ABSTRACT

DREWNOSKI, MARY ELIZABETH. Long-term agronomic performance and animal performance on stockpiled Jessup tall fescue with varying endophyte status. (Under the direction of M. H. Poore.)

The objective of this study was two fold. The first objective was to evaluate the long term agronomic performance of endophyte-infected (E+), endophyte-free (E-) and novel endophyte-infected (EN) tall fescue when stockpiled and intensively grazed in the winter. The second objective was to evaluate the performance of growing cattle when intensively grazing stockpiled E+, E- and EN fescue. Stands were established in fall, 1999. In August of each year, plots (1 ha, 4 per trt) were harvested for hay, and in early-September they were fertilized. Forage was allowed to accumulate from August until December. In December of each year, 48 Angus-cross tester cattle (4 per plot) were grazed on one of three treatments for 86 days (yr 1), 70 days (yr 2), 86 days (yr 3), 72 days (yr 4), and 56 days (yr 5). Steers were used the first year and heifers were used in subsequent years. Cattle were given a daily allotment of forage, under strip-grazing management, with a target residual height of 5 cm. Total pre-graze forage mass was determined in mid-November using a falling plate meter. Forage samples were taken every two weeks. Pasture ADG of animals did not differ among the treatments ($P = 0.13$). Gains were 0.52, 0.59 and 0.56 kg/d for E+, E- and EN, respectively. Forage disappearance (DM Basis) did not differ among treatments and was 4.76, 4.72 and 4.97 kg $\cdot hd^{-1} \cdot d^{-1}$ (SE ± 0.27) for E+, E-, and EN, respectively. Endophyte-free had lower (P = 0.01) percent of total fescue (82.5%) in the sward than did the E+ (90.7%) or EN (88.4%) which did not differ (P = 0.16). Slight differences among the treatments in nutritive composition of the sward were found. The IVTDMD of the total sward differed among treatments ($P < 0.01$) with E- having the lowest digestibility (70.7%), EN being intermediate (72.53%) and E+ being the highest (73.94%). Crude protein of the total sward was higher ($P < 0.01$) for the E- (11.81%) than E+ (10.86%) and EN (11.07%) which did not differ ($P = 0.13$). Endophyte status did not influence leaf senescence over the winter. Total forage mass of E- (3508 kg/ha) was lower than E+ (3979 kg/ha; $P < 0.01$) and EN (3829 kg/ha; $P = 0.01$), while E+ and EN did not differ ($P = 0.16$). The gain per ha was higher ($P \leq 0.05$) on E+ (257 kg) than on E- (220 kg) or EN (228 kg). Novel endophyte-infected fescue appears to have agronomic performance similar to E+ under these conditions. The use of stockpiled E+ as a source of low cost winter feed is a viable option for producers.

Long-term agronomic performance and animal performance on stockpiled Jessup tall fescue with varying endophyte status

by

Mary Elizabeth Drewnoski

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APPROVED BY:

Dr. Jim Green	Dr. Mitch Hockett

Chair of Advisory Committee
Dr. Matt Poore

BIOGRAPHY

Mary Elizabeth Drewnoski was raised in the mountains of western North Carolina in the rural community of Marshall. After graduating with honors from Madison high school in 2000, she moved to the small town of Berea, Kentucky to obtain further education. While, attending Berea College she was employed at the college farm where she managed the cattle operation and in 2004, she received a Bachelor of Science degree in Agriculture and Natural Resources. Mary was then given the opportunity to pursue a Masters degree in Animal Science at North Carolina State University, in Raleigh. Upon completion of her Masters degree, she will begin pursuit of her Doctor of Philosophy degree in Animal Science at North Carolina State University, continuing under the guidance of Dr. Matthew Poore.

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Introduction

The perennial bunchgrass tall fescue [*Lolium arundinaceum* (Schreb.) Darbysh] is one of the most abundant forage crops in the United States and is the dominant cool-season forage in the north-south transition zone of the southeastern United States. Its predominance is mainly due to its ease of establishment, wide range of adaptation, pest resistance and tolerance to poor management (Thompson et al., 1993). However, consumption of tall fescue has been linked to poor animal performance (Paterson et al., 1995). The presence of an endophytic fungus *Neotyphodium coenophialum* (Glenn et al., 1996) in tall fescue has been associated with both the desirable agronomic traits and the undesirable animal responses associated with tall fescue (Joost, 1995).

Alkaloids produced by the fungus or by the plant in response to the fungus are biologically active compounds that suppress consumption of tall fescue by livestock and pests (Porter, 1994). The endophyte also seems to induce morphological changes, in the plant that are responsible for increased resistance to drought (Arachevaleta et al., 1989) and mineral (Malinowski et al., 1998) stress. The alkaloids are responsible for tall fescue toxicosis syndrome seen in cattle, sheep and horses consuming endophyte infected fescue (E+) (Paterson, 1995). Cattle with fescue toxicosis experience reduced average daily gain (Hoveland et al., 1983; Paterson et al., 1995) and feed intake (Hemken et al., 1981) as well as intolerance to heat including increased body temperature (Hannah et al., 1990), excessive salivation, panting, and retention of winter hair coat (Paterson, 1995). Endocrine and reproductive effects of fescue toxicosis in cattle include delayed puberty

(Washburn and Green, 1991), reduced prolactin levels (Hurley, 1981) and decreased calving rates (Gay et al., 1988). Less common symptoms of toxicosis include loss of circulation and necrosis of the extremities and abdominal fat (Bush, 1979).

Two alternatives for producers in the fescue belt are endophyte-free fescue (E-) and non-toxic endophyte infected fescue (EN). The negative effects of E+ are not seen on cattle grazing E- (Hoveland et al., 1983; Chestnut et al., 1991). Animal performance on E- fescue can rival that of animals on orchardgrass (Peters et al., 1992). However, competition from both warm and cool season grasses is a problem (Bouton et al., 1993; Hill et al., 1998) due to decreased pest and drought tolerance (Malinowski et al., 1999).

Recently an endophyte that did not produce ergot alkaloids was discovered and inserted into endophyte-free fescue (Bouton et al., 2002). Research on this non-toxic endophyte infected fescue has shown favorable results. Animal performance on EN has been equal to that of E- (Nihsen et al., 2004; Parish et al., 2003). In a short term study the persistence and yield of EN has been greater than E- and equal to E+ (Bouton et al. 2002).

Tall fescue-endophyte mutualistic association

Association of *Neotyphodium coenophialum* and tall fescue has been termed mutualistic symbiosis because both the fungus and the plant derive benefits from the association (Bacon, 1993). *Neotyphodium coenophialum* is a clavicipitaceous fungi of the tribe Balansieae (Bacon, 1993). The fungus exists within the intercellular spaces of supporting and assimilative tissues of the leaf sheaths and flowering stems but is not found in the leaf blades or the roots (Bacon and Siegel, 1988). The greatest concentration of the endophyte is in the base of the leaf sheath and in the seed, between the scutellum of the embryo and the starchy endosperm (Bacon et al., 1986).

The fungus completes its life cycle in the plant, relying entirely on tall fescue for dissemination and proliferation (Bacon et al., 1986). The infection of seed serves as the primary disseminating agent. The fungus grows as the seed germinates, invading the seedling quickly after germination. As the grass grows the fungus spreads into new tillers and leaf sheaths. When the grass is vegetative the fungus is located in the meristem tissue of the leaf sheath. The mycelium enters the flowering stem in the spring where it penetrates tissues of the ovule (Bacon and Siegel, 1988).

Neotyphodium coenophialum imparts positive agronomic qualities on its host by enhancing tolerance to biotic and abiotic stresses. These attributes include increased drought tolerance (Thompson et al., 2001), improved plant vigor (Studemann and Hoveland, 1988), improved growth (Read and Camp, 1986; Clay, 1984), and insect, nematode and disease resistance (West et al., 1988).

Bioprotective alkaloids

The main mechanism of enhancing tolerance to biotic stress is through the antiherbivore attributes (Malinowski and Belesky, 2000) of alkaloids produced by the plant in response to the fungus or by the fungus itself. In endophyte-infected fescue, five main classes of compounds have been found: diazaphenanthrene alkaloids, ergot alkaloids, lolitrem alkaloids, pyrrolizidine alkaloids and pyrrolopyrazine alkaloids as well as other miscellaneous alkaloids (Porter, 1995). Ergot alkaloids have been shown to negativly effect mammalian herbivores and are thought to be the main cause of toxicosis symptoms found in livestock grazing endophyte infected fescue (Porter, 1994). Pyrrolizidine and pyrrolopyrazine alkaloids have been shown to have insect deterrent properties (Dahlman et al., 1997; Breen, 1994).

Pyrrolopyrazine alkaloids. Peramine has been shown to be produced by endophyte infected fescue (Siegel et al., 1990) and has insect deterrent properties (Breen, 1994). However, it does not appear to have negative activity against mammalian herbivores (Bush et al., 1997).

Pyrrolizidine (loline) alkaloids. Loline alkaloids (N-formal and N-acetyl) are synthesized by endophyte infected tall fescue (Siegel et al., 1990; Porter, 1994). Loline has been found in the plant where the fungus is known to exist as well as in the leaf and roots. This suggests that these alkaloids or the signal for their synthesis are translocated to the leaf blade and roots of the plant. They have been shown to be found in concentrations as high as 8,253 ug/g of plant dry wt. Loline alkaloids have been shown to increase 2 to 3 fold when plants were subjected to water stress but concentrations did not change in response to nitrogen application (Kennedy et al., 1983).

Loline alkaloids have been shown to be potent insecticides (Dahlman et al., 1997) and appear to have negligible effects on livestock (Strickland et al., 1996; Fletcher et al., 2000). Loline alkaloids have also been shown to have allelopathic properties such as reducing the germination rate of monocot and dicot seeds (Petroski et al., 1990). Loline alkaloids are thought to be of plant origin and, when produced at high enough concentration, has been shown to be detrimental to other plants grown with infected tall fescue. Malinowski et al. (1999b) showed that E+ genotype DN2 had higher loline alkaloid concentrations in the root than E+ genotype DN11 (1083 ug/g and 536ug/g, respectively). When grown in a mixture with red clover, DN2 had a relative yield total that was less than one, suggesting an alleopathic interaction. The E- DN2 and DN11 as well as the E+ DN11 had a relative yield total of one suggesting full competition between the tall fescue and red clover in the mixture.

Diazaphenanthrene alkaloids. Perloline is a diazaphenathrene alkaloid that was initially thought to be the cause of fescue toxicosis (Gentry et al., 1968). However work by Hemken et al. (1979) caused this theory to be dismissed. The idea that perloline was not the cause of negative animal performance was further confirmed by the finding that concentrations of perloline of fescue stands with a high endophyte infection rate were similar to stands with a low endophyte infected rate (Strahan et al., 1987).

Lolitrem (indole diterpene) alkaloids. Lolitrems and paxilline were first identified in endophyte infected ryegrass and is related to livestock neurotoxicities of ryegrass staggers. Lolitrems and paxilline have been shown to be produced in vitro in cultures of *N. coenophialum* (Penn et al., 1993) as well as in vivo in endophyte infected tall fescue (Garthwaite, 1997). It is unknown whether lolitrem alkaloids contribute to fescue toxicosis in livestock.

Ergot alkaloids. The similarity of fescue foot to chronic ergotism suggested that the vasoconstrictive ergot alkaloids might be involved. However, which ergot alkaloid or combination of alkaloids is responsible for fescue toxicosis in livestock is still up for debate. The bioavailability and metabolism of ergot alkaloids in ruminants is not well understood (Hill et al., 2001). Only three "ergot alkaloid" classes have been isolated from *N. coenophialum.* These include ergopeptines, simple lysergic acid amides, and clavines (Porter, 1994).

Phosphorus seems to be involved in ergot alkaloid synthesis and accumulation in E+ fescue. In a green house study of four tall fescue clones, concentration of ergot alkaloids increased with increasing soil phosphorus availability from 17 to 50 mg per kg but declined at 96 mg per kg of P in the soil (Malinowski et al., 1998). In an in vitro study the activity of the first enzyme in the biosynthesis pathway of ergot alkaloids, dimethylallyl tryptophan synthase has been shown to be restricted in response to high P concentrations (Robbers, 1984; Flieger et al., 1991). This may explain the reduced ergot alkaloid concentration observed at the 96 mg per kg in the study by Malinowski et al. (1998).

Ergot alkaloid concentrations have been shown to be lowered by making the grass into hay (Roberts et al., 2002). This may be due to the several cycles of drying and rehydration that occur when forage is sun cured. Alkaloids have been shown to be unstable when photolysis, oxidation, and rehydration of plant tissue happens (Gardner et al., 1993; Porter, 1995).

Ergot alkaloids have been shown to affect lipid metabolism by lowering fat stores and blood concentration of cholesterol and triglycerides (Cincotta and Meier, 1989; Barnett et al., 1991). Floss et al. (1973) reported that ergot alkaloids stimulated dopamine receptors, antagonized α-adrenergic and serotonin receptors and altered thermoregulatory and vasoregulatory mechanisms. Physiological effects of ergot alkaloids are associated with monoamine-affected systems (Muller-Schweiniter and Weidmann, 1978).

Stuedemann et al. (1998) found that the approximately 96% of ergot alkaloids consumed were excreted in the urine. Appearance or disappearance of ergot alkaloids in the urine occurred 12 hours after steers were moved to or removed from E+ pastures. Autoclaved ruminal fluid decreased the ergot alkaloid concentration in the pellet and increased the ergot alkaloids in the supernatant by 33 and 59% after 12 and 48hr, respectively. While unautoclaved ruminal fluid did not effect the alkaloid concentration in the pellet, suggesting ergot alkaloids are liberated from forage by ruminal microorganisms and absorbed into the blood stream (Stuedemann et al., 1998).

Ergopeptines. Studies have shown that ergopeptines cause some of the physiological responses associated with endophyte infected fescue. In a study conducted on rat anterior pituitaries using α-ergocryptine, Strickland et al. (1992) suggested that the ergopeptine group was responsible for reduced serum prolactin levels seen in cattle. Erogpeptines have been reported to exhibit dopamine-like activity at physiological ranges (Browning et al., 2000; Larson et al., 1999). When ergotamine was injected into steers at heat stress temperatures, plasma concentrations of prolactin and LH were reduced (Browning et al., 1997).

Total concentrations of ergopetide alkaloids in E+ tall fescue vary with season, amount of nitrogen fertilization and part of plant (Rottinghaus et al., 1991; Arachevaleta et al., 1992). Higher rates of N increase the total concentration of ergopeptide alkaloids (Arachevaleta et al., 1992; Belesky et al., 1988).

Belesky et al. (1988) measured the ergopeptine alkaloid concentration of ky-31 tall fescue from late February until mid December. The pasture was managed under a put and take system with forage availability maintained at 1800 kg per ha. The alkaloid levels began to increase in early April and peaked around mid to late May at 550 mg/kg. Alkaloid concentrations were lowest (400 mg/kg) during the summer when the fescue was dormant. Concentrations began to rise again in early September and continued to increase until early November peaking at 1150 mg/kg.

Ergovaline is the major ergopeptine alkaloid produced by *N. coenophialum.* Of the ergopeptine alkaloid concentration, ergovaline has been shown to make up 80 to 90% of the total (Belesky et al., 1988; Rottinghaus et al., 1991). Due the high content of ergovaline, its concentration has been used as an indicator of total ergot alkaloid content (Rottinghaus et al., 1991).

Rottinghaus et al. (1991) measured the ergovaline concentrations in the stem with leaf sheath, leaf blade and seedhead of infected tall fescue plants during a two year study conducted from April to the beginning of July. Even though the endophyte was not present in the leaf blade, significant quantities of ergovaline was found in this part of the plant. It was theorized that ergovaline must be translocated from the stem or the leaf sheath to the blade (Rottinghaus et al., 1991).

Concentrations of ergovaline increased from late April to early May in the leaf blade and stem. The concentration in the leaf blade in late April was less than 250 ug/kg but in May increased to 450 to 500 ug/kg by early-May. In the stem, the concentration increased from around 500 ug/kg to between 800 to 1300 ug/kg. However, once the seedhead began to develop in mid May, concentrations in the leaf and stem declined. Concentrations of ergovaline increased in the seedhead as it matured and peaked around mid June. Peak concentrations were highly variable, ranging from 5000 ug/kg in year one to 1700 ug/kg in year two. After seedhead maturation the concentration in the leaf and stem remained between 200 to 400 ug/kg. These data suggests that the mature seedhead is the most toxic part of the plant and that management to reduce the presences of mature seedheads would reduce potential toxicity.

Kallenbach et al. (2003) measured the ergovaline content of stockpiled KY-31 tall fescue from mid December until mid March for two years. Ergovaline concentration varied among the years but in both years there was an 85% decrease in ergovaline by mid March. Ergovaline concentrations in mid December were approximately 450 and 190 ug/kg in year 1 and 2, respectively.

When ergovaline was injected into steers prolactin levels were reduced (McCollough et al., 1994). In an in vitro study, ergovaline was shown to be the more potent inhibitor of the D2 dopamine receptor in comparison with two other ergopeptides and lysergic acid amides (Larson et al., 1999).

In a study by Gadberry et al. (2003), lambs were fed E- seed, E+ seed containing 520 ug/kg of ergovaline and E- seed with ergovaline (EF+EV) added at a concentration similar to the E+ seed. Feed intake of lambs fed E- seed and EF+ EV did not differ but were higher than those fed E+ seed. Prolactin levels of the sheep fed EF+ EV were reduced when compared to the E- diet but was higher than the sheep fed E+ seed. The authors suggested that the E+ seed may have other compounds that strengthen the reduction of PRL levels. Skin temperature was lower for the E+ diet than the E- and the EF+ EV diet, which did not differ. Consistent with the decrease in skin temperature, body temperature of E+ was higher than E- and EF+ EV, which did not differ. However, the thermocirculation index for EF+ EV was lower than E- and equal to that of E+. This suggests that EV has vasconstrictive properties when fed to lambs. In a second trial lambs were fed EF seed, E+ seed and endophyte infected ryegrass seed (R+). The E+ and R+

diets had 530 ug/kg and 610 ug/kg of EV, respectively. However, the E+ had a higher concentration of ergot alkaloids 2690 ug/kg vs 1320 ug/kg for E+ and R+, respectively. The lambs on the R+ had prolactin levels that were lower than the E- but higher than the E+. Rectal temperatures were higher for lambs on the E+ than the E- while R+ was intermediate. Skin temperature was lower on E+ than E- and R+, which did not differ. When lambs were exposed to heat stress (temperature maintained at 33˚C) lambs on E- and R+ had higher intakes than E+. Lambs on E+ had lower alkaline phosphatase (AP) higher aspartate transaminase (AST), lactate dehydrogenase (LDH), cholesterol, and triglyceride levels in the blood than those on E-. Lambs on R+ had intermediate AP, LDH and triglyceride concentrations while AST and cholesterol levels similar to E-. Overall, the data suggest that EV alone can cause some negative effects but other compounds in E+ fescue work in combination with EV to cause fescue toxicosis. Therefore, toxicosis may be most augmented by the total concentration of ergot alkaloids (Porter, 1994).

Lysergic acid amide. Lysergic acid amide (LAA) can also exist in high quantities in E+ fescue and has been reported to approach 45% of the concentration of ergovaline (Oliver et al., 1993). Lysergic acid amide may be the product of solvolytic cleavage of lysergylmethylcarbinolamide and therefore is not considered a true natural product (Floss, 1976). Both LAA and lysergylmethylcarbinolamide have biological activity (Oliver., 1993).

Lysergic acid amides (ergine and ergonovine) have been reported to bind and agonize with the D_2 dopamine receptors; however, they were 1/100th as potent as ergovaline (Larson et al., 1999). Eronovine is a lysergic acid amide and has been shown to have vasoconstrictive effects (Oliver et al., 1992). Ergonovine has also been shown to reduce prolactin levels, but for a shorter time than the ergopeptide ergotamine (Browning et al., 1997).

Lysergic acid amide has been shown to produce vasoconstriction of veins in vitro at concentrations that seem to be within the range that animals grazing E+ tall fescue would consume (Oliver et al., 1993). It is likely that lysergic acid amides play a role in fescue toxiosis but to what extent is unclear. Even though in many studies the same concentrations of lysergic acid amides seems to be less potent than the ergopeptides, ergovaline or ergotamine, Hill et al. (2001) suggested that lysergic acid may play a greater role in fescue toxicosis than ergovaline because it had the greatest transport potential both in the omasum and rumen when compared to two other lysergic acid amides (lysergol and ergonovine) and two ergopeptines (ergotamine and ergocryptine). However, transport of ergovaline was not tested in this study.

Clavine alkaloids. The clavine alkaloids chanoclavine(s), penniclavine, elymoclavine and agroclavine have also been isolated from *N. coenophialum* infected tall fescue (Lyons et al., 1986). These clavinet alkaloids are precursors in the biosynthesis of the simple lysergic acid amides and the ergopeptines (Floss, 1976; Gardner et al., 1993, Porter, 1994).

Other alkaloids. Several other alkaloids have been found in endophyte infected tall fescue including: ergosterol, ergostatetraeneone, ergosterol-peroxide, harman, norharman, and halostachine (Latch, 1993). It is possible that they may play a role in animal toxicities and/or insect deterrence.

Pasture and grazing management

Certain management techniques can be utilized to decrease the effect of E+ tall fescue on animal performance. One pasture management technique that has been shown to be effective is to interseed E+ pastures with clover. Studies with steers (McMurphy et al., 1990; Chestnut et al., 1991) and cow-calf pairs (Stricker et al., 1979; Ellis et al., 1983) have shown increased performance. Fescue toxicosis can be partially alleviated by having legumes mixed in the pasture at a rate of 10 to 25% (Fribourg et al., 1991).

As Rottinghaus et al. (1991) has shown, alkaloid content is most concentrated in the seedhead. Two possible ways of reducing seedhead formation are by increasing stocking rate (Ball et al., 1993; Bransby et al., 1988) or by mowing the tall fescue before the seedhead matures to prevent cattle from consuming mature seedheads with high alkaloid content. Bransby et al. (1988) studied the effects of increasing stocking rate and found that increasing stocking rate on E+ resulted in the ADG increasing or maintaining while the same changes in stocking rate decreased ADG on E- pastures.

Another technique is to remove cattle from E+ pastures during the summer when ambient temperatures are increased and graze them on warm season grass. Forcherio et al. (1992) found that switching steers from E+ tall fescue to Caucasian Bluestem in early summer improved ADG over those left on E+ (0.72 vs. 0.37 kg/d).

Kallenbach et al. (2003) suggested that by delaying the use of stockpiled fescue until mid to late winter a producer could minimize negative effects because alkaloid content decreases over the winter while nutritional content remains above the requirements of a gestating beef cow.

Endophyte-free tall fescue

Neotyphodium coenophialum has been removed from several cultivars of tall fescue in an effort to eliminate the reduction in animal performance. Results from several studies showed that ADG of steers grazing endophyte free fescue was increased anywhere from 30 to 100% compared to endophyte infected (Hoveland et al., 1983; Read and Camp, 1986, Chestnut et al., 1991). However, E- fescue is less persistent due to drought (West et al., 1993) and pests (Kimmins et al., 1990; Muegge et al., 1991), and as a consequence has a higher incidence of other grass species invading stands (Hill et al., 1991).

Responses of tall fescue to endophyte infection

Many changes in morphology and growth of tall fescue due to endophyte infection are variable and dependent on plant and/or endophyte genotypes (Boesky et al., 1987, 1989; Hill et al., 1990). Characteristics such as forage yield and tillering have been shown to be highly dependent on plant genotype and therefore responses to endophyte infection have been variable (Wilhelm and Nelson, 1978; Hill et al., 1990). Other effects of endophyte infection, such as higher DM production per tiller and a lower crown depth seem to be more consistent among differing plant genotypes (Hill et al., 1990).

Plant vigor and growth. In the field endophyte infected plants have been shown to be larger (Clay, 1984) as well as having greater tiller survival and growth than their endophyte free counter parts (Read and Camp, 1986; Clay, 1984). When grown in a mixture, Hill et al. (1991) found E+ plants were larger and more competitive than E- plants.

Clay (1987) reported that under ideal conditions E+ seed germinated at an approximately 10% higher rate than E- seed. Endophyte infection increased final germination rates in five out of 10 genotypes when compared to E- (Pinkerton et al., 1990). Increased germination of E+ seed may be due to reduced relative water gain during imbibition (Rice et al., 1990).

Cheplick et al. (1989) studied the effect of nitrogen application on a heterogeneous population of KY-31 tall fescue and found that E+ plants produced more biomass at each applied nutrient level. Using one genotype of KY-31, Archevaleta et al. (1989) found that E+ had a higher nitrogen utilization efficiency. Higher glutamine synthetase activity of E+ may be responsible for the ability of E+ to better utilize available nitrogen (Lyons et al., 1990). Glutamine synthetase is responsible for reassimilation of ammonia inside plants.

Insect and nematode resistance. Insect herbivory is reduced in E+ fescue due to an accumulation of deterrents, toxins and their synergists. In Louisiana, Cercopidae (leaf hoppers) were found to be the most prevalent insect pests of tall fescue (Muegge et al., 1991). Throughout the growing season E+ had less total number of leafhoppers than E- (Muegge et al., 1991).

The numbers of root feeding nematodes were higher in the soil and on the roots of E- than E+ tall fescue 7 wks after inoculation with nematodes (Pedersen et al., 1988). In a green house study, Kimmons et al. (1990) showed that the number of egg masses and eggs per mass of one species of nematode (*Meloidogyne graminis*) were reduced in E+ infected fescue. The reason for E+ apparent resistance to nematodes is not clear. West et al. (1988) suggested that while the fungus itself is not found in the roots alleochemicals synthesized by the endophyte may be translocated to the roots to deter nematodes. Evidence that lolines are present in the roots of E+ (Malinowski et al., 1999b) support this theory. The resistance to nematodes may also be due to the endophyte stimulating the thickening of the roots inner endodermal cell walls, making it difficult for juvenile nematodes to establish feeding sites (Gwinn and Bernard, 1993).

Drought tolerance. Drought stress has been identified as one of the most limiting factors to the adaptation and persistence of tall fescue, particularly in the southern portion of its range (Bates et al.,1990; Bacon, 1993; Bouton et al., 1993). Advantages of endophyte infection can be seen during times of severe water deficits. *Neotyphodium coenophialum* appears to induce adaptations that help the plant reduce transpiration losses (Elmi and West, 1995), improve water uptake and maintain critical growing points during drought (Elmi et al., 1989).

Populations of E+ are more stable than E- during drought stress (West et al., 1993; Read and Camp, 1986). At low and medium irrigation rates E+ had higher stand density and forage yield than E-. However, at high irrigation rates E+ did not differ from E- (West et al., 1989).

Stomatal closure is the most common water conserving response of plants. This reduces water loss via transpiration. Bunyard and McInnis (1990) found that in response to drought stress, E+ produced more of the phytohormone abscisic acid, more quickly than E-. Abscisic acid is thought to play a regulatory role in stomatal function and may be a messenger that stimulates stomatal closure when moisture is deficit in the root zone (Lachno and Baker, 1986). It has been shown that the stomatal conductance of water-stressed, E+ declines earlier and faster than E- (Elmi and West, 1995). However, other researchers have reported variable responses of stomatal function to drought stress in respect to plant endophyte status (Malinowski and Belesky, 2000). It has been suggested that stomatal conductance in response to drought stress is under the control of the plants genetic makeup and it seems that a great degree of genotypic variability exists.

Endophyte infection has been shown to cause morphological changes that result in reduced water loss and therefore enhance plant survival during drought stress. Endophyte infection has been reported to affect plant morphology in many genotypes by causing increased leaf rolling in response to drought stress when compared to uninfected tall fescue (Arachevaleta et al., 1989; Belesky et al., 1989; Hill et al., 1990). Greater leaf senescence and a reduction in leaf expansion rate in E+ has also been reported under conditions of drought (Belesky et al., 1989).

During drought stress, maintenance of cell turgor in critical growing points protects these regions from desiccation and therefore allows regowth to occur when adequate moisture returns. Cell turgor is maintained through the accumulation of solutes resulting in the reduction of osmotic potential (Hellebust, 1976). Infected tall fescue has been reported to show a greater degree of osmotic adjustment than E- in leaf blades and tiller bases (Elmi et al., 1989). Within an E+ tall fescue plant the greatest adjustment is occurring in the basal meristem (West et al., 1990). Young merstematic and elongating leaf tissue showed greater osmotic adjustment than matured leaf tissue (West et al., 1990). The authors suggested that osmotic adjustment in immature leaves and young tissue allows E+ tall fescue to persist during drought stress.

Drought stressed E+ shows an increased regrowth rate in comparison to E- (Arechavaleta et al., 1989; West et al., 1990) and may be due to increased turgor which allows for rapid elongation (Meyer and Boyer, 1972). Another possibility is that increased indole acetic acid (IAA) may be responsible for increased growth response observed in E+ (Debattista et al., 1990).

Nutrient stress resistance. In marginal resource environments E+ tall fescue often performs better than E- tall fescue. Endophyte infection appears to benefit the plant during conditions of low P availability. Endophyte infection benefits the plant by causing altered root morphology in response to phosphorus deficiency. At low levels of available P in the soil, concentrations of P, magnesium and calcium in the roots and shoots were higher in E+ tall fescue than E- (Malinowski et al., 1998). This may be due in part to E+ fescue having a greater specific root length than E- at low and medium P levels (17 and 50 mg per kg) in the soil (Malinowski et al., 1998). At low soil P levels, endophyte infection increased root DM, relative growth rate and P uptake rate of two tall fescue clones (DN2 and DN4) when plants were grown with phosphate rock but did not effect these parameters when plants were grown with a commercial P fertilizer (Milinowski and Belesky, 1999). Another mechanism of increased mineral uptake in response to P deficiency may be due to alterations in activity of root exudates (Malinowski and Belesky, 2000). In one of the clones (DN2) endophyte infection increased root exudate activity in dissolving P from the phosphate rock, by 100%. While in the other clone (DN4) endophyte infection had no effect on root exudate activity.

Non-ergot alkaloid producing infected tall fescue

Agronomic performance. Tall fescue endophyte strains that did not produce ergot peptide alkaloids but produced pest-deterring alkaloids were isolated from field-grown plants that were collected from around the world (Latch, 1993). Bouton and colleagues infected Jessup endophyte-free and Georgia 5 endophyte-free tall fescue with five strains of non-ergot alkaloid producing *N. coenophialum* that had been found. Of these only AR502, AR510 and AR542 transmitted a high frequency in the Jessup and only AR542 transmitted at a high frequency in the Georgia 5 (Bouton et al., 2002)

In a 2 year trial Bouton et al. (2002) tested the stand survival of non-ergot alkaloid producing endophyte strains AR502, AR542 in Jessup tall fescue, and AR542 in Georgia 5 tall fescue cultivars planted in a bermduagrass sod. The stands were then continuously grazed close to the ground from April until November. After two years only the Jessup AR542 was found to be equal to Jessup E+ in terms of stand percentage. However, the coefficient of variation percentage was high and the Jessup AR542 was always numerically lower leading the authors to believe that it may be difficult to achieve the same level of agronomic performance of E+ with a reinfected novel endophyte.

The same cultivar-strain combinations as used to test stand survival along with the addition of Jessup AR510 were drilled into plowed and disked soil to asses the forage yield. The Jessup AR542 yields were not statistically different from Jessup E+ while the Georgia-5 AR542 was less than the Georgia-5 E+.

Barker et al. (2005) studied the resistance to reinfection of Jessup E- and EN by E+. Endophyte-free was readily contaminated by volunteer E+ fescue at a rate of 12.8% while the EN rate of contamination was only 2.1%. The authors suggested that because the EN is less susceptible to reinfection, establishment of EN stands could be varied from the recommendations for establishing E- stands.

Effect on animal performance. Animal toxicity was tested using Jessup (AR502), Jessup (AR542) and Georgia 5 (AR542). During 3yrs of spring and 2yrs of fall grazing, lambs (n = 4/trt/yr) on AR542 and AR502 had similar ADG to those on E- and higher than E+. Blood prolactin (PRL) levels were reduced in animals grazing the E+ when compared to the novel endophyte and the endophyte free. Prolactin levels were similar among the novel endophyte infected fescue and endophyte free. These results suggested that it was possible to produce strain-cultivar combinations of tall fescue with non-ergot alkaloid producing endophyte that could provide the agronomic advantage of E+ while not producing negative effects on animal performance.

Parish et al. (2003) further studied the effects of two strains of non-ergot alkaloid producing endophyte (AR502 and AR542) that were inserted into three cultivars of tall fescue (Jessup, Kentucky-31, and Georgia-5) on stocker cattle performance. During both spring and fall cattle grazing the E+ had lower PRL concentrations, ADG, and gain/ha than the cattle grazing EN and the E-. Rectal temperatures at the end of the spring were higher for cattle grazing the E+ than for those on the EN and E- however, rectal temperature did not differ among the trts in the fall.

The grazing behavior of steers on Jessup E+, E- and ANR542 was also studied. Steers on EN and E- exhibited grazing behavior that supported superior growth in comparison to steers grazing E+. Both idling time (not grazing or ruminating) and standing time was higher on the E+ than for the EN or the E- during April through June. It has been suggested that cattle attempt to maximize evaporative cooling by spending more time standing during heat stress (Low et al., 1981). During autumn (October and November) no difference in the amount of daily ruminating was observed among the trts. During both seasons steers on the E- and EN took more prehensions per day and had a higher biting rate (prehension/min of grazing) than steers on the E+. During both seasons bite size did not differ among trts. In the spring daily DM intake was greater for steers grazing EN and E- than for those grazing E+. During the fall DMI was higher for steers grazing E- than for those on E+.

Nihsen et al. (2004) showed similar results to Parish et al. (2003). When steers grazed from April until October on HiMag EN pastures ADG was similar to HiMag E- and higher than steers on Ky-31 E+. Steers on E+ also had higher rectal temperatures and lower PRL than those on EN or E-.

Watson et al. (2004) conducted a 3 year study from March until August with cow-calf pairs grazing Georgia-5 tall fescue. Cows on the EN (AR542) pastures had higher serum PRL, gave birth to heavier calves and had higher BCS at the end of the grazing period than those on E+. The suckling calves on the EN pastures had higher ADG and higher weaning weights than the calves on E+. However, calving rate and calving interval did not differ between the treatments.

Stockpiled tall fescue

Stockpiling is the accumulation of autumn growth for grazing during the winter (Mays and Washko, 1960). In a two year study conducted by Poore et al. (2006) in the Piedmont of North Carolina, CP levels of stockpiled tall fescue fertilized in September averaged 16.8 and 12.6% and ADF levels averaged 25.9 and 30.7% during the winter (early December to late February).   In a three year study Burns and Chamblee (2000b) found similar CP levels during winter. Crude Protein averaged 15.7, 9.8 and 10.2 % from December to early March in years 1, 2 and 3, respectively.

In general, accumulation and fertilization of stockpiled fescue earlier in the summer increases dry matter yield (Burn and Chamblee, 2000a). However, earlier accumulation dates reduces the quality of forage (Burn and Chamblee, 2000b). Delaying forage accumulation from June to September resulted in a linear increase in IVDMD. In vitro dry matter disappearance in December were 59.5% and 69.9% for June and September accumulation start dates, respectively (Burn and Chamblee, 2000b).

The potential loss of nutrients in stockpiled E+ during the winter that occurs through age and weather has been evaluated in several studies. Most indicators of forage quality decline slightly as winter progresses (Ocumpaugh and Matches, 1977; Burns and Chamblee, 2000b; Poore et al., 2006). The decline in nutritive value is due to leaf senescence and leaching of digestible fractions due to cell rupture caused by freezing (Ocumpaugh and Matches, 1977).

In contrast to E+ very little research has been conducted on the herbage mass and nutritive value of stockpiled EN fescue. In a two year study by Kallenbach et al. (2003) the herbage mass of EN and E- HiMag fescue did not differ but was 20% lower than E+ Ky-31 fescue. However, the difference in herbage mass may be due to a cultivar effect. Joost and Mattas (1996) showed that Ky-31 often produces more fall growth than other cultivars. Endophyte status did not affect tall fescue's ability to maintain nutrient content over the winter. Nutritive value decreased slowly throughout the winter but did not differ among the treatments. In this study, herbage mass did not decline over the winter. For a more thorough review of the research that has been conducted on stockpiled fescue see Oliphant (2004).

Fescue Toxicosis

Fescue foot, bovine fat necrosis and summer slump are three endophyte-related conditions associated with animals grazing endophyte infected fescue. Fescue foot occurs during the winter and its presence is usually determined by the presence of a red line at the coronary band of the hoof (Bush et al., 1979). Vasoconstriction of extremities (hooves, ears and tails) can cause death of the soft tissues and lead to partial or complete loss the extremities (Bush et al., 1979; Ball et al., 2002). Bovine fat necrosis is characterized by the presence of hard, necrotic masses of fat in the adipose tissue of the abdominal cavity. This can lead to gastrointestinal disorder, kidney failure and difficult births. Summer fescue toxicosis is the most common and costly syndrome associated with E+ infected fescue (Hoveland, 1993). Cattle displaying signs of summer fescue toxicosis exhibit increased respiratory rates, increased rectal temperature, rough hair coats and excessive salivation (Paterson et al., 1995).

Weight Gain. Reduced ADG of cattle consuming E+ fescue has been shown consistently (Hoveland et al., 1983; Chestnut et al. 1991; Paterson et al., 1995). However, the amount of the reduction has been variable. Generally, ADG of steers grazing E- fescue increased by 30 to 100% when compared to steers grazing E+ (Paterson et al., 1995). Beef cows consuming E+ lose more weight, have lower milk production and wean lighter calves than those on E- (Peters et al., 1992; Keltner et al., 1989). The extent to which performance is reduced depends on many factors including: infection rate, ergot alkaloid content of the grass (Chestnut et al., 1991; Crawford et al., 1989), ambient temperature (Aldrich et al., 1993), and species composition of the stand (Fribourg et al., 1991).

Intake. Several trials have been conducted to determine the effect of endophyte on intake. Ergot alkaloid consumption and elevated ambient temperature (31˚C or above) interact and produce a negative feedback on animal intake. In a study by Hemken et al. (1981) steers were fed less toxic tall fescue, more toxic tall fescue or orchardgrass soilage (green chop). Compared to orchardgrass the less toxic strain of fescue did not reduce intake and the more toxic strain only reduced intake when environmental temperatures were maintained above 32˚C in environmental chambers.

In a grazing study by Peters et al. (1992) forage intakes of cows grazing E+ were not reduced when the environmental temperature was constantly below 32˚C but did decrease when environmental temperature was consistently above 32˚C.

In a study by Aldrich et al., (1993) in which a seed based diet containing 381 ug/kg of ergovaline was fed, the intake of beef heifers was not effected by trt when the environmental temperature was varied diurnally between 22˚C and 32˚C. However, when steers were fed E+ diet containing 285 ug/kg of ergovaline and environmental temperature was maintained at 22˚C intake was 10% lower than steers on E-. Further reduction of intake was observed when the environmental temperature was maintained at 32˚C. Under heat stressed conditions intake of E+ was 22% lower than E-.

In a study by Mathews et al. (2005) in which ambient temperature was maintained below 27˚C the intake of steers did not differ between E- and EN hay but was about 10 % lower for E+ hay. The hay contained 3, 0 and 120 ug/kg of ergovaline for E-, EN and E+, respectively.

Diet Digestibility. Although chemical measures of forage quality are usually reported to be similar between E+ and E- fescue stands (Bush and Burrus, 1988; Arachevaleta et al., 1989; Ball et al., 2002) studies of digestibility has been shown mixed results (Aldrich et al. 1993a,b; Hannah et al., 1990; Goetsch et al., 1987; Westendorf et al., 1993).

When steers were fed ad libitum tall fescue hay containing 100, 75, 50, 25 or 0% E+ mixed with E- hay total tract DM, NDF and N digestibility increased with increased E+ (Goetsh et al. 1987). The DM intake and passage rate decreased with increasing E+ mixed into the diet. The lower digestibility seen with E- diets may be due to the increased feed intake which elevates outflow of potentially fermentable substrate from the rumen.

Hannah et al. (1990) fed sheep a seed-based diet in which DMI was equalized between the E+ and E- treatments. The results showed decreased ruminal and total tract OM, NDF and cellulose digestibility in the E+ diet containing 0.19 mg/kg BW $^{0.75}$ of ergovaline. Similar results were reported by Fiorito et al. (1991) when lambs were fed equal amounts of high endophyte (>95%) or low endophyte (<1%) Ky-31 hay. Total tract DM, NDF, and ADF digestibility was reduced by the high endophyte treatment. Westendorf et al. (1993) fed sheep 1200 g/d of seed-based diet. The E+ diet contained 1400 ug/kg of ergot alkaloid. Total tract DM, ADF and CP as well as ruminal NDF, ADF and CP digestibility were reduced by endophyte.

However, in a study with sheep Aldrich et al. (1993a) reported DM digestibility was not different between E+ and E- when intake was equalized to 1.5 % BW and the E+ diet contained 0.09 mg/kg BW $^{0.75}$ of ergovaline. In a study with steers Aldrich et al. (1993b) reported lower DM and OM digestibilities on the E+ diet containing 0.04 mg/kg BW$^{0.75}$ but NDF digestibility were similar.

Recently two studies have been conducted to look at the digestibility of EN hay in comparison with E+ and E-. In a study by Mathews et al. (2005) IVTDMD did not differ among the three treatments, but when the hay was fed to steers at a restricted level digestibility was lower for E+ than E- or EN. Dry Matter and CP digestibility did not differ between E- and EN but was lower for E+. Organic Matter and ADF digestibility was lower for E+ when compared to E-. Digestibility of ADF was intermediate for EN. Cellulose and NDF digestibility did not differ among treatments.

Burns and Fisher (2005) fed goats and sheep ad libitum E+, E- and EN hay with 221, 103 and 110 ug/kg of ergot alkaloids. The goats' intake of E+ hay was lower than the intake of EN and E-. The hay intake by the sheep did not differ among treatments. In both species apparent DM, CP, ADF and NDF digestion were similar among the treatments.

Temperature regulation. During heat stress conditions, rectal temperatures have been shown to be increased by 0.4℃ to 1.2℃ in animals consuming endophyte infected diets (Hannah et al., 1990; Schmidt et al., 1982). Cattle and sheep have also exhibited increased respiration rate and excessive salivation when fed E+ fescue (Hemken et al., 1981, Burke et al., 2001, Schmidt et al., 1982).

The reduced intake, higher rectal temperatures, and higher respiration rates of animals consuming endophyte infected fescue at increased temperatures is most likely due to the reduced ability to dissipate heat through skin vaporization. Consumption of E+ decreased the ability of steers and wethers to regulate their body temperature through surface vaporization at elevated ambient temperatures (Aldrich et al., 1992; Aldrich et al., 1993).

Aldrich et al. (1993) found that while consumption of E+ reduced prolactin levels regardless of environmental temperature, the ability to dissipate body heat was impaired only at elevated environmental temperatures. The authors suggested that while consumption of E+ may physiologically prime the animal for fescues toxicosis, elevated environmental temperature is needed for the condition to manifest itself.

Serum analytes

Prolactin. Ingestion of E+ fescue by cattle and sheep results in reduced serum prolactin levels (Elasassor and Bolt, 1987; Hurley, 1981; Porter et al. 1990). Reduced secretion of prolactin is one biological measure of fescue toxicosis. It is often monitored to assess the effects of the endophyte on the animal. In a study by Chestnut et al. (1991) prolactin levels reflected the difference of infection levels of pastures. Prolactin levels were highest for steers consuming E- fescue, intermediate for 22% infected stands and lowest for the steers consuming 35% infected stands. Changes in PRL levels occur relatively quickly. Pituitary secretion of prolactin in sheep was decreased within two to three days after consumption of E+ fescue (Bolt, 1987). A similar amount of time is required for recovery of prolactin secretion when stock are removed from E+ pastures.

The main inhibitory regulation of prolactin is the suppressive effects of the neurotransmitter dopamine (Lamberts and Macleod, 1990). The ergoline ring structure possessed by ergot peptide alkaloids shares structural similarities to dopamine and serotonin (Berde, 1980). It has been shown that ergot peptide alkaloids decrease PRL through dopaminergic-like activity; the ergot alkaloids bind to dopamine receptors on the pituitary and inhibit the secretion of prolactin (Berde and Schild, 1978; Goldstein, 1980).

Circulating PRL concentrations vary according to photoperiod; they are highest during periods of light and are lower during periods of darkness (Critser et al., 1988; Tucker et al., 1984). Other factors known to increase PRL secretion are serotonin and elevated environmental temperature (Porter, 1990; Smith 1977). Consumption of E+ has been shown to reduce the ability to increase secretion of PRL in response to photoperiod, elevated environmental temperature, and serotonin (Hurley, 1981; Thompson et al. 1987).

Cattle consuming E+ fescue also have reduced melatonin levels (Porter, 1990). The decreased circulating prolactin and melatonin levels may be related to the long hair coat retention seen in cattle grazing E+ fescue (Porter, 1990) by affecting normal hair follicle function (Milne et al., 1990). Maintaining a long hair coat during the summer may contribute to the elevated body temperatures of cattle grazing E+ fescue.

Enzymes, protein and cholesterol. Schltze et al. (1999) found that serum analyte values of cattle grazing E+ tall fescue had lower alanine aminotransferase, total protein and cholesterol than cattle grazing E- tall fescue. Creatinine levels were higher in cattle grazing E+ than those grazing E-, while lactate dehydrogenase (LDH) and alkaline phosphatase were not statistically different between trts. Other studies (Thompson and

Stuedemann, 1993; Oliver et al., 1997) have indicated that alkaline phosphatase activity is suppressed in cattle suffering from fescue toxicosis.

In a 2 yr study Nihsen et al. (2004) evaluated the effect of grazing novel endophyte infected fescue on prolactin, alkaline phosphatase (ALP), lactate dehydrogenase (LDH), cholesterol, triglyceride, and creatinine of growing beef steers. Consumption of E- and EN had the same affects on the serum analytes measured with the exception of serum triglyeride concentration. Serum triglyeride was highest in EN, intermediate for E- and lowest in cattle on E+. Serum prolactin and cholesterol levels and LDH and ALP activities were similar for steers grazing EN and E- but were higher than those of the steers grazing E+. Creatinine concentrations were higher in steers grazing E+ than those grazing EN and E-, which did not differ.

Reproduction

Studies of the effect of E+ fescue on pregnancy rate have been conducted with variable results. Some studies have reported cattle consuming E+ have reduced pregnancy rates (Beer and Piper, 1987; Gay et al, 1988; Schmidt et al., 1986) while others have reported no difference (Burke et al., 2001; Burke and Rorie, 2002; Fanning et al., 1992). Discrepancies in E+ effect on pregnancy rate and hormonal response may be related to differences in experimental conditions. Some of the factors that could contribute to the difference in results observed include: amount of ergot alkaloid consumed, environmental temperature and the animals stage of maturity.

Heifers. Several studies have been conducted to look at the effect of consuming E+ fescue on the estrous cycle and ovarian function of heifers. The age and reproductive status of the heifers may play an important role in their response to consuming E+ fescue. Heifers exposed at an early age seem to show more detrimental effects. Beef heifers raised on highly infected fescue had lower conception rates than those raised on low endophyte infected fescue, 55% vs. 96% conception for high E+ and low E+, respectively (Schmidt et al., 1986). McKenzie and Erickson (1991) reported a 23% reduction in basal LH and reduced estradiol-stimulated LH, PRL, FSH and folliculogenesis in 3 month old heifers fed E+ hay. In a study by Washburn and Green (1991) puberty of heifers raised on E+ was delayed. However, this may be due to the lower gains of heifers on E+. Estienne et al. (1990) reported altered luteal function of heifers on E+ fescue. Of the heifers with a Corpus luteum (CL), 62% of the E+ trt had reduced circulating progesterone concentrations. Ahmed et al. (1990) reported that heifers grazing E+ exhibited alteration in the development and cellularity of the CL. The CL of the heifers consuming E+ had fewer nuclei and a greater number of large luteal cells with increased diameter. These large luteal cells had increased cellularity with a greater number of mitochondria, lipid droplets and secretory granules. Changes in the cellularity of the CL in rats fed ergot alkaloids has also been observed (Fitzhugh et al., 1944). The effects of E+ on the CL of heifers may reduce their ability to maintain pregnancy.

Mahmood et al. (1994) conducted a 2 yr grazing study on the growth and ovarian function of weanling (n = 40) and yearling (n = 40) beef heifers. Heifers were put on E- or E+ pasture in April and grazed through mid-July. The E+ pasture caused reduced ADG for both the weanling and yearling heifers when compared to the E-. The ovarian function of the yearling heifers did not differ due to trt. However, ovarian activity and luteal function were adversely altered in weanling heifers, with subsequent decrease response to estrus synchronization. The decreased estrus response after synchronization may be due to the induction of hormonal imbalance. In both years the weanling heifers on E+ had lower progesterone concentrations than their E- counterparts. The results suggest that weanling heifers may be more sensitive to the effects of E+ than yearling heifers. The ovarian effects on the weanling heifers may have been due to a delay in the onset of puberty.

In a study by Rahe et al. (1991) beef heifers grazing E+ fescue had decreased embryo survival. The lower fertility of heifers grazing E+ pastures may be due to decreased luteal function resulting in early embryonic loss. Burke et al. (2001a) found changes in follicular and luteal function of 350 kg beef heifers fed E+ seed. Serum progesterone concentrations were not affected by diet under thermoneutral conditions but under heat stress heifers on the E+ diet had lower serum progesterone concentrations. The lack of negative response of heifers fed endophyte infected fescue under thermoneutral conditions may explain the lack of consistent results to treatment reported in the literature. During both thermoneutral and heat stress conditions heifers consuming E+ had a decreased number of large follicles (>10mm) during the estrus cycle. Similarly, the pre-ovulatory serum estradiol concentrations were lower in heifers consuming E+.

Fanning et al. (1992) reported suppressed serum PRL levels and decreased ADG, but observed no effect of grazing E+ pastures on ovarian activity, synchronized estrous response or pregnancy rate of heifers exposed to E+ for 100d prior to insemination. However, this study had limited animal numbers (n = 32) and was conducted in the winter (November to March) when alkaloid concentrations are usually low (Belesky et al., 1988) and ambient temperature was cool.

Cyclic heifers exposed to E+ tall fescue seem to be less susceptible to the negative effects than pre-pubertal heifers. Cyclic heifers placed on E+ pastures showed no disruption in their estrous cycle (Bond and Bolt, 1986). In a 40 day trial (April to May) 2 yr old cycling Angus heifers (n = 8) and 4 yr old angus cows (n = 8) were pair fed E+ or E- seed (Mizinga et al., 1992). Luteinizing hormone (LH) concentration, LH pulse amplitude and frequency were similar between trts. Cattle on E- seed had higher ADG (.70 vs. .32 kg/day) than cattle fed E+ seed. However, PRL levels did not differ among the trts. Cattle consumed between 1,100 and 1,800 μg/d of ergovaline for the first 15 days and between 2,600 and 3,200 ug/d of ergovaline for the remaining 25 days. The heifers were receiving fescue seed as 10 to 20% of their diet. The high energy intake of the heifers may have attenuated some of the effect of endophyte that has been seen in grazing animals.

Cows. Results have shown that heifers may be more susceptible to alkaloids in E+ than mature cows. In early post-partum beef cows little difference in follicle dynamics and estradiol production between cows on E- (n = 20) and E+ (n = 30) pastures was observed (Burke and Rorie, 2002). Diameter of the CL and serum progesterone concentration did not differ between trts. Serum estradiol concentrations over the entire estrus cycle were not different. However, the diameter of the largest follicle present on the ovary tended to be smaller for cows grazing E+ compared to E- and estradiol concentrations of cows on E+ were lower between days 5 and 8 of the estrus cycle. Pregnancy rates and calving interval did not differ. In another study by Burke et al. (2001b) postpartum beef cows were put on E+ (n = 30) or E- (n = 20) pastures in April and were bred from May to June. The authors monitored fetal development weekly until

60 days of pregnancy. No difference in pregnancy rate or embryonic losses between the trts was detected.

Luteinizing hormone concentration and LH pulse amplitude and frequency of postpartum primiparous Angus cows (n = 16) pair fed E+ or E- seed from May to mid July did not differ (Mizinga et al., 1992). However, by the end of the trial (day 70) were PRL levels lower for cattle consuming E+ compared to those fed E- seed. Weight loss was higher for cows consuming E+ than for cows consuming E- seed. Cows consuming E+ seed received 677μg/d during the first 42 days and 2,788 μg/d of ergovaline during the last 28 days of the trial. Intake of ergovaline during the first 42 days may not have been high enough to elicit fescue toxicosis.

Consumption of ergot alkaloids may indirectly impair reproduction though increased body temp and/or decreased energy consumption. Elevated environmental temperatures during breeding may contribute to the decreased reproductive performance. The inability to regulate body temperature predisposes animals on E+ to heat stress which, itself, is associated with reduced conception. Small follicles can be damaged by heat stress (Wolfenson et al., 1995). In a study by Burke et al. (2001b) heat stress led to reduced diameter of the CL and progesterone levels when compared to thermoneutral conditions. Feed intake has also been shown to be reduced under heat stress conditions (Burke et al., 2001b). The reduction of feed intake may indirectly impair reproduction by decreasing energy consumption. In postpartum beef cows it has been shown that inadequate energy intake can cause decreased serum LH concentration (Lishman et al., 1979; Echternkamp et al., 1982).

Bulls. Little research has been done on the effects of consuming E+ on bull fertility. Holstein bulls fed E+ hay and grain (n = 9) from the age of 2 months until 13 months had lower PRL than bulls on E- hay and grain (n = 9) but did not differ in body weight, testicular and seminal vesicle weight, daily sperm production potential, sperm maturity, and blood testosterone levels (Evans et al., 1988). Alamer and Erickson (1990) reported that gonadotrophin-releasing hormone stimulated testosterone release differed in 3 month old bulls but not in 8 or 12 month old bulls. The number of sertoli cells was lower for bulls on E+ at 3, 8 and 12 months of age.

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LONG-TERM AGRONOMIC PERFORMANCE AND ANIMAL PERFORMANCE ON STOCKPILED JESSUP TALL FESCUE WITH VARYING ENDOPHYTE STATUS

M.E. Drewnoski, E.J. Oliphant, M.H. Poore, M.E. Hockett and J.T. Green, Jr.

Department of Animal Science, North Carolina State University

Raleigh, NC 27695-7621

Introduction

Autumn growth of tall fescue [*Lolium arundinaceum* (Schreb.) Darbysh] can be accumulated to extend grazing into the winter (Matches, 1979) and reduce feed costs (Bishop-Hurley and Kallenbach, 2001). The presence of the endophytic fungus *Neotyphodium coenophialum* in tall fescue has been shown to improve agronomic traits such as stand persistence and increased growth. However, presence of the endophyte is also the cause of poor animal performance due to its production of ergot alkaloids. Growing cattle grazing endophyte-infected fescue during the winter have low gains despite the apparently good nutritive value of the forage (Poore et al., 2000). Ergovaline concentration (often used as a marker for ergot alkaloids) has been shown to be in the toxic range during December but drops to below toxic levels over the course of the winter (Kallenbach et al., 2003). If low performance while grazing stockpiled fescue is due to ergot alkaliods, grazing stockpiled endophyte-free fescue or non-toxic endophyte infected should eliminate any livestock health problem associated with ergot alkaloids and may therefore increase animal performance. Infection of tall fescue with non-toxic endophyte has the potential to improve plant persistence when compared to endophyte-free tall fescue (Latch, 1998) while still not causing reduced animal performance. However, animal performance while grazing stockpiled non-toxic endophyte infected fescue and long term agronomic performance of non-toxic endophyte infected stands when intensively grazed during the winter have not been evaluated. The objective of this study was two-fold. The first objective was to evaluate the long term agronomic performance of endophyte-infected (E+), endophyte-free (E-) and novel endophyte-infected (EN) tall fescue when stockpiled and intensively grazed in the winter. The second objective was to evaluate the performance of growing cattle when intensively grazing stockpiled E+, E- and EN fescue.

Materials and Methods

The experiment was replicated over 5 winters in a randomized complete block design with four field replications of three treatments (trt). Treatments consisted of Jessup tall fescue that was either infected with wild-type toxic endophyte (E+), infected with a non-toxic (AR542) novel endophyte (EN) or endophyte-free (E-).

Pasture establishment and management

The plots were planted in November, 1999 at the Butner Research Field Laboratory in Butner, NC. Each plot averaged 1.01 ha. The soil was a Georgeville silt loam (clayey, kaolinitic, thermic Typic Hapludults). Each yr, plots were cut for hay in August and then were allowed to accumulate for winter grazing. In early September, plots were fertilized with 84.5, 94.9, 84.2, 94.3 and 56.2 kg/ha of nitrogen from 30% urea ammonium nitrate in yr 1 through 5, respectively. Grazing of the plots was initiated in early December and lasted for 86, 70, 86, 72, and 56 d in yr 1 through 5, respectively.

Tiller samples (60 per plot) were taken in November, 2001 and again in August, 2006 to determine infection levels and percent of infected tillers producing ergot alkaloids. Tiller infection was detected by Agrinostics, Ltd. (Watkinsville, GA) using the procedure of Hiatt et al., (1999). Ergot alkaloid production was determined according to the procedure of Adcock et al., (1997).

Animals

The protocol for this study was approved by the Institutional Animal Care and Use Committee at North Carolina State University. Each yr, 48 Angus-cross tester cattle, steers in yr 1 and heifers in subsequent years, were randomly assigned to graze on one of the three trt. Extra “grazer” heifers were used in yr 3 (n = 12) and yr 4 (n = 10) in an effort to more uniformly utilize the available forage across replications. Animals were given a daily allotment of grass through strip-grazing management. The target residue grazing height was 5 cm.

Animals were given free choice access to a mineral supplement (Ru-Min 1620®, Southern States Cooperative, Inc., Richmond VA) containing 1780 mg/kg monensin; 10.5 % Ca; 6.0 % P; 26.0% Salt; 3,200 ppm zinc; 1,000 ppm copper (from sulfate); 3,000 ppm Mn; and 26 ppm Se. The supplement was labeled to contain 68,182 IU/kg, 18,182 IU/kg and 68 IU/kg of Vitamin A, D, and E, respectively.

Prior to the initiation of the study, cattle were vaccinated for protection against respiratory diseases (Titanium 5, Agri Labs, St. Joseph MO), clostridial organisms (Vision 7, Intervet, Millsboro, DE) and pinkeye (Piligard ® Pinkeye-1 trivalent, Schering-Plough, Omaha NE). Animals were also treated for internal and external parasites using Cydectin (Fort Dodge Animal Heath, Overland Park, KS) in yr 1, Safegard drench (Intervet, Millsboro, DE) in yr 2 and Bovimec pour on (Vibac, Fort Worth TX) in yr 3, 4 and 5.

Prior to the start of the grazing period all animals were given ad libitum orchardgrass hay and water for 10 d and then weighed on two consecutive days to determine initial full barn weights. Access to feed and water was then removed and animals were reweighed after 24 h to determine initial shrunk weights. Animals were stratified by body weight and randomly allotted to 12 groups. The groups were randomly assigned to replicate and trt. Subsequently, animals were placed on the plots and after 7 d animals were weighed on two consecutive days to determine pasture-adjusted start weights. Interim weights were taken every 14 d during the grazing period. At the end of the grazing period animals were weighed on two consecutive days to determine pasture end weights. Animals were then given ad libitum orchardgrass hay for 10 d. Final barn weights and shrunk weights were determined using same protocol as initial barn and shrunk weights. Prior to the initiation and at the conclusion of the grazing period an independent evaluator that was blind to treatment assigned body condition scores. Body condition score (BCS) was based on a 9 point scale (1 = emaciated; 9 = extremely obese) (NRC, 1996).

Blood samples

Blood samples were collected via jugular venipuncture 7 d prior to placement on plots to determine baseline level of serum urea nitrogen (SUN) and were collected every 28 d during the grazing period. Blood was collected in vacuum tubes without additive (Becton Dickinson, Franklin Lakes, NJ) and put on ice for no more than 6 h before transport to the laboratory, centrifuged at approximately 1,900 *g* for 20 min and serum was extracted. Serum samples were then stored at -15°C until assayed. Serum urea nitrogen was analyzed in duplicate by colorimetry using an auto-analyzer (Technicon Industrial Systems, Tarrytown, NY) using the diactyl monoxime method (Marsh et al., 1965).

Forage samples

At the start of the grazing period and every two wks thereafter, forage samples were clipped 5 cm from ground level using hand held battery operated grass shears (Black and Decker, Inc., Town, MD), at 10 randomly selected areas (0.09 m^2) per pasture, within the area that was estimated to be grazed in the next two wk. Forage was sub-sampled and divided into two portions. One portion was analyzed for nutrient content of the total sward and the other portion was used to determine species composition by hand separation. Forage samples used to determine nutritive composition of the total sward were frozen at -15˚C until dried in a 60˚C forced air oven for 48 h. During yr 1, two pasture replications of the portion used to determine species composition were pooled and then separated into 3 fractions consisting of green fescue, brown fescue and non-fescue species. During yrs 2 through 5, forage for hand separation were not pooled among replications and were separated into 4 fractions consisting of green fescue, brown fescue, green non-fescue and brown non-fescue. Following hand separation samples were frozen at -15˚C until dried in a forced air oven at 60˚C to determine the percent of each fraction in the sward on a DM basis. In yrs 2 though 5, nutritive value of hand separated fractions was determined on samples from wks 1, 5, 9 and 13 of the grazing period. Due to very small amounts of tissue, the brown non-fescue samples were pooled across replication by trt within date and green non-fescue samples were pooled across replication and trt within date for chemical analysis. In yr 4 and 5, a third portion of the forage sampled was frozen, freeze dried and analyzed for alkaloid content using the procedure of Adcock et al. (1997).

Forage nutrient analyses

Samples were ground in a Wiley Mill (Thomas Scientific, Swedesboro, NJ) to pass though a 1-mm screen and stored at room temperature in whirl-pack bags (NASCO, Modesto, CA). Dry Matter, ash and CP (Kjeldahl N*6.25) were analyzed by methods outlined by AOAC (1999). Total N was determined by auto-analyzer (Technicon Industrial Systems, Tarrytown, NY). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin were determined sequentially according to procedures outlined by Van Soest et al. (1991), using a batch processor (Ankom Corp., Fairpark, NY). Hemicellulose was determined as the difference between NDF and ADF, and cellulose was determined as the difference between ADF and the 72% sulfuric acid residue.

In vitro true dry matter digestibility (IVTDMD) was determined by a 48 h in vitro fermentation using an Ankom II Daisy IV 100 batch fermenter (Ankom Corp., Fairport, NY). Vessels contained McDougal's buffer and strained ruminal fluid (Tilley and Terry, 1963) obtained from a mature Hereford steer fed alfalfa hay (*Medicago sativa* L.), and digestion was terminated with NDF extraction.

Forage measurements

Initial forage mass was determined in mid-November of each yr. Indirect estimates of forage mass were taken using a 0.25m^2 falling plate meter (Vartha and Matches, 1977; Mueller et al. 1990) at 20 to 30 randomly selected sites in each pasture. Nine sample sites (0.25m^2) were clipped at ground level using battery-operated electric sheep shears (Sunbeam, Botany, New South Wales, Australia). The nine sample sites represented the range of forage mass present, with three sites representing low forage mass, three representing medium and three representing high forage mass. Forage harvested from the sites was dried at 60˚C for 72 h and weighed hot to determine the DM per acre. Regression was used to develop an equation relating the harvested forage from the nine sample sites with falling plate meter readings taken at each of the sites (Macoon et al., 2003). This equation was used to predict the mass available in each pasture using the 20 to 30 falling plate meter readings.

Forage offered and forage disappearance

Measurements for pre-grazing and post-grazing forage mass were taken every two wks during yr 3, 4 and 5 of the study. To determine pre-grazing mass falling plate meter readings were taken at 20 random sites within the area that was to be grazed within the subsequent two wk interval. Regression was calibrated using 7 harvested sites from within the pre-graze area. To determine post-grazing mass, falling plate meter readings were taken at 15 sites within the area that had been grazed in the previous two days. Regression was calibrated using 5 harvested sites within the post-graze area. A GIS TSCe field device with a PRO XRS receiver and Trimble survey controller software (Trimble Navigation Limited, Sunnyvale, CA) was used to measure the area grazed during the two wk intervals.

Forage offered ($kg \cdot hd^{-1} \cdot d^{-1}$) during each two wk period was determined using the following formula: [pre-grazing mass (DM/ha) x area offered during period (ha)] / [number of animals grazing (hd) x length of grazing period (d)]. Pasture disappearance ($kg \cdot hd^{-1} \cdot d^{-1}$) during each two week period was determined using the following formula:

[[pre-graze forage mass (DM/ha) - postgraze forage mass (DM/ha)] x area offered (ha)] / [number of animals grazing (hd) x length of grazing period (d)].

Gain per ha and animal grazing days

During each year the gain per ha and the number of animal grazing days per ha was calculated for each plot. Gain per ha (kg/d) was calculated using the following formula: [pasture ADG ($kg \cdot hd^{-1} \cdot d^{-1}$) x animals grazing (hd) x length of grazing period (d)] / [total area grazed (ha)]. Animal grazing days (d/ha) was calculated using the following formula: [animals grazing (hd) x length of grazing period (d)] / [total area grazed (ha)].

Statistical analysis

Data were analyzed using the PROC MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). Nutritive composition of total sward and forage fractions and species composition were analyzed using repeated measures. The model included the fixed effects of trt and yr and the trt by yr interaction. The effect of time on the nutritive composition of the forage was evaluated by testing for linear and quadratic effects of sample date as well as associated interactions. Interactions that were not significant ($P >$ 0.20) were removed from the model. The experimental unit was for all animal data was group within trt and replication. The error term for all data except SUN was replication by trt by yr. Error term for SUN data was animal within replication by trt by yr, in order to account for variation due to individual animals. Species composition during year 1 was analyzed separately from subsequent years due to differences in the method by which samples were separated. Significant differences were defined as $P \leq 0.05$ and tendencies at $P \leq 0.10$.

Results and Discussion

Tiller samples

The percent of tillers that were endophyte infected differed among trts ($P < 0.01$) but did not differ by yr ($P = 0.12$). A trt by yr interaction was observed ($P = 0.01$; Table 1). In yr 1, the percent of infected tillers of EN was higher than E+ ($P = 0.03$) and E- ($P < 0.01$), while the percent of infected tillers of E+ was higher than E- ($P < 0.01$). From yr 1 to yr 5, the percent of infected tillers of E+ increased ($P < 0.01$) while the percent of infected tillers of EN tended to decrease ($P = 0.08$) causing E+ to have a higher infection rate than EN ($P < 0.01$) in yr 5. The percent of infected tillers of E- did not change ($P = 0.70$) from yr 1 to 5 maintaining at approximately 5% infectivity. Both E+ ($P < 0.01$) and EN ($P < 0.01$) had a higher percent of infected tillers than E- in yr 5. Of the endophyte infected tillers the percent producing ergot alkaloids differed by trt ($P < 0.01$) and by yr ($P < 0.01$). A yr by trt interaction was also observed ($P = 0.04$; Table 1). All of the infected tillers in E+ and E- in yr 1 produced ergot alkaloids in contrast to less than 4% of EN infected tillers. From yr1 to yr 5, there was a decrease in ($P < 0.01$) the percent of infected tillers producing ergot alkaloids in E+ while tillers producing ergot alkaloid in EN tended ($P = 0.08$) to decrease and E- did not change ($P = 1.0$). Over the five yr period neither E- nor EN appeared to be invaded by E+.

Climatological data

Rainfall and temperature (Table 2) during the forage-accumulating phase (August through November) influences the growth of tall fescue and can therefore have a large impact on the dry matter yield. In yr 1, rainfall was below average from August to November. In yr 2, rainfall was above average in August, October and November, but slightly below average in September. In yr 3, rainfall was above average during the first half of the accumulation phase but below during the second half. In August of yr 4, rainfall was almost double that of the 30-yr average after which rainfall was below average thru November. During August, October and November of yr 5 rainfall was above average but below average in September.

Temperature while the cattle were on pasture (December through February) was above the 30-yr average in yr 1, 4 and 5 while in yr 2 and 3 it was below the 30-yr average (Table 2). The average temperature from December through February was 6.2, 2.8, 2.9, 5.3 and 5.2˚C in yr 1 through 5, respectively.

Forage mass

Initial forage mass. Initial forage mass differed by trt ($P < 0.01$; Table 3) and by yr ($P < 0.01$; Table 4) but trt by yr was not significant ($P = 0.11$). Initial forage mass of E- was lower than E+ ($P < 0.01$) and EN ($P = 0.01$). Forage mass of E+ and EN did not differ ($P = 0.16$). In contrast, previous research indicated that the addition of a novel-endophyte to HiMag tall fescue did not improve fall growth over HiMag E- (Kallenbach et al., 2003).

Animal grazing days. Animal grazing days differed among trt ($P < 0.01$; Table 3) and by yr ($P < 0.01$; Table 4). The animal grazing days on E+ were higher than on EN ($P = 0.02$) or E- ($P < 0.01$) which did not differ ($P = 0.12$). The trt by year interaction was significant ($P > 0.01$; Table 5). In yrs 1, 3 and 4, E+ had a higher ($P \geq 0.05$) number of animal grazing days than E- and EN. In yr 2, E+ was higher than E- ($P = 0.04$) and tended to be higher ($P = 0.08$) than EN. In years 1, 2, and 4 E- and EN did not differ ($P > 0.50$); however, in yr 3 EN was higher than E- ($P < 0.01$). In yr 5, endophyte status did not affect animal grazing days ($P > 0.20$).

Stand composition

Percentage of fescue in the sward and green fescue fractions. Due to the replications in yr 1 being pooled data from yr 1 was analyzed separately from subsequent yrs. In yr 1, the percent of fescue in the sward did not vary ($P = 0.55$) by trt and was 87.9, 87.1 and 86.7 % (SE ± 1.70) for E+, E- and EN, respectively. However, the amount of fescue in the sward in yr 2 through 5 varied by yr ($P < 0.01$; Table 6) and among the trts ($P < 0.01$; Table 7). The interaction between trt and yr was not significant ($P = 0.23$). In yrs 2 through 5, the percent of total fescue in the sward of E- was lower ($P = 0.01$) than E+ or EN which did not differ ($P = 0.16$).

The percent of fescue that was green in yr 1 differed among trt ($P < 0.01$). The E- sward had the least green fescue ($P < 0.01$), EN was intermediate ($P < 0.01$) and E+ had the highest amount of green fescue ($P < 0.01$). The percent of fescue that was green was 48.9, 31.2, and 41.0 % (SE ± 3.82) for E+, E-, and EN, respectively. There was also a wk by trt interaction in yr one ($P = 0.05$). In early December the percent of fescue that was green was 72.8, 55.2, and 61.4 % for E+, E-, and EN, respectively. While E+ had more green fescue at the beginning of the winter, the decrease in fescue that was green was higher for E+ than E- and EN. By the end of the winter the percent of green fescue in the sward was 26.2, 16.2 and 23.3 % for E+, E- and EN. The lower percent of fescue that was green present in E- and EN during yr 1 may have been due to drought stress (Table 2) and was not noted in subsequent yrs.

The percent of fescue that was green in yr 2 through 5 did not differ among the trt (P = 0.71; Table 7) but did differ among yr (P = 0.05; Table 6). The trt by yr interaction was not significant (P = 0.24). The percent of fescue that was green in the sward decreased linearly (P = 0.04) over the winter. In yr 2 through 5, the percent fescue that was green in wk 1 was 81.4 % and it decreased to 42.8 % by wk 11. There was a wk by yr interaction (P = 0.04). In yrs 2 through 5, the trt by wk interaction was not significant (P = 0.15) suggesting that endophyte infection does affect leaf senescence over the winter.

Percentage green and brown non-fescue fractions. The percentage of green and brown non-fescue fractions was not determined in yr 1. In yrs 2 through 5, the brown non-fescue species consisted mainly of crabgrass (*Digitaria sanguinalis*) and johnsongrass (*Sorghum halepense*). The amount of brown non-fescue species differed by yr (P > 0.01; Table 6) and by trt (P = 0.01; Table 7).

The green non-fescue in yrs 2 through 5 was mainly Kentucky bluegrass (*Poa pratensis*). The amount of green non-fescue species differed by trt (P = 0.01; Table 7) and by yr (P = 0.01; Table 6). There was a trt by yr interaction (P = 0.02; Figure 1). In yr 2 of the study E+, E-, and EN did not differ (P > 0.20) in the amount of green non-fescue in the sward. However, in yr 3, 4, and 5 the amount of green non-fescue in E- was higher than E+ and EN ($P < 0.02$) which did not differ ($P > 0.20$). The percent of green non-fescue in yr 2 was 2.8, 2.0 and 1.9 % (SE ± 0.46) for E+, E-, and EN. In yr 5 the green non-fescue was 3.0, 6.4, and 3.0 % for E+, E-, and EN, respectively. Endophyte-free stands showed an increase in green non-fescue from yr 2 to 5 ($P < 0.01$) while E+ and EN did not ($P = 0.15$, $P = 0.11$, respectively). There was a significant ($P < 0.01$) wk by trt interaction.

There were wk by yr interactions for the percent of fescue ($P < 0.01$), brown non-fescue ($P = 0.06$) and green non-fescue ($P = 0.04$) in the sward. However, this may be reflective of differences in the area sampled in the field and may not necessarily reflect changes in composition over the course of the winter.

Forage nutrient composition

Green and brown fescue fractions. Nutritive composition of the fractions was not analyzed in yr 1. All measures of nutrient content in green and brown fescue in yrs 2 through 5 differed by yr ($P < 0.01$; Table 8). The IVTDMD of green fescue differed among the trt ($P = 0.05$; Table 9). The IVTDMD of E- was higher ($P = 0.01$) than E+ but did not differ ($P = 0.11$) from EN, while E+ and EN did not differ ($P = 0.22$). Consistent with higher IVTDMD, NDF, and cellulose were lower ($P < 0.01$) for E- than E+ and EN. Acid detergent fiber ($P = 0.35$) and lignin ($P = 0.71$) of green fescue did not vary among the trt and were 21.6 and 1.5 %, respectively. In contrast, Burns et al. (2006) did not report a difference due to endophyte status in ADF, NDF, cellulose, lignin, or CP of the green leaf but did report that IVTDMD was slightly higher (less than one percentage unit) for E- than EN. While statistical differences in the IVTDMD, NDF, and cellulose of the green fescue fractions were observed in this study these differences were less than one percentage unit and would not be reflected in animal performance. While the amount of fescue that was green declined over the winter, the tissue that remained green retained high IVTDMD (85% or greater) throughout the winter and was not affected by trt ($P > 0.20$).

Most of the nutrient content parameters measured (IVTDMD, ADF, NDF, cellulose, and lignin) on the brown fescue fraction of the sward did not vary among trt ($P > 0.10$; Table 9). However, there was a trt by yr interaction ($P < 0.01$) for CP of both green and brown fescue fractions but all differences were small (Table 10). Burns et al. (2006) reported no differences in nutritive composition due to endophyte status of dead fescue tissue. As expected, brown fescue was nutritionally inferior to the green fescue having lower CP and IVTDMD as well as higher ADF and NDF. As observed previously by Burns and Chamblee (2000) and Poore et al. (2006), the increase in brown tissue was the main contributor to the decrease in nutritive value over the winter.

Green and brown non-fescue fractions. All measures of nutritive content of the brown non-fescue in yrs 2 through 5 varied by yr ($P < 0.01$; Table 11). The IVTDMD, CP, NDF, cellulose, and lignin of the brown non-fescue fraction did not differ ($P > 0.05$) among trt. However, the ADF was lower ($P < 0.05$) for E- (38.03 %) than for E+ and EN (39.02 and 38.75 %, respectively) which did not differ ($P = 0.44$). This difference is probably not biologically significant. All of the measures of nutrient content of the green non-fescue with the exception of IVTDMD ($P = 0.23$) differed by yr ($P \leq 0.05$; Table 11).

Total sward. Nutritive composition (IVTDMD, CP, ADF, NDF, cellulose, and lignin) of the total sward varied by yr ($P < 0.01$; Table 12) but were within the range observed in other studies conducted in North Carolina (Burns and Chamblee, 2000; Burns et al., 2006; Poore et al., 2006). In general, yr 1 and 3 had the lowest nutritive composition while yr 2 and 5 had the highest. The low forage quality in yr 1 and 3 may be due in part to the higher yield noted in these yr. Balasko (1977) reported that high yielding stockpiled fescue (two to three metric tons/ha) had a higher rate of leaf senescence and decomposition. The higher nutritive content during yrs 2 and 5 may have been due to delayed accumulation. Burns et al. (2006) reported that higher IVTDMD is associated with delayed accumulation. While forage was given the same amount of time to accumulate during each yr, weather would have effected growth and could have delayed accumulation in yr 2 and 5. This is supported by the lower yields that occurred in yr 2 and 5.

The IVTDMD of the total sward differed by trt ($P < 0.01$; Table 13) and by yr ($P < 0.01$; Table 12). There was a trt by yr interaction ($P = 0.03$). In yr 1, IVTDMD of E+ was higher ($P < 0.01$) than E- and EN, while EN was higher (P < 0.01) than E-. In yr 1, IVTDMD was 67.7, 59.9 and 65.5% for E+, E-, and EN, respectively. The differences in digestibility are most likely related to the higher content of brown fescue in the EN and E- swards throughout the winter. In yr 2, 4, and 5 the IVTDMD did not differ by trt ($P > 0.05$). However, in yr 3 IVTDMD of E+ (67.7 %) was higher ($P \leq 0.01$) than E- (63.6 %) and EN (64.4 %) which did not differ ($P = 0.57$). In contrast, Burns et al, (2006) did not report a difference in the IVTDMD of stockpiled fescue due to endophyte status.

Acid detergent fiber tended ($P = 0.10$) to differ by trt (Table 13). There was also a trend ($P = 0.08$) for a trt by yr interaction. In yr 1, the ADF of E- (33.7 %) was higher ($P < 0.01$; $P = 0.05$) than that of E+ (32.2 %) and EN (32.9 %) which did not differ ($P = 0.13$; SE ± 0.51). The ADF in yr 2 through 5 did not differ among the trt ($P > 0.05$). The NDF content of the total sward differed ($P < 0.01$) by trt (Table 13). The E+ sward had a lower ($P < 0.01$) NDF than E- or EN which did not differ ($P = 0.56$).

The cellulose content of the total sward did not vary ($P = 0.64$) among the trt (Table 13). There was a trend for a trt by yr interaction ($P = 0.09$). Cellulose content did not differ by trt in yr 2, 3, and 5 ($P > 0.05$). However, in yr 1 the cellulose content of E+ (27.7%) was lower ($P = 0.02$) than cellulose content of E- (28.4 %), while EN (27.9%) did not differ ($P > 0.05$) from E+ or E-. Whereas in yr 4, the cellulose content of E+ (27.6%) was higher ($P = 0.01$) than cellulose content of E- (26.8 %), while EN (27.4%) did not differ ($P > 0.05$) from E+ or E-. The lignin content of the total sward differed ($P < 0.01$) among the trt (Table 13). Lignin content of E- was higher ($P = 0.05$) than EN and E+, while the lignin content of EN tended to be higher ($P = 0.06$) than E+. The higher lignin observed in E- sward is most likely due to the higher content of brown non-fescue in the sward.

In this study, the fiber content and digestibility of the total sward varied slightly due to trt, and was caused by E- having an increased amount of brown fescue in yr 1 as well as higher amounts of brown non-fescue species in the sward during yr 2 through 5. However, Burns et al., 2006 reported no differences in IVTDMD, NDF, ADF, cellulose or lignin of stockpiled E+, E- and EN Jessup tall fescue and Kallenbach et al. (2003) also reported no difference in ADF or NDF of stockpiled Ky-31 E+, HiMag E- and HiMag

EN. The difference seen in this study compared to other studies may be related to length of the study and age of the stand.

Both lignin and cellulose increased linearly ($P < 0.01$) over the winter and correspond to a linear ($P < 0.01$) increase in ADF over the winter (Table 14). While NDF showed both linear ($P < 0.01$) and quadratic ($P = 0.03$) wk effects, with the NDF content of the sward increasing as the winter progressed but decreasing slightly toward the end of the winter. As expected with an increase in the fiber content of the forage, there was a linear decrease ($P < 0.01$) in IVTDMD over the winter (Table 14). The linear decrease in digestibility and linear increase in ADF and NDF over the winter are consistent with the literature (Burns et al., 2006; Kallenbach et al., 2003). Burns and Chamblee (2000) noted a general decline in IVDMD and CP and an increase in NDF as the proportion of green tissue in the sward declined as the winter progressed.

Crude protein of the total sward (Table 13) was higher ($P < 0.01$) for the E- than the E+ and EN which did not differ ($P = 0.13$). There was a trend ($P = 0.10$) toward a trt by yr interaction (Table 9). The higher CP content of the total sward of E- is presumably due to the higher composition of green and brown non-fescue (Table 8) in the stand which had a higher CP content than the fescue fractions. Averaged across trt, yr and time the CP was 12.7 % and 8.5 % for the green and brown fescue, respectively, compared to 16.4 % and 12.7 % for the green and brown non-fescue, respectively. The slightly higher CP content of the E- green and brown fescue fractions (Table 8) would have also contributed to the higher CP observed in the total sward of E-. In contrast other research on stockpiled E+, E- and EN fescue (Kallenbach et al., 2003; Burns et al., 2006) reported no differences in CP of the total sward. The CP content of the sward showed both linear

($P < 0.01$) and quadratic ($P < 0.01$) effects over the winter. The slight increase in CP as well as the slight decrease in NDF of the total sward at the end of the winter is probably due to new growth occurring. New growth of fescue in mid to late February in accumulated forage has been reported to occur when temperature reach 27°C for 10 to 14 d at a time (Burns and Chamblee, 2000).

Ergot alkaloid content of total sward. Ergot alkaloid concentration in early-December did not vary between yr 4 and 5 ($P = 0.16$) but did differ by trt ($P < 0.01$). Total sward ergot alkaloid concentration in early-December of E+ was higher ($P < 0.01$) than E- or EN which did not differ ($P = 0.50$). Ergot alkaloid levels at the beginning of the winter were 2350, 291 and 190 ug/kg (SE ± 144.7) for E+, E-, and EN. Ergot alkaloid content of E+ decreased ($P < 0.01$) to 533 ug/kg by early-February. Ergot alkaloid concentration in stockpiled fescue had not been previously reported but the 77 % decline from early-December to late-February in ergot alkaloid concentration observed in this study is similar to the decrease in ergovaline reported in other studies (Kallenbach et al. 2003; Burns et al., 2006). Kallenbach et al. (2003) observed an 85 % decrease in ergovaline concentration in stockpiled Ky-31 from mid-December to mid-March. Burns et al. (2006) showed that the decrease in ergovaline is correlated to the decrease in ergovaline of the green leaf.

Animal performance

Weight gain. Initial body weights did not differ among the trt ($P = 0.94$). Initial body weights were heavier ($P < 0.01$; SE ± 5.37) in yr 1 and 2 (270 kg) than in yr 3 through 5 (255 kg). Pasture ADG did not differ ($P = 0.12$) among trt while barn ADG tended to differ ($P = 0.07$) among trt (Table 14). Shrunk ADG differed ($P = 0.02$) among the trt (Table 14); animals on E+ had lower shrunk AGD than both E- and EN ($P < 0.01$, $P = 0.04$, respectively), while E- and EN did not differ ($P = 0.47$). However, the reduction in gain due to grazing E+ was small. Nutrient composition of stockpiled E+ often over predicts gains observed for grazing heifers (Poore et al., 2000). Data from this trial suggests that the presence of endophyte in E+ is not the cause of the lower than expected gains observed by cattle grazing stockpiled fescue.

There was a yr effect ($P < 0.01$) for all three ADG measurements (Table 15). Pasture ADG in yr 1 and 2 were the lowest with gain increasing in subsequent yr. However, barn and shrunk ADG in yr 3 and 4 were much lower than the pasture ADG recorded in those years. This is most likely due to the low quality of the orchardgrass hay fed to the heifers after being removed from pasture during yr 3 and 4, which decreased body wt during the 10 d period. The IVTDMD of the orchardgrass hay was 46.4 % in yr 3 and in 57.3 % yr 4.

The high fiber content and low digestibility of the sward in yr 1 and 3 may have been partly responsible for the lower pasture ADG compared to yr 4 and 5 (Table 15). Poore et al. (2006) reported pasture ADG of unsupplemented heifers intensively grazing stockpiled E+ from late-December to late-February of 0.56 kg/d (yr.1) and 0.41 kg/d (yr.2). The IVTDMD and gains reported by Poore et al. (2006) are comparable to the

IVTDMD and gains observed in yr 1 and 3 of this study. The invitro true organic matter disappearance (IVTOMD) reported by Poore et al. (2006) during yr 2 was 72.3 % in early December and decreased to 67.3 % by early February. The IVTDMD of the total sward in this study was 73.6 % (yr 1) and 72.8 % (yr 3) in early-December and decreased to 58.9 % (yr 1) and 61.2 % (yr 3) by mid-February.

The differences in temperature may also account for some of the yr to yr variation in animal gains while on pasture. Cattle have been reported to have reduced efficiency of production when environmental temperature is below thermoneutral zone (Delfino and Mathison, 1991). In yr 2 and 3, the average air temperature while on pasture was below 4˚C. In yr 2 and 3 the lower temperature may have decreased dietary energy available for gain as compared to yr 1, 4 and 5 when air temperature was higher.

Body condition score. Initial body condition scores (BCS) differed across yr (P = 0.01) but were not different among trt (P = 0.71). Mean BCS were 5.05, 5.13, 4.95, 5.05 and 4.99 (SE ± 0.05) for yr 1 through 5, respectively. Change in body condition score (CBCS) over the course of the study did not differ (P = 0.89) among the trt (Table 14) but did differ across yr (P < 0.01; Table 15). The CBCS did not differ ($P \geq 0.25$) among yr 1, 2 and 3, but was greater in yr 5 (P < 0.01), while yr 4 had the highest CBCS ($P \leq 0.01$). Poore et al. (2006) reported CBSC of -0.03 (yr 1) and 0.13 (yr 2), which is similar to what was observed in yr 1, 2 and 3 of this study. The gains and CBCS during yr 4 and 5 of this study were much greater than those in previous yr of this study as well as those reported by Poore et al. (2006). However, the higher CBCS is consistent with the higher pasture ADG observed in yr 4 and 5.

Gain per ha. Gain per ha differed by trt ($P = 0.04$; Table 3) and by yr ($P < 0.01$; Table 4). There was no trt by yr interaction ($P = 0.14$). The gain per ha on E+ was higher than E- ($P = 0.02$) and EN ($P = 0.05$).

Serum urea nitrogen. Serum urea nitrogen of animals grazing during the winter differed by yr (P < 0.01; Table 15) and by trt (P = 0.03; Table 14). The SUN concentration of cattle grazing E- were higher (P < 0.01) than cattle grazing E+ and EN, which did not differ (P = 0.82). The SUN during yrs 1, 2, and 3 did not differ (P > 0.15) but were lower (P < 0.01) than in yr 4 and 5. While yr 4 was lower (P < 0.01) than yr 5. Hammond et al. (1994) indicated that blood urea nitrogen levels of cows on an all forage diet that are below 9.0 mg/dL indicate protein deficiency. Irregardless, SUN concentrations in yrs 1 through 3 were 6 mg/dL or below, suggesting that protein intake was limiting ADG. In yr 4, SUN concentrations were higher than in pervious yrs (approximately 7 mg/dL) but still below protein adequate level.Whereas, in yr 5 SUN was approximately 11 mg/dL suggesting that protein intake was not limiting growth. Year differences in SUN observed may partially explain the higher gains in yrs 4 and 5. There was a significant trt by yr interaction (P = 0.02; Table 16). In yrs 1 and 3, SUN did not differ among trt (P > 0.20). In yr 2, E+ did not differ (P > 0.10) from E- or EN while E- was higher (P = 0.02) than EN. In yrs 4 and 5, cattle on E- had higher (P < 0.05) SUN than those on E+ or EN, which is consistent with higher CP content in the sward. There was also a date by yr interaction (P < 0.01). In yrs 1, 2 and 3, SUN decreased over the winter averaging 6.72, 5.82 and 4.83 mg/dL in wk 3, 7 and 11, respectively. However, in yr 4, SUN increased from 7.01 mg/dL in wk 3 to 7.97 mg/dL in wk 7 and then decreased to 6.80 mg/dL in wk 11. In yr 5, SUN increased from 8.30 mg/dL in wk 3 to 11.30 mg/dL in wk7. In a study by Poore et al. (2006) heifers grazing stockpiled E+ had SUN concentrations of 9.44 (yr 1) and 7.82 (yr 2) mg/dL and when supplemented with whole cottonseed heifers had increased SUN and gains, with the response to supplementation being higher in yr 2 when CP in the forage was lower.

Estimated intake

Forage offered. The forage offered to the heifers in yr 3, 4 and 5 differed by yr (P = 0.05) but did not differ by trt (P = 0.96; Table 3). Forage offered in yr 3 did not differ from yr 4 (P = 0.23) or yr 5 (P = 0.11). Forage offered in yr 5 was higher (P = 0.02) than in yr 4. There were significant linear (P = 0.03) and quadratic (P < 0.01) effects of week as well as wk by yr (P < 0.01) and wk by wk by yr (P < 0.01) interactions.

Residual forage mass. Residual forage mass did not differ among trt (P = 0.14; Table 3) but did differ by yr (P < 0.01). Residual forage mass was higher (P =0.02) in yr 5 (1628 kg/ha) than in yr 3 (1476 kg/ha) while residual forage mass in yr 4 (1541 kg/ha) did not differ from yr 3 (P = 0.31) or yr 5 (P = 0.14). The trt by yr interaction was not significant (P = 0.58). There were significant wk by yr and wk by wk by yr interactions (P < 0.01).

Forage disappearance. Forage disappearance (DM basis) differed by yr (P > 0.01) but did not differ among the trt (P = 0.75; Table 3). Forage disappearance tended to be higher in yr 3 than in yr 4 (P = 0.07) and 5 (P = 0.09), which did not differ (P = 0.62). Forage disappearance was 5.48, 4.33 and 4.56 kg $\cdot hd^{-1} \cdot d^{-1}$ (SE ± 0.31) in yr 3 through 5, respectively. The increase in estimated intake in yr 3 may be due to the cooler weather in yr 3 when compared to yr 4 and 5. Increased feed intake has been reported when ambient temperature falls below thermoneutral (NRC, 1996). The forage disappearance observed in this study was higher than the forage organic matter disappearance reported in a two yr study by Poore et al. (2006), which observed forage disappearance of 3.29 (yr 1) and 4.16 (yr 2) kg $\cdot hd^{-1} \cdot d^{-1}$. There were significant linear ($P = 0.02$) and quadratic ($P = 0.02$) effects of week as well as a wk by wk by yr interaction ($P < 0.01$).

Implications

Under the conditions in this study, EN appears to have agronomic performance similar to E+ while E- has lower forage production and stand persistence. Gains of growing cattle strip-grazing stockpiled fescue from December to March, did not appear to be greatly affected by endophyte status. The lower forage production of E- and the higher cost associated with establishing EN combined with little increase in gains, suggests that E+ is a good option for a low cost source of winter feed, while grazing of EN may be more beneficial during the spring and fall when more severe negative effects of ergot alkaloids produced by E+ have been observed.

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SUMMARY

Endophyte status did not affect the rate of leaf senescence over the winter and caused only slight variation in nutritive content of the total sward. The IVTDMD of the total sward was slightly lower and CP was higher for E- than E+ and EN. These differences were mainly caused by differences in the amount of non-fescue species in the sward and not by differences in the fescue itself. A small difference in animal performance due to endophyte status was also noted. A 0.08 kg/d reduction in the gain in growing cattle grazing E+ when compared to E- was observed. Forage offered and forage disappearance did not differ among trts. Total forage mass of E+ and EN did not differ but were higher than E-. Animal grazing days of EN and E- did not differ and were lower than E+. Even though daily gain of cattle grazing E- and EN was slightly higher, gain per ha on E+ was higher.

Table 1. Percent of tillers infected with endophyte and percent of infected tillers that produced ergot alkaloids of stands with varying endophyte status during yr 1 and yr 5

		Treatment[†]				*P*-value	
Item	E+	E-	EN	SEM	trt	yr	trt x yr
Endophyte infected tillers, %							
yr 1	85.8^{c}	4.6^{d}	91.3^{b}	2.38	< 0.01	0.12	0.02
yr 5	97.5^{a}	5.4^{d}	87.1^{bc}				
Producing ergot alkaloids[‡],%							
yr 1	100^{a}	100^{a}	3.2^{c}	1.35	< 0.01	< 0.01	0.04
yr 5	94.0^{b}	100^{a}	0.5^{c}				

[a-d] means with differing superscripts differ
[†] E+ = endophyte infected; E- = endophyte-free; EN = non-toxic endophyte-infected
[‡] Percent of infected tillers producing ergot alkaloids

Table 2. Mean precipitation (precip.) and temperature departure from long-term monthly mean during yr 1 through 5[†]

	30-yr mean		Departure from 30-yr mean									
			Yr 1		Yr 2		Yr 3		Yr 4		Yr 5	
Month	Precip	Temp	Percip	Temp	Precip	Temp	Precip	Temp	Precip	Temp	Precip	Temp
	mm	˚C	mm	˚C	mm	˚C	mm	˚C	mm	˚C	mm	˚C
August	111	25.2	-21	-1.0	61	-0.4	91	-0.4	103	-2.6	31	0.7
September	111	21.5	-66	2.6	-2	-0.1	76	-1.1	-10	-1.0	-74	1.9
October	94	15.0	-59	-1.8	128	0.3	-25	-0.8	-44	0.2	3	0.8
November	86	9.8	-76	1.4	8	-2.0	-38	2.5	12	1.1	72	0.2
December	87	5.3	-59	2.8	-2	-1.7	10	-1.6	-28	-0.4	20	-1.6
January	113	3.6	-31	0.8	-31	-2.2	-82	-1.8	-9	1.7	-50	3.2
February	94	5.3	-67	0.8	53	-1.8	-35	-1.9	-38	0.3	-32	-0.1

[†] Data recorded in Durham Co. and reported by the National Oceanic Atmospheric Administration (approx. 20km from research site).

Table 3. Forage mass, gain per hectare, animal grazing days and estimated intake of heifers strip-grazing stockpiled fescue with varying endophyte status

	Treatment†				
Item	E+	E-	EN	SEM	*P*-value*
Initial forage mass, kg	3979[a]	3508[b]	3829[a]	95.5	0.01
Gain per hectare, kg	257[a]	220[b]	228[b]	16.0	0.04
Animal grazing days, d/ha	511[a]	399[b]	440[b]	22.7	< 0.01
Forage offered‡, $kg·hd^{-1}·d^{-1}$	8.52	8.60	8.73	0.52	0.96
Forage disappearance‡, $kg·hd^{-1}·d^{-1}$	4.76	4.72	4.97	0.27	0.75
Forage residual‡, kg/ha	1614	1491	1541	78	0.14

[a-b] Treatment means with differing superscripts differ ($P \leq 0.05$).
† E+ = endophyte infected; E- = endophyte-free; EN = non-toxic endophyte-infected
* *P*-value of treatment effect
‡ mean of yrs 3,4 and 5

Table 4. Forage mass, gain per hectare and animal grazing days of stockpiled fescue with varying endophyte status as influenced by year

	Year						
Item	1	2	3	4	5	SEM	*P*-value*
Initial forage mass, kg	5570[a]	2280[d]	4736[b]	3759[c]	2518[d]	112	< 0.01
Gain per hectare, kg	199[c]	176[c]	274[ab]	297[a]	229[bc]	13.3	< 0.01
Animal grazing days per hectare, d/ha	534[a]	445[b]	569[a]	426[b]	277[c]	27.3	< 0.01

[a-b] means with differing superscripts differ ($P \leq 0.05$).

* *P*-value of year effect

Table 5. Animal grazing days of stockpiled fescue as influenced by endophyte status and year

Item		Treatment†		SEM	*P*-value*
	E+	E-	EN		
Animal grazing days per hectare, d/ha					
yr 1	591[a]	499[b]	513[b]	34.16	< 0.01
yr 2	492[a]	414[b]	428[ab]		
yr 3	686[a]	444[c]	576[b]		
yr 4	494[a]	380[b]	404[b]		
yr 5	292	260	280		

[a-b] Treatment means with differing superscripts differ within yr ($P \leq 0.05$).
† E+ = endophyte infected; E- = endophyte-free; EN = non-toxic endophyte-infected
* *P*-value of treatment by year effect

Table 6. Percent of fescue and non-fescue tissue (DM basis) of stockpiled fescue sward as influenced by year

	Year					
Item	2	3	4	5	SEM	*P*-value*
Total fescue in sward, %	86.6^{b}	81.8^{c}	91.7^{a}	88.8^{ab}	1.31	< 0.01
Green fescue‡, %	68.0^{a}	53.1^{c}	56.9^{b}	57.0^{b}	1.02	0.05
Green non-fescue of sward, %	2.23^{c}	1.95^{c}	3.01^{b}	4.11^{a}	0.28	<0.01
Brown non-fescue of sward, %	12.3^{b}	16.1^{a}	5.2^{d}	8.5^{c}	1.11	<0.01

$^{a\text{-}c}$ means with differing superscript differ
* *P*-value of yr effect
‡ Percent of fescue in sward that is green

Table 7. Percent of fescue and non-fescue tissue (DM basis) of stockpiled fescue sward with varying endophyte status in year 2 through 5

	Treatment[†]				
Item	E+	E-	EN	SEM	*P*-value*
Total fescue in sward, %	90.7[a]	82.5[b]	88.4[a]	1.3	< 0.01
Green fescue[‡], %	60.1	57.9	58.2	0.77	0.71
Green non-fescue of sward[1], %	1.95[b]	4.40[a]	2.14[b]	0.26	< 0.01
Brown non-fescue of sward, %	7.94[b]	14.02[a]	9.78[b]	1.11	< 0.01

[a-c] means with differing superscript differ
[1] trt by yr interaction ($P < 0.05$)
[†] E+ = endophyte infected; E- = endophyte-free; EN = non-toxic endophyte-infected
* *P*-value of treatment effect
[‡] Percent of fescue in sward that is green

Table 8. Nutritive composition (DM basis) of green and brown fescue tissue in stockpiled fescue

	Year					
Item	2	3	4	5	SEM	*P*-value*
Green fescue						
IVTDMD, %	90.5^{a}	85.0^{d}	86.9^{c}	88.2^{b}	0.23	< 0.01
CP, %	11.5^{c}	11.5^{c}	12.5^{b}	15.1^{a}	0.26	< 0.01
ADF, %	19.6^{d}	22.7^{b}	23.5^{a}	20.8^{c}	0.32	< 0.01
NDF, %	42.8^{c}	48.7^{b}	50.2^{a}	50.2^{a}	0.38	< 0.01
Cellulose, %	18.3^{c}	21.0^{b}	22.1^{a}	18.6^{c}	0.41	< 0.01
Lignin, %	0.89^{d}	1.36^{b}	1.09^{c}	2.49^{a}	0.07	< 0.01
Brown fescue						
IVTDMD, %	67.3^{a}	53.2^{c}	60.7^{b}	58.1^{b}	1.74	< 0.01
CP, %	7.7^{c}	7.6^{c}	9.1^{b}	9.8^{a}	0.30	< 0.01
ADF, %	34.1^{c}	37.2^{a}	35.9^{b}	33.5^{c}	0.33	< 0.01
NDF, %	66.9^{c}	72.7^{a}	69.6^{b}	70.6^{b}	0.67	< 0.01
Cellulose, %	30.1^{c}	32.3^{a}	31.5^{b}	27.9^{d}	0.28	< 0.01
Lignin, %	2.79^{d}	4.05^{b}	3.45^{c}	6.35^{a}	0.26	< 0.01

$^{a-c}$ means with differing superscript differ

* *P*-value of year effect

Table 9. Nutritive composition (DM basis) green and brown fescue fractions of stockpiled fescue sward with varying endophyte status in years 2 through 5

	Treatment†				
Item	E+	E-	EN	SEM	*P*-value*
Green fescue					
IVTDMD, %	87.3[b]	88.1[a]	87.6[ab]	0.25	0.05
CP[1], %	12.3	13.2	12.5	0.24	0.14
ADF, %	22.2	21.1	21.6	0.27	0.35
NDF, %	48.2[a]	47.5[b]	48.2[a]	0.36	< 0.01
Cellulose, %	20.5[a]	19.4[b]	20.1[a]	0.27	< 0.01
Lignin, %	1.44	1.47	1.47	0.04	0.71
Brown fescue					
IVTDMD, %	61.1	59.6	58.9	0.82	0.07
CP[1], %	8.3	8.9	8.4	0.21	0.39
ADF, %	35.5[a]	34.6[b]	35.4[a]	0.28	< 0.01
NDF, %	70.1[a]	69.2[b]	70.5[a]	0.42	< 0.01
Cellulose, %	30.8[a]	29.8[b]	30.8[a]	0.23	< 0.01
Lignin, %	4.12	4.18	4.17	0.12	0.79

[a-c] means with differing superscript differ ($P < 0.05$)
[1] treatment by yr interaction ($P < 0.05$)
† E+ = endophyte infected; E- = endophyte-free; EN = non-toxic endophyte-infected
* *P*-value of treatment effect

Table 10. Crude protein content (DM basis) of total sward and green and brown fescue fractions of stockpiled fescue sward as influenced by endophyte status and year

	Treatment†				
Item	E+	E-	EN	SEM	*P*-value*
CP of total sward, %				0.28	0.10
yr 1	10.7[b]	11.5[a]	10.9[b]		
yr 2	11.0[ab]	11.2[a]	10.5[b]		
yr 3	9.4[b]	10.2[a]	9.7[b]		
yr 4	10.6[c]	12.0[a]	11.1[b]		
yr 5	12.7[b]	14.2[a]	13.1[b]		
CP of green fescue, %				0.28	< 0.01
yr 1	ND‡	ND	ND		
yr 2	11.8[a]	11.6[ab]	11.2[b]		
yr 3	11.1[b]	12.2[a]	11.4[ab]		
yr 4	12.3[b]	13.1[a]	12.3[b]		
yr 5	14.2[c]	16.0[a]	15.2[b]		
CP of brown fescue, %				0.39	< 0.01
yr 1	ND‡	ND	ND		
yr 2	7.8	7.8	7.4		
yr 3	7.2[b]	8.1[a]	7.5[ab]		
yr 4	8.5[b]	9.7[a]	9.0[ab]		
yr 5	9.6	10.1	9.8		

[a-c] treatment means with differing superscript within yr differ ($P \leq 0.05$)

† E+ = endophyte infected; E- = endophyte-free; EN = non-toxic endophyte-infected

* *P*-value of treatment by yr effect

‡ Not determined

Table 11. Nutritive composition (DM basis) of green and brown non-fescue tissue in stockpiled fescue sward as influenced by year

	Yr					
Item	2	3	4	5	SEM	*P*-value*
Green non-fescue						
IVTDMD, %	89.9	81.8	83.6	78.6	4.1	0.28
CP, %	15.3^{a}	15.1^{a}	17.9^{b}	17.4^{b}	0.67	0.01
ADF, %	19.3^{a}	24.2^{b}	22.2^{c}	19.5^{ac}	0.87	0.01
NDF, %	43.1^{b}	52.4^{a}	46.2^{b}	46.4^{ab}	2.16	0.03
Cellulose, %	17.4^{b}	22.1^{a}	19.6^{a}	17.3^{b}	8.67	0.01
Lignin, %	1.7^{c}	2.0^{bc}	2.1^{b}	3.3^{a}	0.18	< 0.01
Brown non-fescue						
IVTDMD, %	54.0^{a}	43.4^{b}	54.0^{a}	53.1^{a}	1.36	< 0.01
CP, %	14.5^{a}	9.8^{c}	14.1^{a}	12.5^{b}	0.39	< 0.01
ADF, %	37.1^{b}	43.0^{a}	36.5^{b}	37.8^{ab}	0.39	< 0.01
NDF, %	70.2^{b}	75.0^{a}	67.2^{d}	68.9^{c}	0.39	< 0.01
Cellulose, %	29.1^{b}	34.9^{a}	29.0^{b}	29.6^{b}	0.6	< 0.01
Lignin, %	6.3^{c}	7.4^{b}	6.0^{d}	9.2^{a}	0.41	< 0.01

$^{a\text{-}d}$ means with differing superscript differ ($P \leq 0.05$)

* *P*-value of yr effect

Table 12. Nutritive composition (DM basis) of stockpiled fescue as influenced by year

Item	Yr					SEM	*P*-value*
	1	2	3	4	5		
Total Sward							
IVTDMD, %	64.4^{c}	80.2^{a}	65.2^{c}	74.0^{b}	78.1^{a}	1.45	< 0.01
CP, %	11.0^{b}	10.9^{b}	9.8^{c}	11.2^{b}	13.3^{a}	0.3	< 0.01
ADF, %	32.9^{a}	25.5^{c}	31.9^{a}	28.8^{b}	26.2^{c}	0.53	< 0.01
NDF, %	65.7^{a}	51.9^{d}	62.4^{b}	57.9^{c}	56.1^{c}	0.86	< 0.01
Cellulose, %	28.0^{a}	22.4^{c}	27.2^{b}	27.3^{b}	22.1^{c}	0.25	< 0.01
Lignin, %	4.6^{a}	2.3^{c}	3.9^{b}	2.5^{c}	4.6^{a}	0.31	< 0.01

$^{a\text{-}d}$ means with differing superscript differ ($P \leq 0.05$)

* *P*-value of yr effect

Table 13. Nutritive composition (DM basis) of total sward of stockpiled fescue sward with varying endophyte status

	Treatment†				
Item	E+	E-	EN	SEM	*P*-value*
Total sward					
IVTDMD[1], %	73.9[a]	70.7[c]	72.5[b]	0.59	< 0.01
CP, %	10.9[b]	11.8[a]	11.1[b]	0.13	< 0.01
ADF, %	28.8[b]	29.3[a]	29.1[ab]	0.21	0.09
NDF, %	58.2[b]	59.2[a]	59.0[a]	0.30	< 0.01
Cellulose, %	25.4	25.3	25.4	0.15	0.64
Lignin, %	3.2[a]	2.9[b]	3.6[a]	0.15	0.01

[a-c] means with differing superscript differ ($P \leq 0.05$)
[1] treatment by yr interaction ($P \leq 0.05$)
† E+ = endophyte infected; E- = endophyte-free; EN = non-toxic endophyte-infected
* *P*-value of treatment effect

Table 14. Nutritive composition (DM basis) of stockpiled fescue sward from early-December to late-February

	Date of sampling†							*P*-value*	
Item	Wk 1	Wk 3	Wk 5	Wk 7	Wk 9	Wk 11	SEM	L	Q
IVTDMD, %	79.35	76.72	72.68	71.50	69.30	68.97	0.56	< 0.01	< 0.01
CP, %	12.44	12.13	11.27	10.84	10.64	10.75	0.15	< 0.01	< 0.01
ADF, %	26.04	26.64	27.70	29.34	30.31	31.55	0.32	< 0.01	0.42
NDF, %	53.40	54.59	55.98	58.89	61.00	63.32	0.30	< 0.01	0.01
Cellulose, %	22.78	23.07	24.02	25.47	26.85	27.68	0.20	< 0.01	0.20
Lignin, %	2.94	3.27	3.41	3.68	3.71	4.01	0.13	< 0.01	0.24

†Wk 1 sampled at beginning of December, wk 11 sampled during mid-February.

*L = Linear; and Q = Quadratic.

Table 15. Performance of growing cattle strip-grazing stockpiled fescue with varying endophyte-status from early-December though late-February

	Treatment†				
Item	E+	E-	EN	SEM	*P*-value*
Pasture ADG, (kg/d)	0.51	0.59	0.56	0.03	0.13
Barn ADG, (kg/d)	0.40[a]	0.46[b]	0.46[b]	0.03	0.08
Shrunk ADG, (kg/d)	0.35[a]	0.43[b]	0.41[b]	0.02	0.02
CBCS‡	0.27	0.24	0.25	0.04	0.89
Serum urea nitrogen, mg/dL	6.90[b]	7.70[a]	6.86[b]	0.18	0.03

[a-b] Treatment means with differing superscripts differ ($P \leq 0.05$).
† E+ = endophyte infected; E- = endophyte-free; EN = non-toxic endophyte-infected
* *P*-value of trt effect
‡Change in body condition score from beginning to end of winter

Table 16. Performance of growing cattle strip-grazing stockpiled fescue from early-December though late-February as influenced by year

	Yr						
Item	1	2	3	4	5	SEM	*P*-value*
Pasture ADG, (kg/d)	0.35[d]	0.40[d]	0.48[c]	0.71[b]	0.83[a]	0.05	< 0.01
Barn ADG, (kg/d)	0.38[c]	0.46[b]	0.29[d]	0.37[cd]	0.68[a]	0.04	< 0.01
Shrunk ADG, (kg/d)	0.35[c]	0.43[b]	0.27[d]	0.34[cd]	0.61[a]	0.04	< 0.01
CBCS[‡]	0.14[c]	0.08[c]	0.06[c]	0.60[a]	0.36[b]	0.07	< 0.01
Serum Urea Nitrogen mg/dL	5.71[c]	6.09[c]	6.01[c]	7.08[b]	10.86[a]	0.34	< 0.01

[a-b] Treatment means with differing superscripts differ ($P \leq 0.05$).

* *P*-value of yr effect

[‡] Change in body condition score from beginning to end of trial

Table 17. Serum urea nitrogen of cattle strip-grazing stockpiled fescue as influenced by endophyte status and year

Item		Treatment†			
	E+	E-	EN	SEM	*P*-value*
Serum urea nitrogen, mg/dL				0.28	0.02
yr 1	5.50	5.66	5.96		
yr 2	6.20^{ab}	6.50^{a}	5.55^{b}		
yr 3	6.31	5.94	5.78		
yr 4	6.52^{b}	7.88^{a}	6.84^{b}		
yr 5	9.95^{b}	12.49^{a}	10.14^{b}		

a-b Treatment means with differing superscripts differ within yr ($P \leq 0.05$).

† E+ = endophyte infected; E- = endophyte-free; EN = non-toxic endophyte-infected

* *P*-value of treatment by year effect

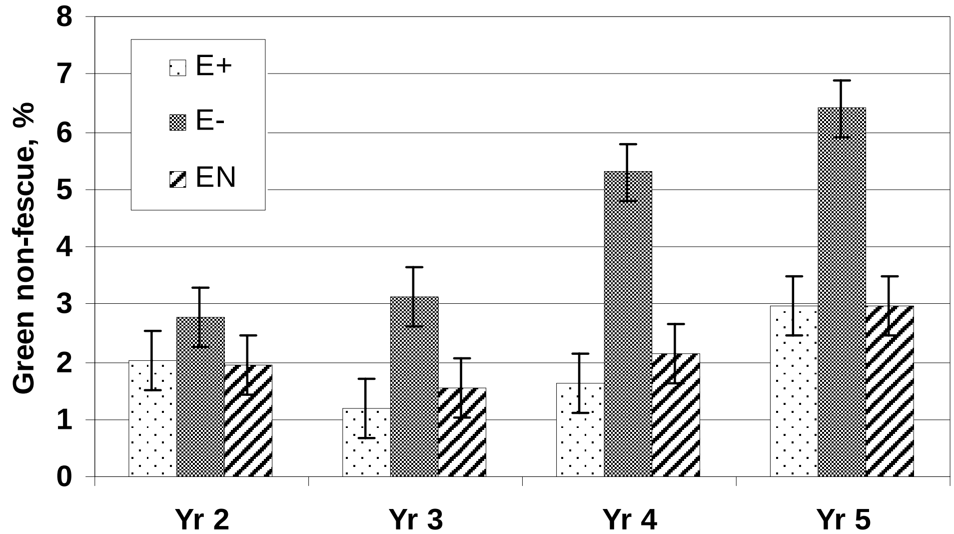

Figure 1. Percent green non-fescue in sward of stockpiled endophyte-infected (E+), endophyte-free (E-) and novel endophyte-infected (EN) stands during yr 2 though 5

APPENDIX

Table 1a. Species composition of stockpiled fescue sward from December through February as influenced by date and year

Item		Date of sampling[1]							SEM	*P*-value*
		Week 1	Week 3	Week 5	Week 7	Week 9	Week 11	Week 13		
Fescue, %									1.28	< 0.01
	yr 2	87.4	92.8	84.9	83.1	91.4	81.1	84.7		
	yr 3	84.2	77.2	79.8	81.3	85.8	79.3	86.4		
	yr 4	90.6	88.6	90.7	93.3	92.3	92.3	94.9		
	yr 5	83.9	87.0	83.3	86.5	88.5	90.5	ND‡		
Green fescue†, %									1.02	0.04
	yr 2	89.3	79.8	75.5	66.0	62.5	49.1	43.7		
	yr 3	76.8	68.5	53.0	55.0	43.4	35.7	30.1		
	yr 4	80.4	76.8	57.9	55.7	47.2	37.3	32.8		
	yr 5	79.2	77.2	64.5	54.6	51.5	49.2	ND‡		
Brown non-fescue, %									1.26	0.06
	yr 2	9.9	11.5	13.2	13.0	6.8	18.0	13.9		
	yr 3	14.2	19.9	17.5	15.6	12.8	19.9	12.8		
	yr 4	4.3	5.9	6.6	4.1	5.5	6.0	3.9		
	yr 5	10.1	8.8	10.0	9.7	8.4	6.7	ND‡		
Green non-fescue, %									0.27	0.01
	yr 2	2.66	2.69	1.92	3.88	1.83	0.94	1.43		
	yr 3	1.62	2.91	2.68	3.07	1.44	0.78	0.77		
	yr 4	5.08	5.51	2.77	2.38	2.14	1.69	1.13		
	yr 5	6.03	4.25	6.74	3.78	3.07	2.8	ND‡		

[1] Wk 1 sampled at beginning of December, wk 13 sampled during late-February.
* *P*-value of wk by yr effect
† Percent of fescue that is green
ND‡ = Not determined

Table 2a. In vitro true dry matter digestibility and crude protein content of stockpiled fescue sward from December through February as influenced by date and year

Item		Date of sampling[1]						SEM	*P*-value[2]	
	Week 1	Week 3	Week 5	Week 7	Week 9	Week 11	Week 13		L x yr	Q x yr
IVTDMD, %								1.01	< 0.01	< 0.01
yr 1	73.5	70.2	63.0	61.0	58.7	58.9	64.7			
yr 2	87.6	83.3	82.0	79.7	81.3	73.9	72.1			
yr 3	72.8	67.5	66.1	64.4	62.8	61.2	61.4			
yr 4	82.8	80.3	70.0	74.4	67.0	75.0	67.8			
yr 5	80.0	82.3	82.3	78.0	76.6	75.8	ND‡			
CP, %								0.22	< 0.01	< 0.01
yr 1	11.3	11.7	11.4	10.8	10.5	10.8	10.6			
yr 2	12.6	12.0	10.8	10.3	9.7	10.5	10.2			
yr 3	11.9	11.0	9.8	9.6	8.8	8.7	8.5			
yr 4	11.1	11.5	11.4	11.3	10.9	11.5	10.8			
yr 5	15.3	14.4	12.9	12.3	13.4	12.2	ND‡			

[1] Wk 1 sampled at beginning of December; wk 13 sampled during late February.
[2] L x yr = Linear week by year effect; Q x yr = Quadratic week by year effect
ND‡ = Not determined

Table 3a. Fiber content (DM basis) of stockpiled fescue sward from December through February as influenced by date and year

	Date of sampling[1]								*P*-value[2]	
Item	Week 1	Week 3	Week 5	Week 7	Week 9	Week 11	Week 13	SEM	L x yr	Q x yr
ADF, %								0.45	< 0.01	< 0.01
yr 1	29.3	30.3	33.4	33.8	35.4	35.2	33.4			
yr 2	21.0	23.5	24.0	25.8	25.2	29.1	31.0			
yr 3	29.6	30.9	30.7	32.0	32.7	33.2	34.3			
yr 4	25.5	25.1	27.9	28.9	30.1	32.4	32.2			
yr 5	24.8	23.4	22.5	26.2	28.2	27.8	ND‡			
NDF, %								0.61	< 0.01	< 0.01
yr 1	58.9	61.9	65.9	67.2	70.6	69.0	66.7			
yr 2	44.2	50.6	49.5	50.9	50.1	58.0	61.4			
yr 3	58.6	61.1	59.5	62.3	63.5	65.0	67.6			
yr 4	52.5	50.1	56.5	58.1	60.5	64.9	63.6			
yr 5	52.8	49.2	48.5	56.0	60.3	59.6	ND‡			
Cellulose, %								0.24	< 0.01	< 0.01
yr 1	25.8	26.1	28.4	28.1	29.9	29.8	28.2			
yr 2	19.2	21.0	21.4	22.9	22.7	24.2	25.9			
yr 3	25.8	26.0	26.0	27.2	28.0	28.3	29.9			
yr 4	22.8	22.7	25.0	26.7	30.1	32.4	32.2			
yr 5	20.2	19.6	19.3	22.4	23.6	23.7	ND‡			
Lignin, %								0.23	< 0.01	< 0.01
yr 1	2.83	4.08	4.84	5.27	5.18	5.12	4.51			
yr 2	1.53	2.05	2.23	2.41	1.97	3.19	3.06			
yr 3	3.17	3.84	4.03	3.91	3.96	4.27	3.81			
yr 4	2.11	2.04	2.40	2.64	2.60	2.95	3.18			
yr 5	5.08	4.36	3.57	4.16	4.83	4.53	ND‡			

[1] Wk 1 sampled at beginning of December, Wk 13 sampled during late February.
[2] L x yr = Linear week by year effect; Q x yr = Quadratic week by year effect
ND‡ = Not determined

Table 4a. Nutritive composition of stockpiled fescue sward as influenced by treatment and year[1]

Item		Treatment†		SEM	*P*-value*
	E+	E-	EN		
IVTDMD, %				1.25	0.03
yr 1	67.69[a]	59.95[b]	65.5[c]		
yr 2	81.0	79.2	80.4		
yr 3	67.7[a]	63.6[b]	64.4[b]		
yr 4	74.2	73.5	74.3		
yr 5	79.1	77.1	78.1		
CP, %				0.28	0.10
yr 1	10.7[c]	11.5[a]	10.9[b]		
yr 2	11.0[a]	11.2[ab]	10.5[b]		
yr 3	9.4[b]	10.2[a]	9.7[b]		
yr 4	10.6[c]	12.0[a]	11.1[b]		
yr 5	12.7[b]	14.2[a]	13.1[b]		
ADF, %				0.51	0.08
yr 1	32.2[a]	33.7[b]	32.9[a]		
yr 2	25.3	26.0	25.3		
yr 3	31.4	32.1	32.0		
yr 4	28.9	28.5	28.9		
yr 5	26.0	26.5	26.3		
Cellulose, %				0.31	0.08
yr 1	27.7[b]	28.4[a]	28.0[ab]		
yr 2	22.4	22.4	22.3		
yr 3	27.2	27.2	27.3		
yr 4	27.6[a]	26.8[b]	27.4[ab]		
yr 5	22.2	21.8	22.2		

† E+ = endophyte infected; E- = endophyte-free; EN = non-toxic endophyte-infected

* *P*-value of trt by yr effect

[1]trt by yr effect not significant for NDF ($P = 0.21$) and lignin ($P = 0.84$)

Table 5a. Serum urea nitrogen of animals grazing stockpiled fescue with varying endophyte status from December to February as influenced by endophyte status, date and year

	Date of sampling[1]				*P*- value[2]	
Item	Week 3	Week 7	Week 11	SEM	L	Q
Serum urea nitrogen[§], mg/dL				0.20	< 0.01	< 0.01
yr 1						
E+	6.10	5.18	4.19			
E-	5.63	5.50	4.81			
EN	5.73	6.07	4.96			
yr 2						
E+	7.31	6.32	4.50			
E-	6.36	6.25	6.69			
EN	5.97	5.77	4.76			
yr 3						
E+	8.14	6.13	4.70			
E-	7.60	5.74	4.52			
EN	7.62	5.39	4.36			
yr 4						
E+	7.02	7.02	5.80			
E-	7.77	8.71	7.77			
EN	6.23	8.17	6.82			
yr 5						
E+	7.40	10.36	ND[‡]			
E-	9.57	12.96	ND			
EN	8.22	10.57	ND			

[§] week by trt ($P < 0.01$) and week by trt by yr ($P < 0.01$)
[1] Week 3 =sampled at mid during December, Week 11 =sampled during mid February.
[2] *P*-value of L = Linear week effect; Q = Quadratic week effect
[3] Not determined

Table 6a. Forage offered, residual forage mass, and forage disappearance (DM basis) in years 3 through 5 as influenced by date and year

		Date of sampling[1]						*P*- value[2]	
Item		Week 3	Week 5	Week 7	Week 9	Week 11	SEM	L	Q
Forage offered[§†], kg•hd^{-1}•d^{-1}							0.51	0.03	< 0.01
	yr 3	6.78	8.38	ND[3]	9.54	7.19			
	yr 4	8.51	8.34	8.01	6.70	7.84			
	yr 5	7.85	10.14	8.90	11.64	10.20			
Residual forage mass[§†], kg/ha							77.9	0.56	0.56
	yr 3	1276	1030	ND	1967	1601			
	yr 4	1867	1824	1249	1229	1480			
	yr 5	1560	1598	1863	1806	1380			
Forage disappearance[†], kg•hd^{-1}•d^{-1}							0.31	0.02	0.02
	yr 3	4.93	6.65	ND	5.75	4.40			
	yr 4	4.06	4.09	5.34	4.40	4.15			
	yr 5	3.75	5.52	2.67	5.77	5.40			

[§]wk by yr interaction ($P \leq 0.05$)
[†]wk by wk by yr interaction ($P \leq 0.05$)
[1] wk 3 is mid-December; wk 11 is mid-February
[2]L = Linear week effect; Q = Quadratic week effect
[3] Not determined