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GRAZING EVALUATION OF A NOVEL ENDOPHYTE TALL FESCUE DEVELOPED FOR THE UPPER TRANSITION ZONE

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ABSTRACT OF DISSERTATION

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The Graduate School
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ABSTRACT OF DISSERTATION

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Plant and Soil Sciences at the University of Kentucky

By
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ABSTRACT OF DISSERTATION

GRAZING EVALUATION OF A NOVEL ENDOPHYTE TALL FESCUE DEVELOPED FOR THE UPPER TRANSITION ZONE

A wild-type endophyte (*Neotyphodium coenophialum* [(Morgan-Jones & Gams) Glen Bacon & Hanlin]) that infects tall fescue [*Lolium arundinaceum* (Schreb.) Darbysh. = *Schedonorous arundinaceum* (Schreb.) Dumort.] imparts tolerances to moisture, heat, and grazing stresses, but also produces ergot alkaloids that adversely affect performance and physiology of cattle. Novel endophytes, developed by AgResearch Ltd. NZ, can sustain fescue persistence and productivity, but do not produce toxic ergot alkaloids. University of Kentucky Plant Breeder, T. D. Phillips Ph.D, developed a tall fescue experimental population (KYFA9301) for the upper transition zone. A 2-yr grazing experiment was conducted with steers to evaluate steer performance and physiology, and forage quality and productivity of KYFA9301 infected with AR584 novel endophyte (AR584) compared with KY31 wild-type endophyte (KY31), endophyte-free KYFA9301 (EF9301) and AR542-‘Jesup’ (MaxQ). Fescue-endophyte combinations were assigned to 1.0-ha pastures in a randomized complete block design with three replications. Pastures were grazed with variable stocking (four testers) from 6 May to 23 July 2008 (76 d), and 2 April to 25 June 2009 (84 d). Shrunk bodyweights were taken at initiation and termination of grazing each year. Average daily gains among MaxQ, AR584, and EF9301 were similar and were greater ($P < 0.10$) than KY31. Rectal and skin temperatures were collected three times each year at approximately days 28, 56, and study completion, along with blood collection for serum prolactin assay. Rectal and skin temperatures among AR584, MaxQ, and EF9301 were similar and were lower ($P < 0.10$) than KY31. Serum prolactin concentrations were similar among the three nontoxic varieties and higher ($P < 0.10$) than KY31. Forage collections were taken at 2 week intervals throughout the study each year and nutritive quality analysis were conducted through wet chemistry to determine forage acid detergent fiber (ADF), neutral detergent fiber (NDF), crude protein (CP), and In-Vitro Dry Matter Digestibility (IVDMD). Nutritive analyses indicated no differences between fescue-endophyte combinations with the exception of EF9301 having higher ADF concentrations ($P = 0.031$) than KY31 during the dry year of 2008. Results indicated steer performance and physiological responses for KYFA9301, with and without AR584 were enhanced compared to KY31 and similar to those for MaxQ.

KEYWORDS: Beef Cattle, Novel Endophyte, Fescue Toxicosis,
Kentucky 31 Tall Fescue, Ergovaline

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May 7, 2010

RULES FOR THE USE OF DISSERTATIONS

DISSERTATION

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To my grandparents, George and Bertelle Johnson, and William Sims, all of whom I lost during this journey, I dedicate this dissertation. Through their teaching I’ve learned I can do anything I put my mind to, I must finish anything I start, and that no wall is too high or too thick to either climb over or bust through. I carry their love, encouragement and stubbornness with me daily.

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Chapter One: Introduction

The most abundant cool-season grass in the United States, tall fescue [*Lolium arundinaceum* (Schreb.) Darbysh. = *Schedonorous arundinaceum* (Schreb.) Dumort] occupies approximately 15 million hectares and is utilized predominately in the region spanning between the subtropical southeast to the temperate northeast and west to the Great Plains; a region most commonly referred to as the "Fescue Belt" (Bacon and Siegel, 1988; Lacefield et al., 2004). Popularity of the grass has been attributed to its exceptional agronomic performance, stress and grazing tolerance, and stand persistence; however, a major concern with tall fescue is the overall poor performance and thriftiness of cattle (Ball, 1984; Lacefield et al., 2004).

Poor cattle performance on tall fescue has been linked to a toxicosis caused by an endophyte, *Neotyphodium coenophialum*[(Morgan-Jones & Gams) Glen Bacon & Hanlin], a fungus found to grow symbiotically with the tall fescue plant that produces ergot alkaloids (Bacon and Seigel, 1988). Fescue toxicosis, a collective term that encompasses symptoms associated with grazing tall fescue, include poor weight gain, reduced conception rates, retention of rough hair coats, depressed serum prolactin concentrations, decreased heat tolerance, increased internal and external temperatures, vasoconstriction (constricted blood flow), as well as behavioral changes in time spent in the shade, grazing, and at water (Bacon and Siegel, 1988; Ball, 1984; Lacefield et al., 2004; Schmidt and Osborn, 1993). Ergot alkaloids produced by the endophyte may also cause 'fescue foot' and fat necrosis; ailments which are more isolated and have less effect than toxicosis. Fescue foot is characterized by tissue necrosis and dry gangrene of the distal extremities, including the tips of the ears and tail, and is most often observed with cold ambient temperatures (Bacon, 1995). Fat necrosis is defined as the presence of hard fat masses resulting in inadequate space in the reproductive tract of cattle, most commonly occurring in the adipose tissue of the abdominal cavity (Schmidt and Osborn, 1993). Annual economic losses can be greater than $600 million to the cattle industry (Hoveland, 1993).

In an attempt to alleviate the negative affects associated with the endophyte, endophyte-free tall fescue cultivars were developed, but these cultivars proved unsuccessful at withstanding grazing pressure or providing stress tolerance (Lacefield et al., 2004). Recent research has combined endophyte-free experimental populations with AgResearch Ltd. NZ novel endophytes in an attempt to develop tall fescue cultivars that can provide the desired animal performance associated with endophyte-free fescue along with the plant performance of toxic endophyte-infected fescue. Research in Georgia led to the commercial release of 'Jesup-MaxQ' after infecting 'Jesup' tall fescue with the novel endophyte strain AR542 was successful in grazing trials (Watson et al., 2004; Parish et al., 2003).

Recent work in the University of Kentucky forage breeding program, by forage breeder Dr. Tim Phillips, has led to the development of a tall fescue experimental population, KYFA9301. A diverse synthetic, KYFA9301 was produced by intermating selected genotypes from 22 half-sib families. Most of the parental clones were from endophyte-free Kentucky 31 and related germplasm. As an endophyte-free variety, KYFA9301 demonstrated excellent seedling vigor, a desirable maturity, high yield potential and good grazing tolerance (T.D. Phillips, Personal communication). The need of a novel endophyte fescue variety adapted to the upper transition zone led to the infection of KYFA9301 with AgResearch Ltd. NZ novel endophyte AR584, a novel endophyte with improved seed viability over extended storage periods (Hill and Roach, 2009).

The objective of this 2-yr experiment was to evaluate steer performance and forage quality and productivity of KYFA9301-AR584 endophyte (AR584) in comparison to 'Jesup'-MaxQ infected with AR542 endophyte (MaxQ), Kentucky 31(KY31) tall fescue naturally infected with toxic wild-type endophyte, and endophyte-free KYFA9301 (EF9301).

Chapter Two: Literature Review

Tall fescue is a cool-season perennial bunchgrass that has been utilized in the United States (US) for decades. In 1931, Dr. E.N. Fergus collected a highly productive strain on the Suiter Farm in Menifee County, KY. While there had been early research with tall fescue in small-plot evaluations before this time, its popularity as a pasture forage began in 1943 with the commercial release of Kentucky 31 tall fescue by the University of Kentucky. Since that time, Kentucky 31 has become the most commonly used and economically important perennial grass in the US. An area commonly referred to as the transition zone or fescue belt spanning from the subtropical southeast to the temperate northeast and west to the Great Plains contains most of the approximate 15 million hectares of tall fescue in the US. Tall fescue is a desirable forage due to its wide adaptation, high persistence under different management systems, good forage yield, long-growing season, excellent seed production, and pest resistance (Bacon and Siegel, 1988; Ball, 1984; Lacefield et al., 2004; Schmidt and Osborn, 1993).

After the commercial release of tall fescue in the 1940's, producers began observing animal performance issues. Early signs were the sloughing of ear tips, tails, and hooves from a gangrenous condition in the extremities, which is now referred to as 'Fescue Foot' and occurs most noticeably in the winter (Jensen et al., 1956). Cattle grazing Kentucky 31 tall fescue reportedly had reduced feed intake, lower weight gains, decreased milk production, higher respiration rates, elevated body temperatures, rough hair coats, more time spent in water and/or shade, less time spent grazing, excessive salivation, low serum prolactin levels, and reduced reproductive performance. All of these signs are collectively referred to as 'Fescue Toxicosis' (Ball 1984; Lacefield et al., 2004; Strickland et al., 1993; Schmidt and Osborn, 1993).

While producers and researchers observed issues with animal performance in cattle grazing Kentucky 31, nutritive composition compared favorably with other cool-season perennial grasses (Bush and Burrus, 1988). Bacon et al. (1977) were among the first to report a causal agent for the animal performance from grazing 'toxic' tall fescue, by isolating the *Epichloe typhina* endophyte from tall fescue cultivars and then testing the frequency among pastures with animals showing performance issues. They found endophyte frequencies to be 100% in those pastures. The presence of this endophyte, re-named *Acremonium coenophialum* (Morgan-Jones &Gams), then later re-named *Neotyphodium coenophialum* [(Morgan-Jones &Gams) Glen Bacon & Hanlin], and its apparent association with animal performance became the main focus of tall fescue research (Bacon et al., 1977; Strickland et al., 1993).

Endophyte

Tall fescue is easily established and tolerant of a wide range of managements and uses. Popularity of tall fescue is due to its persistence and ability to withstand drought and stress situations, heavy grazing, pest tolerance, adaptation to poor soil and good winter growth (Hemken et al., 1984; Lacefield et al., 2004; Stuedemann and Hoveland, 1988). These superior agronomic qualities have been associated with the presence of an endophytic fungus, *N. coenophialum* (Bush and Burrus, 1988). The endophyte mycellium is found in leaf sheaths, stems, seedheads, and to a lesser extent, leaf blades. Ergovaline concentrations in tall fescue fluctuate throughout the plant, with concentrations reported highest in seed heads, followed by stems and sheaths, and lowest in leaf blades (Rottinghaus et al., 1991). Rottinghaus et al. (1991) reported that initial growth of plant tissues have ergovaline concentrations of 300 to 500 μg kg^{-1} d, but as the season progresses and the plant matures developed seedheads have higher concentrations of ergovaline of 1000 to 5000 μg kg^{-1} d, while leaf sheaths and blades remain at the lower concentration of 300 to 500 μg kg^{-1} d. The endophyte is a seed-borne, systemic fungus that forms a symbiotic mutualistic relationship with the host plant. The endophyte initiates enhanced tillering and root growth, drought and stress tolerance, and protection against certain nematodes, fungal pathogens, insects, and herbivores (Bacon and Seigel, 1988; Schardl and Phillips, 1997). Drought tolerance has been associated with improved osmotic adjustment, greater sugar accumulation, better root growth and leaf rolling to conserve water in endophyte-infected (E+) fescue (West, 1994). These qualities have made E+ tall fescue a superior forage with exceptional fitness and low management requirement.

Herbivory deterrence is one protective effect of the alkaloids produced by E+ tall fescue. Four main alkaloid classes have been linked to fungal endophytes and their protective qualities. These include the ergot alkaloids, the indolediterpenes, a pyrrolopyrazine, and the saturated aminopyrrolizidines (collectively termed 'lolines'). The loline and peramine alkaloids have been related more to insect deterrence. The main alkaloids producing negative effects on grazing animals have been the indolediterpenes and ergot alkaloids, more specifically lolitrem B in *N. lolli,* the endophyte found in perennial ryegrass (*Lolium perenne*), and ergovaline in tall fescue. The *N. coenophialum* endophyte is reproduced asexually and spread by the seed of infected plants (Porter, 1994; Schardl and Phillips, 1997).

Research indicates that the ergot alkaloids produced by the endophyte are both present in the endophyte cultures and in E+ tall fescue (Bush and Burrus, 1988) and that fescue toxicosis results from ingesting ergot alkaloids derived from the endophyte association (Hill et al., 1994).

Forage Quality of Endophyte-Infected Fescue

In the purest of definitions, forage quality, as defined by Mott and Moore (1969), is some product of digestibility and intake of the diet, with intake being the more important of the two aspects. Forage quality is best measured by animal performance, and intake of digestible organic matter (intake x in-vitro digestible organic matter (IVDOM)) is the best indicator of performance (Mott and Moore, 1969). Many parameters have been set to determine forage digestibility based on nutritive values which include crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), and in-vitro dry matter digestibility (IVDMD). According to Bush and Burrus (1988) forage quality parameters, CP, ADF, NDF, and IVDMD are not changed by the presence of the endophyte in tall fescue. Schmidt et al. (1982) found that E+ hay tested superior for IVDMD when compared to endophyte-free (E-) hay. Steers ingesting E+ hay had lower average daily gain (ADG), and intake, and increased body temperature, and respiration (symptoms which will be discussed in detail later), compared to those ingesting E- hay, but IVDMD percentages were higher for E+ (65.8%) than E- (62.6%) (Schmidt et al., 1982). Read and Camp (1986) found no difference in IVDMD percentages due to infection rate when comparing tillers collected from E+ and E- tall fescues. Fritz and Collins (1991) determined that evaluations of NDF digestion and digestion lag time in a 3-yr evaluation were not affected by endophyte status, and support the hypothesis that the presence of the endophyte has no direct effect on tall fescue herbage digestibility. While digestibility may not be an underlying factor for reduced intake, residence time in the rumen, rumen fluid dilution rate, and rate of passage as influenced by intake can affect digestibility within the animal (Schmidt and Osborn, 1993).

An evaluation by Aldrich et al. (1993) of E+ tall fescue consumption on diet in relation to temperature was conducted due to noticeable reductions in grazing activity during the summer months. Results indicated that at high ambient temperature (32^{o}C), forage intakes by all cattle were reduced by 22%, and water consumption was increased by 62%. Consumption of the E+ fescue at the increased temperature reduced intake by 10% without additionally affecting water consumption (Aldrich et al., 1993). Therefore, suggesting that reductions in intake are no doubt exacerbated by external factors such as environment. These findings are confirmed by McClanahan et al. (2008) who reported a negative correlation in grazing frequency (percentage of cattle actively grazing) and ambient temperature, as greater frequency of grazing occurred during the early morning associated with lower ambient temperatures, and frequency declined as ambient temperature increased throughout the day. While dry matter (DM) intake may not be easily estimated through digestibility measurements, it can be concluded that the presence of an endophyte has no direct effect on tall fescue herbage digestibility, and reductions in intake may be due to combinations of confounding environmental, fungal, and physiological factors which will be discussed further in the Fescue Toxicosis section.

Fescue Toxicosis

Fescue toxicosis, a condition occurring from grazing E+ tall fescue, has a detrimental impact on animal performance and is of great economic importance to the beef industry. Economic loss in the US has been estimated to be greater than $600 million annually (Hoveland, 1993). It was not until the 1970’s that researchers found an association between poor animal performance and the endophyte found in tall fescue (Bacon et al., 1977). Much research has been conducted to better understand the role and impact of ergot alkaloid ingestion in altering animal physiology, as well as developing management approaches to alleviating the associated performance issues.

Bush and Burrus (1988) reported that forage nutritive value comparisons (ADF, NDF, CP, etc) of E+ fescue were not limiting animal performance. Therefore, the limiting factor on animal performance such as sickness may be the toxic effects from the endophyte resulting in lower intake thus lower weight gain (Schmidt and Osborn, 1993; Strickland et al., 1993). As previously stated, increases in ambient temperature have reportedly led to reductions in DM intake of ruminants that were further confounded by the presence of the endophyte (Aldrich et al., 1993). Many studies comparing the gains of beef calves have consistently reported lower weight gain when grazing E+ fescue. While among them there are differences in the levels of loss of gain due to climate, time of year, and management conditions, reports are consistent in showing that yearling cattle grazing E+ tall fescue have lower weight gains than those grazing E- fescue (Boling, 1985; Chestnut et al., 1991; Crawford et al., 1989; Evans et al., 1989; Goetsch et al., 1988; Hoveland et al., 1983; McMurphy et al., 1990; Read and Camp, 1986; Schmidt et al., 1982; Stuedemann et al., 1986; Tulley et al., 1989).

Studies have indicated that these animals will retain rough hair coats into the spring and summer periods (Hemken et al., 1984; Schmidt and Osborn, 1993; Stuedemann and Hoveland, 1988). While a definite source has not yet been identified, speculation indicates that the retention of a rough hair coat may be directed at nutrient deficiency due to vasoconstriction and peripheral ischemia, or to altered hormonal levels at hair follicles (Oliver, 2005). Results of McClanahan et al. (2008) indicated that steers grazing toxic fescue not only retain their rough hair coats but exhibit continued hair growth late into the summer months.

Cattle ingesting ergot alkaloids are vulnerable to thermal stress, or hyperthermia. Cattle grazing E+ fescue exhibit behavioral changes which include spending more time in shade or in/around water to alleviate heat stress associated with dysfunctional thermoregulation. Thermoregulation, the control of body temperature through vascular dilation and constriction, is needed to provide physiologic stability (Spiers et al., 2005). Vasoconstriction is the narrowing or increased muscle contractility in blood vessels known to reduce blood flow to peripheral tissue for conserving core body temperatures.

It is a physiological response that can occur naturally through environmental stimuli, such as a response to temperature, or can be influenced by other factors. Ingestion of ergovaline leads to a contractile response of vasoconstriction to isolated tissues or cells by interactions with adrenergic, serotonin, and dopamine receptors (Oliver, 2005), making the animal less effective at dissipating heat and leading to an increase in core body temperature (Hill, 2005; Klotz et al., 2008). A study by Klotz et al. (2007) determined that ergovaline and ergotamine are potent vasoconstrictors in the bovine lateral saphenous vein, and that the binding strength of ergovaline and its abundance within the plant suggests even small exposure over extended periods of time can lead to bioaccumulation, making ergovaline the most potent ergot alkaloid. Ergovaline concentrations as low as $1 \times 10^{-8} M$ elicit an in-vitro contractile response (Klotz et al., 2007). Another in-vitro study by Klotz et al. (2009) suggested there is bioaccumulation of ergot alkaloids in the vasculature of cattle. The discovery of this bioaccumulation and suggestion that the vasoconstriction contractile response to ergot alkaloids may be intensified and persistent with recurring exposure to ergovaline implies greater potential for it's role in inducing toxicosis (Klotz et al., 2009) and offers explanation for lingering toxicosis effects once animals are removed from E+ pastures. Early research by Lyons et al. (1986) evaluated the occurrence of peptide and clavine egrot alkaloids in tall fescue and found ergovaline to consistently be the predominant ergot alkaloid.

Studies have suggested a loss of ear and tail tips and sloughing off of hooves due to gangrene and vasoconstriction have also been associated with animals grazing Kentucky 31 tall fescue (Ball, 1984; Hemken et al., 1984; Stuedemann and Hoveland, 1988). “Fescue foot” is characterized by tissue necrosis and dry gangrene of the distal extremities, including the tips of the ears and tail, and is most often observed with cold ambient temperatures during the late fall and especially the winter but can occur year round (Bacon, 1995). Fescue foot is associated with vasoconstriction of blood vessels which result in inadequate blood supply to the extremities and skin (Aiken et al., 2007). The reduction in heat transfer from core body tissues to the skins surface is due to a lack of blood flow consistent with vasoconstriction caused by ergot alkaloid ingestion (Strickland et al., 2008).

The increase in core body temperature, indicated by an elevated rectal temperature, can be detrimental to cattle grazing in high ambient temperatures. Visible signs of increased respiration and excessive salivation as well as decreased grazing time are all highly indicative of toxicosis stressed animals. Research has found that animals exhibiting fescue toxicosis spend less time grazing during the hot parts of the day, more time grazing at night during the summer months, and overall less time grazing in general (Bond et al., 1984; McClanahan et al., 2008; Paterson et al., 1987). Parish et al. (2003) evaluated grazing behavior of cattle grazing toxic and nontoxic fescue and found significant differences among cattle grazing E+ cultivars versus nontoxic cultivars. Steers grazing E+ cultivars spent 36.1% of their daily activity grazing, 32.4% ruminating, and 31.5% idling in the months of April, May and June in 2001, whereas steers grazing E- cultivars during the same time period spent a greater amount of time grazing (41.9%) and ruminating (34.7%) and less time idling (23.4%). Steers grazing novel endophyte cultivars spent more time ruminating (39.7%) and grazing (38.6%), and less time idling (21.7%) than steers grazing E+ tall fescue.

A research marker for fescue toxicosis has been the consistent expression of a low prolactin concentration (Rice et al., 1997; Strickland et al., 1993). Work by Hurley et al. (1981) indicated that consumption of tall fescue depressed serum prolactin regardless of ambient temperature. There has been an expressed relationship between circulating ergopeptines and low prolactin levels (Aiken et al., 2006; Hurley et al., 1981; Strickland et al., 1993). Production and secretion of prolactin occurs in the anterior pituitary, and in vitro studies indicate that ergovaline has dopaminergic activity (Oliver, 2005). Binding of dopamine receptors by alkaloids results in a decrease in circulating prolactin that causes agalactia (Spiers et al., 2005). Agalactia, the decrease in milk production, ultimately affects suckling calf weight gain (Hill, 2005; Spiers et al., 2005). A recent grazing study evaluated the productivity of cow-calf pairs grazing tall fescue cultivars with differing endophytes (Watson et al., 2004). Cows and calves grazing E+ tall fescue had greater serum prolactin depression than those grazing Jesup-MaxQ, suggesting that these animals could be suffering from toxicosis. Results from this study found calves on E+ tall fescue pasture with lower average daily gains, and weaning weights as compared to those on Jesup-MaxQ pasture. When evaluating reproductive performance, E+ cows reported a reduction in birth weight of E+ calves of approximately 5 kg less than calves born to cows grazing Jesup-MaxQ. Low calf growth rate was attributed to decreased dry matter intake as well as depressed milk production (Watson et al., 2004).

Endophyte-Free Tall Fescue

To alleviate the problems associated with the *N. coenophialum* endophyte, research efforts in the 1980's focused primarily on the development and promotion of E- cultivars of tall fescue. Early grazing trials with E+ fescue found cattle grazing presumably infected pastures sometimes showed high performance (Hoveland et al., 1980; Robbins, 1983). Further investigation of these pastures, and with the discovery of the endophyte relationship by Bacon et al. (1977), found that endophyte infection levels in these pastures were 10% or less, making them seemingly endophyte free. Therefore, both studies demonstrated higher ADG with steers grazing fescue with low infection percentages (Hoveland et al., 1980; Robbins, 1983). These results initiated further research into the endophyte/plant relationship and animal performance studies, but also the possibility of utilizing E- fescues as an alternative to E+ tall fescue. Commercially available 'Forager', 'Kenhy', 'AU Triumph', 'Johnstone', and 'Missouri 96', were E- seed cultivars that were marketed in the early to mid 1980's as an alternative to E+ fescue (Ball, 1984).

Research was conducted testing animal performance on E- fescue cultivars. Several E- cultivars were highly promoted and marketed after a quick release in the 1980's. Unfortunately, persistence of these E- cultivars was low, and producers began reverting back to E+ fescue (Lacefield et al., 2004). Research determined that, without the endophyte, these tall fescue cultivars lacked stand persistence, drought/stress tolerances, or an ability to withstand heavy grazing (Bouton et al., 1993). In Georgia it was determined that summer drought resulted in the greatest loss of E- tall fescue stands (Bouton et al., 2001). While animal performance on these E- tall fescues showed much promise, the ultimate outcome was that E- cultivars did not have the persistence of E+ cultivars under stressful conditions, and would require more intensive management (Bacon and Siegel, 1988; Bouton et al., 1993 & 2001).

Poor persistence of E- cultivars resulted in researchers looking for alternative ways to alleviate toxicosis. One of the most recommended options has been the renovation of pastures with the use of legume species. Studies were conducted that found interseeding legumes such as white clover, red clover, annual lespedeza, or alfalfa can dilute the effects of toxicosis, and improve animal and pasture performance (Ellis et al., 1983; Fribourg et al., 1991; Hoveland et al., 1997; McMurphy et al., 1990). Dilution of E+ tall fescue has become another alternative. Rather than planting another species into E+ pastures, studies found that diluting alkaloids in the diet with supplemental feeding of grains, hay, and by-product feeds can dilute toxicosis symptoms (Aiken and Piper, 1999; Aiken et al., 1998). Aiken et al. (1998) reported that by supplementing the diets of steers grazing E+ fescue with a mixture of corn and broiler litter fed ad libitum, steer ADG was comparable to those grazing E- fescue with no supplementation (0.67 kg/d). Removal of grazing animals during the most stressful times of spring/summer from E+ pastures to warm-season forages has been found to be an alternative to reduce symptoms (Joost, 1995). Aiken and Piper (1999) found that animal performance can be greatly improved when steers grazing E+ fescue are moved to eastern gamagrass (*Tripsacum dactyloides)* pastures during the hot summer months. Steers moved to eastern gamagrass had greater ADG and serum prolactin concentrations than those that remained on E+ fescue (Aiken and Piper, 1999).

Novel Endophyte Tall Fescue

AgResearch Ltd. NZ patented seven strains of tall fescue endophytes in 1997 (Latch et al., 2000). These endophytes were selected based on improved resistance to insect pests as compared to E- cultivars, and reduced toxicity to livestock as compared to the wild-type endophyte in E+ tall fescue. *Neotyphodium* endophyte strains were isolated from fescues from Mediterranean locations for a presence of peramine and an absence of ergovaline; naturally occurring, therefore not considered a genetically modified organism. The seven strains have been divided into two taxonomic groups; group one containing five endophyte strains AR501, AR502, AR510, AR572, and AR577, which form colonies on potato dextrose agar (PDA), and the second group containing strains

AR542 and AR584 that conformed to the species description of *N. coenophialum* (Latch et al., 2000).

The concept of novel endophytes is to utilize a naturally occurring endophyte that can maintain the agronomic benefits of the toxic endophyte without adversely affecting grazing animals. The AR542 was inserted into Jesup and Georgia 5 tall fescue cultivars in a University of Georgia study (Bouton et al., 2002). Stand survival of E+ cultivars were significantly greater than E- cultivars with an average of 64% survival across three sampling dates at two locations, as compared to 42% for E- cultivars. Cultivars with the AR542 strain were comparable to both E+ and E- cultivars at 59% stand survival with the same parameters. The best combination of Jesup with AR542 possessed greater yield and better survival than the E- cultivars and equal to the E+ cultivars. Fescue cultivars combined with the novel endophyte AR542 produced the yield and stand survival desired by producers accustomed to using E+ tall fescue (Bouton et al., 2002).

The combination of novel endophyte AR542 with University of Georgia tall fescue cultivars, and the success reported from previous studies led to the release of Jesup-MaxQ tall fescue. Parish et al. (2003) studied the effects of novel endophytes AR542 and AR502 combined with 'Jesup' and 'Georgia-5' tall fescues as compared to E- and E+ Jesup on steer weight gain and physiology. Steers grazing E+ cultivars had significant prolactin depression, from initial to post-treatment, averaging reductions of 93% across all seasons and sites. Rectal temperatures were elevated during the months of February to June and averaged $39.8^{o}C \pm 0.2$ for E+ steers, which were greater than those grazing non-toxic cultivars. Steers grazing non-toxic cultivars had higher ADG, approximately 0.84 kg across all sites and dates, and approximately 295 kg gain ha^{-1}, where those grazing the E+ fescue had an ADG of 0.45 kg and a gain ha^{-1} of 157 kg. Similar to Bouton et al. (2002), this study concluded that novel endophyte strains provided cattle performance comparable to E- cultivars and higher than E+ cultivars making them a promising alternative for utilization by producers (Parish et al., 2003).

A two-year grazing study conducted in southwest Missouri compared the growth rate and physiology of steers grazing 'HiMag' tall fescue with their Strain-4 novel endophyte (Nihsen et al., 2004). Steers grazing E+ fescue exhibited fescue toxicosis with a depression in serum prolactin concentrations, ADG, DM intake, and elevated core body temperatures than steers on other cultivars. Steers grazing Strain 4-HiMag tall fescue had higher ADG and DM intake than E+, and had rectal temperatures and serum prolactin concentrations similar to those grazing E- tall fescue. It was concluded that that novel endophyte Strain 4-HiMag fescue provided animal performance as good as E-, while also being able to protect the plant from environmental stressors (Nihsen et al., 2004).

Hopkins and Alison (2006) compared stand performance and grazing animal performance among tall fescue cultivars with different fungal endophyte combinations in the South Central US. Results suggested that novel endophyte cultivars were a viable option for alleviation of animal performance issues created from grazing E+ fescue while maintaining stand longevity. The novel endophytes could be an important factor in the success of non-toxic tall fescue cultivar survival in this region, and breeder selection should now be focused on improved persistence in the South Central US for broader tall fescue use (Hopkins and Alison, 2006).

Several studies indicated that the combination of a novel endophyte with E- tall fescue cultivars provide the positive animal performance producers desire even though more available forage was produced from E+ pastures (Bouton et al., 2001; Hopkins and Alison, 2006; Nihsen et al., 2004; Parish et al., 2003). The discovery of E- cultivars was more happen-stance than calculated, but allowed researchers to understand that endophyte infection in stored seed is short-lived rather than permanent. With the ambient temperatures and humidity of the south, the wild-type endophyte usually has a lifespan of one year in normal seed storage. The development of novel endophyte strains has required as much attention on seed viability as pasture performance, as endophyte survival in the seed is crucial to plant persistence. Seed handling and storage can have an impact on the endophyte viability if harsh conditions or time constraints become a factor (Hill and Roach, 2009). Hill and Roach (2009) observed the viability of different endophytes in the seed of a common tall fescue cultivar and different cultivars utilizing a common endophyte, and whether endophyte viability during seed storage was a heritable trait. Novel endophyte AR542 was inserted into different tall fescue cultivars representing diverse genetic backgrounds, and both AR542 and AR584 were inserted into Jesup tall fescue. Survival of AR542 in seed varied greatly when compared across all cultivars. These results suggest there is an inherent relationship between endophyte survival and host plant genetics since all cultivars were treated the same and variables that might create differences during storage were controlled. When comparing Jesup-AR542 and Jesup-AR584, AR584 was 24% viable compared to AR542 at 10% viability after 18 months of storage. This indicates that besides a plant genetic-endophyte strain interaction, there also is an endophyte genotype effect on seed viability during storage. It is apparent that the utilization of novel endophytes to alleviate the affects of fescue toxicosis is a viable option for producers, but attention should also be in finding the best endophyte-plant genotype combination and improving the agronomic traits through traditional plant breeding (Hill and Roach, 2009).

Since the release of Jesup-MaxQ jointly by AgResearch Ltd. NZ and the University of Georgia, much work has been conducted to develop more novel endophyte cultivars with a broader range of utilization. This research includes but is not limited to: the advanced testing of a selected variety derived from endophyte AR584 in Georgia-5 at the University of Georgia, experimental testing of different fescue cultivars adapted to the Oklahoma region by the Noble Foundation, and development of novel endophyte cultivars in private industry by both Barenburg USA and DLF International (Phillips and Aiken, 2009).

University of Kentucky plant breeding research has led to the development of many tall fescue cultivars. Some of the recent work by Tim Phillips has been to develop an E- tall fescue variety currently labeled as KYFA9301. A diverse synthetic, KYFA9301, was produced by intermating selected genotypes from 22 half-sib families. The original source material came from an old variety trial, harvested in 1987-88 for 18 consecutive months, at monthly intervals. Most of the parental clones were from E-Kentucky 31 and related germplasm. As an E- accesion, KYFA9301 has demonstrated excellent seedling vigor, a desirable maturity, approximately 5 days later than Kentucky 31, high-yield potential, and good grazing tolerance (T. D. Phillips, Personal communication).

The development of a novel endophyte variety adaptable to the upper transition zone led to the combining of KYFA9301 with novel endophyte AR584. This fescue-endophyte combination was developed by AgResearch Ltd. NZ, inserting the endophyte into germinating seedlings of KYFA9301, then producing seed from the infected plants.

The seed from New Zealand was grown in Oregon, in a field along with endophyte-free KYFA9301 to ensure open pollination between the two lines, but keeping them separate to have E- and /AR584 versions of the same cultivar (T. D. Phillips, Personal communication).

Studies have indicated that the most successful technique for replacing E+ tall fescue is with the utilization of both herbicide and cover-crop rotation before new stand establishment. Existing stands should be sprayed out with a 'burndown' herbicide such as glyphosate (Roundup®, Monsanto, St. Louis, MO) or paraquat (Gramoxone®, Syngenta, Greensboro, NC) at a high enough rate to kill the fescue sod as the label recommends. Herbicide application should be followed by planting an annual cover crop; some studies have utilized legumes for their fertility additions, and others have chosen annual row crops to gain additional use from the land area. A second application of herbicide is important to ensure a greater than 80% eradication of the previous E+ fescue, before no-till planting of an E- or novel endophyte fescue variety (Defelice and Henning, 1990; Fribourg et al., 1988; Munson et al., 1991). While an exact number is difficult to quantify, after conversing with forage specialists, area extension agents and local seed salesmen, it has been projected that approximately 30,000 acres of E+ fescue have been replaced by novel endophyte or E- fescue in Kentucky since the emergence of non-toxic fescue cultivars in 1980's and the marketing of now available novel endophyte cultivars, such as Jesup-MaxQ (G.D. Lacefield, Personal communication)

Economics of Replacement

While a definite number is hard to reach, the economic impact of fescue toxicosis on the beef cattle industry is apparent. The majority of fescue acres in production to date are still highly infected with the wild-type endophyte, therefore still negatively impacting the beef cattle industry. Not only does fescue toxicosis result in reduced weight gain, which consequently reduces profitability, but the reduction in reproductive performance and milk production also have a heavy impact. As stated in a report by Hoveland (1993) "economic losses from reduced conception rates and weaning weights of beef cattle grazing tall fescue in the US have been conservatively estimated at $609 million annually"; thereby demonstrating a definite need for an alternative to grazing toxic tall fescue.

Replacement of E+ pastures with non toxic tall fescue cultivars can be a costly venture and one that producers must determine if the added benefit of animal performance outweighs the costs. Zhuang et al. (2005) concluded that endophyte infection levels on previously established pastures must be greater than 74% for replacement to be feasible in generating greater annual returns than maintaining wild-type infected stands. Cattle grazing stands with infection levels between 70 - 80% reflected larger differences in reproductive fertility, or calving rate, than with lower infection rates. Other variables needing to be considered are a decrease in stocking rate and pasture stand life when switching to an E- cultivar and a fluctuation in discount rate with cattle exposed to E+ fescue (Zhuang et al., 2005).

In 2004, research indicated that the costs of establishment of E- cultivars do not outweigh the overall gains in animal performance. A study by Gunter and Beck (2004) determined that it would take approximately 7 years to pay establishment costs and have a net return for cow-calf enterprises that replace E+ pastures with novel endophyte pastures if a market discount on cattle exhibiting fescue toxicosis symptoms is not accounted, and approximately 3 years if the market discount is accounted. The economic value of the southeastern forage systems for beef cattle and calves, which includes many hectares of Kentucky 31 tall fescue, has been estimated at approximately $11.6 billion annually. Improved forage cultivars that limited or even eliminated toxicity and increased productivity would benefit farmers, but the development of E- cultivars did not meet the needs of cattle producers, as indicated by the lack of overall sales of E- seed in the tall fescue market seed shares. Two suggested approaches to decrease the toxicosis impact on the beef cattle industry have been management adjustments and the introduction of novel endophyte fescue cultivars (Bouton, 2007). The improved animal performance and stand persistence of novel endophyte tall fescue demonstrated in grazing trials indicate that they are a feasible alternative to wild-type Kentucky 31 E+ tall fescue (Bouton et al., 2002; Parish et al., 2003). Andrae and Lacy (2004) suggested that producers experiencing depressed reproduction due to cows grazing infected fescue could generate improved net returns of $55 to $107 per cow per year, or $36 to $87 per cow per year when considering establishment costs if they replaced infected pastures with novel endophyte tall fescue Jesup-MaxQ. With a realistic expectation of improved calving performance, and depending on previous infection levels, establishment of a Jesup-MaxQ pasture has the potential to pay for itself in 3 years (Andrae and Lacy, 2004).

Chapter Three: Materials and Methods

Experimental Site

The grazing experiment was conducted at the University of Kentucky Animal Research Center (UK-ARC) in Woodford County. Grazing in each year was from 6 May to 23 July in 2008 (76 d) and from 2 April to 25 June in 2009 (84 d). The experiment utilized crossbred (predominately Angus) yearling steers (*Bos taurus*) as 'testers' obtained from a collaborator. The steers (8 to 10 mo of age) were purchased locally through the Certified Preconditioned Health Program that is sponsored by the Kentucky Cattlemen's Association (CPA-48; weaned at least 48 days prior to sale). Steers grazed 12, 1.0-ha tall-fescue [*Lolium arundinaceum* (Schreb.) Darbysh. = *Schedonorous arundinaceum* (Schreb.) Dumort.] monoculture pastures that were planted on 27 September, 2006 at a seeding rate of 28 kg ha^{-1}. Soils at the site were either Maury (fine, mixed, semiactive, mesic Typic Paleudalfs) or McAfee (fine, mixed, active, mesic Mollic Hapludalfs) silt loams.

The experimental site had a 25+ yr old stand of Kentucky 31 tall fescue before research preparation. A "burn-smother-burn" approach to removing fescue and impeding emergence of fescue seed in the seed-bank was utilized. Two applications of glyphosate (Roundup®, Monsanto, St. Louis, MO) were applied at a 2-wk interval at a rate of 4.68 L ha^{-1} to the pastures in October 2005 followed by no-till planting of winter wheat (*Triticum aestivum* L.) at a seeding rate of 135 kg ha^{-1}. Wheat fields were closely grazed in early spring of 2006, and corn (*Zea mays* L.) was no-till planted at a seeding rate of 62,000 seed ha^{-1} with 20.3 cm row spacing on 1 May, 2006. Following harvest of silage on 22 September, 2006, a second application of glyphosate (Roundup®, Monsanto, St. Louis, MO) was applied at a rate of 4.68 L ha^{-1} to kill any residual fescue. The remaining stubble near fences was mowed before planting the fescue-endophyte combinations on 26 September, 2006. Rainfall data were collected from the national weather service station, located approximately 13.5 km from the experimental site at UK-ARC, at the Bluegrass Airport in Lexington, KY.

Experimental Design and Grazing Methodologies

Four tall fescue-endophyte combinations were assigned at planting to 1.0-ha pastures in a randomized complete block design with three replications. Combinations were Kentucky 31(KY31) tall fescue infected with the wild-type endophyte, KYFA9301 Endophyte Free (EF9301), KYFA9301 infected with AR584 endophyte (AR584), and Jesup-MaxQ infected with AR542 endophyte (MaxQ). Forty-eight tester steers were blocked by body weight for assignment to pastures (4 testers per pasture). In 2008, tester steers with initial mean BW of 304 kg ± 34 kg were placed on pastures on 5 May to 23 July, and in 2009 steers with initial mean BW of 277 kg ± 24 kg were placed on pastures on 2 April to 25 June. During the study, put-and-take stocking (Mott and Lucas, 1952) was used to maintain forage mass at approximately 2500 kg ha^{-1} ± 200 kg, to minimize effects of forage mass on steer performance and to measure carrying capacity (steers d ha^{-1}). Therefore, stocking rates were varied among pastures to maintain comparable forage masses. Average body weights were determined separately for tester steers and put-and-take steers for calculating stocking rates on a kg body weight ha^{-1} basis. Put-and-take steers were more of mixed breeding than the testers, but their body weights were similar to those of the testers. Put-and-take steers were added or removed from pastures as determined by disk meter height measurements (4 to 6-cm; described in detail in pasture responses section) taken one day prior to collecting rectal and skin temperatures when stocking adjustments were made. However, some stocking adjustments were made during the last month of grazing in both years after fescue growth rates had declined. When not being utilized in the research project, put-and-take steers were maintained on bermudagrass pastures. Pastures were continuously grazed.

Animal Management

Cattle were received 15 April, 2008 and 24 March, 2009 at the UK-ARC in Woodford County. Steers were placed on 5 ha of bermudagrass, fed free-choice tall fescue hay, and allowed an adjustment period of two to three wks before study onset each year.

The experimental protocol was reviewed and approved by the University of Kentucky Institutional Animal Care and Use Committee (2008-0289). Steers were provided water and mineral (Burkmann Mills, Danville, KY) free-choice throughout the experiment. On the day that grazing was initiated in each year, steers were treated for internal and external parasites with Moxidectin dewormer (1 mL/10 kg BW pour-on) (Cydectin®, Fort Dodge Animal Health, Fort Dodge, IA). All steers were ear implanted with Synovex-S (200 mg progesterone-20 mg estradiol; Fort Dodge Animal Health, Fort Dodge, IA) implants on 6 May in 2008 and 2 April in 2009. Steers exhibiting pinkeye symptoms were injected with oxytretracylcine (Liquamycin® LA-200® Pfizer Animal Health, Exton, PA, 4.5 mL/100 lbs body weight), and Shut-Eye (American Animal Health Inc., Wisner, NE) patches were applied to steers suffering severe infections.

Pasture Management

Following planting of the fescue-endophyte combinations in September 2006, 56 kg N ha^{-1} was applied approximately 3-wk after seedling emergence. Hay was harvested in late June 2008, and cows grazed the area to an 8 to 12-cm stubble in early Fall to promote tillering. Prior to grazing in 2008, the 1.0-ha pastures were fenced and watering sources were installed. Pastures were fertilized 21 March, 2007 and 18 March, 2008 with 67 kg N ha^{-1} using aqueous nitrogen, and on 16 March, 2009 N was applied at 67 kg ha^{-1} with polycoated urea (Agrium Inc; Calgary, Alberta). Following establishment, herbicide applications were utilized each spring for weed control. On 6 April, 2007, pastures were applied with 5.68 L ha^{-1} of 2,4-D amine. Aminopyralid (Milestone®, Dow AgroSciences, Indianapolis, IN) was sprayed for weed control each spring at a rate of 355 g ha^{-1} on 10 April in 2008, and 283 g ha^{-1} on 4 April in 2009.

Animal Responses

Cattle were fasted from feed and water for 14 hrs prior to weighing. Cattle were weighed shrunk at 0700 h on 6 May and 23 July in 2008 and on 2 April and 25 June in 2009, the initial and terminal dates of the experiment. Rectal and skin temperatures were recorded on 5 June, 1 July, and 22 July in 2008, and on 5 May, 1 June, and 24 June in 2009. Steers were removed from pastures at approximately 1630 hrs as the daily ambient temperatures were declining from daily maximums. Rectal temperatures were measured using the TM99A digital temperature instrument (Cooper-Atkins Corporation, Middlefield, CT). Skin temperatures were measured using a Raytek® Raynger ST60 ProPlus infrared thermometer (Raytek Corporation, Santa Cruz, CA) on a 2- x 2-cm area over the shoulder that was clipped to the skin surface with a Oster® Powerpro Ultra cordless clippers (Oster Professional Products, McMinnville, TN) (#40 blade). Approximately 7 mL of jugular blood was collected from each tester steer. Blood samples were centrifuged at (3,000 x g for 15 minutes) to collect serum that was stored frozen (0^{o}C). Serum prolactin concentrations were assayed by radioimmune assay procedures described by Bernard et al. (1993).

Pasture Responses

Forage mass (kg DM ha^{-1}) was determined using a disk meter similar to one described by Bransby et al. (1977). The disk meter used in the present experiment had a weight of 1.9 kg and was 45-cm in diameter. Disk meter heights were collected at approximately 4-wk intervals on 5 May, 30 May, 27 June, and 23 July in 2008 and 8 April, 28 April, 27 May, and 25 June in 2009. Disk meter heights were taken every 5 steps for 2 lengths of each pasture. Over the experiment, total disk meter height measurements per pasture ranged from 100 to 175. Disk meter calibrations were taken by clipping forage below the disk meter plate to the soil surface at 3 random locations throughout each pasture on 30 May and 3 July in 2008, and 1 June, 2009. Samples were dried in a forced-air oven at 60^{o}C for 48 hrs and then weighed to regress kg DM ha^{-1} over disk meter heights as a calibration equation for estimating mean DM.

Forage nutritive value was estimated by harvesting forage from 3 randomly chosen locations within each pasture at approximately 2 wk intervals: 19 May, 6 June, 18 June, 30 June, and 16 July in 2008 and 16 April, 30 April, 21 May, 2 June, and 15 June in 2009. Forage was harvested from 0.25-m^2 quadrats with a stubble height of 5 cm, dried in a forced-air oven at 60^{o}C for 48 hrs. Subsequent hand-plucked samples, plucked from the upper third of the canopy, were also collected to estimate nutritive value of herbage selectively grazed by steers in 2009. Harvested forage was dried at 60°C for 48-h and ground in a Wiley mill (Thomas® Scientific, Swedesboro, NJ) to pass a 1-mm sieve and stored for analysis.

Samples were analyzed for ADF, NDF, IVDMD, and CP. Neutral detergent fiber and ADF were determined using the ANKOM^{200} Fiber Analyzer (ANKOM Technology Corp., Macedon, NY). Percentage nitrogen in dry matter was determined by combustion in a Leco FP-528 nitrogen/protein determinator (Leco Corp., St. Joseph, MO) and then multiplied by 6.25 for adjustment to CP. *In vitro* dry matter digestibility determination was performed using a Daisy II incubator (ANKOM Technology Corp., Macedon, NY) and followed procedures for estimating true DM digestibility.

Single tall fescue tillers were randomly chosen and collected from 25 plants within each pasture for testing of endophyte infection on 9 June and 7 July, 2009. Endophyte infection levels were assayed on collected tillers using an immunoblot procedure (Gwinn et al., 1991).

Single tillers were also collected to determine ergrovaline concentrations on 10 June in 2008 and on 8 June in 2009. Tiller samples were clipped at the tiller base. Samples were placed on ice and subsequently frozen until being analyzed. Tiller samples were freeze-dried, ground through a 1-mm screen, and assayed for ergovaline and ergovalanine concentrations by HPLC florescence using modified procedures described by Yates and Powell (1983). Separation was conducted with an Alltima C18 150 mm x 4.6 mm column with a 3µ particle size (Grace Davison Discovery Science, 2051 Waukegan Rd., Deerfield, IL). Elution solutions were 75 mM ammonium acetate (A) in water:acetonitrile (3:1, v/v) and acetonitrile (B). Elution gradient was 95:5 (A:B) for 1 min; linear change to 60:40 (A:B) during next 15 min and maintained for 5 min; changed to 0:100 (A:B) in 1.5 min and maintained for 5 min; changed to 100:0 (A:B) in 1 min and maintained for 6 min before returning to 95:5 (Yates and Powell, 1983).

It was apparent in 2009 that there was significant encroachment of Kentucky bluegrass (*Poa pratensis* L.*)*. Botanical composition of tall fescue and bluegrass were determined on 24 June in 2009 using a double sampling procedure (Ortega-Santos et al., 1992). Two observers visually estimated DM composition of each grass within 25, 0.16-m^2 rings, tossed at random for each pasture (n = 50). Herbage within 3 rings for each observer was clipped to the soil surface for subsequent separation into the two grass components. Components were dried at 60°C for 48 h and weighed for calculating DM composition for each sample. A linear regression equation was calculated for estimating

DM percentage of tall fescue by regressing actual DM composition over estimated DM percentage of tall fescue (Figure 3.1).

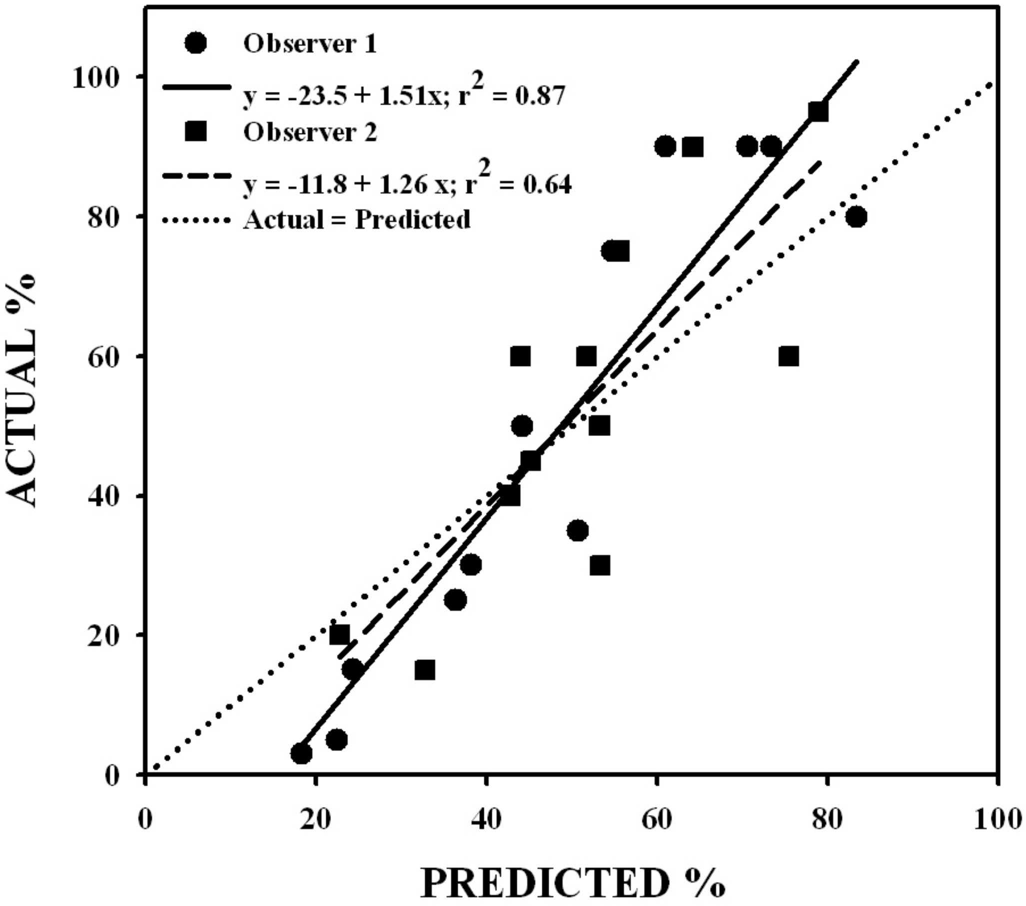

Figure 3.1. Botanical composition of tall fescue and Kentucky bluegrass collected on June 24, 2009 using a double sampling procedure (Ortega-Santos et al., 1992). Equations calculated for calibrating the visual estimates by regressing actual DM percentages of tall fescue in hand separated samples over visual composition estimates of DM percentages from two observers.

Calculations and Statistical Analysis

Animal responses were ADG, BW gain ha^{-1}, rectal and skin temperature, and serum prolactin concentrations. Pasture responses were herbage mass and allowance, carrying capacity, stocking rate (steers ha^{-1} and mean BW ha^{-1}), and nutritive value (CP, NDF, ADF and IVDMD concentrations). Herbage allowance was determined by dividing mean pasture forage mass by mean BW for testers x mean stocking rate (steers ha^{-1}) on a per 100 kg BW basis. Carrying capacity is the summation of the daily stocking rates (steer per hectare). Stocking rate (steers ha^{-1}) was calculated by dividing carrying capacity by the total days of grazing for the year of study (76 d in 2008, 84d in 2009). Stocking rate on a kg BW ha^{-1} basis was calculated for each pasture by multiplying mean BW of testers [(initial + final BW)/2) x mean stocking rate (steers ha^{-1}). Stocking rates at initial, midpoint, and final days of grazing were estimated for each pasture using the calculation: kg BW ha^{-1} = (initial mean BW + (ADG x days) x stocking rate. Average daily gain was for the full duration of grazing and stocking rate (steers ha-1) was the total number in the pasture at that point of time during the grazing experiment.

Data were analyzed as a randomized complete block design with four steers in each of three replications of each treatment. Animal and pasture responses were analyzed using PROC MIXED of SAS (SAS Inst. Inc., 2002). Pasture was used in all analyses as the experimental unit. Fixed effects for animal response were year, fescue-endophyte combination, and year x fescue-endophyte combination interaction. For statistical models without measurement date analyzed as an effect (ADG, BW gain ha^{-1}, carrying capacity, stocking rates, and herbage mass and allowance), block x year was the error term (a) for analyzing year effects, and residual error (b) was used to evaluate fescue x endophyte combination and year x combination effects. For statistical models with date within year effects (rectal and skin temperatures, serum prolactin, and forage nutritive values) and for time effects on stocking rate, block x year was the error term (a) for analyzing year effects, block x fescue-endophyte combination (year) was the error term (b) for evaluating fescue x endophyte combination and year x combination effects, and residual error (c) was used to evaluate date within year and combination x date (year) effects. Single degree orthogonal contrasts were used to compare toxic versus non-toxic fescues, EF9301 versus AR584 and MaxQ, and AR584 versus MaxQ. Data collected over dates (rectal and skin temperatures, serum prolactin concentrations, and forage nutritive values) were analyzed as repeated measures using the first-order autoregressive covariance structure. Least square means were analyzed using the PDIFF option of SAS to analyze date within year effects or fescue-endophyte combination x date within year interactions.

Chapter Four: Results and Discussion

Tall fescue is widely known for its drought tolerance and persistence in the upper transition zone, qualities that have been attributed to the endophyte and witnessed through the recent drought years suffered by Kentucky pastures. Precipitation amounts varied greatly throughout both years of the study. Year one (2008) was representative of a very dry year in Kentucky. Precipitation levels declined steadily from study initiation in May to conclusion in July of 2008 and fell below the 39-yr average for the region (Figure 4.1). The second year of the study was unusually wet; with precipitation levels that were exceptionally high in the spring from study initiation in April and remained high throughout the study to conclusion in June. Precipitation in 2009 was higher than the 39-yr average for the region, and the added moisture led to visual pasture variations, increased forage growth and required more adjustment to variable stocking at disk meter reading dates than were required in year one of the study. Precipitation data were collected from the national weather service station located at the Bluegrass Airport in Lexington, KY. This weather station, which is approximately 13.5 km from the experimental site, was used for precipitation data because of inaccuracies in precipitation data collected for the UK-ARC weather station in 2009.

It should be noted that the steers used in this study only underwent a brief period of non-tall fescue exposure before study initiation. Both years steers were obtained from a local collaborator and were all native Kentucky cattle that likely had been exposed to KY31 wild-type endophyte for much of their existence. This being the case, these steers likely entered the study with high alkaloid saturation. Watson et al. (2004) suggested that while no quantifiable research has been conducted, data indicate that calves exposed to grazing E+ pastures early in life may exhibit long-term carryover effects which may permanently impair their development later in life. As suggested by Klotz et al. (2007, 2009) ergovaline can be a potent vasoconstrictor that, with even small exposure over extended periods of time, has been found to lead to bioaccumulation in the vasculature of cattle. This suggests there is a carryover effect; therefore, there likely is no threshold ergot alkaloid concentration in the diet that is needed to be reached for inducing toxicosis symptoms.

Endophyte infection percentages, averaged over two dates in the second year, were 67 ± 4.1, 81 ± 2.1, 86 ± 2.7, and 3 ± 0.9 for KY31, MaxQ, AR584 and EF9301 pastures, respectively (Table 4.1). The low endophyte infection in EF9301 was below 5% and likely of no consequence to animal responses. Previous threshold research has indicated that toxicosis effects are evident when endophyte infection levels are above 22% (Fribourg et al., 1991). This low percentage of infected plants had likely emerged from the existing seed bank. While KY31 pastures had lower infection percentages than the novel endophyte pastures, toxicosis was clearly evident in animal performance measures and ergot alkaloid measurements. The endophyte infection percentages of KY31 pastures used in this study were high enough to induce visible, as well as measureable, toxicosis in grazing steers.

Ergovaline is an ergot alkaloid that is present in high concentrations in E+ tall fescue (Lyons et al., 1986), and has shown to be potent in causing vasoconstriction (Klotz et al, 2007). Tillers collected in the study were tested for concentrations of ergovaline and ergovalanine, an epimer of ergovaline (Table 4.1). Ergovaline and ergovalanine concentrations were lower than expected, but the tillers were collected on a single date in each year and ergot alkaloids can be highly variable during the growing season (Carter, 2008). Although there was dilution of these concentrations from non-infected tillers, ergovaline and ergovalanine concentrations were high enough to elicit responses in line with toxicosis.

Forage mass throughout the study was measured monthly with a disk meter for making decisions in varying stocking rates. Mean forage mass was greater in 2009 (2,773 ± 25 kg DM ha^{-1}) than 2008 (2,471 ± 26 kg DM ha^{-1}) ($P = 0.038$), but still remained near our target of 2,500 kg DM ha^{-1}, an amount of available forage that was assumed to maximize selective grazing. Using put-and-take grazing management, forage mass did not differ between fescue-endophyte combinations ($P = 0.437$) and there was no year x fescue-endophyte combination interaction ($P = 0.999$). Temperature and drought stress are known factors to influence forage mass. Cool-season grasses prefer average daily temperatures around 20^{o}C for optimal growth, and express rapid reductions in growth rate when temperatures reach about 25^{o}C (Nelson and Moser, 1994). The dry year and above average ambient temperatures reported for 2008 were apparently major factors that influenced observed differences between the years.

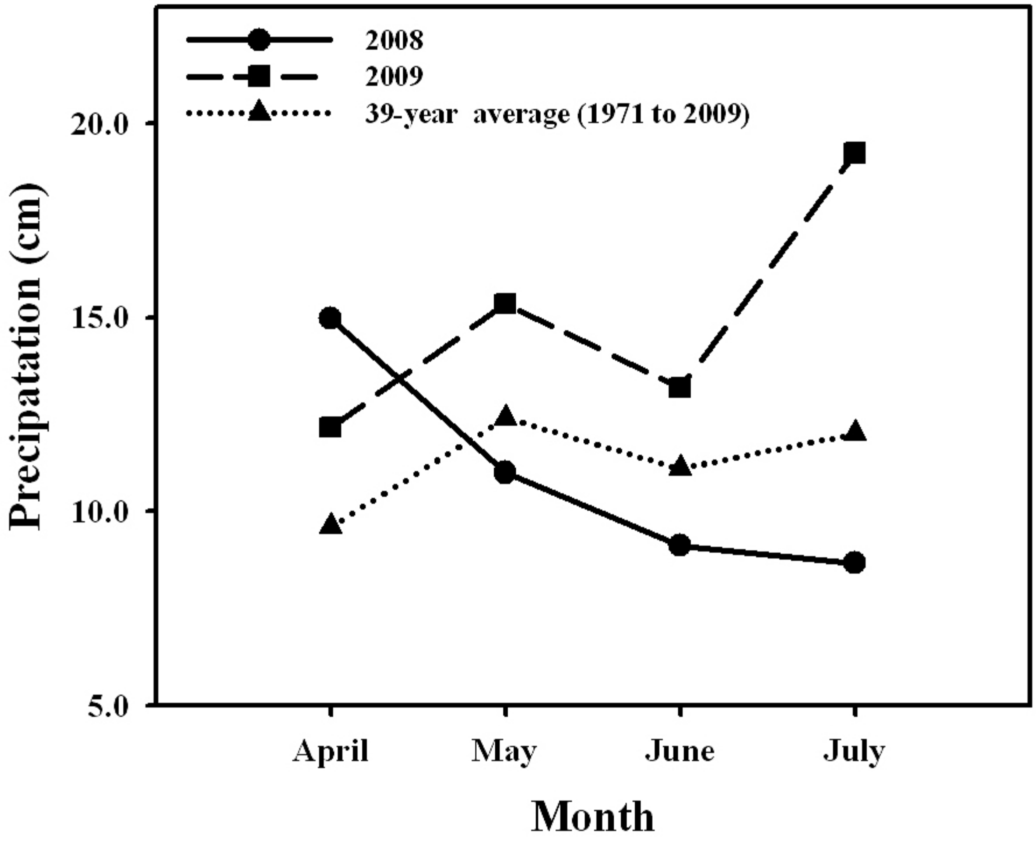

Figure 4.1 Precipitation data averaged for the months of April to July, for the grazing periods in 2008 and 2009, and the 39-yr average, collected from the National Weather Service Station (Bluegrass Airport in Lexington, KY) located approximately 13.5 km from the experimental site.

Table 4.1. Ergovaline / ergovalanine concentration (±SEM), and endophyte infection percentages (±SEM) in Kentucky-31 tall fescue infected with wild-type endophyte (KY31), 'Jesup'-MaxQ infected with AR542 endophyte (MaxQ), KYFA9301 infected with AR584 endophyte (AR584), and KYFA9301 endophyte-free (EF9301) tall fescue-endophyte combinations in a 2-yr grazing evaluation.

Entry	**Mean ergovaline / ergovalanine concentration †**	**Mean endophyte infection rate ‡**
	mg kg^{-1} DM	**%**
KY31	0.35 ± 0.03	66.8 ± 4.1
MaxQ	0.02 ± 0.01	81.2 ± 2.1
AR584	0.07 ± 0.01	86.3 ± 2.7
EF9301	0.02 ± 0.01	2.8 ± 0.9

† Combined means collected on June 10, 2008 and June 8, 2009.

‡ Combined means collected on June 9, 2009 and July 7, 2009.

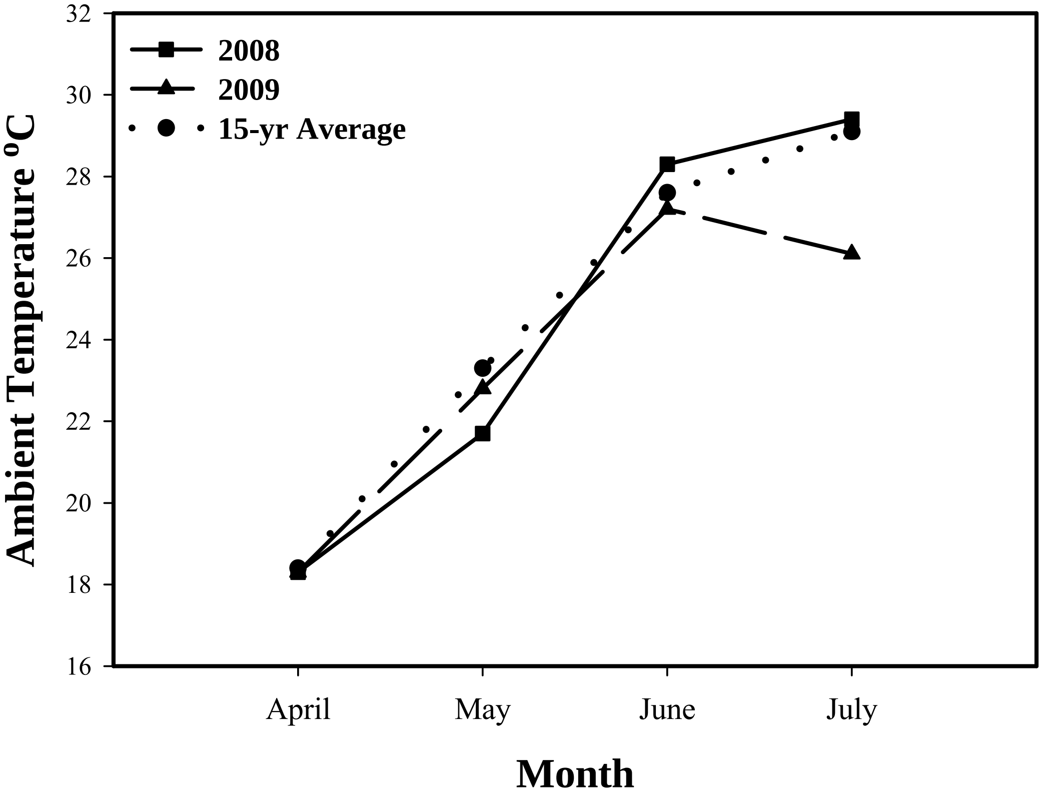

Figure 4.2 Mean ambient temperature for 2008 and 2009 and the 15 year average for the months of April through July, collected from the UK-ARC weather information center in Woodford County.

Bluegrass Percentage

Kentucky bluegrass percentages among fescue-endophyte combinations differed (P = 0.098) (Table 4.2). Toxic KY31 pastures had lower percentages (P = 0.040) of Kentucky bluegrass (32.3%) than the nontoxic fescues. Toxic KY31 pastures could have been more competitive than the nontoxic pastures, but since above ground forage mass was controlled this competiveness would have been expressed at the root zone and was not measured. There were no differences between EF9301 pastures and the novel endophyte-fescue combinations (P = 0.959), but MaxQ tended (P = 0.124) to have a lower percentage of bluegrass.

Kentucky bluegrass encroachment likely occurred from dry weather in 2008, followed by adequate rainfall in the fall of 2008 and spring of 2009 leading to germination of dormant bluegrass seed in the seed bank. Kentucky bluegrass, in the specified climate zone for Lexington, reaches physiological maturity during mid-April to early May (Ball et al., 2007). The progressive maturity of Kentucky bluegrass occurred near simultaneously with the initiation of the 2009 study in April. It was observed visually that, while encroachment was occurring and much bluegrass was present, bluegrass seedheads remained throughout the study due to selective grazing of tall fescue by steers. Steers were visually observed to selectively graze tall fescue over mature Kentucky bluegrass in all pastures. One factor affecting forage preference by grazing animals is palatability. The external form of a plant, presence of awns, spines, hairiness, leaves, stems, seedheads, etc. can affect its palatability (Heady, 1964). At the conclusion of grazing in June 2009, it was observed that the tall fescue across all pastures had been grazed harder than Kentucky bluegrass.

Table 4.2. Percentages of Kentucky bluegrass (*Poa pratensis*) in 2009 for Kentucky-31 tall fescue infected with wild-type endophyte (KY31), 'Jesup'-MaxQ infected with AR542 endophyte (MaxQ), KYFA9301 infected with AR584 endophyte (AR584), and KYFA9301 endophyte-free (EF9301) tall fescue-endophyte combinations in a 2-yr grazing evaluation. Botanical composition measurements were taken on 24 June.

Entry	**Kentucky Bluegrass Percentage**
	%
KY31	32.3
MaxQ	38.2
AR584	46.5
EF9301	42.1
SEM†	2.0
Contrasts:	
KY31 vs. Nontoxic	P = 0.0402
EF9301 vs. MaxQ, AR584	P = 0.9587
MaxQ vs. AR584	P = 0.1238

†SEM, standard error of the mean.

Pasture Responses

Carrying capacities (steer days ha^{-1}), differed among fescue-endophyte combinations (P = 0.003), but there was a tendency for an interaction between year and fescue-endophyte combinations (P = 0.147). Orthogonal contrasts indicated that KY31 pastures provided higher carrying capacities than non-toxic pastures, 2008 (P = 0.010), and 2009 (P = 0.040) (Table 4.3). These expected results can be attributed to the negative effect of ergot alkaloids on DM intake. In 2008, EF9301 provided higher (P = 0.0033) carrying capacity than the two novel endophyte combinations. Differences were not found between the two novel endophyte combinations (P = 0.1638). Carrying capacities in 2009 were more uniform, and no differences (P > 0.15) were detected between EF9301 and the novel endophyte combinations, or between the AR584 and MaxQ. Differences in 2008 can be attributed to low rainfall as compared to 2009, and the time of study initiation and conclusion. Allowing the 2008 study to run into July put the forage under more stress than the second year when grazing was terminated in June and the overall precipitation was greater allowing for more overall forage in general.

Mean stocking rate (kg BW ha^{-1}) differed among fescue-endophyte combinations (P = 0.024), and there was no interaction between year x fescue-endophyte combination (P = 0.195). Higher stocking rates were provided by KY31 than for the three nontoxic combinations (P = 0.017), as well as by EF9301 pastures over the two novel endophyte (P = 0.049). No differences (P = 0.290) were found between AR584 and MaxQ combinations (Table 4.3). Stocking rate also was calculated as the number of steers grazing per hectare. In this expression, stocking rate again differed among fescue-endophyte combinations (P = 0.004) and there was a tendency for year x fescue-endophyte combination interaction (P = 0.127). Orthogonal contrasts showed KY31 toxic pasture combinations to provide higher stocking rates each year, 2008 (P = 0.011) and 2009 (P = 0.060) than the three ‘nontoxic’ combinations, and EF9301 provided higher stocking rates over the two novel endophyte pasture entries in 2008 (P = 0.003). In 2008, AR584 tended to provide a higher stocking rate (P = 0.137) than MaxQ (Table 4.3). In 2009 stocking rate did not differ (P > 0.15) between EF9301 and the novel endophytes or between AR584 and MaxQ. Stocking rate generally increased overtime each year in KY31 pastures. Similar results have been reported in grazing studies by

Parish et al. (2003) and Peters et al. (1992) in which both studies found highest stocking rates on toxic endophyte pastures. While overall means indicated EF9301 exhibiting a significantly higher stocking rate than the novel endophytes, contrasts indicated that stocking rates for EF9301 were greater than for AR584 and MaxQ, but these differences were not observed in 2009. Stocking rate (kg BW ha^{-1}) over times initial, midpoint, and final did not differ by year ($P = 0.246$) (Table 4.4), year by fescue-endophyte combination ($P = 0.534$), or year by time by fescue endophyte combination interactions ($P = 0.233$). Mean stocking rate over time differed among year by time ($P < .0001$), and time by fescue-endophyte combination ($P = 0.029$). Mean stocking rate did not differ initially and at midpoint of grazing ($P > 0.10$). Final stocking rates were greater for KY31 ($P = 0.0001$) versus nontoxic pastures, and EF9301 ($P = 0.096$) versus novel endophyte pastures. There was also a tendency for difference between MaxQ and AR584 ($P = 0.123$). Mean stocking rates for 2008 were initially high (1898 ± 117 kg BW ha^{-1}), remained high at midpoint (1923 ± 117 kg BW ha-1), and then dropped significantly ($P = .0001$) at final (1560 ± 117 kg BW ha-1). Mean stocking rates for 2009 were initially low (1412 ± 117 kg BW ha-1), rose significantly ($P < .0001$) at midpoint (1797 ± 117 kg BW ha-1), and remained high at final (1814 ± 117 kg BW ha-1). Initial and final stocking rates for 2008 and 2009 differed significantly ($P = .003$, $P = .043$), respectively. The expressed year differences can be attributed to the delayed initiation of the study in 2008. The delayed study date of May 5 in 2008, as compared to April 5 in 2009, allowed for excessive forage accumulation at the beginning of the study and required higher implementation of initial stocking rates to maintain our targeted forage mass. Excessive precipitation in 2009 allowed for increased stocking rates throughout the study as forage growth was not limited by environmental stress as compared to the dryness experienced at the final date in 2008 which is represented by decreased stocking rate. The higher stocking rate on KY31 pastures was expected, and the higher stocking of EF9301 pastures suggests an ability of survival without an endophyte, but stocking rates were not pressed enough to stress pastures, so this conclusion cannot be made from this study. The tendency for higher stocking in AR584 pastures than MaxQ later in the season suggests the ability of this novel endophyte fescue to withstand grazing and carry more animals later in the season and gives reason to believe this variety is a viable option over MaxQ for utilization in this region.

Herbage allowance (kg DM 100 kg^{-1} BW) differed between the two years of the study ($P = 0.018$). In 2009 mean herbage allowance (167 kg DM 100 kg^{-1} BW) was greater than the allowance (136 kg DM 100 kg^{-1} BW) in 2008. No differences were expressed among herbage allowance fescue-endophyte combinations ($P = 0.867$) and there was not a year x fescue-endophyte combination interaction ($P = 0.835$). Herbage allowance was not a limiting factor among combinations. Mean herbage allowance for fescue-endophyte combined over both years were 149, 153, 152, and151 kg DM 100 kg^{-1} BW, for KY31, MaxQ, AR584, and EF9301 respectively. Through put-and-take stocking, available herbage was maintained at a high level and consistent among the combinations. Between year differences can be explained by the high precipitation levels from 2009 precipitation as compared to 2008 precipitation levels, but neither year expressed limitations in overall herbage allowance.

Table 4.3. Pasture responses to grazing Kentucky-31 tall fescue infected with wild-type endophyte (KY31), 'Jesup'-MaxQ infected with AR542 endophyte (MaxQ), KYFA9301 infected with AR584 endophyte (AR584), and KYFA9301 endophyte-free (EF9301) tall fescue-endophyte combinations in a 2-yr grazing evaluation.

Entry	**Carrying Capacity**		**Stocking Rate**		
	2008	**2009**	**2008**	**2009**	**Combined**
	steer d ha^{-1}		**steer ha^{-1}**		**kg BW ha^{-1}**
KY31	462	479	6.1	5.7	1833
MaxQ	391	441	5.1	5.3	1665
AR584	415	452	5.5	5.4	1719
EF9301	456	450	6.0	5.4	1784
SEM†	19	9	0.3	0.1	46
Contrasts:					
KY31 vs. Nontoxic	$P = 0.0100$	$P = 0.0396$	$P = 0.0112$	$P = 0.0595$	$P = 0.0172$
EF9301 vs. MaxQ, AR584	$P = 0.0033$	$P = 0.8121$	$P = 0.0032$	$P = 0.8571$	$P = 0.0490$
MaxQ vs. AR584	$P = 0.1638$	$P = 0.5280$	$P = 0.1372$	$P = 0.5360$	$P = 0.2902$

† SEM, standard error of the mean.

Table 4.4. Stocking rate over time of Kentucky-31 tall fescue infected with wild-type endophyte (KY31), 'Jesup'-MaxQ infected with AR542 endophyte (MaxQ), KYFA9301 infected with AR584 endophyte (AR584), and KYFA9301 endophyte-free (EF9301) tall fescue-endophyte combinations in a 2-yr grazing evaluation**.**

Entry	**Stocking Rate**		
	Initial	**Midpoint**	**Final**
	----------------------**kg BW ha^{-1}** -------------------		
KY31	1600	1895	1978
MaxQ	1625	1854	1446
AR584	1658	1875	1623
EF9301	1736	1816	1700
Contrasts:			
Toxic vs. Nontoxic	P = 0.4269	P = 0.6168	P = 0.0001
EF 9301 vs. MaxQ, AR584	P = 0.3359	P = 0.6230	P = 0.0957
MaxQ vs AR584	P = 0.7681	P = 0.8572	P = 0.1230

Nutritive Values

The major source of dietary fiber for animals are plant cell walls. The fiber is converted to energy in ruminant animals, and measured as forage ADF and NDF. Forage NDF is an estimate of primary-cell wall components, usually accounting for 60-80% of the organic matter of forage crops. Estimates of ADF are comprised of plant cell wall materials of cellulose and lignin plus ash. The lignin in the cell walls is highly indigestible, therefore, creating a negative correlation between fiber concentration and digestibility. Estimates of neutral detergent fiber are comprised of cell wall materials hemicellulose, cellulose, and lignin, or hemicellulose plus ADF (Goering and Van Soest, 1970; Buxton and Redfearn, 1997).

There was a year x fescue endophyte combination interaction ($P = 0.035$) on ADF concentration in quadrat samples. Higher ADF concentrations were found in EF9301 than in KY31 for 2008 ($P = 0.031$), and there was a trend for difference in 2009 ($P = 0.138$). There were no differences among nontoxic combinations in 2008 or 2009 ($P > 0.15$). Forage ADF concentrations taken from hand plucked samples in 2009 did not differ by endophyte combination ($P = 0.209$), but there was a date effect ($P < .0001$) (Figure 4.3).

Forage NDF concentrations taken from quadrat analyses were not different among endophyte combination ($P = 0.678$) but did have a tendency for a year x endophyte combination interaction ($P = 0.133$). Forage NDF concentrations differed by year ($P = 0.035$) and date within year ($P <.0001$). Concentrations of NDF for 2008 were higher (600 g kg^{-1}) than 2009 NDF concentrations (591 g kg^{-1}). Forage NDF concentrations taken from hand plucked samples in 2009 did not differ among combinations ($P = 0.662$), but did differ among dates ($P = 0.005$) (Figure 4.4).

Digestibility of perennial cool-season grasses is generally highest in spring and declines in mid-to-late summer, but is also affected by climatic changes; drought stress or excessive moisture can cause decreased or improved digestibility. Temperature and water affect forage quality. Cell wall materials are less lignified and more digestible when deposited at lower temperatures, hence, increases in temperatures lead to increased lignification and decreased digestibility (Nelson and Moser, 1994). In year one (2008) of the study, ambient temperature reached and exceeded 25^{o}C (temperature at which plant growth is rapidly reduced (Nelson and Moser, 1994)) during the experimental period. Comparisons of ADF and NDF concentrations (Figure 4.3 and 4.4) between the two years suggested that water stress combined with increased temperature in 2008 could have led to variations in NDF and ADF content, cell wall lignin, and overall digestibility. Fritz and Collins (1991) reported similar findings in fiber concentrations, with greater concentrations in 1987 than 1986, and rainfall levels 26 and 16% below the average, respectively.

Forage CP concentrations taken from quadrat analyses were not different among combinations ($P = 0.360$) or year x endophyte combination interaction ($P = 0.735$). Forage CP concentrations differed by year ($P < .0001$), and date within year ($P <.0001$). Crude protein concentrations in 2008 (89 g kg^{-1}) were lower than 2009 CP concentrations (153 g kg^{-1}). Forage CP concentrations in hand-plucked samples in 2009 did not differ among combinations ($P = 0.529$), but did exhibit a tendency for a date effect ($P = 0.142$) (Figure 4.5).

Forage IVDMD concentrations taken from quadrat analyses were not different for endophyte combination ($P = 0.623$) or year x endophyte combination interaction ($P = 0.658$). Forage IVDMD concentrations differed among year ($P = 0.059$) and date within year ($P <.0001$) (Figure 4.6). In vitro digestible DM concentrations were lower in 2008 (694 g kg^{-1}) than 2009 concentrations (742 g kg^{-1}). Forage IVDMD concentrations taken from hand-plucked samples in 2009 were not different among endophyte combination ($P = 0.328$), but there was a date effect ($P <.0001$). Pitman and Holt (1982) reported IVDOM is negatively correlated with ambient temperature and that it is positively correlated with moisture conditions. Forage digestibility of stems and leaves declined from spring to summer, as temperatures and day length increased and available moisture decreased (Pitman and Holt, 1982). As previously stated, in this study temperature and moisture conditions lead to variations between years and expected declines in digestibility over time with increasing maturity.

Forage concentrations increased in ADF and NDF and decreased in CP and IVDMD over time as expected with plant growth and maturity. Also, hand-plucked samples, representative of the top 7 cm of the canopy and more representative of what cattle selectively graze, provided higher CP and IVDMD, and lower ADF and NDF concentrations than quadrat samples which include all forage from one inch above the ground.

Forage nutritive quality of tall fescue declines with advancing maturity in spring. Forage quality parameters established by the NRC (1996) for ADF, NDF, CP, and TDN concentrations in fresh tall fescue were 344 ± 44, 622 ± 84, 150 ± 20, and 610 g kg^{-1} DM, respectively. As illustrated in Figures 4.3 through 4.6, forage nutritive values were all within or exceeded the expected ranges for tall fescue in 2009, and were near the ranges in 2008. It should be restated that the 2008 values are for quadrat samples and more representative of the whole plant and not the top portions that are typically grazed by cattle, and that the 2008 sample collections began a month later and were taken in a hotter and dryer environment than the 2009 samples. These reported analyses were still such that digestibility during the time of the experimental study were likely not a limiting factor of intake. Further, forage nutritive values of hand-plucked concentrations reported in this study were greater in the early collection dates than those suggested by the NRC. Digestibility is negatively correlated with cell wall concentrations (Merchen and Bourquin, 1994). Therefore, these low NDF concentrations suggest higher digestibility in the top 7-cm of the canopy, which also corresponds with higher CP and IVDMD concentrations and further confirms that digestibility of the forage was not a major limiting factor.

These results follow previous tall fescue research that indicate increased ADF and NDF concentrations and decreased CP and IVDMD concentrations with forage maturity in the spring. Howard et al. (1992) evaluated steers grazing E+ (Kentucky 31) and E- (Johnstone) tall fescue from May to September and determined ADF, NDF, and CP concentrations of the forage were similar across all periods of collection. An increase over the grazing season was detected for ADF and NDF concentrations, and CP concentrations from extrusa samples showed a decrease with experiment progression. McCracken et al. (1993) reported ADF and NDF concentrations increased during the spring months of May and June, and IVOMD decreased as forage matured. Kallenbach et al. (2003) evaluated nutritive values of stock-piled tall fescue cultivars and determined that nutritive value declined slowly and steadily with increased ADF and NDF, and decreased CP. Diversity in the weather between years added to expressed differences in overall results between the years (Kallenbach et al., 2003). Hedrick et al. (1982) observed significant correlations between forage quality parameters and steer performance. In a 3-yr grazing evaluation, it was determined that a strong correlation resulted from forage ADF, NDF, CP, and IVDMD concentrations and steer ADG, concluding that a high percentage of ADG variation was due to forage quality parameters (Hedrick et al, 1982).

From the nutritive value concentrations reported in this study, it can be determined that the forage quality could be considered high enough to meet the energy requirements of the animal. The relationship of NDF and energy described by Mertens, (1994) suggests that DM intakes of steers in the present experiment were limited by gut fill or environmental factors. Therefore, toxicosis-related heat stress is likely a major factor in limiting the performance of cattle grazing E+ KY31.

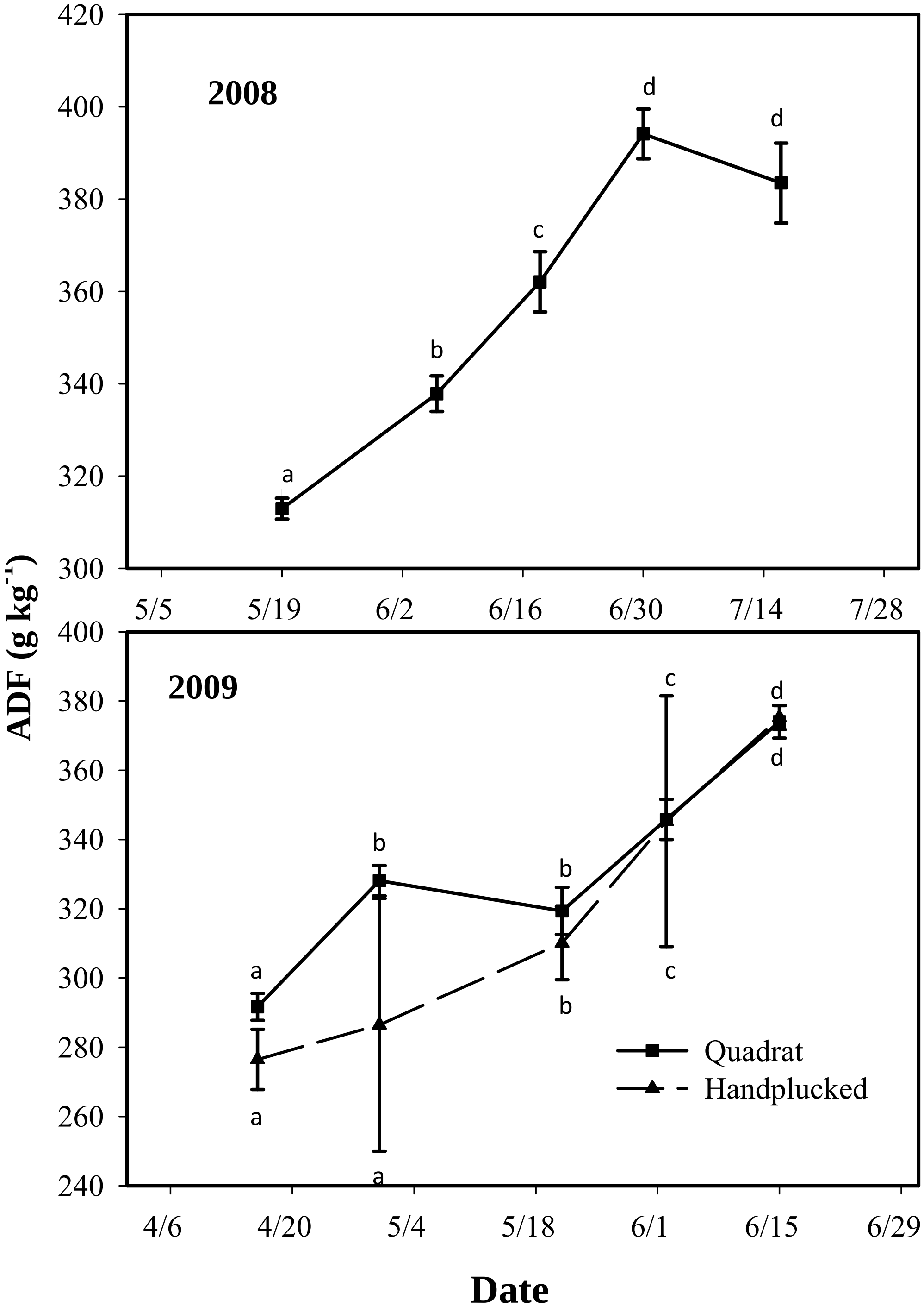

Figure 4.3 Acid Detergent Fiber (ADF) concentrations with standard error bars from quadrat and hand-plucked samples over sample dates in 2008 and 2009. Date within year differences signified by letters (abcd). Hand-plucked samples were only conducted in 2009.

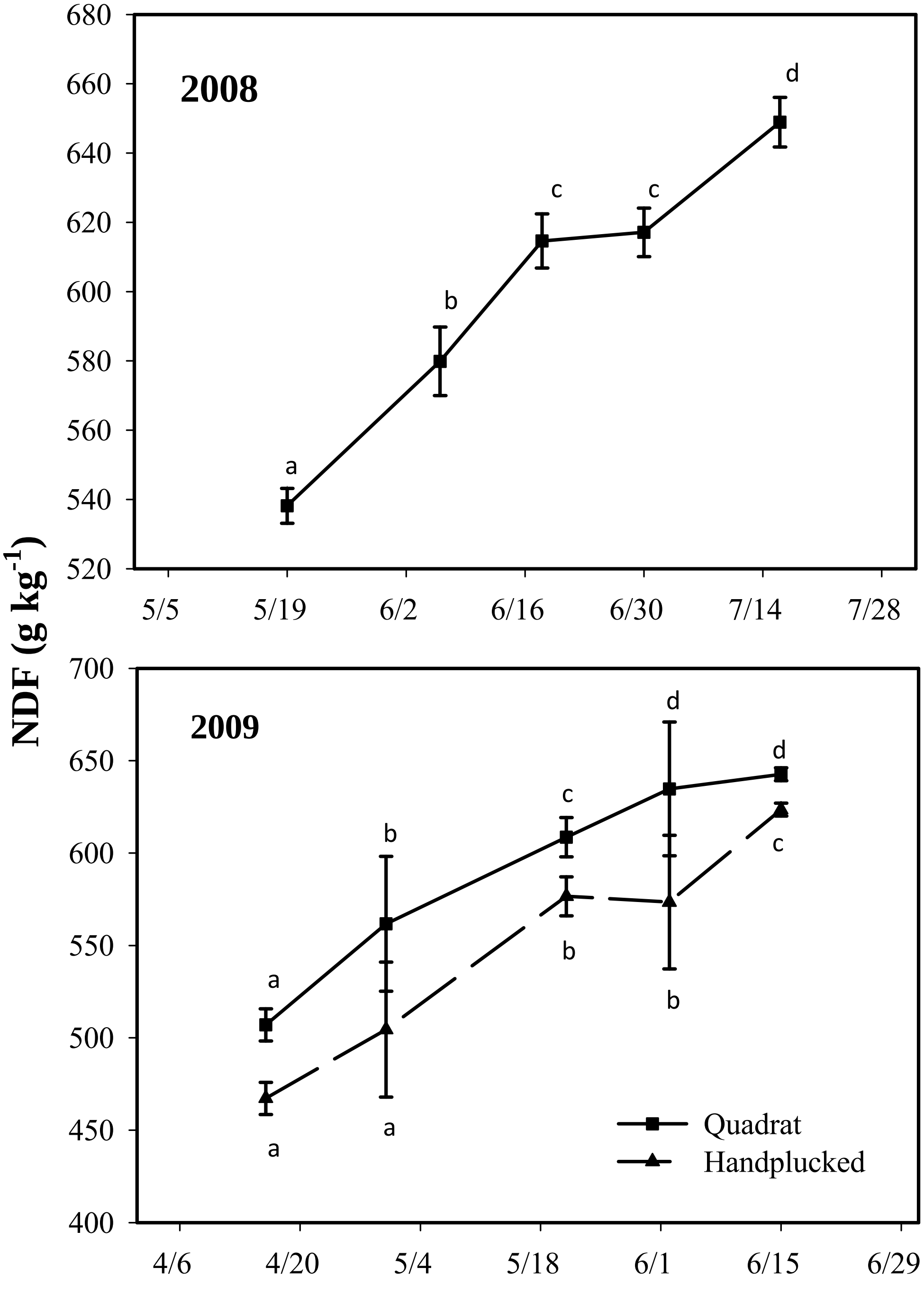

Figure 4.4 Neutral Detergent Fiber (NDF) concentrations with standard error bars from quadrat and hand-plucked samples over sample dates in 2008 and 2009. Date within year differences signified by letters (abcd). Hand-plucked samples were only conducted in 2009.

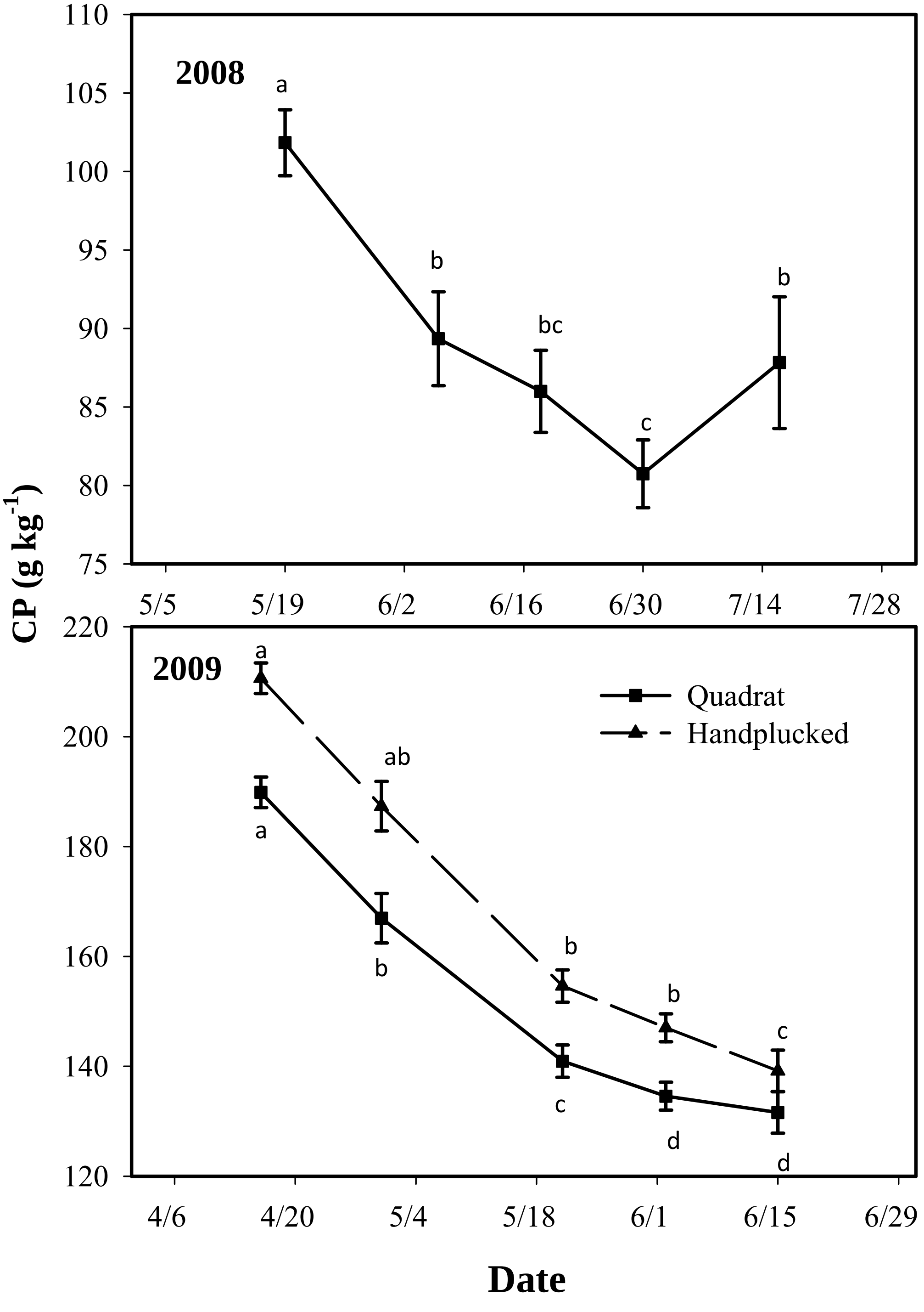

Figure 4.5 Crude Protein (CP) concentrations with standard error bars from quadrat and hand-plucked samples over sample dates in 2008 and 2009. Date within year differences signified by letters (abcd). Hand-plucked samples were only conducted in 2009.

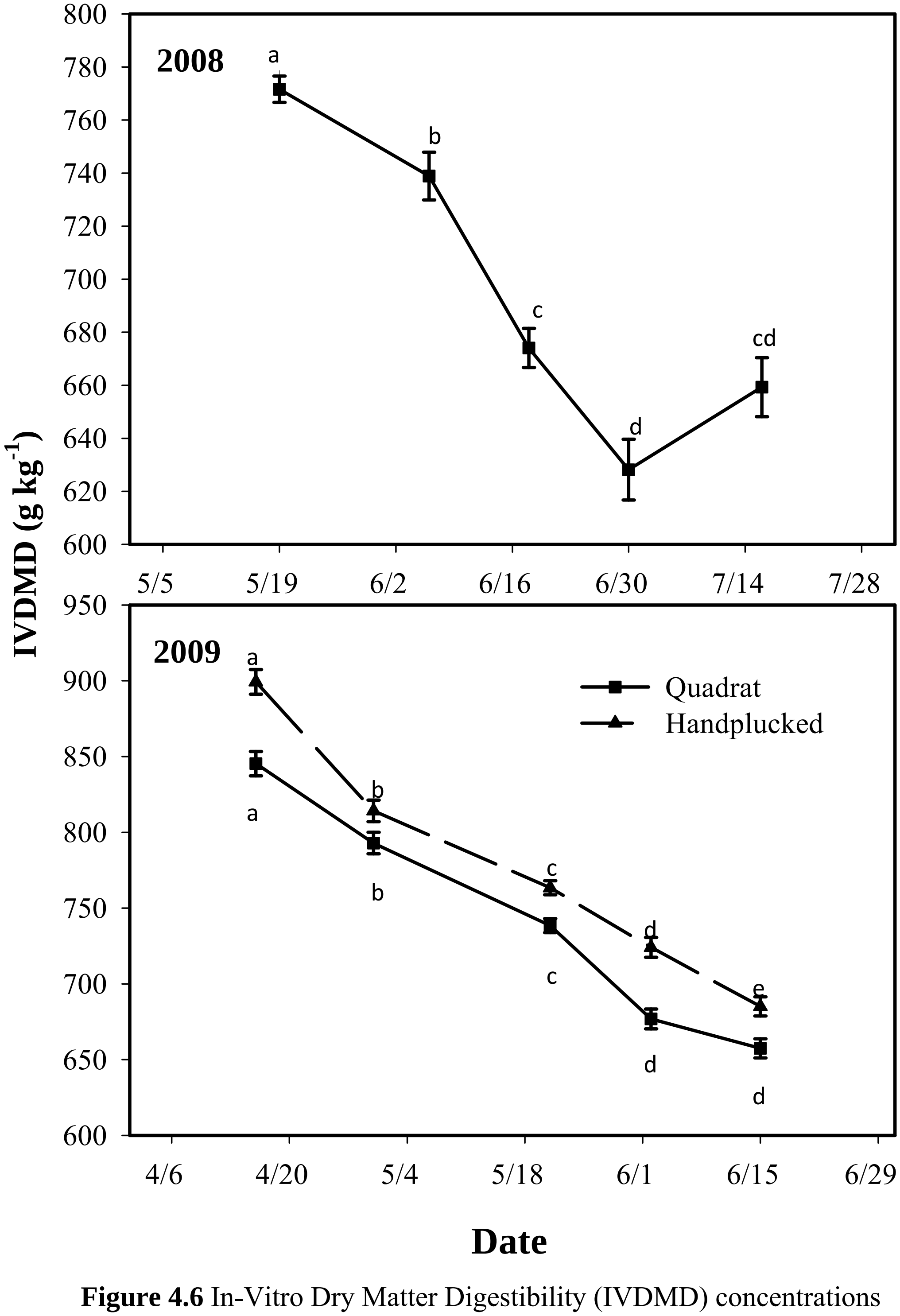

Figure 4.6 In-Vitro Dry Matter Digestibility (IVDMD) concentrations with standard error bars from quadrat and hand-plucked samples over sample dates in 2008 and 2009. Date within year differences signified by letters (abcd). Hand-plucked samples were only conducted in 2009.

Steer Weight Gain

Average daily gain (ADG; kg d^{-1}) differed among fescue-endophyte combination (P = 0.025), but did not exhibit a year x fescue-endophyte combination interaction (P = 0.961) (Table 4.5). Steers grazing toxic KY31 pastures had lower ADG (P = 0.002) than steers grazing the three nontoxic combinations. Average daily gain for EF9301 was not different from those for the novel endophyte pastures (P = 0.450), nor did ADG differ between AR584 and MaxQ (P = 0.264). Daily gain of steers grazing nontoxic pastures were 20% greater than those grazing KY31 pastures (Table 4.5) There was a year effect (P = 0.115) with 2009 showing higher ADG than 2008; 0.84 ± 0.4 kg d^{-1} and 0.73 ± 0.3 kg d^{-1}, respectively. Total BW gain per ha did not differ among fescue-endophyte combination (P = 0.159), and there was not a year x fescue-endophyte combination interaction (P = 0.822). However, there was a strong year effect (P = 0.001) on total BW gain per ha. Body weight gain per ha was higher in 2009 (383 ± 1.6 kg ha^{-1}) than in 2008 (312 ± 3.2 kg ha^{-1}). This difference in year could be attributed to the overall abundant available forage across all pastures as well as herbage allowance in 2009. Orthogonal contrasts did show KY31 pastures provided lower BW gains than nontoxic pastures (P = 0.008). No differences were detected among nontoxic combinations (P > 0.10).

Average daily gain was lowest for steers grazing KY31 fescue-endophyte combinations, an expected result from reduced intake and less time grazing, but correlates with the higher carrying capacity of these pastures. The higher carrying capacity of KY31 pastures, however, did not compensate for the low reported ADG of the steers grazing KY31 fescue-endophyte combinations, and therefore did not provide a higher BW gain per hectare for these pastures. Concluding that KY31's ability to tolerate more grazing and a higher carrying capacity cannot compensate for loss expressed in lower gains.

These results are consistent with similar grazing trials which reported higher ADG on nontoxic endophyte and endophyte free tall fescue compared to toxic E+ tall fescues. Parish et al. (2003) reported stockers grazing non-toxic cultivars exhibited higher ADG at approximately 0.84 kg across all sites and dates, and approximately 295 kg gain/hectare, as compared to stockers grazing the E+ cultivars which exhibited an approximate ADG of 0.45 kg and a gain/hectare of 157 kg. A two-year study by Nihsen et al. (2004) determined that steers grazing nontoxic fescue (i.e. EF KY31 and Strain 4-HiMag fescue) pastures had higher ADG than those grazing toxic E+ fescue pasture.

The presence of Kentucky bluegrass in 2009 was a confounding factor that could lead to an increase in gain but, as previously discussed, visual observation found grazing preference being tall fescue over KY bluegrass as the study progressed. This occurred even in KY31 pastures, which is further explained by the obvious toxicosis symptoms expressed by these steers. It can be assumed that forage consumption was not limited by lack of availability, and high rainfall in 2009 likely generated rapid forage growth that contributed to higher ADG and body weight for that year.

Table 4.5. Steer weight gain responses to grazing Kentucky-31 tall fescue infected with wild-type endophyte (KY31), 'Jesup'-MaxQ infected with AR542 endophyte (MaxQ), KYFA9301 infected with AR584 endophyte (AR584), and KYFA9301 endophyte-free (EF9301) tall fescue pastures. Means represent the two years combined.

Entry	**Average Daily Gain (ADG)**	**Gain/Ha**
	kg d^{-1}	**kg ha^{-1}**
KY31	0.64	297
MaxQ	0.88	363
AR584	0.81	351
EF9301	0.80	364
SEM†	0.03	13
Contrasts:		
KY31 vs. Nontoxic	P = 0.0016	P = 0.0078
EF9301 vs. MaxQ, AR584	P = 0.4364	P = 0.7520
MaxQ vs. AR584	P = 0.2508	P = 0.6250

† SEM, standard error of the mean.

Rectal and Skin Temperature

Rectal temperatures differed among years ($P = 0.073$), fescue-endophyte combinations ($P = 0.002$), and dates within year ($P = 0.001$), and there was not a year x fescue-endophyte combination interaction ($P = 0.218$). Rectal temperature for KY31 was greater than for the nontoxic fescues ($P = 0.0004$), but there were no differences ($P > 0.15$) among the three nontoxic fescues (Table 4.6). Mean rectal temperature for 2008 were lower ($P = 0.073$) than in 2009 (Figure 4.7).

Similar to rectal temperatures, skin temperatures, taken over the shoulder, also differed among years ($P = 0.056$), dates within year ($P = 0.0005$), and fescue-endophyte combination ($P = 0.0003$), and there was not a year x fescue-endophyte combination ($P = 0.337$) interaction. Steers grazing KY31 had higher ($P < .0001$) skin temperatures than those grazing the three nontoxic entries. No differences were observed between EF9301 and AR584 and MaxQ ($P = 0.569$); nor were differences found among AR584 and MaxQ ($P = 0.945$) (Table 4.6). Steer skin temperatures were significantly lower in 2008 (36.3^{o}C) than 2009 (36.9 ^{o}C) ($P = 0.059$) (Figure 4.7).

Unlike previous grazing studies of this nature, body temperature measurements were recorded in the late afternoon rather than in the early morning hours. Therefore, these temperatures were collected after maximum ambient temperature occurred in the early afternoon heat of the day rather than in the mornings after minimum ambient temperatures relieved heat stress symptoms. The time of temperature collection in this study likely improved detection of effects as compared to morning measurements.

Ambient temperatures varied on collection dates in both years and Figure 4.7 illustrates maximum ambient temperatures for days collected to coincide more accurately with the actual temperature at the time body temperature measures were collected. Ambient temperatures in 2008 on collection dates ranged from 27 to 32^{o}C and from 21 to 30^{o}C in 2009. Expected differences between KY31 pastures and nontoxic pastures coincided with visual observations of toxicosis. Date within year and year effects are closely related to differences expected from fluctuations in ambient temperatures across dates and years.

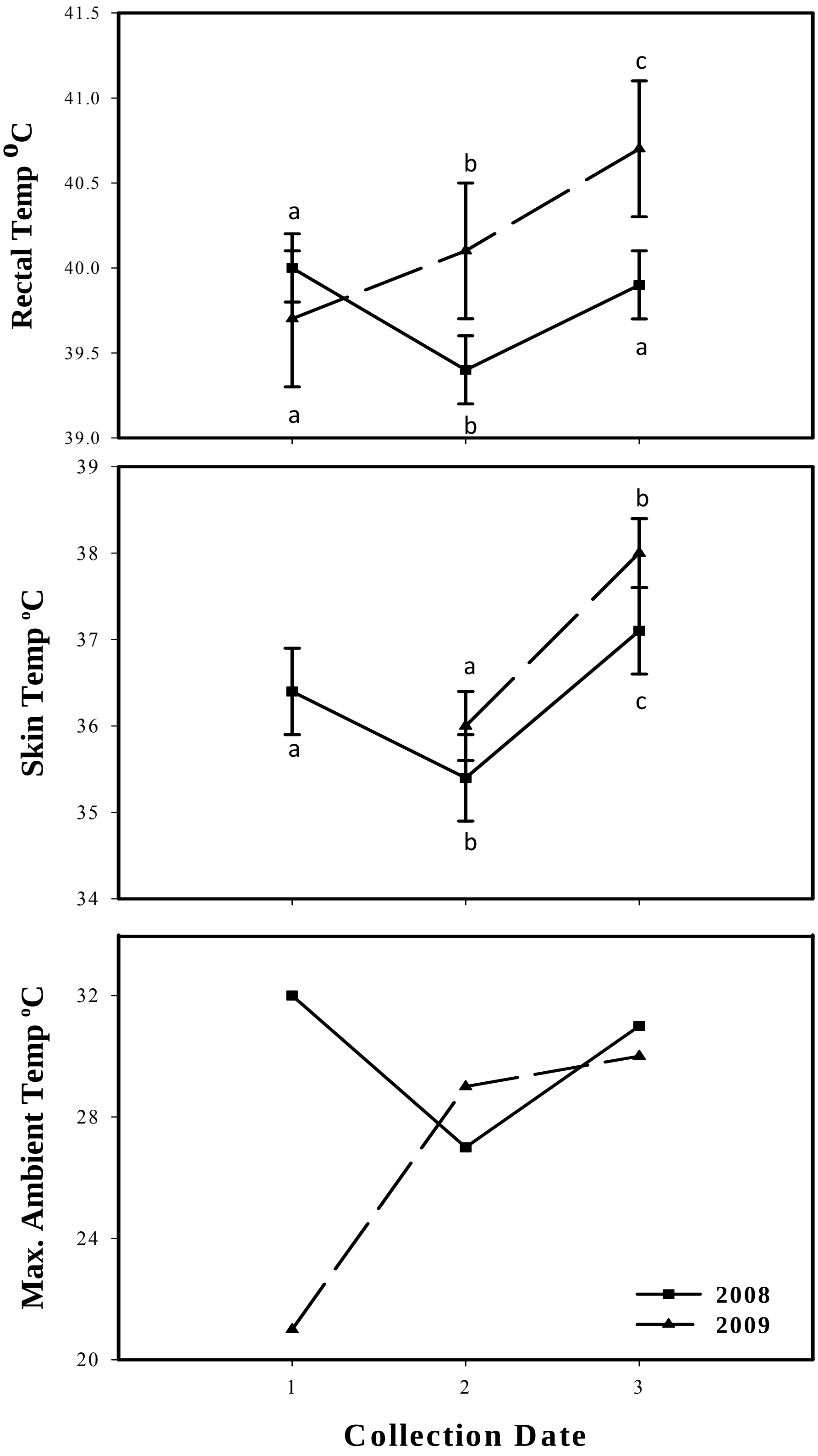

Figure 4.7 Rectal, skin, and maximum ambient temperatures with standard error bars, at approximately 1600 hr. for 5 June, 1 July, and 22 July, 2008 and 5 May, 1 June, and 24 June, 2009. Differences between dates within year signified by letters near standard error bars. Ambient temperature data collected at UK-ARC weather information center.

Serum Prolactin

Concentrations of serum prolactin (ng ml^{-1}) differed among year (P = 0.050), date within year (P = 0.016), and combination (P <.0001). There were no year x combination (P = 0.509) or combination x date within year (P = 0.530) interactions. Serum prolactin concentrations were not different between steers grazing AR584 and MaxQ (P = 0.228), nor were these different from steers grazing EF9301 (P = 0.7533), but these were all higher (P < .0001) than the concentrations of steers grazing KY31 toxic pastures (Table 4.6). Serum prolactin levels in 2008 were higher than in 2009 (P = 0.050), 192 ± 12.8 ng ml^{-1} and 165 ± 11.5 ng ml^{-1}, respectively.

A research marker for fescue toxicosis has been low prolactin concentrations due to its consistency in animals exhibiting toxicosis symptoms (Rice et al., 1997; Strickland et al., 1993). Hurley et al. (1981) concluded that consumption of tall fescue will depress serum prolactin regardless of ambient temperature. Changes in serum prolactin levels could be attributed to the pre-study carry-over alkaloid saturation in grazing steers and dissipation of these lingering effects throughout the study with the steers consuming nontoxic tall fescue having declining alkaloid accumulations. Previous tall fescue grazing research has reported similar results. Nihsen et al. (2004) found that while differences were not exhibited between endophyte free and their novel endophyte Strain 4-HiMag fescue combination endophyte variety, steers grazing E+ fescue expressed lower serum prolactin concentrations.

Table 4.6. Steer physiological responses to grazing Kentucky-31 tall fescue infected with wild-type endophyte (KY31), 'Jesup'-MaxQ infected with AR542 endophyte (MaxQ), KYFA9301 infected with AR584 endophyte (AR584), and KYFA9301 endophyte-free (EF9301) tall fescue pastures. Means represent the two years combined.

Entry	Rectal Temperature	Skin Temperature	Serum Prolactin
	----------------------°C------------------		ng ml^{-1}
KY31	40.3	37.2	82
MaxQ	39.9	36.5	230
AR584	39.8	36.5	207
EF9301	39.8	36.4	194
SEM†	0.1	0.1	9
Contrasts:			
KY31 vs. Nontoxic	P = 0.0004	P < .0001	P < .0001
EF9301 vs. MaxQ, AR584	P = 0.1803	P = 0.5688	P = 0.7533
MaxQ vs. AR584	P = 0.8205	P = 0.9449	P = 0.2282

† SEM, standard error of the mean.

Chapter Five: Conclusions

Growth performance measures, steer ADG and BW gain per ha on AR584, MaxQ, and EF9301, were superior to that of cattle on wild-type endophyte-infected KY31 fescue pastures. Steers grazing AR584, MaxQ, and EF9301 did not exhibit decreased serum prolactin concentrations or increased rectal and skin temperatures that are indicative of fescue toxicosis, as compared to steers grazing KY31 pastures. In the dry year of 2008, pasture responses, carrying capacity and stocking rate, of AR584 tended to be higher than MaxQ. In 2008 AR584 and MaxQ carrying capacity and stocking rates were lower than EF9301 pastures, but in 2009 no differences were detected. This suggests that AR584 pasture performance is comparable to that of EF9301 and slightly greater than that of MaxQ in drought years in this region. Higher carrying capacities in 2008 for the tall fescue accession KYFA9301 without the endophyte, should not go unnoticed. However, grazing in the 2-yr experiment was somewhat lenient and there were only two environments; therefore, it is not possible to conclude that an endophyte is not needed in this accession, or in this region, for prolonged tall fescue survival. Stocking rates of AR584 tended to be greater than MaxQ at final grazing days, indicating that the pasture productivity of the later maturing AR584 is high enough to carry more animals than MaxQ into the later grazing season (late June, early July) in this region.

While the pasture measurements (i.e. carrying capacity, stocking rate, herbage allowance) provided valid conclusions for forage productivity and quality under controlled grazing conditions, stand persistence of these fescue-endophyte combinations could not be ascertained. Presence of a novel endophyte in KYFA9301 would be recommended until more research is conducted to determine the value of the AR584 novel endophyte in providing stand persistence with harsher environments.

Tall fescue pastures infected with novel endophytes (AR584 or AR542) supported animal production as effectively as endophyte-free tall fescue pastures. Nutritive analyses among fescue-endophyte combinations were comparable. Steer performance and physiological responses for KYFA9301, with and without AR584, were enhanced compared to KY31 and similar to those for MaxQ. Based on results of the 2-yr grazing experiment, it can be concluded animal performance and physiology and pasture performance with KYFA9301-AR584 makes it a viable option for alleviating fescue toxicosis in the upper transition zone.

Future Implications

If repeating this study the following suggestions might enhance the findings of the current evaluation. Collection of tillers for ergot alkaloid evaluations at two week intervals throughout the study, rather than one time each year. This would show the concentrations of alkaloids at any given time throughout, as well as, the fluctuations of alkaloids in differing environments and across different fescue-endophyte combinations. Following this same thinking, collecting hand-plucked samples in both years would have given better representation of what the animals were grazing in 2008 rather than the total forage nutritive values as represented by the quadrat analyses presented in the study.

The study ended each year near the same time that producers would normally be rotating off of cool-season pasture if utilizing this forage in a rotation system, or during which time the plant would be expected to enter summer slump. Extending grazing beyond the later June, early July date could further test the late maturity of KYFA9301 with and without the endophyte, but having MaxQ as one of the four fescue-endophyte combinations could limit the ability to continue this evaluation later into the season and risk of grazing out this fescue-endophyte combination.

Another option would be the inclusion of extra fescue-endophyte combinations. Keep the current Jesup-MaxQ with AR542 endophyte, and include newly developed Jesup-MaxQ with AR584 endophyte. Also the utilization of a rotational grazing scheme to determine the extent of grazing into the summer, the utilization of each fescue-endophyte combination in a long-term grazing system, and to more replicate what producers are recommended in management of cool season grasses.

Appendix

A. 1 Table of 2008 mean forage mass and herbage allowance for individual pastures.

Pasture	Entry	Block	Forage Mass	Herbage Allowance
			kg DM ha^{-1}	kg DM 100 kg BW^{-1}
25-1	Max-Q	A	2183	149
25-2	KY31	A	2399	137
25-3	AR584	A	2520	149
26-1	EF	B	2526	149
26-2	Max-Q	B	2192	132
26-3	AR584	B	2192	135
27-1	KY31	B	2520	143
27-2	AR584	C	2605	131
27-3	KY31	C	2721	117
28-1	EF	C	2715	127
28-2	Max-Q	C	2642	132
28-3	EF	A	2432	127

A. 2 Table of 2009 mean forage mass and herbage allowance for individual pastures.

Pasture	Entry	Block	Forage Mass	Herbage Allowance
			kg DM ha^{-1}	kg DM 100 kg BW^{-1}
25-1	Max-Q	A	2696	179
25-2	KY31	A	2695	174
25-3	AR584	A	2745	171
26-1	EF	B	2733	164
26-2	Max-Q	B	2679	162
26-3	AR584	B	2705	164
27-1	KY31	B	2833	160
27-2	AR584	C	2796	160
27-3	KY31	C	3015	164
28-1	EF	C	2779	157
28-2	Max-Q	C	2821	164
28-3	EF	A	2773	182

A. 3 Table of 2008 mean for pasture responses carrying capacity and average stocking rate for individual pastures.

Pasture	Entry	Block	Carrying Capacity	Average Stocking Rate	Average Stocking rate by BW
			steer d ha^{-1}	steers ha^{-1}	kg BW ha^{-1}
25-1	Max-Q	A	320	4	1469
25-2	KY31	A	416	5	1751
25-3	AR584	A	398	5	1691
26-1	EF	B	398	5	1695
26-2	Max-Q	B	376	5	1657
26-3	AR584	B	376	5	1627
27-1	KY31	B	416	5	1762
27-2	AR584	C	472	6	1988
27-3	KY31	C	554	7	2327
28-1	EF	C	523	7	2138
28-2	Max-Q	C	476	6	2009
28-3	EF	A	443	6	1914

A. 4 Table of 2009 mean for pasture responses carrying capacity and average stocking rate for individual pastures.

Pasture	Entry	Block	Carrying Capacity	Average Stocking Rate	Average Stocking rate by BW
			steer d ha^{-1}	steers ha^{-1}	kg BW ha^{-1}
25-1	Max-Q	A	403	5	1508
25-2	KY31	A	430	5	1549
25-3	AR584	A	433	5	1606
26-1	EF	B	453	5	1665
26-2	Max-Q	B	450	5	1655
26-3	AR584	B	443	5	1648
27-1	KY31	B	497	6	1772
27-2	AR584	C	480	6	1752
27-3	KY31	C	510	6	1835
28-1	EF	C	480	6	1770
28-2	Max-Q	C	470	6	1722
28-3	EF	A	417	5	1523

A.5 Table of 2008 quadrat nutritive value concentrations for individual pastures and collection dates.

Entry	Block	Date	ADF	NDF	Protein	IVDMD
			----------------------------g kg^{-1}---------------------------			
Max-Q	A	5/19	308	537	96	761
KY31	A	5/19	313	534	119	789
AR584	A	5/19	323	547	97	772
EF	B	5/19	306	551	104	758
Max-Q	B	5/19	315	528	100	776
AR584	B	5/19	305	530	109	786
KY31	B	5/19	297	496	105	803
AR584	C	5/19	309	554	96	781
KY31	C	5/19	320	533	107	772
EF	C	5/19	320	555	95	740
Max-Q	C	5/19	319	531	95	760
EF	A	5/19	319	561	98	760
Max-Q	A	6/6	326	553	86	754
KY31	A	6/6	349	571	86	759
AR584	A	6/6	341	588	94	712
EF	B	6/6	339	571	83	752
Max-Q	B	6/6	328	575	96	743
AR584	B	6/6	329	590	99	744
KY31	B	6/6	324	574	83	763
AR584	C	6/6	337	591	88	751
KY31	C	6/6	352	576	92	727
EF	C	6/6	336	595	93	745
Max-Q	C	6/6	349	587	88	716
EF	A	6/6	343	586	85	701
Max-Q	A	6/18	355	582	75	643
KY31	A	6/18	359	621	85	656
AR584	A	6/18	359	618	91	684
EF	B	6/18	358	626	86	670
Max-Q	B	6/18	354	635	82	648
AR584	B	6/18	375	613	94	703
KY31	B	6/18	357	617	83	818
AR584	C	6/18	361	602	85	665
KY31	C	6/18	348	620	96	664
EF	C	6/18	361	607	100	645
Max-Q	C	6/18	365	614	89	656
EF	A	6/18	395	622	67	637

A.5 (continued) Table of 2008 quadrat nutritive value concentrations for individual pastures and collection dates.

Entry	Block	Date	ADF	NDF	Protein	IVDMD
			--------------------g kg^{-1}-----------------------			
Max-Q	A	6/30	382	626	75	603
KY31	A	6/30	401	621	73	612
AR584	A	6/30	399	619	73	642
EF	B	6/30	401	655	86	665
Max-Q	B	6/30	401	612	86	634
AR584	B	6/30	364	601	90	632
KY31	B	6/30	357	620	85	596
AR584	C	6/30	401	520	87	656
KY31	C	6/30	407	638	86	618
EF	C	6/30	386	643	75	597
Max-Q	C	6/30	417	638	75	634
EF	A	6/30	410	611	78	649
Max-Q	A	7/16	399	641	76	634
KY31	A	7/16	358	660	92	637
AR584	A	7/16	412	661	95	645
EF	B	7/16	385	642	101	94
Max-Q	B	7/16	378	653	74	603
AR584	B	7/16	365	619	85	674
KY31	B	7/16	371	641	92	667
AR584	C	7/16	404	670	82	618
KY31	C	7/16	371	629	87	673
EF	C	7/16	423	688	85	526
Max-Q	C	7/16	336	646	94	646
EF	A	7/16	401	634	91	651

A.6 Table of 2009 quadrat nutritive value concentrations for individual pastures and collection dates.

Entry	Block	Date	ADF	NDF	Protein	IVDMD
			-------------------------g kg^{-1}----------------------			
Max-Q	A	4/16	308	531	185	869
KY31	A	4/16	277	476	187	889
AR584	A	4/16	277	494	174	891
EF	B	4/16	293	503	192	862
Max-Q	B	4/16	293	519	196	789
AR584	B	4/16	313	548	184	774
KY31	B	4/16	314	521	185	808
AR584	C	4/16	312	535	194	801
KY31	C	4/16	285	504	218	890
EF	C	4/16	278	477	189	871
Max-Q	C	4/16	299	469	176	847
EF	A	4/16	252	506	200	853
Max-Q	A	4/30	344	555	181	809
KY31	A	4/30	330	553	168	799
AR584	A	4/30	316	541	151	792
EF	B	4/30	316	562	157	770
Max-Q	B	4/30	309	589	199	797
AR584	B	4/30	361	592	178	789
KY31	B	4/30	342	577	171	815
AR584	C	4/30	323	544	173	796
KY31	C	4/30	302	561	165	817
EF	C	4/30	334	545	155	785
Max-Q	C	4/30	329	550	150	770
EF	A	4/30	331	574	155	776
Max-Q	A	5/21	321	621	141	700
KY31	A	5/21	327	620	142	705
AR584	A	5/21	319	601	128	711
EF	B	5/21	314	607	151	719
Max-Q	B	5/21	298	589	133	754
AR584	B	5/21	297	595	153	752
KY31	B	5/21	324	622	150	774
AR584	C	5/21	317	606	143	769
KY31	C	5/21	316	602	153	758
EF	C	5/21	328	624	143	734
Max-Q	C	5/21	339	586	133	762
EF	A	5/21	331	630	123	723

A.6 (continued) Table of 2009 quadrat nutritive value concentrations for individual pastures and collection dates.

Entry	Block	Date	ADF	NDF	Protein	IVDMD
			-----------------------g kg^{-1}-----------------------			
Max-Q	A	6/2	357	650	128	699
KY31	A	6/2	363	651	126	601
AR584	A	6/2	342	658	134	678
EF	B	6/2	334	620	156	713
Max-Q	B	6/2	329	626	150	682
AR584	B	6/2	322	618	129	694
KY31	B	6/2	342	617	125	697
AR584	C	6/2	374	626	126	651
KY31	C	6/2	386	632	133	678
EF	C	6/2	326	635	142	705
Max-Q	C	6/2	341	645	136	650
EF	A	6/2	333	640	131	674
Max-Q	A	6/15	373	651	127	675
KY31	A	6/15	386	643	132	644
AR584	A	6/15	361	650	146	617
EF	B	6/15	382	650	123	671
Max-Q	B	6/15	379	640	126	660
AR584	B	6/15	364	629	133	669
KY31	B	6/15	372	640	132	669
AR584	C	6/15	374	663	140	628
KY31	C	6/15	382	650	130	624
EF	C	6/15	373	637	146	674
Max-Q	C	6/15	368	651	122	664
EF	A	6/15	375	606	122	694

A.7 Table of 2009 hand-plucked sample nutritive value concentrations for individual pastures and collection dates.

Entry	Block	Date	ADF	NDF	Protein	IVDMD
			---------------------------	**g kg^{-1}**	-----------------------	
Max-Q	A	4/16	290	456	220	917
KY31	A	4/16	274	447	207	916
AR584	A	4/16	268	479	217	911
EF	B	4/16	265	468	215	911
Max-Q	B	4/16	272	515	220	888
AR584	B	4/16	304	510	220	854
KY31	B	4/16	281	505	223	840
AR584	C	4/16	291	475	198	895
KY31	C	4/16	266	441	203	931
EF	C	4/16	258	429	203	914
Max-Q	C	4/16	266	444	196	890
EF	A	4/16	283	437	204	925
Max-Q	A	4/30	288	575	197	767
KY31	A	4/30	271	108	188	830
AR584	A	4/30	308	546	179	792
EF	B	4/30	294	540	203	805
Max-Q	B	4/30	274	542	205	836
AR584	B	4/30	276	538	176	846
KY31	B	4/30	263	499	190	847
AR584	C	4/30	283	517	201	817
KY31	C	4/30	287	547	159	819
EF	C	4/30	307	558	206	788
Max-Q	C	4/30	308	553	165	798
EF	A	4/30	278	530	182	825
Max-Q	A	5/21	302	572	143	759
KY31	A	5/21	297	583	152	778
AR584	A	5/21	292	579	156	759
EF	B	5/21	312	587	156	769
Max-Q	B	5/21	288	591	165	789
AR584	B	5/21	305	545	166	781
KY31	B	5/21	287	474	175	773
AR584	C	5/21	319	601	145	750
KY31	C	5/21	301	579	150	755
EF	C	5/21	307	595	149	763
Max-Q	C	5/21	343	597	158	757
EF	A	5/21	367	616	141	728

A.7 (continued) Table of 2009 hand-plucked sample nutritive value concentrations for individual pastures and collection dates.

Entry	Block	Date	ADF	NDF	Protein	IVDMD
			-------------------------g kg^{-1}-----------------------			
Max-Q	A	6/2	317	613	154	715
KY31	A	6/2	340	598	154	738
AR584	A	6/2	329	624	141	721
EF	B	6/2	317	177	144	753
Max-Q	B	6/2	341	617	149	725
AR584	B	6/2	344	605	147	741
KY31	B	6/2	355	599	150	755
AR584	C	6/2	349	618	141	695
KY31	C	6/2	336	592	166	737
EF	C	6/2	369	611	136	725
Max-Q	C	6/2	383	629	133	680
EF	A	6/2	363	599	149	705
Max-Q	A	6/15	371	634	132	704
KY31	A	6/15	400	632	144	669
AR584	A	6/15	369	615	145	666
EF	B	6/15	372	630	149	714
Max-Q	B	6/15	358	622	132	689
AR584	B	6/15	377	632	158	701
KY31	B	6/15	339	601	156	725
AR584	C	6/15	377	620	153	669
KY31	C	6/15	379	626	131	664
EF	C	6/15	374	601	126	677
Max-Q	C	6/15	398	634	125	656
EF	A	6/15	389	634	120	685

A. 8 Table of 2008 mean animal rectal and skin temperature, ADG, and weight per pasture for individual pastures.

Pasture	Entry	Block	Rectal Temp	Skin Temp	ADG	Weight per pasture
			^{o}C	**^{o}C**	**kg d^{-1}**	**kg ha^{-1}**
25-1	Max-Q	A	39.5	35.7	0.94	317
25-2	KY31	A	40.1	37.4	0.50	303
25-3	AR584	A	40.1	36.6	0.62	315
26-1	EF	B	39.8	36.6	0.77	312
26-2	Max-Q	B	39.8	36.1	0.69	312
26-3	AR584	B	39.9	36.1	0.63	317
27-1	KY31	B	40.1	36.5	0.64	299
27-2	AR584	C	39.6	36.1	0.70	310
27-3	KY31	C	40.2	36.8	0.63	303
28-1	EF	C	39.3	35.7	0.70	314
28-2	Max-Q	C	39.6	36.1	0.60	311
28-3	EF	A	39.6	36.1	0.44	308

A.9 Table of 2009 mean animal rectal and skin temperature, ADG, and weight per pasture for individual pastures.

Pasture	Entry	Block	Rectal Temp	Skin Temp	ADG	Weight per pasture
			^{o}C	**^{o}C**	**kg d^{-1}**	**kg ha^{-1}**
25-1	Max-Q	A	40.9	37.3	0.91	351
25-2	KY31	A	40.7	37.7	0.73	327
25-3	AR584	A	40.2	37.5	0.82	331
26-1	EF	B	40.4	36.9	0.92	332
26-2	Max-Q	B	40.2	36.9	0.78	346
26-3	AR584	B	40.1	36.3	0.77	338
27-1	KY31	B	40.6	37.6	0.49	329
27-2	AR584	C	40.1	36.8	0.81	326
27-3	KY31	C	40.1	37.2	0.73	323
28-1	EF	C	39.8	36.7	0.83	309
28-2	Max-Q	C	39.8	37.2	0.84	327
28-3	EF	A	40.2	36.6	0.63	335

A.10 Table of 2008 serum prolactin concentrations for individual pastures and collection dates.

Entry	Block	Date	Collection	Serum Prolactin
				ng mL^{-1}
Max-Q	A	6/5	1	242
KY-31	A	6/5	1	80
AR584	A	6/5	1	237
EF	B	6/5	1	193
Max-Q	B	6/5	1	193
AR584	B	6/5	1	154
KY-31	B	6/5	1	79
AR584	C	6/5	1	151
KY-31	C	6/5	1	64
EF	C	6/5	1	176
Max-Q	C	6/5	1	280
EF	A	6/5	1	151
Max-Q	A	6/30	2	324
KY-31	A	6/30	2	62
AR584	A	6/30	2	245
EF	B	6/30	2	254
Max-Q	B	6/30	2	243
AR584	B	6/30	2	184
KY-31	B	6/30	2	107
AR584	C	6/30	2	190
KY-31	C	6/30	2	99
EF	C	6/30	2	208
Max-Q	C	6/30	2	329
EF	A	6/30	2	216
Max-Q	A	7/22	3	287
KY-31	A	7/22	3	69
AR584	A	7/22	3	325
EF	B	7/22	3	232
Max-Q	B	7/22	3	268
AR584	B	7/22	3	203
KY-31	B	7/22	3	107
AR584	C	7/22	3	209
KY-31	C	7/22	3	140
EF	C	7/22	3	208
Max-Q	C	7/22	3	275
EF	A	7/22	3	216

A.11 Table of 2009 serum prolactin concentrations for individual pastures and collection dates.

Entry	**Block**	**Date**	**Collection**	**Serum Prolactin**
				ng mL^{-1}
Max-Q	A	5/5	1	212
KY-31	A	5/5	1	86
AR584	A	5/5	1	236
EF	B	5/5	1	237
Max-Q	B	5/5	1	205
AR584	B	5/5	1	249
KY-31	B	5/5	1	27
AR584	C	5/5	1	205
KY-31	C	5/5	1	52
EF	C	5/5	1	157
Max-Q	C	5/5	1	295
EF	A	5/5	1	162
Max-Q	A	6/1	2	243
KY-31	A	6/1	2	118
AR584	A	6/1	2	190
EF	B	6/1	2	254
Max-Q	B	6/1	2	209
AR584	B	6/1	2	273
KY-31	B	6/1	2	60
AR584	C	6/1	2	148
KY-31	C	6/1	2	85
EF	C	6/1	2	132
Max-Q	C	6/1	2	169
EF	A	6/1	2	170
Max-Q	A	6/24	3	236
KY-31	A	6/24	3	86
AR584	A	6/24	3	132
EF	B	6/24	3	203
Max-Q	B	6/24	3	118
AR584	B	6/24	3	187
KY-31	B	6/24	3	68
AR584	C	6/24	3	209
KY-31	C	6/24	3	87
EF	C	6/24	3	194
Max-Q	C	6/24	3	109
EF	A	6/24	3	134

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