci. Soc. Am., Madison WI..

Church, D.C. 1988. The Ruminant Animal. Digestive Physiology and Nutrition. Prentice-Hall Inc. Englewood Cliffs, NJ.

Clay, K. 1988. Fungal endophytes of grasses: A defensive mutualism between plants and fungi. Ecology. 69:1:10-16.

Cordes, C.S., K.E. Turner, J.A.Paterson, J.G.P. Bowman, and J.R. Forwood. 1988. Corn gluten feed supplementation of grass hay diets for beef cows and yearling heifers. J. Anim. Sci. 66:522.

Cox, K.R., J.A. Gibson, C.S. Sowers, G. Scaglia, R.K. Shanklin, and J.P. Fontenot. 2003. A comparison of *in situ* dry matter digestibility of alfalfa hay, fescue hay, and barley straw in beef cattle. www.gsa.vt.edu/projects/papers/AS1paper.pdf. Accessed on June 12, 2004.

Crawford, R. J., J.R. Forwood, R.L. Belyea, and G.B. Gardner. 1989. Relationship between level of endophyte infection and cattle gains on tall fescue. J. Prod. Agric. 2:147.

Danilson, D.A., S.P. Schmidt, C.C. King, L.A. Smith, and W.B. Webster. 1986. Fescue toxicity and reproduction in beef heifers. J. Anim. Sci. 63(Suppl. 1):296.

Ensley, S., and D. Larson. 2001. A field investigation of tall fescue toxicosis in beef cattle in Southern Iowa. 2001 Beef Research Report- Iowa State University. pp. 138-139.

Evans, K.L., P.M. Zavos, R.W. Hemken, and J.A. Jackson Jr. 1988. Effects of feeding endophyte infected (Acremonium coenophialum) KY-31 fescue hay on reproductive development of Holstein bulls. Theriogenology. 30:169.

Fergus, E.N. 1972. A short history of Kentucky-31 fescue. In: Proc. 29th South Pasture Crop Imp. Conf., 16-18 May. Clemson, SC, USDA/ARS, US Govt. Print Office, Washington DC pp. 136-139.

Figroid, W., W.H. Hale, and B. Thuerer. 1972. An evaluation of the nylon bag technique for estimating rumen utilization of grains. J. Anim. Sci. 35:113-120.

Fiorito, J.M., L.D. Bunting, G.M. Davenport, and J.A. Boling. 1991. Metabolic and endocrine responses of lambs fed Acremonium coenophialum infected or non-infected tall fescue hay at equivalent nutrient intake. J. Anim. Sci. 69:2108-2114.

Fleck, A.T., and K.S. Lusby. 1986. Corn gluten feed or soybean meal as winter supplements for pregnant beef cows grazing native range. In: Okla. State Univ. Anim. Sci. Res. Rep., MP-118, p. 196.

Fleck, AT., K.S. Lusby, and F.T. McCollum. 1987. The value of corn gluten feed as a supplement for beef cattle grazing native range. In: Okla. State Univ. Anim. Sci. Res. Rep. MP-119, p. 256.

Fletcher, L.R., C.G. Fletcher, and B.L. Sutherland. 2000. Non-toxic tall fescue endophyte associations. pp. 36 in Tall Fescue Toxicosis Workshop. Southern Extension Research Activity Info. Exchange Group-8. Chapel Hill, TN.

Fribourg, H.A., J.C. Waller, G.C.M. Latch, L.R. Fletcher, H.S. Easton, and A.E. Stratton. 2002. Evaluation under grazing of tall fescue cultivars infected with different endophytes. pp. 91-92 in Tall Fescue Toxicosis Workshop. Southern Extension Research Activity Info. Exchange Group-8. Wildersville, TN.

Fribourg, H., and J. Waller. 2004. Current Trends in Neotyphodium Research and Application-USA. In: C. Roberts, D. Spiers, and C. West. (Ed.) Neotyphodium in Cool-Season Grasses: Current Research and Applications. Blackwell Publishing. Ames Iowa.

Garner, G.B., R. Morrow, T. Fairbrother, J. Gerrish, C. Nelson, V. Jacobs, and W. Hires. 1984. Nursing cows, calves, and steers response to simultaneously grazed tall fescue pastures with varying levels of endophyte *E. typhina*. In: Proc. Am. Forage and Grassl. Conf. Jan. 23-26, Houston, TX. Am. Forage and Grassland Council, Lexington, KY.

Garner, G.B., G.E. Rottinghaus, C.E. Cornell, and H. Testereci. 1993. Chemistry of compounds associated with endophyte/grass interaction: Ergovaline- and ergopeptine related alkaloids. pp. 65-80 in Int. Symp. Acremonium/Grass interactions. Elsevier Science Publishers, Amsterdam, The Netherlands.

Gay, N., J.A. Boling, R. Dew., and D.E. Miksch. 1988. Effects of endophyte-infected tall fescue on beef cow-calf performance. Appl. Agr. Res. 3:182.

Hannaway, D.B. 2005. Tall Fescue Information Systems, Oregon State University. http://forages.oregonstate.edu/is/tfis/enmain.cfm?PageID=94 Accessed on April 12, 2005.

Hill, N.S., and W.C. Stringer. 1985. Surveying for the fungal endophyte. Acremonium coenophialum in tall fescue pastures in South Carolina. p. 18. In Agronomy abstracts. ASA Madison, WI.

Hill, N.S., W.C. Stringer, G.E. Rottinghaus, D.P. Belesky, W.A. Parrott, and D.D. Pope. 1990. Growth, Morphological, and Chemical Component Responses of Tall Fescue to *Acremonium coenophialum*. Crop Sci. 30:156-161.

Hill, N.S., D.P. Belesky, and W.C. Stringer. 1991. Competitiveness of tall fescue as influenced by *Acremonium coenophialum.* Crop Sci. 31:185-190.

Hill, N.S. 1994. Ecological relationships of Balansiae-infected graminoids. p. 59-71. In: C.W. Bacon and J.F. White Jr. (eds.). Biotechnology of endophytic fungi of grasses. CRC Press, Boca Raton, Fla.

Hill, N.S., F.N. Thompson, J.A. Stuedemann, G.W. Rottinghaus, H.J. Ju, D.L. Dawe, and E.E. Hiatt III. 2001. Ergot alkaloid transport across ruminant gastric tissue. J Anim. Sci. 79:542-549.

Hoveland, C.S., S.P. Schmidt, Cocking Jr., J.W. Odom, E.M. Clark, J.A. McGuire, L.A. Smith, H.W. Grimes, and J.L. Holliman. 1983. Steer performance and association of *Acremonium coenophialum* fungal endophyte on tall fescue pasture. Agronomy Journal. 75:821-824.

Hoveland, C.S. 1993. Economic importance of Acremonium endophytes. Agric. Ecosystem. & Environ. 44:3.

Hoveland, C. 2003. The fescue toxicosis story- An Update. Beef Improvement Federation Annual Meeting and Convention Proceedings. Lexington, KY. http://www.beefimprovement.org/03proceedings/Hoveland.pdf. Accessed on June 4, 2004.

Lane, G.A., M.J. Christensen, and C.O. Miles. 2000. Coevolution of fungal endophytes with grasses: The significance of secondary metabolites. pp. 342-388 in Microbial Endophytes, Bacon, C.W., J.F. White Jr. Eds.; Marcel Dekker, New York.

Lardy, G. 2002. Feeding Corn to Beef Cattle. AS-1238. http://www.ext.nodak.edu/extpubs/ansci/beef/as1238w.htm Accessed on June 6, 2004.

Latch, G.C.M. 1997. An Overview of Neotyphodium-grass interactions. pp. 1-11. *In* C. W. Bacon and N. S. Hill (ed.) Neotyphodium/Grass Interactions. Plenum Press, NY.

LECO Corporation. Nitrogen Analyzer. St. Joseph, MI.

Lindberg, J.E. 1985. Estimation of rumen degradability of feed proteins with the *in sacco* technique and various *in vitro* methods. A review. Acta Agric. Scand. Sect. A-Anim. Sci. Suppl. 25:64-97.

Lyons, P.C., and R.D. Plattner, and C.W. Bacon. 1986. Occurrence of peptide and clavine ergot alkaloids in tall fescue grass. Science 232:487-489.

Malinowski, D.P., and D.P. Belesky. 2000. Adaptations of endophyte infected cool season grasses to environmental stresses: Mechanisms of drought and mineral stress tolerance. Crop Sci. 40:923-940.

McCollum, T. 1997. Supplementation strategies for beef cattle. Texas Agricultural Extension Service. http://animalscience.tamu.edu/ansc/publications/beefpubs/b6067-supplementationstrategies.pdf. Accessed on June 6, 2004.

Michalet-Doreau, B., and P. Cerneau. 1991. Influence of foodstuff particle size on *in situ* degradation of nitrogen in the rumen. Anim. Feed Sci. Technol. 35:69-81.

Michalet-Doreau, B., and M.Y. Ould-Bah. 1992. *In vitro* and *in sacco* methods for the estimation of dietary nitrogen degradability in the rumen: A review. Anim. Feed Sci. Technol. 40:57-86.

Mizinga, K.M. F.N. Thompson, J.A. Stuedemann, and T.E. Kiser. 1992. Effects of feeding diets containing endophyte-infected fescue seed on leutenizing hormone secretion in postpartum beef cows and in cycling heifers and cows. J. Anim. Sci. 70:3483-3489.

Moyer, J.L., N.S. Hill, S.A. Martin, and C.S. Agee. 1993. Degradation of ergoline alkaloids during *in vitro* ruminal digestion of tall fescue forage. Crop Sci. 33:264-266.

Muller-Schweinitzer, E., and H. Weidmann. 1978. Basic Pharmacological Properties. In B. Berde and H.O. Schild (ed.) Handbook of Experimental Pharmacology, V. 49 Ergot Alkaloids and Related Compounds. pp. 87-232. Springer-Verlag, Berlin.

Nihsen, M., T. Denard, Z. Johnson, and R. Crawford. 2000. The effects of grazing steers on tall fescue which contained a non-toxic endophyte. pp. 5-7 in Tall Fescue Toxicosis Workshop. Southern Extension Research Activity Info. Exchange Group-8. Chapel Hill, TN.

Nocek, J.E. 1988. *In situ* and other methods to estimate ruminal protein and energy digestibility: A review. J. Dairy Sci. 71:2051-2069.

NRC. 1996. Nutrient Requirements of Beef Cattle (7th Ed.). National Academy Press, Washington, D.C.

Oliver, J.W., L.K. Abney, J.R. Strickland, and R.D. Linnabary. 1993. Vasoconstriction in bovine vasculature induced by the tall fescue alkaloid lysergamide. J. Anim. Sci. 71:2708-2713.

Olsen, K.C. 1991. Diet sample collection by esophageal fistula and rumen evacuation techniques. J. Range Manage. 44:515.

Parish, J.A., M.A. McCann, R.H. Watson, N.N. Paiva, C.S. Hoveland, A.H. Parks, B.L. Upchurch, N.S. Hill, and J.H. Bouton. 2003. Use of non-ergot alkaloid-producing endophytes for alleviating tall fescue toxicosis in stocker cattle. J. Anim. Sci. 81:2856-2868.

Paterson, J., C. Forcherio, B. Larson, M. Samford, and M. Kerley. 1995. The effects of fescue toxicosis on beef cattle productivity. J. Anim. Sci. 73:889-898.

Peters, C.W., K.N. Grigsby, C.G. Aldrich, J.A. Paterson, J. Lipsey, M.S. Kerley, and G.B Garner. 1992. Performance, forage utilization, and ergovaline consumption by beef cows grazing endophyte fungus-infected tall fescue, endophyte fungus-free tall fescue, or orchardgrass pastures. J. Anim. Sci. 70:1550.

Porter, J.K., C.W. Bacon, and J.D. Robbins. 1979. Ergonosine, and chanoclavin I from *Epichloe typhina*. J. Agric. Food Chem. 27:595-598.

Porter, J.K. 1995. Analysis of endophyte toxins: Fescue and other grasses toxic to livestock. J. Anim. Sci. 73:871-880.

Porter, J.K., and F.N. Thompson Jr. 1992. Effects of fescue toxicosis on reproduction in livestock. J. Anim. Sci. 70:1594-1603.

Pugh, B. 2003. Soybean hull supplementation to pasture-based beef cattle finishing diets. Masters Thesis at The University of Tennessee, Knoxville, TN.

Quicke, B.V., O.G. Bentley, H.W. Scott, R.R. Johnson, and A.L. Moxon. 1959. Digestibility of soybean hulls and flakes and the *in vitro* digestibility of cellulose in various milling by-products. J. Dairy Sci. 42:185.

Rhodes, M.T., C.G. Aldrich, J.A.Paterson, and M.S. Kerley. 1989. The effect of endophyte-infected tall fescue and a dopamine antagonist on relative blood flow in sheep. J. Anim. Sci. 67(suppl.1):286.

Rhodes, M.T., J.A. Paterson, M.S. Kerley, M.E. Garner, and M.H. Laughlin. 1991. Reduced blood flow to the peripheral and core body tissue in sheep and cattle induced by endophyte-infected tall fescue. J. Anim. Sci. 69:2033-2043.

Robbins, J.D., S.R. Wilkinson, and D. Burdick. 1973. Loline alkaloids of tall fescue seed and forage. pp. 98-107 in Proceedings of fescue toxicity conference, G.B. Garner, H. Tookey, and D.R. Jacobson. University of Missouri, Columbia, MO.

Roberts, C. 2000. Tall fescue toxicosis. MU Guide. MU extension, University of Missouri-Columbia, Dept. of Agronomy. http://muextension.missouri.edu/explore/agguides/crops/g04669.htm. Accessed on June 6, 2004.

Rottinghaus, G.E., G.B. Garner, C.N. Cornell, and J.B. Ellis. 1991. HPLC method for quantitating ergovaline in endophyte-infested tall fescue: seasonal variation of ergovaline levels in stems with leaf sheaths, leaf blades, and seed heads. J. Agric. Food Chem. 39:112-115.

Rutschmann, J., and P.A. Stadler. 1978. Chemical background. In: B. Berde and H.O. Schlid (ed.) Ergot Alkaloids and Related Compounds. pp. 30-85. Springer Verlag, Berlin.

SAS. 2002. Statistics Software: Version 9.0. SAS Inst., Inc., Cary, NC.

Schmidt, S.P., C.S. Hoveland, E.M. Clark, N.D. David, L.A. Smith, H.W. Grimes, and J.A. Holliman. 1982. Association of an endophytic fungus with fescue toxicity in steers fed Kentucky-31 tall fescue pastures. J. Anim. Sci. 55:1259.

Schmidt, S.P., D.A Danilson, J.A. Holliman, H.W. Grimes, and W.B. Webster. 1986. Fescue fungus suppresses growth and reproduction in replacement beef heifers. Alabama Agric. Exp. Sta. Highlights Agric. Res 33(4):15.

Schmidt, S.P., and T.G. Osborn. 1993. Effects of endophyte-infected tall fescue on animal performance. Agric. Ecosyst & Environ. 44:233.

Schroeder, J.W. 1997. Corn Gluten Feed: Composition, Storage, Handling, Feeding and Value. NDSU Extension Dairy Specialists. AS-1127. http://www.ext.nodak.edu/extpubs/ansci/dairy/as1127w.htm. Accessed on June 6, 2004.

Schuenemann, G.M., J.L. Edwards, F.M. Hopkins, N.R. Rohrbach, H.S. Adair, F.N. Scenna, J.C. Waller, J.W. Oliver, A.M. Saxton, and F.N. Schrick. 2005. Fertility aspects in yearling beef bulls grazing endophyte-infected tall fescue pastures. Reproduction, fertility and development. 17:479-486.

Seman, D.H., J.A. Stuedemann, and N.S. Hill. 1999. Behavior of steers grazing monocultures and binary mixtures of tall fescue. J. Anim. Sci. 77:1402-1411.

Shelby, R.A., and L.W. Darymple. 1987. Incidence and distribution of tall fescue endophyte in the United States. Plant Dis. 71:783-786.

Stern, M.D., A. Bach, and S. Calsamiglia. 1997. Alternative Techniques for Measuring Nutrient Digestion in Ruminants. J. Anim. Sci. 75:2256-2276.

Strahan, S.R., R.W Hemken, J.A. Jackson Jr., R.C. Buckner, L.C. Bush, and M.R. Siegel. 1987. Performance of lactating dairy cows fed tall fescue forage. J Dairy Sci. 70:1228-1234.

Strickland, J.R., J.W. Oliver, and D.L. Cross. 1993. Fescue toxicosis and its impact on animal agriculture. Vet Human Toxicol. 35:454-456.

Stuedemann, J.A., and C.S. Hoveland. 1988. Fescue endophyte: History and impact on animal agriculture. J. Prod. Agric. 1:39-44.

Stuedemann, J.A., N.S. Hill, F.N. Thompson, R.A. Fayrer-Hoskin, W.P. Hay, D.L. Dawe, D.H. Seman, and S.A. Martin. 1998. Urinary and biliary excretion of ergot alkaloids from steers that grazed endophyte-infected tall fescue. J. Anim. Sci. 76:2146-2154.

Thompson, F.N., J.A. Stuedemann, and N.S. Hill. 2001. Anti-quality factors associated with alkaloids in eastern temperate pasture. J. Range Manage. 54:474-489.

Tilley, J.M.A., and R.A. Terry. 1963. A two-stage technique for the *in vitro* digestion of forage crops. J. Br. Grassl. Soc. 18:104.

Tudzynski, P., T. Correia, and U. Keller. 2001. Biotechnology and genetics of ergot alkaloids. Applied Microbiology and Biotechnology. Springer-Verlag, Berlin.

Van Hellen, R.W., and W.C. Ellis. 1977. Sample container porosities for rumen *in situ* studies. J Anim. Sci. 44:141-146.

Van Keuren, R.W., and W.H. Heinemann. 1962. Study of a nylon bag technique for *in vivo* estimation of forage digestibility. J Anim. Sci. 21:340-345.

Vanzant, E.S., R.C. Cochran, and E.C. Titgemeyer. 1998. Standardization of *in situ* techniques for ruminant feedstuff evaluation. J. Anim. Sci. 76:2717-2729.

Varga, G.A., and W.H. Hoover. 1983. Rate and extent of neutral detergent fiber degradation of feedstuffs *in situ*. J. Dairy Sci. 66:2109-2115.

White, J.F., and G.T. Cole. 1986. Endophyte-host associations in forage grasses. V. Occurrence of fungal endophytes in certain species of *Bromus and Poa Mycologia.*78:852-856.

White, J.F. 1987. The widespread distribution of endophytes in the *Poaceae*. Plant Disease 71:340-342.

Williams, M.J., P.A. Backman, M.A. Crawford, S.P. Schmidt, and C.C. King. 1984. Chemical control of the tall fescue endophyte and its relationship to control performance. N.Z.J. Exp. Agric. 12:165.

Willms, C.L., D.B. Faulkner, D.F. Parrett, L.L. Berger, and P.D. George. 1992. Effect of supplementing corn stalkage diets with dried corn gluten feed or soybean meal-corn fed to gestating cows and heifers. In: Univ. Illinois Beef Rep., p. 55.

Yates, S.G. 1983. Tall fescue toxins. p. 249 in CRC Handbook of Naturally Occurring Food Toxicants. M. Richcigl, Jr. (Ed,), CRC Press, Boca Raton, Fl.

Yates, S.G., R.D. Plattner, and G. B. Garner. 1985. Detection of ergopeptine alkaloids in endophyte infected toxic KY-31 tall fescue by mass spectrometry/mass spectrometry. J. Agric. Food Chem. 33:719-722.

APPENDICES

APPENDIX A
TABLES

Table 1. Effects of grazing a novel endophyte-infected tall fescue selection

Parameter Means	Treatment		
	E+	Hi Mag	E-
Average Daily Gain (lb)	0.73[a]	1.26[b]	1.30[b]
Mean Hair Score	3.67[a]	2.44[b]	2.17[b]
Mean Respiration Rate (R/M)	114.80[a]	90.40[b]	77.40[b]
Mean Rectal Temperature (ºF)	105.80[a]	104.70[b]	103.70[c]
Serum Prolactin (ng/ml)	9.60[a]	50.80[b]	90.40[c]

[a, b, c]Means with no common letter differ, $P < 0.05$ (Nihsen et al., 2000).

Table 2. Nutrient composition of selected feedstuffs[a]

	DM	DE	ME	TDN	CP	NDF	ADF	Ash
	%	%	%	%	%	%	%	%
		---------- On a Dry Matter Basis----------						
Alfalfa, dehydrated	90.6	2.65	2.17	60.0	18.6	43.9	33.8	8.57
Corn, grain	90.0	3.92	3.22	90.0	9.8	10.8	3.3	1.46
Corn gluten feed	90.0	3.53	2.89	80.0	23.8	36.2	12.7	6.90
Soybean hulls	90.3	3.40	2.98	77.0	12.2	66.3	49.0	4.90

[a]Nutrient Requirements of Beef Cattle (1996)

Table 3. Nutrient composition of the feedstuffs used as substrates

	Alfalfa	Corn	Corn Gluten Feed	Soybean Hulls
DM, %	92.52	88.17	89.05	89.90
OM, %	90.02	98.47	93.81	95.29
NDF, %	43.29	13.45	33.35	62.01
ADF, %	31.96	3.63	10.75	45.82
CP, %	20.38	9.77	21.28	11.32

[a]Nutrients presented on DM basis

Table 4. Serum prolactin concentrations in steers (ng/ml)

	Forages		
Sample Date	KY-31/E+	Jesup/E-	Jesup/MaxQ
June 4	6.55[a]	101.58[b]	94.88[b]
June 25	21.04[a]	114.74[b]	134.56[b]

[a, b]Means within sample date not sharing the same superscript differ, $P < 0.05$.

Table 5. Nutrient composition of rumen contents[a]

Time	Pasture	DM %	OM %	NDF %	ADF %	CP %
0700	KY-31/E+	9.56[b]	87.99[d]	66.16[f]	34.62	19.40
	Jesup/E-	10.45[b]	87.15[d]	66.07[f]	34.20	19.31
	Jesup/MaxQ	9.69[b]	88.29[d]	65.85[f]	34.66	18.57
1900	KY-31/E+	10.28[c]	88.52[e]	63.16[g]	33.95	18.55
	Jesup/E-	10.62[c]	89.13[e]	63.62[g]	33.15	18.87
	Jesup/MaxQ	10.63[c]	90.91[e]	64.00[g]	33.32	19.02

[a]Nutrients presented on DM basis.
[b, c]Means within forage not sharing the same superscript differ, $P < 0.05$.
[d, e]Means within forage not sharing the same superscript differ, $P < 0.05$.
[f, g]Means within forage not sharing the same superscript differ, $P < 0.05$.

Table 6. Nutrient composition of masticates[a]

Time	Pasture	DM %	OM %	NDF %	ADF %	CP %
0700	KY-31/E+	7.80[b]	87.64[e]	61.86	31.88[f]	17.58[h]
	Jesup/E-	7.69[b]	88.81[e]	61.2	31.38[f]	19.38[h]
	Jesup/MaxQ	8.44[b]	87.96[e]	62.84	31.74[f]	19.16[h]
1900	KY-31/E+	8.93[c]	88.13[d]	62.47	30.31[g]	17.50[i]
	Jesup/E-	8.34[c]	89.78[d]	58.94	29.70[g]	17.64[i]
	Jesup/MaxQ	9.01[c]	90.09[d]	58.53	29.80[g]	17.31[i]

[a]Nutrients presented on DM basis.
[b, c]Means within forage not sharing the same superscript differ, $P < 0.05$.
[d, e]Means within forage not sharing the same superscript differ, $P = 0.11$.
[f, g]Means within forage not sharing the same superscript differ, $P < 0.05$.
[h, i]Means within forage not sharing the same superscript differ, $P < 0.05$.

Table 7. *In situ* alfalfa disappearance during two incubation times in steers consuming tall fescue pastures that differ in endophyte status[a]

Incubation Time	Pasture	DM %	NDF %	ADF %	CP %
0700-1900	KY-31/E+	55.49	15.68	10.70	73.42
	Jesup/E-	61.28	23.42	17.61	80.74
	Jesup/MaxQ	62.51	24.36	18.50	82.64
1900-0700	KY-31/E+	58.41	19.34	14.15	76.11
	Jesup/E-	60.02	20.59	14.55	80.04
	Jesup/MaxQ	61.47	22.66	16.70	81.89

[a]Nutrients presented on DM basis.

Table 8. *In situ* corn disappearance during two incubation times in steers consuming tall fescue pastures that differ in endophyte status[a]

Incubation Time	Pasture	DM %	NDF %	ADF %	CP %
0700-1900	KY-31/E+	53.60[b]	45.23	46.58	41.68
	Jesup/E-	65.29	50.54	50.04[d]	50.14
	Jesup/MaxQ	65.29	49.18	48.44	49.37
1900-0700	KY-31/E+	59.75[c]	44.05	45.00	44.91
	Jesup/E-	67.06	46.59	43.91[e]	51.46
	Jesup/MaxQ	67.06	50.08	48.84	51.26

[a]Nutrients presented on DM basis.
[b,c]Means within forage not sharing the same superscript differ, $P = 0.08$.
[d, e]Means within forage not sharing the same superscript differ, $P = 0.06$.

Table 9. *In situ* corn gluten feed disappearance during two incubation times in steers consuming tall fescue pastures that differ in endophyte status[a]

Incubation Time	Pasture	DM %	NDF %	ADF %	CP %
0700-1900	KY-31/E+	62.79	27.14	31.09	72.00
	Jesup/E-	68.60	34.80	39.58	77.53
	Jesup/MaxQ	68.20	32.65	37.49	77.75
1900-0700	KY-31/E+	65.83	30.84	35.14	75.05
	Jesup/E-	69.18	35.29	40.51	79.00
	Jesup/MaxQ	70.19	36.89	42.58	70.65

[a]Nutrients presented on DM basis.

Table 10. *In situ* soybean hulls disappearance during two incubation times in steers consuming tall fescue pastures that differ in endophyte status[a]

Incubation Time	Pasture	DM %	NDF %	ADF %	CP %
0700-1900	KY-31/E+	42.62	21.45	15.17	52.78[b]
	Jesup/E-	43.29	21.97	15.53	54.21
	Jesup/MaxQ	44.91	23.55	17.27	53.80
1900-0700	KY-31/E+	46.95	26.33	20.10	59.38[c]
	Jesup/E-	44.74	22.82	17.30	56.57
	Jesup/MaxQ	46.74	25.44	18.74	54.92

[a]Nutrients presented on DM basis.
[b,c]Means within forage not sharing the same superscript differ, $P = 0.12$.

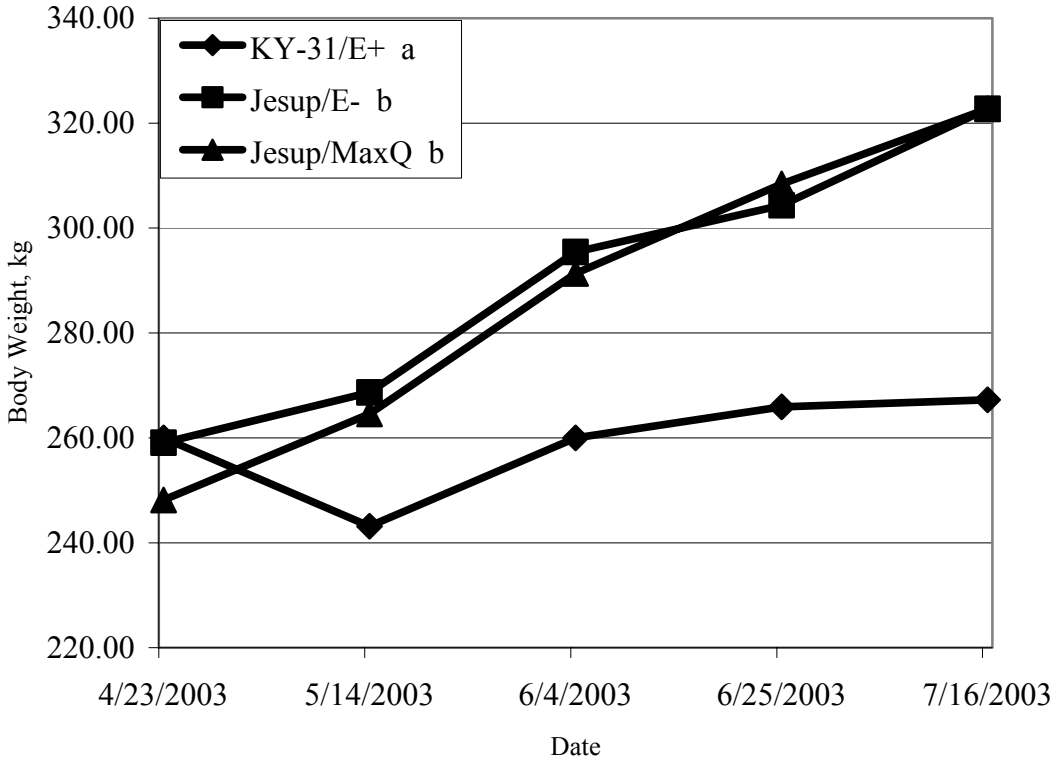
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Table 11. *In vitro* DM disappearance of substrates during two incubation times in rumen fluid from steers consuming pastures that differ in endophyte status[a]

Incubation Time	Pasture	Alfalfa %	Corn %	Corn Gluten Feed %	Soybean Hulls %
0700-1900	KY-31/E+	52.32	81.71	71.02	80.87
	Jesup/E-	52.42	79.78	67.10	80.07
	Jesup/MaxQ	52.12	80.80	69.49	82.64
1900-0700	KY-31/E+	54.22	85.94	75.05	81.85
	Jesup/E-	53.49	83.16	72.04	82.10
	Jesup/MaxQ	54.17	82.91	73.60	83.85

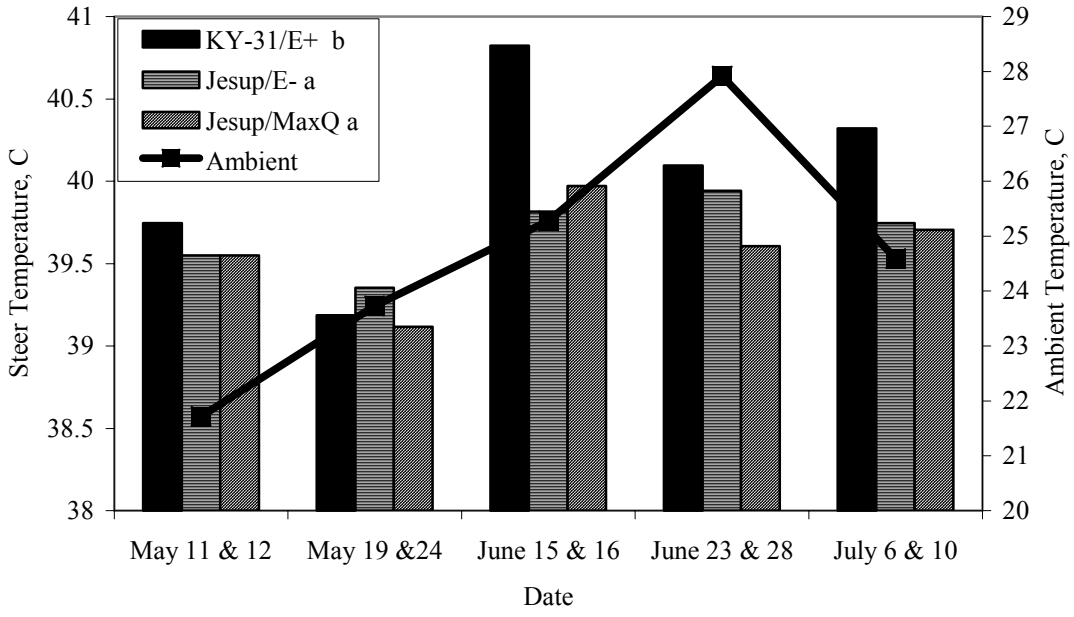
[a]Nutrients presented on DM basis.

APPENDIX B
FIGURES



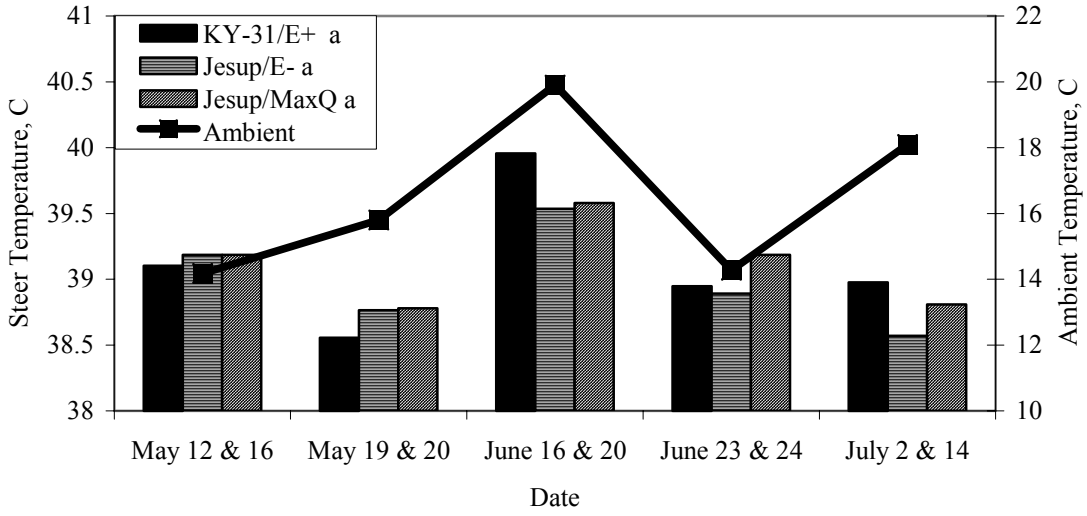
[a, b]Pastures not sharing the same superscript differ, $P < 0.05$.

Figure1. Performance of steers consuming tall fescue pastures that differ in endophyte status.



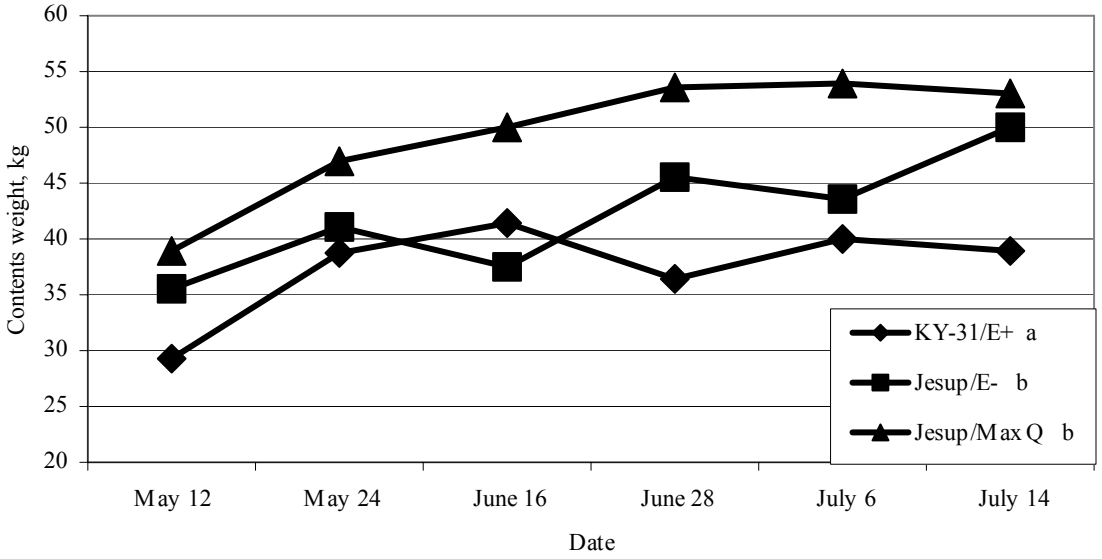
[a, b]Pasture temperature means not sharing the same superscript differ, $P < 0.05$.

Figure 2. Rectal temperatures at 1900 hours of steers consuming tall fescue pastures that differ in endophyte status.



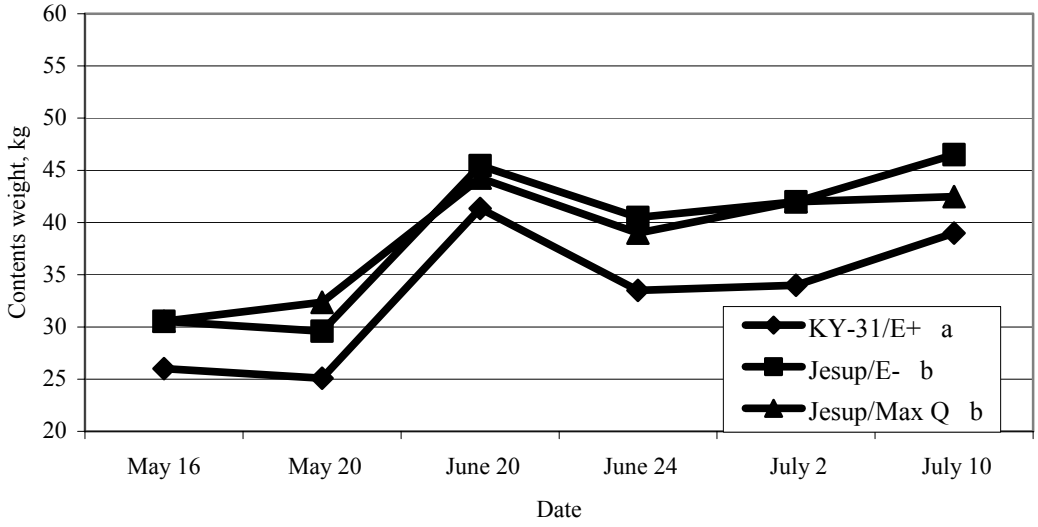
[a, b]Pasture temperature means not sharing the same superscript differ, $P < 0.05$.

Figure 3. Rectal temperatures at 0700 hours of steers consuming tall fescue pastures that differ in endophyte status.



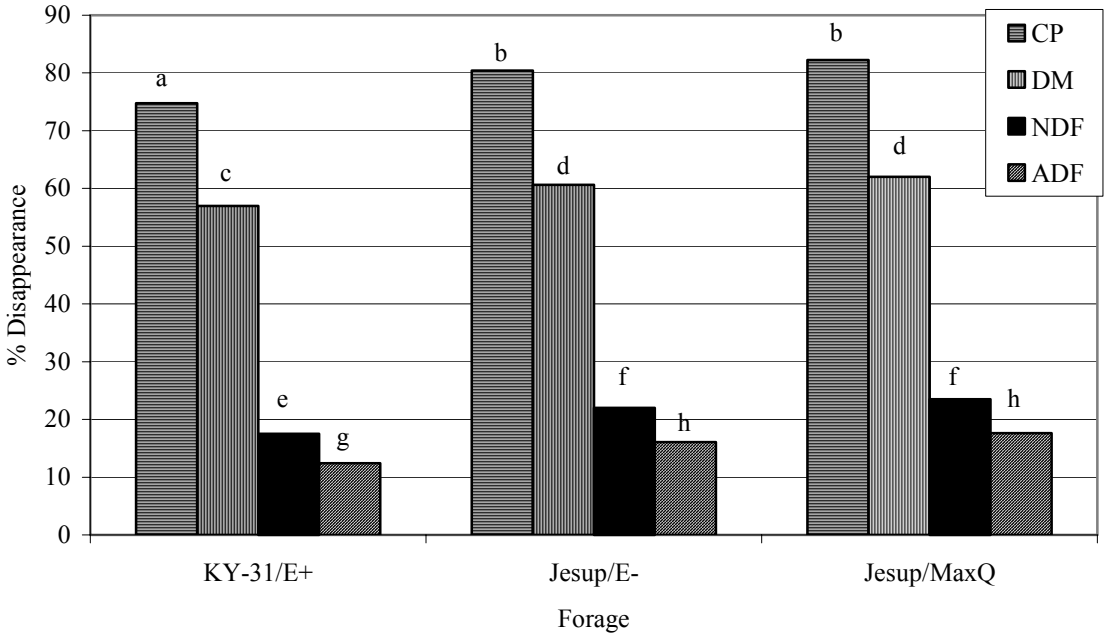
[a, b]Pastures not sharing the same superscript differ, $P < 0.001$.

Figure 4. Weight of rumen contents at 0700 hours of steers consuming tall fescue pastures that differ in endophyte status.



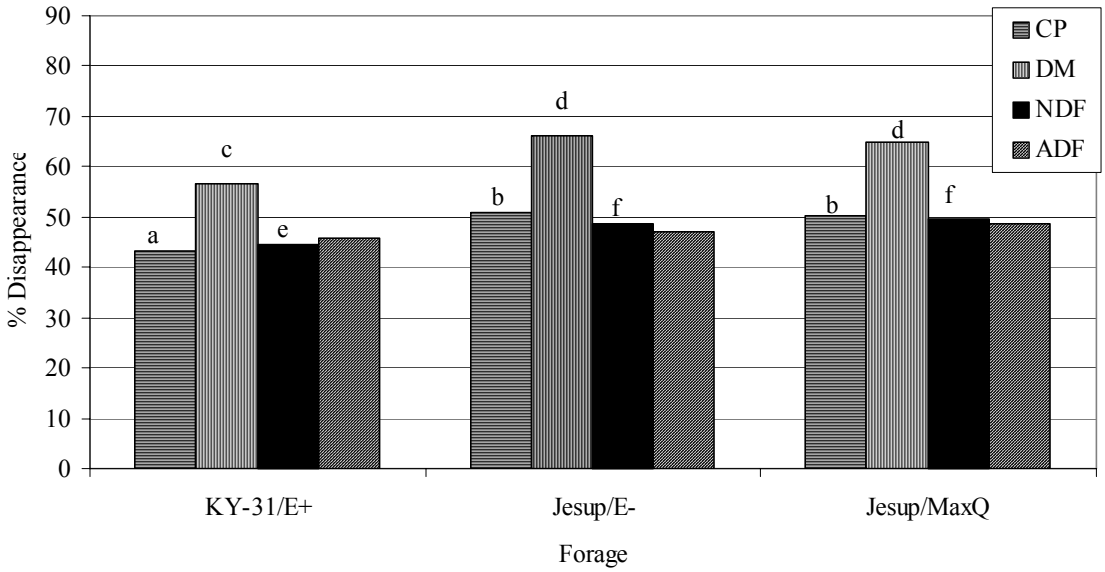
[a, b]Pastures not sharing the same superscript differ, $P < 0.0001$.

Figure 5. Weight of rumen contents at 1900 hours of steers consuming tall fescue pastures that differ in endophyte status.



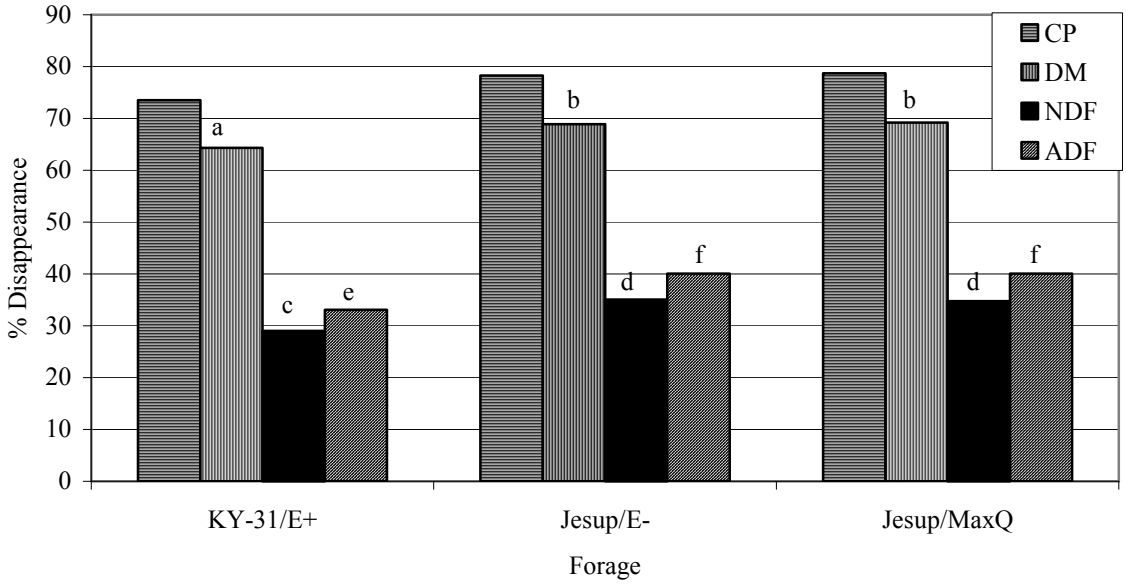
[a, b]Means not sharing the same superscript differ, $P < 0.05$.
[c, d]Means not sharing the same superscript differ, $P < 0.13$.
[e, f]Means not sharing the same superscript differ, $P < 0.13$.
[g, h]Means not sharing the same superscript differ, $P < 0.15$.

Figure 6. *In situ* alfalfa disappearance in steers consuming tall fescue pastures that differ in endophyte status.



[a, b]Means not sharing the same superscript differ, $P < 0.05$.
[c, d]Means not sharing the same superscript differ, $P < 0.05$.
[e, f]Means not sharing the same superscript differ, $P < 0.05$.

Figure 7. *In situ* corn disappearance in steers consuming tall fescue pastures that differ in endophyte status.



[a, b]Means not sharing the same superscript differ, $P < 0.09$.
[c, d]Means not sharing the same superscript differ, $P < 0.13$.
[e, f]Means not sharing the same superscript differ, $P < 0.11$.

Figure 8: *In situ* corn gluten feed disappearance in steers consuming tall fescue pastures that differ in endophyte status.

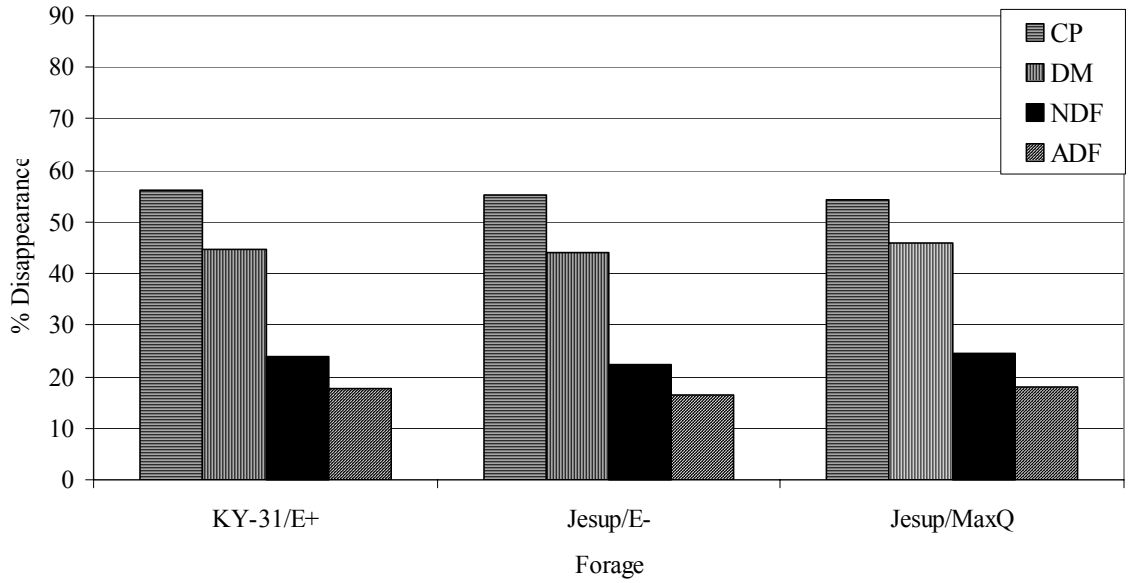


Figure 9. *In situ* soybean hull disappearance in steers consuming tall fescue pastures that differ in endophyte status.

APPENDIX C
CALENDAR OF EVENTS

Period	Dates	AM/PM	Performance	Start	Finish Date	Finish time
1	5/11/2003	PM	In Vitro	7:00 PM	5/15/2003	7:00 PM
1	5/11/2003	PM	In situ	7:00 PM	5/12/2003	7:00 AM
1	5/12/2003	AM	In situ	7:00 AM	5/12/2203	7:00 PM
1	5/12/2003	PM	Rumen evac	7:00 PM		
1	5/12/2003	PM	Masticate	7:00 PM		
1	5/16/2003	AM	In Vitro	7:00 AM	5/20/2003	7:00 AM
1	5/16/2003	AM	Rumen evac	7:00 AM		
1	5/16/2003	AM	Masticate	7:00 AM		
2	5/19/2003	AM	In situ	7:00 AM	5/19/2003	7:00 PM
2	5/19/2003	PM	In situ	7:00 PM	5/20/2003	7:00 AM
2	5/20/2003	AM	In Vitro	7:00 AM	5/24/2003	7:00 AM
2	5/20/2003	AM	Rumen evac	7:00 AM		
2	5/20/2003	AM	Masticate	7:00 AM		
2	5/24/2003	PM	In Vitro	7:00 PM	5/28/2003	7:00 PM
2	5/24/2003	PM	Rumen evac	7:00 PM		
2	5/24/2003	PM	Masticate	7:00 PM		
3	6/15/2003	PM	In Vitro	7:00 PM	6/19/2003	7:00 PM
3	6/15/2003	PM	In situ	7:00 PM	6/16/2003	7:00 AM
3	6/16/2003	AM	In situ	7:00 AM	6/16/2003	7:00 PM
3	6/16/2003	PM	Rumen evac	7:00 PM		
3	6/16/2003	PM	Masticate	7:00 PM		
3	6/20/2003	AM	In Vitro	7:00 AM	6/24/2003	7:00 AM
3	6/20/2003	AM	Rumen evac	7:00 AM		
3	6/20/2003	AM	Masticate	7:00 AM		
4	6/23/2003	AM	In situ	7:00 AM	6/23/2003	7:00 PM
4	6/23/2003	PM	In situ	7:00 PM	6/24/2003	7:00 AM
4	6/24/2003	AM	In Vitro	7:00 AM	6/28/2003	7:00 AM
4	6/24/2003	AM	Rumen evac	7:00 AM		
4	6/24/2003	AM	Masticate	7:00 AM		
4	6/28/2003	PM	In Vitro	7:00 PM	7/2/2003	7:00 PM
4	6/28/2003	PM	Rumen evac	7:00 PM		
4	6/28/2003	PM	Masticate	7:00 PM		
5	7/2/2003	AM	Rumen evac	7:00 AM		
5	7/6/2003	PM	Rumen evac	7:00 PM		
5	7/10/2003	PM	Rumen evac	7:00 PM		
5	7/14/2003	AM	Rumen evac	7:00 AM		
5	7/14/2003	AM	Masticates	7:00 AM		

VITA

Alison Marie Corrigan was born in Belleville, New Jersey on February 22, 1979, and is the daughter of Newton Jr. and Arleen Corrigan. She received her grammar school education from Queen of Peace in North Arlington, New Jersey. She graduated from Queen of Peace High School, North Arlington, New Jersey in May of 1997. She then, attended Cook College, Rutgers, The State University of New Jersey in August 1997 working toward a Bachelor of Science degree in Animal Science. She received this degree in May 2001. Alison began her graduate work at The University of Tennessee, Knoxville in August 2001, while working under Dr. John C. Waller. She received her Master of Science degree with a major in Animal Science in August 2005.