ABSTRACT

LYONS, SARAH ELIZABETH. Fescue Toxicity and Beef Heifer Growth and Reproduction. (Under the direction of Matthew H. Poore and Daniel H. Poole.)

Kentucky-31 Tall Fescue, a forage variety that occupies over 15 million hectares and utilized by over 20% of beef cattle herds in the United States, is involved in a symbiotic relationship with an endophytic fungus, *Epichloë coenophiala.* The fungus produces ergot alkaloids, secondary metabolites that contribute to forage durability and stress tolerance. Although ergot alkaloids provide many benefits to the plant, these compounds are toxic to livestock and hinder animal welfare and production potential. Symptoms of fescue toxicosis in beef cattle include vasoconstriction, retained winter hair coat, decreased heat tolerance, increased respiration, body temperature, and salivation, and reduced reproductive performance. Two studies were conducted in the North Carolina Piedmont to observe the severity of fescue toxicosis in beef heifers in varying environmental conditions. In the first study, it was hypothesized that an increased plane of nutrition through protein supplementation and/or extra forage would aid heifer development leading to improved conception rates. While conception rates were not directly affected by the treatments ($P>0.05$), additional forage and supplemental protein increased average daily gain and body condition ($P<0.05$). Heifers closer to their percent mature body weight were more likely to successfully conceive from artificial insemination, therefore an advanced plane of nutrition was determined to be beneficial. The second study aimed to observe fescue toxicity symptoms in beef heifers naïve to endophyte-infected (E+) tall fescue by feeding ground fescue seed in a total mixed ration under the influence of heat stress. It was hypothesized that heifers consuming E+ fescue seed would present symptoms of fescue toxicity, including vasoconstriction to the reproductive organs. Av-

erage daily gain was lower for the E+ group vs. the endophyte-free (E-) group (*P*<0.05; 0.8 kg/d and 1.0 kg/d for E+ and E-, respectively), and body condition scores tended to be higher for the E- group (*P*=0.053). Hair coat and hair shedding scores were also higher in the E+ treated animals (*P*<0.05), indicating rough hair coats often seen in animals under the influence of toxic fescue. Heart rate, rectal temperature, respiration rate, and blood pressure did not differ between treatments (*P*>0.05). Caudal artery area was smaller for the E+ group vs. the E- group (*P*<0.05; 10 mm^2 and 9 mm^2 for E- and E+, respectively), however caudal vein area did not differ between treatments (*P*>0.05). This supports previous findings suggesting that ergot alkaloids stimulate involuntary muscle contraction, thereby influencing the smooth muscles surrounding arterial blood vessels and reducing blood flow to the peripheral tissues. Uterine and ovarian blood vessel areas were measured in order to detect vasoconstriction to the reproductive organs. There was a treatment effect on ovarian artery area (*P*<0.05; 13 mm^2 and 11 mm^2 for E- and E+, respectively). Additionally, there was treatment x sample time interactions on all reproductive blood vessel areas, with E- group areas being larger than the E+ group in late July (*P*<0.05). Based on these results, vasoconstriction around the reproductive organs may be partly responsible for the disrupted circulating reproductive hormones in cattle consuming E+ tall fescue, contributing to lower reproductive performance. While some parameters observed in these studies align with results found in previous research, others such as heart rate and circulating progesterone in the summer study were not different between treatments. This exemplifies the complex interactions between ergot alkaloids and environmental factors contributing to a wide variety of physiological responses in beef cattle. Further research is needed in order to fully understand these interactions so that fescue toxicity and its negative effects on animal performance and welfare can be avoided.

Fescue Toxicity and Beef Heifer Growth and Reproduction

by
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DEDICATION

This thesis is dedicated to my teachers, who have provided endless amounts of love, support, and guidance throughout my academic career. Primarily Mr. Mike Lemmons, Coach Frank Gerard, Mrs. Amy Zalevskiy, Dr. Wade Worthen, Dr. Carmela Epright, Dr. Laura Thompson, Mr. Johnny Rogers, Dr. Matt Poore, and Dr. Daniel H. Poole. Most especially, though, Sue Lyons, the best teacher, mother, and friend I could have ever asked for.

BIOGRAPHY

Sarah Elizabeth Lyons was born in West Palm Beach, Florida, where she lived in the suburbs for nearly thirteen years before moving to the mountains of Western North Carolina. She graduated 4th in her class from West Henderson High School before continuing on to study biology at Furman University in Greenville, SC. While completing her undergraduate education, Sarah volunteered on a small farm and became interested in sustainable livestock production. After graduating with honors and earning a Bachelor of Science degree in biology, she was offered the opportunity to pursue a Master of Science degree in Animal Science at North Carolina State University with a focus on fescue toxicity in beef cattle. Following the completion of her Masters work, she will pursue a Ph.D. in sustainable livestock production at Cornell University.

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graduate school. Their passion for life and selfless love for others inspire me every day to grow into a better person and make a difference in the community. A special thanks goes to Johnny Rogers, my most unexpected and treasured friend. Thank you for your patience, understanding, and steadfast friendship.

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Above all else, I would like to thank my family. They have never ceased to believe in me despite not really ever knowing what I'm doing in graduate school, and have dealt with my embarrassing amount of emotional breakdowns and love me anyways. Without them, I would have never had the confidence to push myself into a program so different than anything else I've ever done. I would have never realized my potential to make a difference in something as intimidating and important as food animal agriculture.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

Literature Review: Fescue toxicity and heifer development

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Introduction

The Southeastern United States is home to a wide variety of agricultural production systems, including poultry, swine, dairy, beef, pastures for hay, and various row crops. The moderate temperatures, fairly consistent rainfall, and production-friendly geographical landscape makes farming in the southeast U.S. feasible and productive. However, as in any agricultural system, there are obstacles to overcome. For example, the most common species of forage planted for pasture in the southeast is Kentucky-31 (KY-31) tall fescue (*Lolium arundinaceum* [Schreb.] Darbysh), a hardy cool-season perennial grass that has numerous beneficial forage properties and is able to overcome many environmental stresses. However, this particular variety is associated with compromising health effects in the livestock that graze it. Cattle can experience what is known as fescue toxicosis, which describes a variety of symptoms associated with decreased performance of cattle grazing infected fescue. Reduced weight gains, conception rates, and decreased heat tolerance are just a few signs of fescue toxicosis in cattle. These health problems have raised concern for regional producers and have been the focus of countless research studies performed by government agencies and the academic community with a goal of understanding why and how this prominent forage negatively affects livestock. The benefits, drawbacks, and potential solutions to KY-31 tall fescue will comprise this paper in attempt to organize the complicated issue that is fescue toxicosis.

Tall Fescue

Tall fescue (*Lolium arundinaceum* [Schreb.] Darbysh) is a cool season perennial grass native to Europe (Siegel et al., 1985). The KY-31 (E+) variety was released from Kentucky in 1943 (Ball 1984) and has become one of the most prominent forages grown in the

Southeastern United States due to it’s long stand life, palatability and nutritive quality, and adaptation to a broad range of soil types, temperatures, and moisture levels. It can also be grazed year-round if appropriate management strategies are employed (NC ARS Technical Bulletin 317). Tall fescue is a semi-erect bunchgrass that is most commonly established in pure stands, though it is sometimes inter-seeded with ladino or red clover. Most of its growth occurs during the spring season with an additional growth period in the fall months. Growth is inhibited outside of the 7-29°C temperature range, during the mid-summer and winter months. Though the forage can still be utilized during the winter and summer, the nutritive quality is reduced (NC ARS Technical Bulletin 317).

Depending on the season, tall fescue ranges from 60-80% in vitro dry matter digestibility (IVDMD), 12-20% crude protein (CP), 45-55% neutral detergent fiber (NDF), 23-38% acid detergent fiber (ADF), and 2-6% lignin (Burns and Chamblee, 1979; Poore et al., 2006). In general, there is reduced IVDMD, CP, and ADF during the summer and winter when the forage is stockpiled and reduced NDF and lignin during the fall and spring when there is new growth. Another source of decreased quality in the winter is damage from frost. Though there is seasonal variation in the nutritive quality of infected tall fescue, there are generally adequate nutrients to support livestock on a maintenance diet. For growing animals, however, CP can be insufficient in the stockpiled forage during the winter months (Burns and Chamblee, 1979). Percent of green tissue in the stockpiled tall fescue also declines during the winter months, from as much as 80% in early fall and decreasing to 24% by late winter/early spring (Poore et al., 2006). Dead tissue has a negative relationship with IVDMD; as the amount of dead tissue in the grazable forage increases, IVDMD decreases (Poore et al., 2006). In general, IVDMD is approximately equivalent to total digestible nutrients (TDN). If intake is not a limiting factor, and percent green material is kept above a certain level, stockpiled forage should be able to result in adequate gains in growing, pregnant, or lactating animals (NRC, 1984).

Endophyte-infected tall fescue is involved in a symbiotic relationship with an endophytic fungus, *Epichloë coenophiala,* which enhances the persistence of this forage through resistance to environmental stresses and increased growth (Clay, 1988; Clement et al., 1994; Leuchtmann, 2003; White and Cole, 1985; White et al., 1992). Endophyte-infected tall fescue has increased resistance to conditions such as drought, extreme temperatures, insects, diseases, and nematodes (Arechavaleta et al., 1989; Arechevaleta et al., 1992; Kuldau and Bacon 2008; Latch, 1998; Malinowski and Belesky, 2000; White et al., 1992). It also aids in the persistence of the forage under intensive grazing conditions (Burke et al., 2006; Hill et al., 1991a; Bouton et al., 1993) and produces indole acetic acid, a growth regulator, which enhances tiller growth (De Battista et al., 1990). Due to the endophyte, E+ tall fescue is often used for winter grazing by stockpiling the forage during the fall months; it has higher durability and persistence during the winter than many other cool season grasses (Agyare and Watkin, 1967; Baker et al., 1965). This forage-endophyte symbiotic relationship is observed in a variety of forage species. While many negative consequences of endophytes are seen in wild varieties, cultivars utilized in agriculture are often associated with properties that are beneficial to the plant and enhance forage growth and vigor. As in the case of E+ tall fescue, these beneficial relationships have been extensively propagated, resulting in millions of hectares of E+ tall fescue grown in the United States.

Clavicipitaceous Endophytes

Clavicipitaceous endophytes, such as *Epichloë coenophiala,* provide forages like E+ tall fescue with the production of secondary metabolites. Grasses are incapable of producing these compounds that aid in their persistence and survival over time. The tall fescue-endophyte relationship represents one of many mutualistic associations between grasses and microorganisms that have increased the ecological fitness of grass species around the world (Kuldau and Bacon, 2008).

Epichloë sp. fungal endophytes colonize healthy plant tissue without harming the host plant and are otherwise known as “symptomless endophytes” (Kuldau and Bacon, 2008). A member of the Clavicipitaceae family, *E. coenophiala* exists only in the above-ground portion of the tall fescue, and is classified as an obligate biographic intercellular symbiont (Kuldau and Bacon, 2008). Unlike *Claviceps*, spore-producing fungi that exist on the external portions of the plant, *E. coenophiala* grows within the plant tissue and can only be spread via infected seed. Because it is not organ-specific, *E. coenophiala* can be found throughout the leaf sheaths, stems, young inflorescences and seed, however it does not colonize the root tissues. The distribution of the fungal hyphae is not uniform throughout the above ground plant material. It is not surprising that the endophyte is more concentrated in parts of the plant that contain the most energy (except for the roots), and its growth follows a gradient that corresponds to the natural growth pattern of the grass. The highest concentration of the fungus is found in the basal regions, where the majority of the energy is stored, and the hyphae become less concentrated as it moves up the leaf. In most cases, the hyphae do not grow past the ligules. The growth of the endophytic fungus within the grass is closely mediated and highly controlled, representing a relationship that is well developed and has high compatibility

(Lindstrom and Belanger, 1994; Moy et al., 2002; Spierig et al., 2005). Such compatibility allows for a long-term association between *E. coenophiala* and tall fescue. The fungus is passed on to the next generation of plants via the seed, and is therefore not contagious to other non-infected plants (Kuldau and Bacon, 2008). Although the endophyte life cycle is closely related to that of the grass it infects, it has been shown that mature plants host endophytic hyphae that are still metabolically active (Schmid et al., 2000; Spiering et al., 2005).

The hyphae of endophytic fungi exist in the apoplasm, or the intercellular, nonliving spaces in grass plants. These spaces are formed from the imperfect line-up of plant cells, make up about 6% of leaf tissue, and are filled with fluids containing potassium, calcium, sulfur, phosphorus, and chlorine (Kuldau and Bacon, 2008). The apoplasm of cool season grasses is a protected, nutrient-rich space in which fungal endophytes can live with minimal competition from other microorganisms (Kuldau and Bacon, 2008). The method of nutrient uptake by the fungal hyphae is not understood, though there is evidence that the endophyte produces hydrolytic enzymes that may aid the fungus in acquiring nutrients (Lindstrom and Belanger, 1994; Moy et al., 2002; Reddy et al., 1996). In other endophytic systems, it has been shown that there is an increase in pH within the apoplast, which causes the concentration of soluble carbohydrates to be reduced (Tetlow and Farrar, 1993). A change in pH alters the enzymatic activity and host cell sugar acquisition of the plant. Kuldau and Bacon (2008) propose that this altered concentration gradient and shift in intercellular chemical conditions is in favor of the endophyte by increasing the flow of soluble carbohydrates to the fungal hyphae within the apoplasm. Though this may affect the nutrient generation and uptake of the plant, the generation of secondary metabolites by the endophyte provides the infected host with some competitive advantages that may be worth sacrificing some of its energy sources for. This association between endophytic fungi and forage grasses has been classified as a defensive mutualism (Clay, 1988).

Host plants also experience increased growth and tiller production due to endophytic residents. Though this reaction is not necessarily a stress response, the growth that results increases plant vigor and aids forage resistance to both abiotic and biotic stresses. The increase in growth rate and forage yield may be in part due to an increase in water and nutrient flux as mentioned above and/or an up-regulation of plant growth hormones, namely indole acetic acid, as seen *in vitro* in *E. coenophiala* by De Battista et al. (1990). This change in growth rate is also seen in endophyte-infected grasses grown in nutrient poor soils (Arechavaleta et al., 1992; Malinowski and Belesky, 1997; Malinowski et al., 2000). Endophyte infected fescue also shows increased growth in drought conditions. Some secondary metabolites produced by the endophyte are responsible for drought resistance, including loline alkaloids, indole acetic acid, and abscisic acid, which affect the osmotic potential inside the plant (Bush et al., 1997; De Battista et al., 1990). Endophyte-infected grasses have also been observed to have an increase in dehydrins (Carson et al., 2004), compounds associated with resistance to drought and temperature stresses, and an increase in root length and growth (Richardson et al., 1990), aiding in water and nutrient uptake.

In addition to assistance with plant vigor and nutrient access, endophytic fungi also aid infected grasses with resistance to biotic stresses, such as diseases and herbivory by insects, nematodes, and mammals. Studies have shown that, in culture, some fungal endophytes produce degradative enzymes and antibiotics that prevent pathogen proliferation (Siegel and Latch, 1991). Secondary metabolites produced by endophytic fungi also deter herbivory through a decrease in palatability of the forage (Panaccione et al., 2006).

There are many positive outcomes of the defensive mutualistic association between tall fescue and the endophyte, however when considering all relationships between grasses and endophytic fungi, especially in the wild, negative consequences are prevalent. For example, while the endophyte in tall fescue prevents degradation by nematodes and other pests in the root system, it also prevents root colonization by beneficial mycorrhizal fungi (Guo et al., 1992; Siegel and Latch 1991) and has harmful effects on earthworm populations (Humphries et al., 2000). However, many of the negative associations observed between grasses and endophytes have been in wild varieties where most of the host and endophyte species diversity resides. More heavily studied agronomic cultivars often lack these types of associations, as they have been selected for over time to produce more desirable traits (Kuldau and Bacon, 2008). The production of ergot alkaloids is one quality that contributes to the improved performance of forage cultivars, such as endophyte-infected (E+) tall fescue, infected with endophytic fungi. Numerous studies have been conducted in an attempt to fully understand the ecological and physiological roles ergot alkaloids play in forage and livestock production. With ongoing research and practice, scientists and producers alike are coming to find that these compounds may not be as beneficial to our production systems as was originally thought.

Ergot Alkaloids

Ergot alkaloids are compounds that are produced by certain fungi, including those of the *Claviceps* and *Epichloë* genera. These anti-herbivorous mycotoxins are naturally produced by the fungal organisms (Bush and Fannin, 2009) and are categorized into three main classes: clavine alkaloids, lysergic acid and derivatives, and ergopeptine alkaloids (Lyons et al., 1986; Porter, 1995; Bush and Fannin, 2009). Of the ergot alkaloids, those that fall into the ergopeptine classification make up 10 to 50 percent of the compounds found in the blades (up to 1.5 mg/kg) and sheaths (up to 14 mg/kg) of E+ tall fescue (Lyons et al., 1986). The ergot alkaloid that has been most thoroughly investigated and understood is ergovaline (Yates et al., 1985; Belesky et al., 1988). Ergovaline comprises 84 to 97 percent of ergopeptide alkaloids in living tall fescue tissue (Lyons et al., 1986). Although ergovaline is thought to be the predominant ergot alkaloid, there are many others, including ergocornine, ergocryptine, ergocrystine, ergonine, ergonovine, ergoptine, ergosine, ergotamine, lysergic acid, and lysergol, that are likely to contribute to endophytic function within the host plant (Belesky et al., 1988; Bond et al., 1984; Boling et al., 1975; Browning et al., 2001, Strickland et al., 2011). Lyons et al. (1986) found three of the ergopeptide alkaloids, ergovaline, ergosine, and ergonine, present in the blades, sheaths, inflorescences, and stems of all E+ tall fescue plants tested. Two other ergopeptide alkaloids, ergoptine and ergocornine, were found in some of the samples and in much lower concentrations (Lyons et al., 1986). Regardless of the classification, all known ergot alkaloids share a tetracyclic ergoline ring structure that is similar to many compounds found in other biological systems, namely those of livestock, which will be discussed below (Berde, 1980).

Every species of infected grass does not contain every type of ergot alkaloid, for the classes and amounts of alkaloids are related to specific combinations of host genotype and endophytic strain. Additionally, the concentrations of certain ergot alkaloids fluctuate throughout the year; some are greater in concentration during certain months and environmental conditions (Belesky et al., 1988; Arechavalia et al., 1992; Bond et al. 1984; Caldwell et al., 2013; Hill et al., 2002). Environmental conditions that favor the grass including warm temperatures, sufficient rainfall, high humidity, and highly fertile soils, support fungal growth and thus ergot alkaloid production (Craig and Hignight, 1991).

The ergot alkaloids that *E. coenophiala* produces in E+ tall fescue provide protection to the plant against herbivory by mammals and other pests. The study conducted by Panaccione et al. (2006) investigated the extent to which certain ergot alkaloids prevent herbivory of perennial ryegrass (*Lolium perenne*) by rabbits. By genetically altering endophytes to produce ergovaline, clavine alkaloids, or no ergot alkaloids, clavine alkaloids reduced the palatability of the infected ryegrass and ergovaline suppressed rabbit appetite. This suppression likely occurred via agonistic activity of ergovaline at serotonin receptors as seen in rats and guinea pigs (Schöning et al., 2001). This suggests that there is some neuroendocrine response to the consumption of ergot alkaloids to affect the consumption behavior of the rabbits. The ergoline ring structure found in ergot alkaloids is similar to some biological compounds found in mammals, namely the biogenic amines serotonin, dopamine, norepinephrine, and epinephrine (Berde, 1980; Weber, 1980). The diversity of biological receptors that ergot alkaloids target in mammals that consume them explains why the toxicological effects of ergot alkaloids in livestock are not yet completely understood. These chemical compounds constitute a wide variety of physiological activities and pose numerous potential routes for metabolism, storage and excretion in livestock (Review by Strickland et al., 2011).

As suggested by the study on rabbits conducted by Panaccione et al. (2006), ergovaline is commonly used as an indicator of endophyte infection in host grasses, including E+ tall fescue. The concentration range of ergovaline found in E+ Tall Fescue seed and fresh forage is 0.1 - 6.0 $\mu g\ g^{-1}$ (Belesky et al., 1988; Hill et al., 1991b), however there are differences in concentration according to time of year and stage of forage. The highest concentration of ergovaline, as well as other ergot alkaloids, in the plant tissue has been seen in the fall months (Belesky et al., 1988; Rogers et al., 2011), increasing from early September through early November in both first stand and regrowth (Rottinghaus et al., 1991). There is also an increase in ergot alkaloid concentration seen in late spring that corresponds with forage reproductive maturity, as the E+ tall fescue seedheads contain up to three times the concentration of ergot alkaloids than the leaf blades (Rottinghaus et al., 1991). Concentrations decrease up to 80% during the winter, from mid-December through mid-March (Ergovaline: Kallenbach et al., 2003; total ergot alkaloid concentration: Drewnoski et al., 2007) when the forage is experiencing dormancy, suggesting that using E+ tall fescue for winter foraging systems may be a viable option if the toxic effects of fescue are to be minimized. Although the concentrations of ergot alkaloids also decrease during the summer time due to forage dormancy (Belesky et al., 1988), it is not to the same extent as during the winter due to the favorable environmental conditions such as warm temperatures. Minimum growing conditions for *Epichloë sp.* are 5ºC warmer than that of E+ tall fescue (Ju et al., 2006).

Studies have shown that ergot alkaloid concentrations also fluctuate according to forage classification. E+ tall fescue is often used for hay in order to be harvested for later use. Roberts et al. (2008) evaluated tall fescue hay for ergovaline and total ergot alkaloid concentration. At harvest, ergovaline concentrations were 578 – 586 μg kg^{-1} and total ergot alkaloid concentrations ranged from 537 – 688 μg kg^{-1}. Within the first month of harvest, both ergovaline and ergot alkaloid concentrations dropped substantially and continued to decrease over time. Ergovaline concentrations remained above 250μg kg^{-1} throughout the storage period of 18 months, which is above the 150μg g^{-1} threshold believed to initiate toxicity symptoms (Stamm et al., 1994).

Another likely suspect for fescue toxicity symptoms is lysergic acid. Although it is not thought to be stored in body adipose tissue and would thus not bioaccumulate in the livestock that consume ergot alkaloids (Strickland et al., 2011), it is found in large quantities in ruminal fluid as well as in the urine of cattle grazing E+ tall fescue (Ayers et al., 2009). Lysergic acid is suspected to play a part in fescue toxicosis due to its structural similarity to lysergic acid diethylamide (LSD), which can cause alterations in serotonin and dopamine activity, leading to impaired prolactin expression (Burt et al., 1976, Buckholtz et al., 1988; Giacomelli et al., 1998).

The physiological effects of ergot alkaloids on livestock are numerous and range in severity. Reduced forage digestibility (Boling et al., 1975), decreased livestock performance, and alterations in hormone concentrations that lead to issues in health and reproductive capacity have all been observed and are to be discussed in the following sections. These negative consequences in livestock are cumulatively referred to as fescue toxicosis (SRIEG-37, 1992), and have posed major problems for livestock producers in the United States. Fescue infected with novel endophytes, or endophytic fungi that do not produce ergot alkaloids, have been developed in order to relieve the effects of fescue toxicosis in livestock and are used for fescue toxicity research, however for the sake of this review these cultivars will not be discussed in depth.

Mechanisms and Physiological Effects of Fescue Toxicosis

The ergot alkaloids produced by *Epichloë coenophiala* have adverse effects on the growth, performance, and reproductive health of the livestock that graze E+ tall fescue (Burke et al., 2006; Burke et al., 2001; Drewnoski et al., 2009a; Drewnoski et al., 2009b;

Fanning et al., 1992; Mahmood et al., 1994; Schmidt and Osborn, 1993; Seman et al., 1997; Watson et al., 2004). These consequences are thought to be due to altered neurotransmitter function in certain regions of the brain as evidenced by improper amounts of dopamine and serotonin in the pituitary and pineal glands (Porter et al., 1990; Strickland et al., 2011). In order for ergot alkaloids to reach sufficient concentrations in the body to elicit a response, the review by Strickland et al. (2011) points out that the compounds must be able to surpass various mammalian protective mechanisms that improve resistance against environmental toxins. These include mechanisms of influx and efflux, biotransformation reactions, transport and/or distribution to molecular target sites, and physical elimination from the body through excrement.

Ergot alkaloids and the rumen. In grazing livestock, the major route of ergot alkaloid intoxication is via ingestion of ergot alkaloid-containing forage. The specific interactions that natural ergot alkaloids have in livestock are not completely understood, however it is generally accepted that ruminants are better able to metabolize and excrete the toxins than non-ruminant and hindgut fermenter species. This is due to the microbial metabolism that takes place in the rumen prior to the main site of gastrointestinal absorption (Strickland et al., 2011). Although the particular mechanisms of ergot alkaloid metabolism are not known, it has been shown that the fermentative processes in the rumen release additional ergot alkaloids over time (Ayers et al., 2009; De Lorme et al., 2007), potentially enhancing the severity of toxicosis. It is also thought that ergovaline is unstable in the rumen (Moyer et al., 1993), and that ergopeptine alkaloids are converted into lysergic acid during microbial fermentation prior to absorption (DeLorme et al., 2007). However, the in vitro study conducted by Ayers et al. (2009) found that the concentration of ergovaline in rumen fluid did not change over incubation times of 0, 6, 12, 24, and 48 hours, though total ergot alkaloid concentrations did increase over time. This study found that lysergic acid made up the greatest percentage of the ergot alkaloids in the rumen fluid, and that this concentration increased from 0 to 48 hours of incubation. Ergovaline, lysergol and ergonovine were also measured but were found in much lower concentrations.

Ayers et al. (2009) found that the presence of ergot alkaloids in the ruminal fluid directly reflects the time of consumption. During the first day of exposure to E+ tall fescue, there was an increase in rumen ergot alkaloid concentrations, which remained high as long as the steers grazed the E+ tall fescue. Once the animals were removed from E+ tall fescue, the ergot alkaloid concentrations in the rumen decreased within the first day, and were undetectable by day three. Interestingly, the rumen fluid samples collected in this study did not contain any detectable ergovaline regardless of fescue infection status or length of E+ tall fescue exposure (Ayers et al., 2009).

Although it is thought that ruminants can more efficiently metabolize and excrete the ergot alkaloids, the loline alkaloids have been found to inhibit microorganism cellulose degradation in the rumen (Bush et al., 1976). This may interfere with both the release of additional ergot alkaloids as well as the animal's ability to obtain all potential nutrients ingested with the feed. In addition, ergot alkaloids may also affect reticulorumen contractility thereby altering digestive activity. Rumen contractions were inhibited in sheep injected intravenously with ergot alkaloids for up to 14 hours after exposure (McLeay and Smith, 2006). These effects could be partially reversed using atropine, suggesting that the ergot alkaloids interact with biogenic amine receptors, such as dopaminergic, alpha$_1$-adrenergic, serotonergic, or acetylcholinergic receptors, in the muscles responsible for cyclic contractions.

Ergot alkaloid absorption and transport. Ruminant absorption of ergot alkaloids occurs in both the forestomach (i.e. rumen, reticulum, omasum) as well as the small intestine. Depending on the physiochemical properties of the ergot alkaloids as well as the conditions of the surrounding environment, the toxins can be absorbed through the gastrointestinal epithelia and rumen papillae by passive diffusion or transport via facilitated or active mechanisms (Strickland et al., 2011). Due to the amphipathic nature of ergot alkaloids, the pH of the digestive environment will affect the rate and extent to which they are absorbed (Eckert et al., 1978). Because the rumen has a near neutral pH in grass-fed livestock and lacks a surface mucosal layer (Russell and Rychlik, 2001), nutrients, as well as ergot alkaloids, are able to be absorbed through the rumen papillae.

A number of studies have been conducted to determine where different classes of ergot alkaloids are absorbed. Hill et al. (2001) determined that ergot alkaloids were actively transported across luminal, reticular and omasal tissues in sheep. However, they found that lysergic acid was absorbed to a greater extent than other alkaloids administered (lysergol, ergonovine, ergotamine, and ergocryptine). An *in vitro* study showed that lysergic acid was absorbed by the rumen, however ergovaline was not absorbed by either the rumen or omasal tissues (Ayers et al., 2009). It is thought that the small intestine is the critical site of absorption of ergopeptine compounds to induce toxicity (Strickland et al., 2011).

Due to evidence of ergot alkaloids in urine, bile, milk, and adipose tissues (Westendorf et al., 1993; Stuedemann et al., 1998; Durix et al., 1999; Schultz et al., 2006), along with studies conducted on mice and related species (Eckert et al., 1978), it is believed that ergot alkaloids reach the circulatory system via the lymphatic system, portal system and liver following absorption. Stuedemann et al. (1998) found that in steers grazing E+ Tall Fescue, 96% of the ergopeptine alkaloids consumed were excreted in the urine, representing an almost complete absorption of the ingested toxins. Ayers et al. (2009) observed an almost immediate rise in ergot alkaloids in the urine of steers following exposure to E+ tall fescue, however no ergovaline was detected. Differing percentages of ergopeptine excretion have been found from animals of varying species and dietary formulations (Westendorf et al., 1993; Schumann et al., 2009), implying that the conditions under which livestock consume ergot alkaloids greatly affect the extent to which they experience toxicosis and how effectively they can eliminate the toxins.

Vasoconstriction and heat stress. Once ergot alkaloids reach the cardiovascular systems of livestock, two known physiological changes have been observed. A temporary decrease in heart rate occurs followed by a gradual increase over 7 days (McLeay et al., 2002; Aiken et al., 2007), and restricted blood flow to body tissues (Rhodes et al., 1991; Aiken et al., 2007). The vasoconstrictive responses to the ergot alkaloids are a result of their ability to interact with biogenic amine receptors in livestock, including serotonergic, dopaminergic and adrenergic receptors (Dyer, 1993; Oliver et al., 1998; Liang et al., 1998).

A common observation of studies looking at cardiovascular performance under the influence of ergot alkaloids is thickening of the intimal layer of small peripheral vessels (Julien et al., 1974; Williams et al., 1975; Garner and Cornell, 1978). This, along with congestion caused by vasoconstriction of the vascular system, leads to reduced blood flow to tissues. Although it is not fully understood how the various alkaloids affect the cardiovascular system, it has been demonstrated that ergovaline and ergotamine have the greatest effect on inducing vasoconstriction, five times more so than lysergic acid (Klotz et al., 2007).

Vasoconstriction in the peripheral blood vessels disallows cattle and other livestock to efficiently cool themselves off in hot environmental temperatures. This conservation of body heat, as well as the retention of a winter hair coat, leads to deceased tolerance to heat stress (Walls and Jacobson, 1970; Solomon et al., 1989; Rhodes et al., 1991). Cattle undergoing fescue toxicosis are often seen experiencing increased respiration rate and salivation (Browning et al., 1998; Burke et al., 2001; Walls and Jacobson, 1970; Bond et al., 1984), two visible signs of heat stress, as well as reduced weight gain and loss of body condition due to decreased feed intake (Hemken et al., 1981, Panaccione et al., 2006; Caldwell et al., 2013; Drewnoski et al., 2009b).

Burke et al. (2001) showed that heifers under heat stress have reduced feed intake regardless of endophyte status. Even though respiration rate and rectal temperatures did not differ between infected and non-infected fescue prior to the addition of heat stress, there were increases in respiration rates and rectal temperatures in the endophyte-infected group after heat stress was introduced. By the end of the study, while heat stress was still imposed, respiration rates once again did not differ between treatments but rectal temperatures remained elevated. Increased heart rate has also been observed in animals experiencing fescue toxicosis (Bond et al., 1984; Walls and Jacobson, 1970), which is expected if an animal also has an elevated respiration rate and body temperature under heat-induced stress.

An average increase in rectal temperature of 0.4 - 1.2 °C has been found in livestock consuming E+ fescue when compared to non-infected fescue varieties (Hemken et al., 1981; Hannah et al., 1990; Burke et al., 2006; Burke et al., 2001; Parish et al., 2003; Walls and Jacobson, 1970). Aldrich et al. (1993) used thermocoupling in the cecum of sheep to determine whether changes in core body temperature can be accurately described by rectal temperature measurements. They found that sheep consuming infected fescue had increased core temperatures but no change in rectal temperatures. However, they suggested that sheep are less sensitive to toxic fescue than are cattle because the sheep had no change in respiration rates between infected and non-infected fescue, whereas increased respiration rates are commonly seen in cattle grazing infected fescue (Browning et al., 1998; Burke et al., 2001; Walls and Jacobson, 1970).

In the Southeastern United States, fescue toxicosis most often occurs in the summer months when the ambient temperatures are high (32° C) and when the animals are pressed to release body heat (Hemken et al., 1981). Both Burke et al. (2001) and Hemken et al. (1981) found that animals fed infected fescue seed showed symptoms of fescue toxicosis only under heat stress conditions, but not under cooler temperatures under a certain amount of ergovaline in the diet. Drewnoski et al. (2009a) conducted a study during the winter months, when ambient temperatures were 2.8°C to 6.2°C, with cattle grazing stockpiled infected fescue or endophyte-free fescue and did not see changes in average daily gain between forage types.

Winter studies have been conducted to investigate the effects of infected fescue on livestock without the element of heat stress. The study conducted by Drewnoski et al. (2009b) investigated whether or not there is a difference in performance of cattle grazing stockpiled Jesup tall fescue that is endophyte infected (E+), endophyte free (E-) or novel endophyte infected (EN) during the winter months. No differences in body weight, average daily gain, or BCS between treatments were found. Since this study was conducted during the winter, when environmental temperatures are cooler and alkaloid concentrations are lower, the symptoms of fescue toxicosis were not observed. Reduced intake in animals grazing E+

fescue was not observed, contrary to what some studies have shown. As shown through a heat-stressed environment by Burke et al. (2001) and a thermoneutral to cool environment by Drewnoski et al. (2009a; 2009b), ambient temperature plays a major part in the level of intake by interacting with ergot alkaloid consumption to produce a negative feedback effect on animal intake (Hemken et al., 1981; Aldrich et al., 1993). On the other hand, Beconi et al. (1995) conducted a grazing study in the fall, when ergot alkaloid concentrations are highest and ambient temperatures are mild (Belesky et al., 1988). The study found that steers grazing endophyte-infected tall fescue had decreased average daily gain (ADG) than those grazing non-infected fescue. Reduced weight gains are likely due to the increase in ergot alkaloid concentrations compared to other times of the year, for it has been found that the presence of the endophyte does not affect the nutritive quality of the fescue. Schmidt et al. (1982) found that the endophyte-infected tall fescue does not have reduced in vitro digestibility compared to non-infected fescue.

Fescue Toxicity and Heifer Development

Replacement heifers comprise a large percentage of beef herds in the Southeastern United States. As tall fescue is the primary forage utilized in this region, considering the physiological effects of E+ tall fescue consumption is a critical component of heifer development programs. Nutrition has a significant impact on age at which puberty is reached in heifers and is the most useful management tool in preparing for a successful breeding season (Hall, 2013). If heifers are able to reach puberty at an early age, conception can occur earlier in the breeding season and they are more likely to have increased productivity and longevity (Lesmeister et al., 1973). Although the particular effects that E+ tall fescue has on heifer development and reproductive performance are not completely understood, research indicates that specific nutrients and their availability affect the hypothalamic-pituitary-ovarian-uterine axis (Schillo et al., 1992), and ergot alkaloids have shown to alter cycling concentrations of reproductive hormones as well as the digestibility of ingested feed (Porter et al., 1990; Bush et al., 1976).

Post-weaning nutrition and heifer performance. Post-weaning nutrition is critical for optimum heifer development and reproductive success. It has been suggested that the most critical aspect of heifer development programs is for the animals to reach a critical body weight by the start of the breeding season (Lamond, 1970), and that the most important nutritional component in reaching this goal is energy. Heifers on high-energy diets reach puberty sooner and have increased pregnancy rates compared to those on low-energy diets (Short and Bellows, 1971; Hall et al., 1995).

Heifers are often assigned a target body weight to reach by the breeding season, defined by a percentage of their expected mature weight (Hall, 2013). Specific body weights and/or adipose stores do not initiate puberty directly (Hall et al., 1997; Hess et al., 2008), however heifers below a certain percent of their mature weight are less likely to reach reproductive maturity (Kiser et al., 1998; Day et al., 1986). It has been thought that a 65% target weight (% mature weight, MW) is necessary for successful breeding (Patterson et al., 1992). This target weight is successful for many different cattle breeds and feeding regimens (Hall, 2013) and has resulted in less incidence of dystocia (Patterson et al., 1992). However, depending on feed costs, achieving the 65% MW may be too expensive and could result in cattle that are overconditioned due to a higher plane of nutrition than is normally provided on a given farm (Perry, 2012). An alternative target weight of 55% MW has been investigated and effects on reproductive performance have been reported. Studies have found reduced production costs and improved longevity in heifers bred at 53-55% MW without negatively impacting pregnancy rates, calving ease, or rebreeding rates (Funston and Deutscher, 2004; Martin et al., 2008; Larson et al., 2009). However, it is possible that breeding heifers at a lower body weight reduces conception rates early in the breeding season (Roberts et al., 2009) and could result in decreased calf weaning weight in 2-year-olds (Creighton et al., 2005). The ideal target body weight for breeding depends on available resources and production goals. If a farm has access to sufficient feedstuffs to support a higher target weight prior to breeding and is concerned about successful conception early in the breeding season, then a 65% mature weight is ideal. In contrast, if feed is limited and longevity is desired, then a 55% mature weight would be more appropriate.

Studies have investigated various strategies involving timing and rate of gain in order to determine the most efficient way for heifers to reach target body weights. Clanton et al. (1983) found that three different patterns of heifer weight gain, including rapid-slow, steady, and slow-rapid, produced similar pregnancy rates. Pregnancy rates have also been similar in trials comparing steady gain with rapid gain during the 60 days before breeding (Lynch et al., 1997) as well as rapid-slow-rapid gain (Poland et al., 1998; Grings et al., 1999). Short and Bellows (1971) fed heifers various levels of feed (low, medium, high) and found that the low-fed group experienced less gain, were older and weighed less at first estrus, had lower conception rates during the first 20 days of the breeding season, and lower overall conception rates than both the medium- and high-fed groups. Regardless of the feeding pattern, these studies emphasize the importance of adequate energy availability in heifer diets.

Energy is critical for the initiation of estrus cycles at puberty as well as the resumption of estrus following pregnancy. Dietary energy is linked to gonadotropin-releasing hormone secretion by the hypothalamus as well as pituitary production of luteinizing hormone and follicle-stimulating hormone (Schillo et al., 1992). Energy may also be involved in ovarian activity, such as hormone production and follicular development (Webb et al., 2004). Energy requirements of developing heifers are provided by the National Research Council and are dependent on heifer weight and desired average daily gain. For example, a 272 kg heifer that is to gain 0.68 kg/d should be provided with 6.5 kg/d dry matter containing 4.2 kg total digestible nutrients (TDN). However, if ergot alkaloids are capable of reducing the digestibility of feedstuffs in the rumen as well as suppressing intake (Bush et al., 1976), then E+ tall fescue could have a major impact on the overall growth and reproductive performance of replacement heifers preparing for the breeding season.

Energy in the form of fat has been used as a management tool to enhance reproductive fitness in cattle. Fatty acids and cholesterol found in dietary fat are important for reproductive hormone synthesis, including progesterone and prostaglandins. Feedstuffs for cattle are usually less than 3% fat, and high-fat diets do not exceed 8% fat in order to prevent hindered rumen function (Hall, 2013). Studies investigating the effects of high fat diets on reproduction have found increased progesterone concentrations (Talavera et al., 1985; Williams, 1989) and follicular growth (Thomas et al., 1997; Ryan et al., 1992; Lammoglia et al., 1997) in cycling heifers. However, there are conflicting reports on the benefit of additional fat on improved pregnancy rates in first calf heifers (Funston, 2004).

Another source of energy that may have an effect on heifer reproduction is protein. The effects that protein has on reproductive performance is closely linked to overall nutrition

(Hall 2013). Overall digestibility of forage improves with protein intake; dietary crude protein (dietary measurement based on nitrogen content) less than 7% can inhibit rumen function and prevent the acquisition of nutrients. Effects of both digestible protein and undegradable intake protein on heifer reproduction, such as decreased age at puberty, improved pregnancy rates, and enhanced longevity in heifers grazing forages deficient in protein have been observed (Oyedipe et al., 1982; Mulliniks et al., 2013). However, there is a limit to how much protein can be fed for improved performance. Excessive protein in the diet can have negative impacts on pregnancy rates in cattle (Blanchard et al., 1990; Sinclair et al., 2000), possibly due to impaired oocyte function or embryo development. However, it is suggested that with sufficient energy intake, excess protein can be eliminated (Garcia-Bojalil et al., 1994). While the mechanism by which dietary protein affects reproductive fitness is not completely understood, it is certain that adequate protein is necessary for general physiological health, and that supplying protein and/or energy supplementation to growing heifers grazing poor quality forage may be beneficial in reaching target body weights.

Ergot alkaloids and reproduction. As with the physiological systems previously discussed, the problems that ergot alkaloids pose on reproductive fitness are not completely understood. Observations have varied depending on factors such as animal age, diet, genotype, environmental temperatures and humidity, and amount of ergot alkaloids ingested. The effects that the toxins have on reproduction can be direct, through interfering with normal function of the ovary, corpus luteum, and developing embryo, or indirect through compromised nutritional status and reduced body condition and fat stores (Review by Strickland et al., 2011). Studies investigating the direct and indirect influences of ergot alkaloids on reproductive tissues and pregnancy rates have found mixed results, however it is generally accepted that the binding of ergot alkaloids to biogenic amine receptors leads to physiological changes that affect the reproductive performance of livestock.

Reproduction in livestock consuming E+ tall fescue has been hindered through disruption of circulating reproductive hormones, such as prolactin, progesterone, and gonadotropins. These changes are linked to altered neuroendocrine function, as studies have shown that ergot alkaloids cause functional changes in the hypothalamus and pituitary and pineal glands of the brain (Sibley and Creese, 1983; Schillo et al., 1988; Porter et al., 1990).

Prolactin. Prolactin is a protein hormone that is produced within the anterior pituitary gland, central nervous system, immune system and uterus (Freeman et al., 2000). This hormone plays a role in promoting lactation and maintaining homeostasis in mammals. Although the mechanism by which prolactin is involved with reproduction is not fully understood, prolactin receptors have been found in certain reproductive tissues, including the corpus luteum and granulosa cells (Poindexter et al., 1979; Lebedeva et al., 2004), and a positive correlation was discovered between follicle diameter and serum prolactin concentrations (Flores et al., 2008). Secretion of prolactin can be inhibited or stimulated by factors in the brain, pituitary gland and peripheral organs, and dopamine has inhibitory control over its release by binding to the D2-dopamine receptor (Freeman et al., 2000; Lamberts and Macleod, 1990). Because of the ergoline ring structure that ergot alkaloids and dopamine share, ergot alkaloids act as dopamine agonists and are able to bind to the D2-dopamine receptors in the pituitary gland and inhibit normal prolactin secretion (Schillo et al., 1988; Goldstein et al., 1980; Porter and Thompson 1992; Hökfelt and Fuxe, 1972; Henson et al., 1987). In vitro, ergot alkaloids have been shown to prevent the release of prolactin from pituitary cells

(Sheeler et al., 1985), which has been demonstrated in vivo through reduced prolactin concentrations in serum as well as pituitary glands (Hurley et al., 1980; Schillo et al., 1988).

Lipham et al. (1989) suggested that supplementing cattle with metoclopramide, a dopamine antagonist, could reverse the effects of fescue toxicosis by inhibiting the ergot alkaloids from suppressing prolactin secretion. Steers supplemented with metoclopramide had increased serum prolactin concentrations as well as increased grazing time and improved average daily gains while grazing E+ tall fescue. Rhodes et al. (1991) also supplemented cattle and lambs with metoclopramide and found an increase in blood flow to peripheral tissues when feeding E+ tall fescue. Aldrich et al. (1993) investigated metoclopramide's effect on lambs grazing E+ tall fescue under heat stress. Supplementing the lambs increased dry matter intake, thereby increasing their ergovaline consumption, however the supplementation did not produce an increase in prolactin secretion. Similarly, when Aldrich et al. (1993) supplemented heifers grazing E+ fescue with metoclopramide there was no resulting increase in prolactin concentrations. These studies suggest that there may be other factors contributing to the decrease in serum prolactin concentrations besides dopaminergic activity prohibiting prolactin synthesis and release.

The study conducted by Burke et al. (2001) attempted to determine whether heat stress had an additional effect on prolactin concentrations in animals consuming E+ tall fescue. Reduced concentrations of serum prolactin in heifers consuming endophyte infected fescue seed while under heat stress were observed, and there was no increase in serum prolactin concentrations when temperatures were brought to thermoneutral conditions. This suggests that ergot alkaloids cause prolactin concentrations to decrease independent of ambient temperatures. In contrast, the study conducted by Mizinga et al. (1992) found no difference in serum prolactin between heifers fed E- fescue seed and E+ fescue seed (2.6-9.75 μg ergovaline kg^{-1} body weight per day) during April and May, before animals would experience significant heat stress. However, when ergovaline concentrations were increased above 9.75μg ergovaline kg^{-1} body weight, there was a decrease in serum prolactin in those heifers consuming E+ fescue seed. Watson et al. (2004) determined that a total ergot alkaloid concentration of 448μg/kg is sufficient to depress prolactin concentrations and average daily gains of cow-calf pairs grazing during the springtime.

The winter study conducted by Drewnoski et al. (2009a) found lower prolactin concentrations in heifers grazing E+ fescue compared to E- fescue. Even though the ergot alkaloid concentrations decreased substantially during the study (2,349 μg/kg in early December to 533 μg/kg in mid January), they were still high enough to depress serum prolactin concentrations (above the 448 μg/kg threshold as determined by Watson et al. (2004)).

Prolactin is also thought to work together with lutenizing hormone in order to maintain the corpus luteum during the estrus cycle and early pregnancy in rats (Tabarelli et al., 1982) and ewes (Kann and Denamur, 1974). Like prolactin, circulating concentrations of luteinizing hormone have shown to be reduced in endophyte-infected fescue-treated cows (Browning et al., 1998; Browning et al., 2001), which in turn, could lead to decreased luteal function. On the other hand, the study conducted by Mizinga et al. (1992) reported no change in luteinizing hormone concentrations in heifers or cows exposed to endophyte-infected fescue, though the concentration of ergovaline administered to the animals in this study were not reported and may not have been great enough to observe a change in luteinizing hormone activity.

Progesterone. Progesterone is a steroid hormone produced by the corpus luteum (CL) that plays a critical role in the establishment and maintenance of pregnancy. Heat stress alone has been shown to reduce CL size and cause decreased serum progesterone concentrations in livestock (Burke et al., 2001), and the addition of dietary ergot alkaloids intensifies this response through impaired tolerance to high temperatures. Reduced serum progesterone concentrations have been seen in horses, ewes, and heifers consuming E+ tall fescue (Monroe et al., 1988; Burke et al., 2006; Jones et al., 2003; Estienne et al., 1990; Mahmood et al., 1994). Because progesterone is so critical in maintaining pregnancy, insufficient progesterone production could result in failure to recognize pregnancy or abortion.

Burke et al. (2001) observed that serum progesterone concentrations were reduced in beef heifers in a heat stress environment, and were further reduced in animals also consuming E+ fescue seed. Burke et al. (2001) also found that diet alone (E+ vs. E-) did not affect the diameter of the CL in beef heifers, however environmental temperature caused reduced CL diameter in animals under heat stress. The addition of E+ intensified heat stress and therefore amplified the issue of impaired CL seen with heat stress alone. In contrast, Burke and Rorie (2002) found no difference in serum progesterone concentrations or CL diameter between mature beef cows grazing E+ and E- tall fescue. However, this study used mature animals and temperatures were not as high as in previous studies that observed decreases in CL diameter and progesterone concentrations. These studies suggest that the CL in heifers may be more susceptible to heat stress than the fescue toxins alone, and that the reduction in CL diameter may be associated with the aggravated heat stress caused by toxic fescue.

In addition to affecting the size of the CL, ergot alkaloids in E+ tall fescue may also lead to impaired CL function (Mahmood et al., 1994; Estienne et al., 1990). Ergot alkaloids have been shown to stimulate uterine smooth muscle movement (Saameli, 1978), suggesting a consequential release of $PGF_{2\alpha}$ that could cause impaired luteal activity. Sheep have showed early embryonic loss due to decreased luteal function while grazing E+ fescue (Bond et al., 1988). It was proposed that this decrease in CL function could explain the fertility problems seen in heifers grazing E+ fescue. Since then, many studies have found that CLs in heifers grazing E+ fescue appear to develop normally, however they are more likely to be dysfunctional, meaning that they do not produce progesterone (Estienne et al., 1990; Mahmood et al., 1994; Ahmed et al. 1990; Burke et al., 2001).

Jones et al. (2003) investigated whether progesterone concentrations in CL tissue changes following ergot alkaloid consumption. Heifers consuming E+ and E- diets had similar concentrations of progesterone in luteal tissue extracts. Because ergot alkaloids have vasoconstrictive effects, these findings led them to believe that vasoconstriction to the reproductive organs, such as the ovaries and CL may be responsible for decreased circulating reproductive hormones in the blood (Jones et al., 2003).

The observed decrease in progesterone production by the CL in heifers consuming E+ fescue may be due in part to reduced cholesterol concentrations. Cholesterol is a precursor molecule for progesterone synthesis (Stuedemann et al., 1985; Talavera et al. 1985; Bond et al., 1984). In ruminants, the CL produces progesterone from cholesterol through steroidogenesis. The cholesterol is derived from low-density lipoproteins and/or high-density lipoproteins in the blood serum (Savion et al. 1982, O'Shaughnessy et al. 1985, Wiltbank et al. 1990). Burke et al. (2001) found that heifers under heat stress and consuming E+ fescue seed, as well as heifers consuming E+ fescue seed under thermoneutral conditions, had reduced serum cholesterol concentrations. This reduction in cholesterol due to E+ fescue consumption has been observed in other studies (Bond et al. 1988, Bond et al. 1984, Stuedemann et al. 1985.) Bond et al. (1984) found that serum cholesterol concentrations were further reduced in July and August as compared to the rest of the year, likely a result of heat stress during the summer months and elevated alkaloid concentrations. If both heat stress and infected fescue cause decreased serum cholesterol concentrations, then a resulting decrease in serum progesterone concentrations are to be expected.

Estradiol. Estradiol is a hormone that plays a role in regulating the estrus cycle. It is produced within ovarian follicles and is biosynthesized from progesterone. Heifers have exhibited decreased concentrations of serum estradiol under both heat stress alone and as a result of consuming E+ tall fescue under thermoneutral conditions (Burke et al., 2001). This supports the evidence that progesterone is decreased in cattle consuming E+ fescue, causing compounds that rely on progesterone for their synthesis to decrease as well.

Follicular function. Normal follicular function is critical for reproductive success in cattle. Decreased follicle size may cause a reduction in conception rates (Bridges et al., 2000) and small follicles are vulnerable to being damaged by heat stress (Burke and Rorie, 2002; Wolfenson et al., 1995). Burke et al. (2001) found an interaction effect between heat stress and toxic alkaloids in infected fescue on follicular function in heifers. Heifers consuming endophyte-infected fescue under heat stress had smaller pre-ovulatory follicles, less large follicles (≥10mm) during estrus as compared to heifers under heat stress alone, consuming endophyte-infected fescue alone, or non-infected forage and thermoneutral conditions. Similarly, Burke and Rorie (2002) found decreased large follicle sizes and class 2 follicle sizes (6-9 mm) in E+ fescue treated cows.

The study conducted by Burke and Rorie (2002) investigated the effects of E+ fescue on mature cows during one synchronized estrous cycle in late spring. In contrast to other studies, there was not much change in follicular characteristics or estradiol concentrations during the estrous cycles of the cows. These results were perhaps due to differing levels of heat stress in this study as compared to other experiments, or because mature cows were used instead of heifers. As seen with other differences in hormone ratios, the reproductive maturity could account for the different outcomes in follicular characteristics and serum hormone concentrations. This could have been due to an adaptation of the dams to daytime heat stress or the cooler temperatures at night allowing for normal follicular function leading to conception in both treatment groups. All of these mature cows also had high enough BCS to support pregnancy. These results suggest that mature cows may be able to handle the effects of the ergot alkaloids, and that heifers may be more susceptible to the reproductive drawbacks of the toxins because energy is needed for reproduction in addition to that for growth (Burke and Rorie, 2002).

Conception rates. A reduction in conception rates is one of the major problems seen in cattle grazing endophyte-infected tall fescue. Heat stress alone is associated with lowered conception rates (Burke et al., 2001; Wolfenson et al., 1995), so when endophyte-infected fescue is involved to further enhance heat stress during the summertime, problems with conception rates are to be expected and have been observed in beef cattle (Caldwell et al., 2013; Bond et al., 1988). Problems with calving and weaning are also associated with fescue toxicosis (Brown et al., 1992; Gay et al., 1998; Burke et al., 2006), and it has been proposed that estrus cycles are shortened due to ergot alkaloids in the diet (Mahmood et al., 1994; Burke et al., 2006). It is thought that these problems are due to issues with the ovaries, specifically luteal and follicular function, possibly because of the abnormal changes in hormone concentrations during estrus cycling due to the ergot alkaloids (Burke et al., 2001; Mahmood et al., 1994; Burke and Rorie, 2002).

A few studies have found that there is no difference in reproductive capacity between cattle consuming infected versus non-infected fescue. Burke and Rorie (2002) and Drewnoski et al. (2009b) found similar pregnancy rates between diets of endophyte-infected fescue and non-infected fescue. However, as noted above, there have been many studies documenting changes in ovary, corpus luteum, and follicular function as well as hormone concentrations with the presence of endophyte-infected fescue in the diet. These changes will all have an effect on reproductive fitness due to their critical role in successful pregnancies. The study by Drewnoski et al. (2009b) may not have found differences in pregnancy rates due to the lack of heat-induced stress or lack of animal numbers (n=144). The study was carried out during the winter months, when ambient temperatures are low and ergot alkaloid concentrations were reduced. The study did, however, find a decrease in serum prolactin and daily gain in animals grazing E+ fescue. Burke and Rorie (2002) observed some fescue toxicosis symptoms in the cattle, such as decreased body weight, body condition, calf growth rate, and serum prolactin concentrations. However, differences in follicular characteristics, estradiol concentrations, CL characteristics, progesterone concentrations, or pregnancy rates were not found. This may be due to the lack of high temperatures in this study as compared to other studies, or because mature cows were used instead of first calf heifers. The conflicting results found in these and other studies emphasized the complexity of the physiological effects of fescue toxicity and how environmental, among many other, factors can influence its severity.

Reproduction and nutritional status. The effects that ergot alkaloid have on nutrient consumption directly affect the reproductive fitness of livestock. Sufficient dry matter intake (DMI) to maintain body energy reserves is necessary in order for an animal to conceive and carry out a pregnancy. Because DMI is often reduced in animals consuming E+ tall fescue (Looper et al., 2007; Parish et al., 2003), the resulting loss of body condition and subsequent reduction in body adipose stores can greatly reduce reproductive fitness in developing animals by affecting follicular growth, conception and calving rates. Beef heifers with limited energy reserves have produced smaller dominant follicles (Rhodes et al., 1995) due to insufficient nutrients throughout a 60 to 80 day follicular growth period (Britt, 1992), and increased serum prolactin concentrations are associated with increased nutrient intake (Wright et al., 1987). Cattle consuming E+ tall fescue have shown greater losses in body condition and decreased calving rates than those grazing non-fescue forage (Looper et al., 2010).

Potential Solutions

Although fescue toxicity has caused many problems in the health of livestock in the southeastern United States and has cost the industry between $600 million and $1 billion on a yearly basis (Roberts and Andrae, 2010), completely ridding production systems of E+ tall fescue is not a realistic short-term solution. The ease of establishment, persistence, and reliability of E+ tall fescue makes an attractive forage for management purposes, especially during the winter months. E+ tall fescue can be stockpiled during the fall and grazed throughout the winter, allowing producers to extend the grazing season and reduce the need for purchasing hay, thereby reducing feed costs (Matches, 1979, Poore et al., 2000). Utilizing stockpiled E+ tall fescue for grazing during the winter when temperatures and ergot alkaloid concentrations are low may be a viable option for producers attempting to prevent fescue toxicosis (Beconi et al., 1995), however further research is needed to establish the risks associated with ergot alkaloid consumption even without the influence of extreme temperatures.

The lack of heat stress in addition to reduced ergot alkaloids during the winter may prevent the abnormal hormone fluctuations associated with fescue toxicosis and support a successful breeding season. However, during the winter, tall fescue also reduces in nutritive quality (Ocumpaugh and Matches, 1977). While the reduced quality forage may be sufficient for mature cattle on a maintenance diet, the amount of total digestible nutrients (TDN) and crude protein (CP) may not be enough to support developing replacement heifers. Cows on all forage diets with serum urea nitrogen (SUN) concentrations below 7.0 mg/dL relative to digestible energy intake have been determined to be protein deficient, and could result in insufficient ADG (Hammond et al., 1994). During the winter study conducted by Drewnoski et al. (2009), SUN concentrations were below 9 mg/dL in years 1-3, a result of limited protein intake, and were correlated with reduced dry matter intake and ADG. They concluded that protein supplementation during the winter may be necessary in order for growing cattle to reach optimal performance and, for fall calving herds, have successful winter breeding seasons.

A number of studies have investigated the effects of CP supplementation on conception rates. Fishmeal, a rich source of fatty acids and protein, was fed to dairy cattle and resulted in improved pregnancy and conception rates (Armstrong et al., 1990; Bruckental et al., 1989) and decreased plasma concentrations of oxytocin-induced prostaglandin-F2α (Thatcher et al., 1997; Mattos et al., 2002). These studies suggest that supplementation with sources of crude protein and/or energy may be a possible way of preventing conception issues seen in livestock consuming ergot alkaloids. Burke et al. (2006) supplemented ewes fed E+ and non-toxic endophyte-infected (NE) tall fescue seed with either fishmeal or corn gluten meal (14% CP) and measured differences in prolactin, progesterone, and $PGF_{2\alpha}$ production. Serum prolactin was reduced in E+ as compared to NE in the ewes as seen in other studies, and the E+ treatment ewes had greater serum prolactin concentrations when supplemented with fishmeal. PGFM and progesterone, however, were not different in the ewes fed fishmeal as compared to the corn gluten meal. This may be because the amount of CP (14%) was the same between the two supplements; differences may have been found through comparing supplements with varying amounts of CP.

Poore et al. (2006) investigated the effects of feeding whole cottonseed (24.4% CP) on the performance of heifers grazing stockpiled E+ tall fescue during the winter. The supplemented heifers had increased performance as compared to the control group, with greater ADG, BCS improvement, and SUN concentrations. However, even with the whole cottonseed supplementation, during one year of the study the animals had SUN concentrations below 9 mg/dL, which reflected the lower protein content of the forage that year. Although reproductive performance was not included in this study, it is likely that the inadequate level of CP in the diet would have limited the heifers reproductive capacity in addition to their overall growth and performance.

Although much work has been done on finding solutions to the fescue toxicity conundrum, one comprehensive answer has not been discovered. This is due to the complex nature of the effects of ergot alkaloids in livestock, for there are countless compounds, target tissues, mechanisms of action, metabolic processes, environmental factors, and ecological interactions involved that are not completely understood. The diet, genetic makeup, and age of the animals that consume ergot alkaloids can also influence the extent to which they are affected by the toxicity. While available technology is capable of detecting ergot alkaloids both in vivo and in vitro, the analytical procedures available are not yet sensitive enough to measure the extent to which these compounds are stored in animal tissues and fluids (Strickland et al., 2011). Further work on classifying the toxic compounds as well as their interaction with livestock physiology is needed in order to determine the best and most practical solutions to this dilemma for both individual producers and the livestock industry as a whole.

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CHAPTER 2

Effect of protein supplementation and forage allowance on heifer growth and reproduction

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Introduction

Stockpiled tall fescue has the potential to be viable forage for beef cattle during the winter, allowing for year-round grazing and reduced outsourced hay (Matches, 1979). However, replacement heifers on these systems without additional supplementation have shown marginal overall performance during the months prior to breeding (Scott, 2000). Multiple studies investigating the performance of heifers on stockpiled forages have shown a connection between inadequate weight gains, low Serum urea nitrogen (SUN) concentrations and reduced conception rates (Beconi et al., 1995; Drewnoski et al., 2009; Poore et al., 2000). A lack of crude protein in the dormant forage may be partly responsible for this outcome, as the nutritive quality of stockpiled forages decreases throughout the winter (Poore et al., 2006; Scruggs, 2010).

Adequate weight gain is critical for heifers reaching reproductive maturity, therefore high quality feed during the months prior to breeding is essential (Hall, 2013). This suggests that a ruminally-degradable protein supplement given to replacement heifers grazing stockpiled tall fescue during the winter may be able to enhance reproductive performance. In addition to supplementing with protein, another possible source of improved nutrition is increased forage allowance. Having a less restricted grazing area would allow the cattle to be more selective in available forage, contributing to a more nutritious diet, increased dry matter intake, and improved weight gains.

Stockpiled KY-31 tall fescue is infected by an endophytic fungus, *Epichloë coenophiala,* that produces ergot alkaloids that are toxic to cattle. Problems associated with vasoconstriction, inability to regulate body temperature, reduced weight gain, and decreased reproductive performance have been observed in cattle grazing infected fescue (Rhodes et al., 1991; Hemken et al., 1981; Caldwell et al., 2013). In addition to the reduced weight gain and body condition, it is speculated that a potential cause of reduced conception rates is vasoconstriction to the reproductive organs, thereby inhibiting proper ovarian development (Jones and King, 2009; Jones et al., 2003). Two hormonal indicators of reproductive maturity, prolactin and progesterone, have found to be reduced in animals grazing infected tall fescue (Browning et al., 1998; Browning et al., 2001; Burke et al., 2001). However, many of these studies have been conducted during times of the year when alkaloid concentrations are high and heat stress is a factor. During the winter, the endophyte reduces its alkaloid production by up to 85% (Kallenbach et al., 2003), suggesting that these issues may not be as prevalent during this time of the year. Evidence of this was found by Drewnoski et al. (2009), who saw no difference in reproductive performance between heifers grazing infected and non-infected fescue during the winter months. This suggests that providing a higher plane of nutrition during the winter may be adequate to support growing heifers in preparation for the breeding season. It is hypothesized that both the addition of a protein supplement and allocating additional forage would lead to improved conception rates of heifers following winter grazing. This study investigated the effects of increasing forage allocation (from a "normal" amount of forage to 125% of "normal" as defined by best grazing management practices (Hoveland, 1996)) and offering a free choice supplemental protein tub with molasses and fat for energy and ruminally degradable protein (Southern States 25% MaxiBeef Tub).

Materials and Methods

The study was conducted over a 3 year period (2011-2014) at the Butner Beef Cattle Field Laboratory in Butner, NC with sampling dates occurring between late October and late

April and grazing days between early November and early January. In each year, groups of 5 Angus x Simmental cattle (including 2 steers as "put and takes" in years 1 and 2, and 1 heifer as a "put and take" in year 3) were grouped by weight and randomly distributed between replications with four plots each (n=220). Years 1 and 2 had four replications, producing a total of 16 groups of 5 animals, and year 3 had three replications, totaling 12 groups of 5 animals. Since year 3 did not include the fourth replication, each animal group only included one heifer as a "put and take". Treatment combinations were assigned in a 2x2 factorial arrangement and included "normal" forage allocation and basic mineral supplement (NM), "normal" forage allocation plus protein supplement (NP), "extra" forage allocation and basic mineral supplement (EM), and "extra" forage allocation and protein supplement (EP). Each treatment combination was randomly assigned to one of the four plots in each replication.

This study was approved by the Institutional Animal Care and Use Committee at North Carolina State University. In the first year 55 heifers and 25 steers were used, 48 heifers and 32 steers in year 2, and 60 heifers in year three.

Initial pasture management and forage measurements. All plots were planted with KY-31 Infected Tall Fescue in October of 2007. Each plot was approximately 0.8 hectares. The soil type is Georgeville silt loam (clayey, kaolinitic, thermic Typic Hapludults) (Scruggs, 2010). Plots were fertilized with 56.2 kg/ha nitrogen and corrected with phosphorus, potassium and pH according to soil testing recommendations in early September of each year. Forage was allowed to accumulate until grazing began in November.

Initial forage mass estimates were determined by using 0.25 m^2 falling plate meters that were calibrated at the beginning of the trial (Vartha and Matches, 1977; Mueller et al., 1990). Twenty plate drops were taken in a zig-zag pattern within each plot. A subsample (10) of these drop heights were clipped to 5 cm with hand held shears within a 0.25 m^2 framed area in order to represent the range of available forage mass in the pasture. Clipped samples were placed in (pre-weighed hot) cloth bags and dried in a 60ºC forced air oven overnight and then weighed hot to determine initial dry matter (DM) yield. A regression equation was formed by combining the representative plate drop heights and sampled forage masses (Macoon et al., 2003). Initial DM yield was 3,375 kg/ha, 3,725 kg/ha, and 4,104 kg/ha for years 1, 2, and 3, respectively. This sampling and regression technique was used every two weeks during the trial to determine pre- and post- grazing forage mass. For pre-grazing mass, plate drops and forage clippings were taken in the strip to be grazed during the following two days (20 plate drops, 10 clippings to 5 cm height, and 10 clippings to ground level) to determine total available forage (clippings to ground) and forage available for cattle consumption (clippings to 5 cm). Two days later, 20 plate drops and 10 clippings to ground level within the grazed strip were taken and compared to the pre-grazing mass to determine how much forage disappeared during the two day period.

Area grazed in each two-day period was measured by using a 60 m tape measure. Animal grazing days (d/ha) was calculated using the following formula: [(animals grazing x length of grazing period (d)) / total area grazed (ha)] (Drewnoski et al., 2009).

Forage offered and consumed. Total forage offered was determined by applying the pre-grazing measurements as stated above to the following formula:

[Pre-graze yield (DM/ha) x area offered (ha)] / [number animals (hd) x grazing duration (d)]

This was calculated to both 5 cm canopy height as well as to the ground level. Total forage consumed was determined by the following formula:

[Pre-graze yield (ground, DM/ha) – post-graze yield (DM/ha) x area offered (ha)] / [number animals (hd) x grazing duration (d)]

Calculated values are presented in Table 6.1.

Percent forage utilization. The proportion of available forage consumed was determined for both total efficiency and grazable efficiency (Table 6.1). Total percent forage utilization was determined by the following formula:

[(Pre-graze yield (ground, kg/ha) – post-graze yield (ground, kg/ha)) / (pre-graze yield (ground, kg/ha))] x 100.

Forage utilization to a grazing height of 5 cm was determined by the following formula:

[Pre-graze yield (5 cm, kg/ha) – (post-graze yield (ground, kg/ha) – (pre-graze yield (ground, kg/ha) – pre-graze yield (5 cm, kg/ha)))] / pre-graze yield (5 cm, kg/ha).

Forage separations. At the initiation of the experiment in each year, random pasture grab samples were selected by throwing tread-in posts and collected from each plot by clipping an approximate 30 x 20 cm rectangle to 5 cm. Samples were stored in zipper sealed plastic bags and brought to lab for species separations. Categories included green fescue, brown fescue, green other, and brown other. Once separated, forage fractions were placed in vented paper bags and dried in a 60ºC forced air oven for 48 hours. Dry weights determined the percent of each forage fraction in the pasture on a dry matter (DM) basis (Table 2.1).

Forage and Hay Analyses. Forty fescue tillers were collected in September of each year from each replication in order to determine endophyte infection rate and percent of tillers infected. Ten tillers were sampled from each plot in a zig-zag pattern and stored on ice for no more than three hours before being brought back to the lab. Samples were then rinsed, wrapped in damp paper towels, and stored in a cooler until delivered to the NCDA laboratory for analysis. Tillers were tested by the Raleigh, NC NCDA lab using the plant tissue stain test (AOSA, 1996). In 2009, the infection rate was found to be 23.8% in the study conducted by Scruggs (2010). In years 1, 2, and 3 of this trial the infection rate was 48.5%, 42.6%, and 69%, respectively (Table 2.1). This increase in infection rate over time has been seen in other studies (Drewnoski et al., 2007).

Every 14 days, 10 forage samples were collected for nutritive value analysis in the sections of pasture to be grazed in the following 14 days. These samples were collected randomly by throwing a tread-in post in a zig-zag pattern across the 14-day section. Samples were taken with hand-held shears within a 25 x 15 cm rectangle to 5 cm from the soil surface, representing biomass above grazing height. Samples were pooled by replication and sub-samples were sent to the North Carolina Department of Agriculture and Consumer Services forage analysis lab for analysis (Table 3.1 and Table 4.1) (Eisemann et al., 2014). A second set of subsamples was freeze-dried (VirTis, SP Scientific) and then analyzed for alkaloid content (University of Missouri Veterinary Medical Diagnostic Laboratory, Columbia, MO) using HPLC (Rottinghaus et al., 1991). Samples were not separated based on species for nutritive value and alkaloid analysis.

A mix of KY-31 tall fescue and crabgrass hay was provided before animals were placed on pasture and after treatments ended. In an effort to produce a similar amount of fill in the cattle before and after treatments, the same hay source was given both before and after the grazing period.

Animals. Each year in early October, all cattle were dewormed with a combination of pour-on ivermectin (Bimectin®, Bimeda, Irwindale, CA) and Safe-Guard® (Merck, Summit, NJ) and vaccinated for pinkeye (Liquamycin® LA-200®, Santa Cruz Biotechnology, Dallas, TX and Bio-Mycin® 200, Boehringer Ingelheim Vetmedica, Inc., Petersburg, VA), clostridial disease (Vision-7, Merck, Summit, NJ), and respiratory/reproductive diseases (BovaShield Gold 5 FP plus VL5, Zoetis, Florham Park, NJ). Excluding grazing data, all values presented herein will represent heifers only (excluding "put-and-take" steers and heifers) (n= 148).

Before being placed on pasture, cattle spent one week in the barn and were fed KY-31 and crabgrass hay, a free-choice basic mineral supplement (Cattleman's Pride Weathershed® 2:1 Beef Mineral, Southern States Cooperative, Richmond, VA), and unlimited access to fresh water. Cattle were then weighed on two consecutive days and assessed for body condition (Ferguson et al., 1994) by a single trained professional before being placed on pasture to obtain the "initial barn weight" (Table 8.1). Average initial barn weights were 267.6 kg, 305.8 kg, and 286.5 kg for years 1, 2, and 3, respectively. The animals were placed on pasture for 9 weeks, including 1 week for adaptation and 8 weeks of data collection. Animals were weighed on two consecutive days at the end of the adaptation week to determine the "initial pasture start weight" (Table 8.1), whose averages were 266 kg, 297.5 kg, and 264 kg for years 1, 2, and 3, respectively. Animals were observed daily and, following the adaptation week, were moved every other day on to fresh forage. Depending on the amount of available forage in the plots, "put-and-take" steers and heifers could be removed from plots to ensure adequate forage to complete the trial period. Final pasture weights, final barn weights, and final BCS were recorded at the end of the trial (Table 8.1).

Grazing management. The initial pasture measurements were used in order to determine the area given to the cattle with sufficient forage for one week of grazing for adaptation. After the adaptation week, animals in each plot were given strips of pasture in 2 day allotments. When treatments began, the two “normal” grazing height groups (N) in each replicate were offered enough pasture so that approximately 15% of grazable material above 5 cm remained after the 2 day grazing period. The remaining two “extra" forage allocation groups (E) in each replicate were given enough forage so as to leave 25% more residual forage than the N groups. These residual percentages were subjectively determined by the same member of the research station crew each week.

During years 1 and 2, back fencing was employed every 28 days in order to limit grazing of re-growth. In year three, back fencing was not used due to a lack of regrowth 28 days post grazing, suggesting that any re-growth would be insufficient to cause changes in consumption. Unlimited fresh water was provided in plastic tubs daily. Tubs were moved around the pasture throughout the trial in order to prevent excess forage and soil disturbance.

Mineral and supplement analysis and feeding protocol. Mineral (M) was provided to all animals pre- and post-treatment period until final pregnancy checks in mid April (Cattleman’s Pride Weathershed® 2:1 Beef Mineral, Southern States Cooperative, Richmond, VA). Mineral only groups remained on this mineral throughout the trial period, fed in covered mineral feeders, while P groups were provided with a supplemental protein tub as is (25% MaxiBeef Tub*,* Southern States Cooperative, Richmond, VA) during the treatment period. The animals were not limited in intake of the basic mineral at the start of year 1 (8 Nov 2011-22 Nov 2011) and consumed a large amount (0.37 kg/hd/d). After the first 14 days of year 1, a limited amount of mineral (0.12 kg/hd/d) was fed every 7 days in order to prevent overconsumption. Samples of both the protein tub and mineral were taken biweekly, composited, and submitted for nutrient analysis (Table 5.1). The protein tub samples were taken using a power drill with a 3.81 cm boring bit. Each sample weighed approximately 100 g prior to being composited. Protein tubs were fed in a heavy truck tire, and mineral supplements were fed in weather vane mineral feeders (Behlen County Windvane, Southern States Cooperative, Richmond, VA). At the beginning of each week, mineral and tubs were weighed to determine weekly and total intake (Table 6.1)

Blood sampling and analysis. Blood samples were taken via jugular venipuncture with 20 gauge needles and sterile vacutainer serum tubes without additive (Becton Dickinson, Franklin Lakes, NJ). Sampling dates included the start of hay feeding (day -14 in year 2, day -15 in years 1 and 3), one week later just before cattle were placed on pasture (day -8 in year 1, day -7 in year 2, day -6 in year 3), one day before beginning treatments after transition week (day -1), twice in the middle of the trial (days 28 and 48), one day before being removed from pasture (day 55 in years 1 and 2, day 54 in year 3), and one week after being removed from pasture (day 62). During collection, blood was placed on ice for no longer than 3 hours before being centrifuged for 20 minutes (3,000 RPM, 1,580 RCF) (Clay Adams DYNAC® Centrifuge) for separation. Serum was extracted and placed in 5 ml polystyrene tubes (BD Falcon, Franklin Lakes, NJ) to be stored at -15ºC at the research station. All serum was transported on ice to the on-campus lab at the end of each year's collection period.

Blood samples were analyzed for progesterone concentrations to determine the estrous cycle status of the heifers using using Immuchem^{TM} Coated Tube Progesterone ^{125}I RIA Kit (ICN Parmaceuticals, Inc., Costa Mesa, CA) and the Cobra II Auto Gamma counter (Packard Instrument Company, Meriden, CT). Animals with serum progesterone concentrations above 1 nl/ml at the time of synchronization were determined to be cycling (Table 8.1). To determine the protein status of the animals, serum urea nitrogen concentrations (SUN) were determined via colorimetry using an auto-analyzer (Technicon Industrial Systems, Tarrytown, NY) and the diacytl monoxide method (Marsh et al., 1965) (Table 7.1). Blood samples for BioPRYN analysis for specific protein B to detect pregnancy were taken in late February (day 105 in years 1 and 2, day 106 in year 3) (Table 8.1).

Reproductive measurements. Reproductive Tract Scores (RTS) were determined via rectal palpation and pelvic area was measured by a single trained professional just before cattle were placed on pasture in early November, and again one week after being taken off of pasture in mid January. The RTS is a subjective measurement that determines reproductive maturity by measuring the size of the uterus and uterine horn as well as certain ovarian characteristics (Andersen et al., 1991). Scores are between 1 and 5, with 1 being reproductively immature and 5 reproductively mature. Pelvic areas were measured in cm using a Rice pelvimeter (Lane Manufacturing, Denver, CO). Pelvic area (cm^2) is an indicator of likely calving ease and increases with maturity (Bennett et al., 2008). Culling decisions are often made on heifers with pelvic areas less than 140 cm^2.

Breeding protocol. The trial calendar was based upon the breeding schedule. A standard Cosynch + 7 day CIDR synchronization protocol was used. Heifers were bred following heat detection and then via timed artificial insemination (AI) on days 70 and 71. Following AI, heifers were introduced to natural service on day 85. Heifers were checked for pregnancy by AI on day 105 and for overall pregnancy on day 161 via ultrasonography. Proportions of heifers bred off of standing heat versus timed AI are presented in Table 9.1.

Statistical analysis. Data were analyzed using the PROC MIXED procedure of SAS with lsmeans (SAS Inst. Inc., Cary, NC). The model included year, replication, and treatment, and parameter of interest = grand mean + treatment effect + random error. The experimental unit for animal data presented was group within each treatment and replication. The

PROC REG procedure of SAS was also used to compare pregnancy rates with percent mature body weight, with year as a random variable. *P* values of ≤ 0.05 represented significant differences, and *P* values of ≤ 0.10 were defined as tendencies.

Results and Discussion

Climatological data. The precipitation during the accumulation phase (August through October) averaged 11.5 cm, and during the grazing phase (November through January) averaged 7 cm. This rainfall was sufficient to produce an adequate stand of fescue for the experimental period each year. Temperatures during both the accumulation phase and the grazing phase were normal for this geographical location (Table 1.1). Historical temperatures during the accumulation phase range from 15°C to 27°C and 1.5°C to 13°C during the grazing period.

Stand composition. Plots varied in forage composition each year (Table 2.1). Year 3 had the highest percent fescue (97%, green and brown fescue), year 1 had 85% and year 2 had 83% fescue. Year 1 had a high percentage of orchardgrass (approximately 5%), and year 2 had a high percentage of crabgrass (17%). There were differences in forage nutritive value between years that could have resulted from the differences in forage composition. These values are presented below as well as in table 3.1.

Forage alkaloid content. Ergovaline was detected in the forage each year (Figure 2.1) however concentrations were lower than those seen in previous studies on the same plots (Drewnoski et al., 2009; Drewnoski et al., 2007). Ergot alkaloid content of tall fescue is dependent on environmental conditions and the age of the stand (Belesky et al., 1988; Arechavalia et al., 1992; Bond et al., 1984; Caldwell et al., 2013; Hill et al., 2002; Drewnoski et al., 2007). The increase in infection rate over time seen in this trial has been observed in previous studies (Drewnoski et al., 2007). Although the observed increase in ergovaline concentrations from year 1 to year 3 was expected due to the rise in infection rate, the concentration of alkaloids in this trial were lower than expected and may not have been high enough to elicit toxicity symptoms. However, further analysis of animal physiological parameters, such as prolactin concentrations, is necessary to make such a claim.

Forage nutritive value. The nutritive quality and mineral composition of the stockpiled tall fescue for each year of the trial are presented in Table 3.1 and Table 4.1, respectively. Total digestible nutrients (TDN) and crude protein (CP) were reduced by the end of the trial, and neutral detergent fiber (NDF) and acid detergent fiber (ADF) were higher at the end of the study. This trend of reduced forage quality during the winter has been seen in other winter grazing studies (Poore et al., 2006; Scruggs, 2010). TDN (%DM) of the stockpiled fescue remained just above the minimum requirement of 63% TDN (Davis, 2002) for the growing heifers throughout the grazing period of each year (Table 3.1). Alternatively, crude protein only remained above the minimum requirement of 10.6% CP (%DM) (Davis, 2002) during year three (Table 3.1). Although the nutrient content of the forage was adequate to support he growing heifers, an additional protein supplementation may be beneficial in aiding growing heifers in reaching an adequate condition for optimum performance and successful reproduction.

Both neutral detergent fiber (NDF) and acid detergent fiber (ADF) increased significantly over the course of the study (%DM, $P<0.05$), except for %ADF in year one which only increased slightly. Similar trends in these values were found from November through January in previous studies (Poore et al., 2006; Burns and Chamblee, 2000). Forage mineral concentrations were fairly consistent throughout the study, however both of the supplements provided the heifers with adequate amounts of these nutrients for the duration of the trial.

Supplement nutritive value. The mineral composition of the supplements (Cattleman's Pride Weathershed® 2:1 Beef Mineral, Southern States 25% Maxi Tub) are presented in Table 5.1.

Forage utilization and dry matter intake. Forage and intake parameters are reported in Table 6.1. Initial average forage biomass did not differ between treatments ($P>0.05$) however there was a difference between years ($P<0.05$). Year one had approximately 4,000 kg/ha to 5 cm and 5,960 kg/ha to ground level, year two had 5,280 kg/ha to 5 cm and 7,990 kg/ha to ground level, and year three had 3,750 kg/ha to 5 cm and 7,490 kg/ha to ground level. This may have been due to differences in temperatures, precipitation, or forage composition, however it did not affect intake ($P>0.05$), as the area provided to the cattle could be adjusted according to how much forage was available each year.

Forage offered differed between the N and E treatment groups ($P<0.0001$), as was expected due to the differences in amounts of forage allocated between treatments. Similarly, the amount of forage disappearance was greater for animals receiving extra forage ($P<0.0001$). Forage utilization was greater for animals receiving the N forage allocation to both a 5 cm grazing height and ground level ($P<0.0001$), suggesting that the execution of forage allocation treatments was successful. As expected, the E groups grazed more hectares and produced less grazing days per hectare ($P<0.01$).

Supplement intake did not differ with forage treatment ($P>0.05$), however was greater for animals in the P groups as compared to the M groups ($P<0.0001$).

Animal performance. There was no difference between treatments in animal weights at the beginning of the trial periods (*P*>0.05, Table 7.1). However, there were main effects of the treatments on average daily gain (ADG, *P*<0.0001), though no interaction effects were seen (*P*>0.05). Alternatively, there was a treatment interaction effect on the change in BCS over the course of the three years (*P*<0.05); the group receiving extra forage in addition to the protein tub (EP) had a greater increase in BCS than all other treatment combinations. The discrepancy between the BCS and weight measurements could be due to the subjective nature of body condition scoring and could differ with time, researcher, rumen fill, etc.

Serum urea nitrogen. Serum urea nitrogen (SUN) concentrations were greater in groups to receive extra forage on day -1 (*P*<0.05, Table 7.1), which may have been due to the high CP values of the stockpiled forage of the respective plots in early November (Table 3.1) Otherwise, SUN did not differ (*P*>0.05) between heifers prior to the initiation of treatments. During the treatment period (days 1-56), heifers provided with the protein tub had higher concentrations of SUN than those with the mineral (*P*<0.05). SUN values did not differ once treatments ended (*P*>0.1). Cows on all forage diets with SUN concentrations below 7.0 mg/dL relative to digestible energy intake have been determined to be protein deficient, and could result in insufficient ADG (Hammond et al., 1994). Heifers supplemented with the protein tubs were better able to maintain sufficient SUN concentrations and had greater ADG (*P*<0.05) than those with the mineral. Additionally, the heifers receiving extra forage had increased SUN concentrations on day 56 of the study (*P*<0.05). This suggests that the heifers were better able to achieve higher nitrogen intake as compared to the more restricted groups.

Reproductive performance. Reproductive measurements are presented in Table 8.1. Initial pelvic areas were different between years (135 cm^2, 146 cm^2, and 119 cm^2 for years 1, 2, and 3, respectively; *P*<0.0001), however did not differ between treatments across all years (*P*>0.05). Treatment had no effect on the change in pelvic area from the start of the trial to time of breeding (*P*>0.05), and there was no difference in final pelvic area between years (158 cm^2, 161 cm^2, and 163 cm^2 for years 1, 2, and 3, respectively; *P*>0.05). There was a year effect on the initial, final, and change in RTS (*P*<0.005). Heifers in year one had the greatest change from an initial RTS of 1.16 to a final score of 3.27, however all heifers were determined to have reached reproductive maturity by the time of breeding according to RTS. There was no treatment effect on the change in RTS from the initial measurement to the time of breeding (*P*>0.05). These results suggest that the forage offered was of high enough quality to aid the heifers in reaching reproductive maturity by the time of breeding. If the available forage were severely protein deficient, then the protein supplement may have had a significant effect on the reproductive development of the heifers.

First service conception rates were not different between treatments over the 3 year trial (*P*>0.05). However, there was an interaction effect in year two (*P*<0.05). In year two, the EP group exhibited 91% conception success via AI as compared to 66% in NM, 42% in EM, and 50% in NP. Year two also had the highest AI conception rates as compared to other years (45%, 63%, and 33% in years 1, 2, and 3, respectively; *P*<0.05). The variation in conception rates could be due to the pre-pubertal state of heifers at the time of breeding. Two consecutive progesterone concentrations greater than 1.0 ng/ml over a 7 day period were used to determine whether heifers were experiencing estrus cycles. Only 9% of heifers in year one, 4% in year two, and 17% in year three were cycling prior to the progesterone based synchronization protocol. Percent of cycling heifers was not different between treatments (*P*>0.1; Table 8.1). The successful conception rates in year two are attributed to the onset of cyclicity by the addition of progesterone via a controlled internal drug release (CIDR®) inserts. Intravaginal progesterone supplementation has shown to improve pregnancy rates (Lucy et al., 2001) and reduce interval to first estrus (Dahlen et al., 2003) in peribuperal heifers. Overall pregnancy rates did not differ between treatments ($P>0.1$; Table 8.1). Overall pregnancy rates were 77%, 95%, and 81% in years one, two, and three, respectively ($P<0.05$).

Higher pregnancy rates in year two could be due to heifer body weights that were closer to a targeted % of mature body weight. It is advised that heifers reach 65% of their mature body weight by the time of breeding (Lamond, 1970; Patterson et al., 1992), however studies have shown that heifers can be as low as 50-55% mature body weight for successful conception and pregnancies (Funston and Deutscher, 2004; Martin et al., 2008; Larson et al., 2009), for this is usually when a heifers first estrous cycle occurs (Martin et al., 2008). In year two, heifers were at 59% of their mature body weight (approximately 544 kg mature weight) prior to synchronization, compared to 53% in year one and 51% in year three. There was a positive linear relationship between first service conception rates and percent mature body weight ($P<0.05$, Figure 3.1) as well as between age (days) and % mature body weight ($P<0.05$, Figure 4.1). The results from this study suggest that heifers closer to their mature body weight are more likely to successfully conceive, and that those heifers born earlier in the breeding season are more likely to reach a higher target weight than younger heifers.

Summary

Stockpiled tall fescue can allow for year-round grazing in the southeastern United States. However, it has been shown that the nutritive quality of stockpiled tall fescue declines over the winter months and may not be adequate for developing heifers preparing for breeding (NC ARS Technical Bulletin 317). This study investigated two potential solutions to this dilemma: increased forage allocation and supplementation with a protein tub. The group receiving extra forage required more land area, resulting in less grazing days/ha, however the heifers had increased ADG and BCS as compared to the group receiving less forage. This is likely because the heifers could be more selective and consume the most palatable parts of the plants, contributing to a more nutritious diet. The animals provided with an additional protein source also had greater ADG than those supplemented with a basic mineral, evidence that the protein supplement was able to help compensate for the decrease in forage CP as the winter progressed.

There was an interaction between forage allocation and protein supplementation on change in BCS, an acceptable indicator of performance and reproductive capacity. Change in pelvic area and RTS are also signs of reproductive maturity, however these did not differ between treatments. Improved conception rates due to treatments were only seen in year two of the three-year trial. Because adequate nutrients were provided during the course of the study, this short come is likely due to the immature body size and reproductive state of the heifers. The majority of subjects were not cycling by the time of breeding. This suggests that the animals were dependent on the CIDRs for ovulation and adequate progesterone concentrations as seen in previous studies. Supplementing protein and/or providing extra forage could aid immature heifers in reaching reproductive maturity prior to the start of the breeding season, however it is unlikely that these treatments had a direct effect on conception rates.

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Table 1.1: Monthly precipitation and temperatures in years 1, 2, and 3[1].

	Yr 1			Yr 2			Yr 3		
Month	Total Precip, cm	Mean Max, °C	Mean Min, °C	Total Precip, cm	Mean Max, °C	Mean Min, °C	Total Precip, cm	Mean Max, °C	Mean Min, °C
August	6.5	31.6	19.5	9.1	28.8	19.1	12.8	28.0	18.3
September	10.3	27.0	16.4	15.1	25.9	14.8	8.9	26.0	14.4
October	7.9	20.7	7.3	6.3	20.2	8.9	8.9	20.9	10.6
November	11.4	18.3	4.3	1.0	14.5	1.0	6.4	14.5	2.0
December	6.8	14.3	1.8	7.1	14.0	3.0	13.7	13.2	1.1
January	4.4	12.6	0.1	8.9	11.6	0.9	8.0	7.9	-5.1
February	5.3	13.6	0.8	9.8	10.4	-0.7	8.0	11.2	-0.3

[1] Precipitation and temperature values are from the National Weather Service station, Durham 11 W, approximately 40 km from the site of research.

Table 2.1: Forage composition (DM basis) of stockpiled tall fescue plots

	Year		
Item	1	2	3
Canopy composition, %DM			
Green fescue	75.3	75.7	73.1
Brown fescue	9.5	7.1	23.7
Green other	8.1	4.0	1.5
Brown other	7.1	13.2	1.7
% Fescue tillers infected	48.5	42.6	69.0

Table 3.1: Nutritive value (DM basis) of stockpiled tall fescue

Item[1]		Date					
Yr 1		Nov. 8	Nov. 22	Dec. 6	Dec. 20		
Yr 2		Nov. 12	Nov. 26	Dec. 11	Dec. 26		
Yr 3		Nov. 11	Nov. 25	Dec. 9	Dec. 23	SEM	*P*-value
DM, %	Yr 1	29.32^{a}	29.17^{a}	35.28^{b}	38.02^{c}	0.52	<0.0001*
	Yr 2	32.67^{a}	41.46^{b}	38.69^{c}	18.52^{d}	0.62	<0.0001*
	Yr 3	34.67^{a}	38.61^{b}	20.46^{c}	18.24^{d}	0.40	<0.0001*
TDN[2], %	Yr 1	69.39^{a}	67.13^{b}	63.57^{c}	66.45^{b}	0.45	<0.0001*
	Yr 2	67.04^{a}	68.27^{a}	64.01^{b}	63.70^{b}	0.50	0.0002*
	Yr 3	71.72^{a}	72.40^{a}	70.19^{a}	67.36^{b}	0.85	0.0139*
CP, %	Yr 1	11.01^{a}	10.26^{b}	10.83^{ab}	9.41^{c}	0.23	0.0035*
	Yr 2	12.26^{a}	10.61^{b}	10.48^{b}	9.77^{b}	0.26	0.0006*
	Yr 3	13.40^{ab}	12.27^{a}	11.86^{bc}	10.63^{c}	0.47	0.035*
NDF, %	Yr 1	51.99	52.19	56.39	54.09	1.52	0.2179
	Yr 2	50.72^{a}	48.63^{a}	54.96^{b}	57.99^{c}	0.79	<0.0001*
	Yr 3	44.52^{a}	47.97^{b}	53.31^{c}	58.49^{d}	0.82	<0.0001*
ADF, %	Yr 1	29.03^{a}	31.86^{b}	36.33^{c}	32.72^{b}	0.57	<0.0001*
	Yr 2	31.97^{a}	30.42^{a}	35.78^{b}	36.17^{b}	0.62	0.0002*
	Yr 3	26.09^{a}	25.24^{a}	28.03^{a}	31.57^{b}	1.07	0.014*

All values are least squared means.
**P*-values <0.05 represent significant differences.
$^{a-d}$ Means with differing superscripts differ ($P<0.05$)
[1] DM: Dry matter; TDN: Total digestible nutrients; CP: Crude protein; NDF: Neutral detergent fiber; ADF: Acid detergent fiber
[2] Equation for TDN: 92.5135 – (0.7965 x ADF)

Table 4.1: Mineral composition (DM basis) of stockpiled tall fescue

Item		Date					
Yr 1		Nov. 8	Nov. 22	Dec. 6	Dec. 20		
Yr 2		Nov. 12	Nov. 26	Dec. 11	Dec. 26		
Yr 3		Nov. 11	Nov. 25	Dec. 9	Dec. 23	SEM	*P*-value
Ca, %DM	Yr 1	0.44	0.42	0.47	0.45	0.01	0.178
	Yr 2	0.38	0.41	0.43	0.43	0.01	0.0808
	Yr 3	0.38	0.37	0.36	0.35	0.01	0.5341
P, %DM	Yr 1	0.317^{a}	0.265^{b}	0.277^{b}	0.237^{c}	0.006	<0.0001*
	Yr 2	0.280	0.277	0.267	0.242	0.011	0.175
	Yr 3	0.286^{a}	0.260^{b}	0.260^{b}	0.236^{c}	0.006	0.0055*
Na, %DM	Yr 1	0.045	0.045	0.060	0.047	0.007	0.4469
	Yr 2	0.037	0.052	0.085	0.040	0.016	0.2098
	Yr 3	0.050	0.043	0.040	0.036	0.005	0.5654
Mg, %DM	Yr 1	0.375^{a}	0.347^{b}	0.362^{a}	0.310^{c}	0.004	<0.0001*
	Yr 2	0.355	0.367	0.380	0.335	0.016	0.3201
	Yr 3	0.323^{a}	0.336^{a}	0.280^{b}	0.250^{b}	0.009	0.0006*
S, %DM	Yr 1	0.207^{a}	0.180^{b}	0.192^{b}	0.162^{c}	0.004	0.0004*
	Yr 2	0.187	0.187	0.187	0.167	0.005	0.0658
	Yr 3	0.226^{a}	0.213^{b}	0.203^{bc}	0.193^{c}	0.003	0.0015*
K, %DM	Yr 1	1.89^{a}	1.69^{b}	1.54^{b}	1.20^{c}	0.056	<0.0001*
	Yr 2	2.28^{a}	2.08^{a}	1.54^{b}	1.23^{b}	0.109	0.0003*
	Yr 3	2.09^{a}	2.04^{a}	1.37^{b}	1.18^{c}	0.048	<0.0001*
Cu, ppm	Yr 1	4.75^{a}	4.75^{a}	5.00^{a}	4.00^{b}	0.186	0.0211*
	Yr 2	5.00	5.00	5.00	5.00	0.204	1.000
	Yr 3	3.66	4.33	4.33	3.00	0.596	0.4609
Zn, ppm	Yr 1	19.25^{ab}	20.25^{a}	18.00^{bc}	17.50^{c}	0.441	0.0068*
	Yr 2	22.25^{a}	17.75^{b}	18.75^{abc}	26.00^{c}	1.369	0.0082*
	Yr 3	17.00^{a}	14.33^{b}	16.33^{a}	16.00^{ab}	0.516	0.0199*

All values are least squared means.

**P*-values <0.05 represent significant differences.

$^{a\text{-}d}$ Means with differing superscripts differ (*P*<0.05)

Table 5.1: Supplement composition

Item	Mineral	Tub
DM (%)	94.9	74.1
CP (%DM)	--	34.3
ADF (%DM)	--	10.5
Crude Fat (%DM)	--	4.8
Ca (%DM)	13.8	5.5
P (%DM)	6.5	1.0
Mg (%DM)	4.2	3.1
K (%DM)	1.9	2.1
Na (%DM)	7.7	1.9
Mn (ppm)	4638	198
Zn (ppm)	3780	191
Cu (ppm)	3915	54.3

Table 6.1: Forage and intake parameters with various supplementation and forage allocation from November through January

	Trt[2]					Forage[3]	Supp[3]	Interaction[4]
Item[1]	NM	NP	EM	EP	SEM	*P*-Value	*P*-Value	*P*-Value
Available forage (5 cm, kg*ha^{-1})	4396	4335	4311	4343	39.6	0.330	0.716	0.246
Available forage (soil surface, kg*ha^{-1})	7224	7099	7096	7176	65.8	0.698	0.732	0.123
Forage offered (soil surface, kg*hd^{-1}*d^{-1})	10.7	10.9	14.4	13.9	0.67	<0.0001*	0.811	0.618
Forage offered (5 cm, kg*hd^{-1}*d^{-1})	6.5	6.6	8.8	8.4	0.43	<0.0001*	0.83	0.566
Forage disappearance (kg*hd^{-1}*d^{-1})	4.74	4.79	5.64	5.43	0.02	<0.0001*	0.52	0.957
FUE[5], % (soil surface)	45.9	45.2	40.1	39.6	0.97	<0.0001*	0.515	0.926
FUE[5], % (5 cm)	75.4	74.2	66.3	65.3	1.6	<0.0001*	0.520	0.957
Total hectares grazed (ha)	0.46	0.51	0.58	0.56	0.03	0.0021*	0.498	0.187
Animal grazing days (d*ha^{-1})	608.7	549.0	482.7	500.0	30.8	0.0003*	0.678	0.356
Supplement intake (DM, kg*hd^{-1}*d^{-1})	0.13	0.35	0.09	0.37	0.03	0.757	<0.0001*	0.346
DMI[6] (kg*hd^{-1}*d^{-1})	4.87	5.14	5.73	5.79	0.27	0.0092*	0.555	0.709

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[1] Values are least square means of years 1, 2, and 3 of study.
[2] NM: normal forage allocation, mineral supplement; NP: normal forage allocation, protein tub; EM: extra forage allocation, mineral supplement; EP: extra forage allocation, protein tub.
[3] *P*-values for main effects of forage allocation and supplementation.
[4] *P*-values for interaction effects of forage allocation and supplementation.
[5] Forage utilization efficiency.
[6] Forage and supplement dry matter intake (DMI).
***P*-values <0.05 determined significant.

Table 7.1: Serum urea nitrogen of heifers grazing stockpiled tall fescue with various supplementation and forage allocation from November through January

	Trt[1]					Forage	Supp	Interaction
Trial Day	NM	NP	EM	EP	SEM	*P*-Value[2]	*P*-Value[2]	*P*-Value
-14	15.3	15.1	15.7	15.2	0.477	0.589	0.478	0.708
-7	6.0	6.1	6.3	6.4	0.204	0.122	0.647	0.916
-1	8.9	9.5	10.0	10.5	0.332	0.002*	0.101	0.825
28	8.5	11.9	8.2	11.0	0.366	0.118	<0.0001*	0.462
48	9.1	11.5	9.2	11.0	0.362	0.577	<0.0001*	0.474
55	6.5	7.9	7.4	9.1	0.330	0.003*	<0.0001*	0.699
62	5.0	5.3	5.2	5.0	0.248	0.825	0.917	0.265

Values are least square means of trial years 1, 2, and 3.

[1] NM: Normal forage allocation + mineral supplement; NP: Normal forage allocation + protein tub; EM: Extra forage allocation + mineral supplement; EP: Extra forage allocation + protein tub.

[2] *P*-values for main effects of forage allocation and supplementation.

**P*-values <0.05 represent significant differences.

Table 8.1: Performance of heifers grazing stockpiled tall fescue with various supplementation and forage allocation from November through January

	Treatment[2]					Forage	Supp	Interaction
Item[1]	NM	NP	EM	EP	SEM	*P*-Value[3]	*P*-Value[3]	*P*-Value
Initial BCS	5.30	5.29	5.30	5.27	0.03	0.522	0.370	0.700
BCS change	0.12[a]	0.10[a]	0.18[a]	0.31[b]	0.04	0.0002[*]	0.087	0.037[*]
Initial pasture weight (kg)	274	274	273	273	1.53	0.533	0.855	0.884
Final pasture weight (kg)	289	298	297	302	1.98	0.005[*]	0.0006[*]	0.376
Pasture ADG (kg/hd/d)	0.28	0.43	0.43	0.51	0.02	<0.0001[*]	<0.0001[*]	0.157
% Mature body weight[4]	53.28	54.87	54.63	55.58	0.004	0.0051[*]	0.0006[*]	0.375
Initial pelvic area (cm^3)	134.0	136.2	130.5	133.1	2.44	0.185	0.326	0.937
Pelvic area change (cm^3)	26.6	28.5	25.1	29.6	3.23	0.941	0.325	0.692
Initial RTS	1.59	1.89	1.51	1.62	0.10	0.087	0.049[*]	0.355
RTS change	1.81	1.73	1.89	2.00	0.13	0.162	0.914	0.451
% Cycling	5.1	15.9	7.8	13.2	5.1	1.00	0.114	0.597
% AI preg. rate	56.3	42.8	42.8	45.5	0.08	0.505	0.505	0.318
% Overall preg. rate	0.86	0.86	0.81	0.86	0.06	0.642	0.642	0.642

[1] Values are least square means of years 1, 2, and 3 of study.

[2] NM: normal forage allocation, mineral supplement; NP: normal forage allocation, protein tub; EM: extra forage allocation, mineral supplement; EP: extra forage allocation, protein tub.

[3] *P*-values for main effects of forage allocation and supplementation.

[4] Final pasture weights were used with mature body weight of 544 kg.

[*] *P*-values <0.05 determined significant.

[a] Means with differing superscripts differ (*P*<0.05).

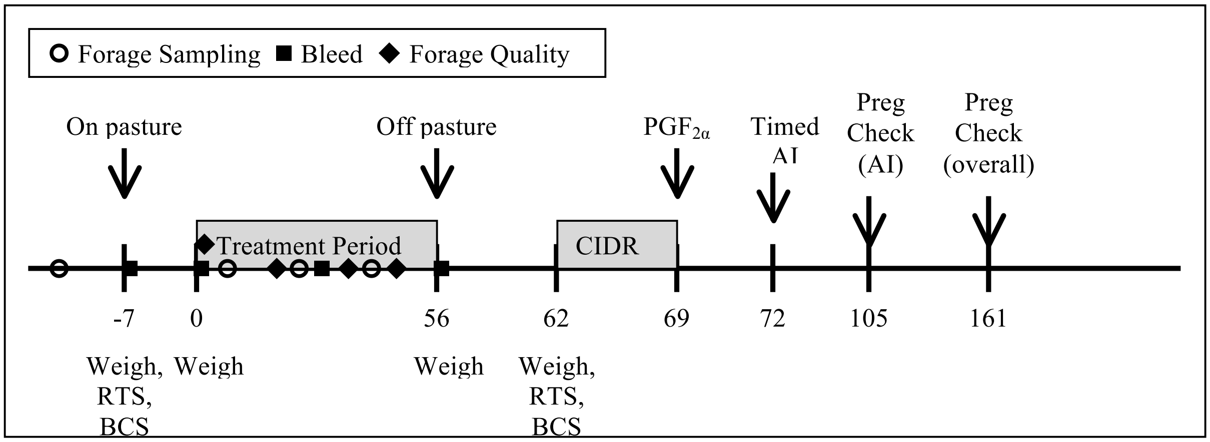

Figure 1.1 Experimental timeline

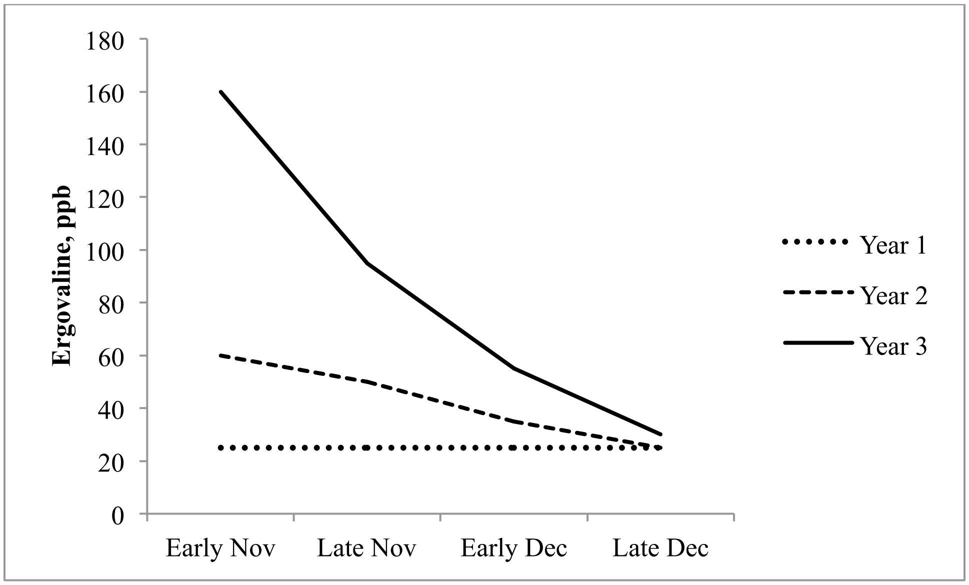

Figure 2.1 Ergovaline in stockpiled K-31 tall fescue

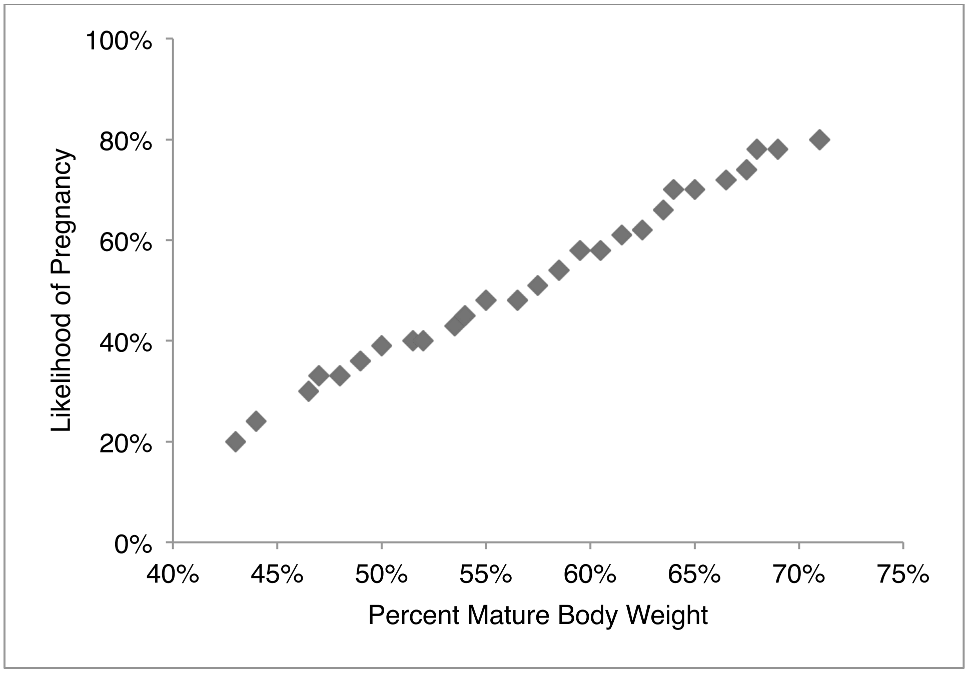

Figure 3.1 Relationship between percent mature body weight and likelihood of pregnancy

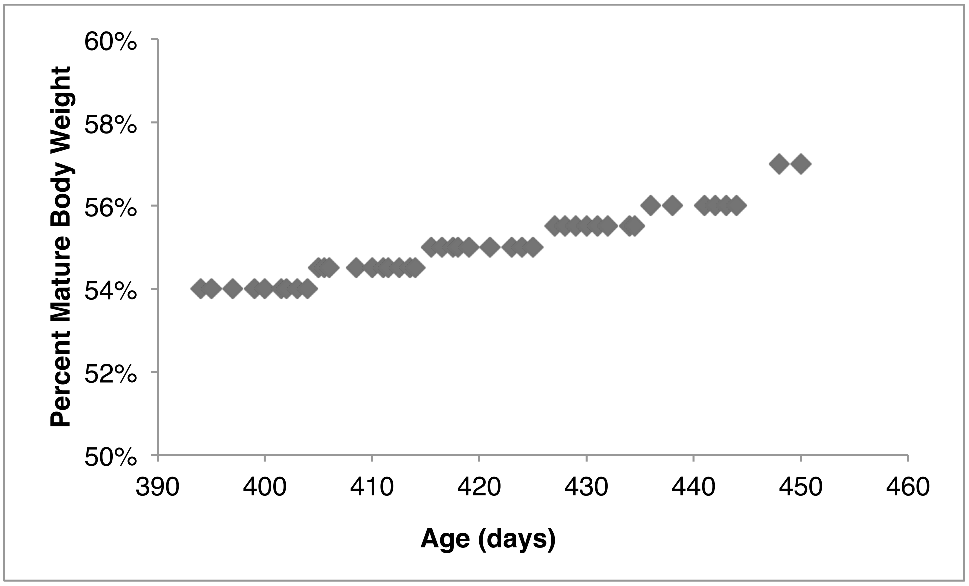

Figure 4.1 Relationship between percent mature body weight and likelihood of pregnancy

CHAPTER 3

The physiological effects of ground infected tall fescue seed on heifers naïve to fescue toxins

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Introduction

Cow-calf operations make up a large portion of the beef production systems in the Southeastern United States. This makes reproductive success in this region a critical component of sustainable and profitable beef herds. Cow-calf producers are largely pasture-based, so optimally managing on-farm resources such as land and forages for year round utilization is essential. Kentucky-31 tall fescue (*Lolium arundinaceum* [Schreb.] Darbysh) is the prominent cool season perennial grass grown in the mid-Atlantic, mid-South and lower mid-West regions of the United States due to its persistence and durability (Clay, 1988; Clement et al., 1994; Leuchtmann, 2003; White and Cole, 1985; White et al., 1992). It is naturally infected with an endophytic fungus, *Epichloë coenophiala*, that produces ergot alkaloids, metabolites that have adverse effects on livestock performance (Burke et al., 2006; Burke et al., 2001; Drewnoski et al., 2009a; Drewnoski et al., 2009b; Fanning et al., 1992; Mahmood et al., 1994; Schmidt and Osborn, 1993; Seman et al., 1997; Watson et al., 2004) cumulatively described as fescue toxicosis (SRIEG-37, 1992). Fescue toxicity symptoms include decreased feed intake and reduced weight gain (Hemken et al., 1981, Panaccione et al., 2006; Caldwell et al., 2013; Drewnoski et al., 2009b), elevated respiration rates and salivation (Browning et al., 1998; Burke et al., 2001; Walls and Jacobson, 1970; Bond et al., 1984), increased body temperatures (Hemken et al., 1981**)**, and vasoconstriction to the extremities (Rhodes et al., 1991). Issues with reproductive performance have also been observed, and include decreased pregnancy rates and reduced circulating hormone concentrations, such as cortisol (Browning et al., 1998), luteinizing hormone, prolactin, and progesterone (Browning et al., 1998; Burke et al., 2006; Drewnoski et al., 2009b). Issues associated with fescue toxicosis have cost the cattle industry between $600 million and $1 billion annually (Roberts and Andrae, 2010), emphasizing the urgent need for understanding the physiological effects of tall fescue on beef cattle so that corrective measures can be taken.

It is well known that the ergot alkaloids in tall fescue cause vasoconstriction and reduced blood flow to the peripheral arteries, however the extent to which vasoconstriction occurs to the internal organs has not been documented. Although decreased serum progesterone concentrations have been seen in cattle consuming E+ tall fescue (Jones et al., 2003; Seals et al., 2005), Jones et al. (2003) extracted luteal tissue from heifers consuming diets either with or without ergot alkaloids and did not observe differences in progesterone concentrations. Thus ergot alkaloids may be causing local vasoconstriction of blood flow to the ovaries, resulting in lower peripheral progesterone concentrations. Adequate blood supply to the reproductive organs is critical for normal reproductive function and development. Without a sufficient blood supply, the ovaries cannot obtain adequate nutrients and steroid precursors, like cholesterol. The delivery of chemical messengers to and from the uterus would also be restricted thereby inhibiting maternal recognition of pregnancy, resulting in pregnancy termination (Roberts et al., 1996). Additionally, a reduced systemic blood flow would negatively affect reproductive cyclicity by hindering ovarian hormonal feedback to the brain (Burke et al., 2001). If ergot alkaloids are causing vasoconstriction to the uterus and ovaries, then the consumption of infected tall fescue could partially explain the reproductive issues seen in cattle grazing it.

Through measuring various physiological parameters that may be altered by the presence of dietary ergot alkaloids, this study aimed to observe the extent to which fescue toxicosis occurs in cattle lacking prior exposure to infected tall fescue by feeding known concentrations of ergot alkaloids. An additional goal of the study was to detect the presence of vasoconstriction to the reproductive organs due to ergot alkaloid consumption.

Materials and Methods

Animals. Under the supervision of the Institutional Animal Care and Use Committee at North Carolina State University (IACUC #13-093), purebred Angus heifers (n=36) were purchased and delivered from KG Ranch in Three Forks, Montana to the Butner Field Research Laboratory in Butner, NC on 3 April 2014. Upon arrival to the research station, the animals, with an average initial weight of 280 kg, were grouped by weight and placed in a freestall barn with Calan gates (American Calan, Northwood, New Hampsire). These individual feeding units enabled us to monitor and regulate feed intake. Heifers were given a transition period of 46 days to adjust to the Calan gate system. Heifers were guided through the chute and head gate system once per week leading up to the trial for conditioning purposes.

Total mixed ration and fescue seed. Animals were fed a total mixed ration (TMR) at 4.6% of the most recent body weight, a slightly restricted amount in order to prevent intake variation. The TMR was formulated according to National Research Council (1996) requirements (Table 2.1). Unlimited fresh water was provided in each pen daily. Following the transition period, heifers remained on the TMR and were randomly assigned to receive either endophyte infected fescue seed (E+, Southern States KY-31) or non-infected fescue seed (E-, Pennington KY-31) for a 60-day trial period. Seed was ground using a hammer mill with a 1.1 cm screen (Meadow Mills, North Wilksboro, NC) prior to being added to TMR. Grab samples of total mixed rations (TMR) and TMR components were sampled biweekly and composited into two subsamples for analysis. The TMR and ground fescue seed were submitted to the North Carolina Department of Agriculture to be analyzed for nutrient content (Table 2.2, Table 2.3) (Eisemann et al., 2014). Each lot of fescue seed was also analyzed for alkaloid concentration (University of Missouri Veterinary Medical Diagnostic Laboratory, Columbia, MO) using HPLC (Rottinghaus et al., 1993) (Table 2.4).

Animal measurements. Measurements were taken on a weekly basis starting two weeks prior to initiation of treatments. Heifers were brought to chute system one pen at a time to reduce time standing in the sun in the exposed lanes. Each pen, containing 12 animals each, took approximately 60 minutes to complete all measurements. Each individual animal took about 10 minutes, allowing the following animal to stand in the shade on the scale for at least 10 minutes before entering the head gate. Behavioral chute scores on a 1-5 scale, 1 being calm and 5 being excited, and body weights were taken just prior to entering the squeeze chute and head gate.

While restrained in the squeeze chute, two blood samples were collected from each animal via jugular venipuncture with 20 gauge needles. Sterile silicone coated glass vacutainer serum tubes without additive (Becton Dickinson, Franklin Lakes, NJ) were used for progesterone analysis. Sterile glass vacutainer serum tubes with liquid K_3EDTA were used for hematocrit measurements. Rectal temperatures were then taken using a basic digital thermometer (Lumniscope®, Graham-Field Health Products). Respiration rates were recorded by visually observing diaphragm movements for 15 seconds to determine breaths per minute. Heart rate and caudal blood pressure was measured with a 16 to 24 cm blood pressure cuff (Lifesource® A&D Engineering, Inc., San Jose, CA) placed around the tail head as close to the anus as possible. Tails were held during this measurement in order to prevent inaccuracies caused by abrupt movements. Three heart rate and blood pressure readings were taken from each animal. Values that exceeded 15% variance were removed from the data set prior to analysis. Caudal vein and artery diameters were measured via doppler ultrasonography (M-Turbo, SonoSite Inc. Bothell, WA) and blood vessel area calculated.

An 18 x 20 cm rectangle was shaved with electric clippers (Oster Professional Care, #10 blade) just behind the left shoulder in preparation for a thermal imaging camera (Fluke Ti45FT IR Flexcam®, Fluke Corporation, Everett, WA) to be used the following week for body surface temperature measurements (Eisemann et al., 2014; Huntington et al., 2012). Images were captured prior to shaving in order to reduce surface heat created by the clippers. A square section of the shaved region in each image was selected, and the highest, lowest, and average temperature of that square was recorded (SmartView® 3.5 Thermal Imager Software). The perimeter of shaved region was avoided during analysis due to slightly higher temperatures around the hairline.

Body condition scores were taken by a single trained professional each week following chute measurements (Bond et al., 1984). Hair coat thickness was evaluated on a scale from 1-5 in order to record the presence of a retained hair coat, 5 representing a thick hair coat and 1 representing a thin coat (Bond et al., 1984). A shedding score was also given using a 1-5 scale, 5 representing a hair coat that had not been shed and 1 representing a completely shed hair coat.

Uterine and ovarian characteristics were observed via rectal ultrasound. Vein and arterial areas of the uterus and ovaries were recorded in order to determine occurrence of vasoconstriction to the reproductive organs.

Synchronization protocol. To ensure that all reproductive measurements were taken at the same stage of the estrous cycle, heifers were synchronized using the standard CO-Synch + 7 day CIDR® protocol during the trial period. The controlled internal drug release insert (CIDR®; 1.38 mg progesterone) was placed in the heifers on day 32 of the study (June 20) along with a 100 µg gonadotropin releasing hormone (GnRH) injection. After one week, the CIDRs® were removed and 25 mg of $PGF_{2\alpha}$ was given. Display of estrous was expected on trial day 47.

Temperature and temperature humidity index. Barn temperatures were collected daily and ambient temperature and humidity were recorded from the National Weather Service station at Durham 11 W, approximately 40 km from the research station (Table 1.1). Temperature-humidity index (THI, Mader et al., 2002) was calculated using the formula:

$$\text{THI} = \text{Tdb} – [0.55-(0.55 \times \text{RH} / 100) \times (\text{Tdb} – 58)$$

where Tdb represents dry bulb temperature (°F) and RH represents relative humidity.

Statistical analysis. Data were analyzed using the PROC MIXED procedure of SAS with (SAS Inst. Inc., Cary, NC) with repeated measures and a lsmeans statement. The model included treatment and sample time, and the experimental unit was animal within each treatment. The model was parameter of interest = grand mean + ergot alkaloid status of fescue seed + random error. *P* values of ≤ 0.05 represented significant differences, and *P* values of ≤ 0.10 were defined as tendencies.

Results and Discussion

Temperature and temperature humidity index. Daily high ambient temperatures and ambient temperatures at 1:00 PM remained above the 21°C thermoneutral housing temperature (Eisemann et al., 2014), and were generally above the ambient temperature stress threshold of 25°C (Hahn, 1999), above which performance is significantly impacted (Figure 1.2). Temperatures greater than 25°C are associated with decreased feed intake (Hahn et al., 1992), and the 21°C threshold is associated with increased respiration rate (Hahn, 1999). However, daily low ambient temperatures were generally below these thresholds, potentially allowing for nighttime recovery. In contrast, daily low temperatures recorded in the barn were generally above 21°C (Figure 2.2), suggesting that the animals were less likely to experience nighttime recovery from heat stress while in the barn. The temperature humidity index (THI) in the barn fluctuated above and below the recovery threshold of 75 throughout the study, and rose above the emergency threshold of 85 on multiple days during the middle and end of the trial period. Experiencing heat stress can result in decreased feed intake, impaired growth and milk production, and reduced efficiency and reproduction (Hahn, 1985). Brief periods of heat stress have minimal influence on these physiological changes, however prolonged exposures can have debilitating effects on cattle health and production. The temperatures and THI values in this study likely caused some level of heat stress in the heifers.

Intake. Intake was not affected by treatment (P>0.05, Table 5.2). This suggests that the addition of the E+ seed to the TMR did not influence the palatability of the ration. Reduced intake has been seen in cattle consuming E+ tall fescue, and this is often attributed to reduced palatability due to the presence of ergot alkaloids (Panaccione et al., 2006; Hemken et al., 1981). High ambient temperatures can also cause reduced intake, however the slightly limited TMR offered appeared to prevent reduction in feed consumption due to environmental influence.

Body weight and body condition score. There was a treatment x day interaction on body weight (kg, $P<0.05$) (Figure 4.2). The E+ group had significantly lower body weights on sampling days during weeks 8-11. While there was not a treatment effect on body weight ($P>0.05$), there was a treatment effect on average daily gain (ADG, $P<0.01$) over the course of the study (Table 5.2). Heifers consuming TMR with E- seed gained more daily than heifers under the influence of toxic seed (1.05 kg/d and 0.81 kg/d for E- and E+, respectively). However, there was no difference in ADG between sample dates ($P>0.05$). Reduced ADG in cattle consuming E+ tall fescue has been seen in other studies, where it is suggested to be due to decreased feed intake (Hemken et al., 1981; Panaccione et al., 2006; Caldwell et al., 2013; Drewnoski et al., 2009b). Because intake was not affected by treatment in this study, one possible explanation for the reduced weight gain is a reduced digestibility of feed due to the presence of ergot alkaloids. One class of ergot alkaloids, the loline alkaloids, can inhibit rumen microorganism cellulose degradation (Bush et al., 1976). We did not test for loline alkaloids in our sample analysis, however this activity may have interfered with the E+ heifers' ability to obtain all available nutrients in the TMR leading to reduced weight gain.

There was a trending difference in body condition scores (BCS) between treatments ($P=0.054$). While both treatment groups maintained acceptable BCS, the E- group had slightly higher BCS overall (5.25 and 5.15 for E- and E+, respectively). When considering the interaction between treatment and sample time, the E- group had a higher BCS than the E+ group on weeks 9 and 11 ($P<0.05$, 7 July and 20 July; Figure 5.2). This was most likely related to the difference in ADG between the two treatment groups. The difference seen between June and July in body condition and body weight may have been due to environmental conditions.

Hematocrit. Blood hematocrit measurements determine the percentage of red blood cells. It is useful when investigating anemic or dehydrated individuals (Nordenson, 2006) as well as determining the oxygen carrying capacity of an animal circulatory system (McWilliams, 2008). Higher hematocrit values indicate increased oxygen carrying capacity, suggesting an enhanced physiological capacity to handle stressful situations (McWilliams, 2008). Reports of hematocrit values in cattle range from 41-85% (Kirk and Davis, 1970; Reeves et al., 1962) depending on the elevation and time of year. In Florida, hematocrit values from normal cattle averaged 55% packed cells in August (Kirk and Davis 1970), while in Colorado cattle on various supplements had approximately 36% packed cells during the summer months (Reeves et al., 1962). This difference may have been due to water intake between the two studies, however this information was not reported. Hematocrit values in the current study ranged from 33 to 35% packed cells for the E- group and 33 to 35% for the E+ group (Figure 6.2). There was not a significant interaction between treatment and sampling date (P>0.05). In order to establish a relationship between fescue toxicity and percent packed red blood cells, however, further research is needed that includes water intake and excretion.

Respiration rate. Although studies have shown that cattle consuming E+ tall fescue have elevated respiration rates (Browning et al., 1998; Burke et al., 2001), there was no statistically significant difference between the two treatments in this study (P>0.05, Figure 7.2). A larger sample size may have produced statistically significant results.

Heart rate and blood pressure. While the E+ treatment group had a slight increase in heart rate (HR) there was no significant difference between groups (P>0.05). HR averaged 72 beats/min for the E- group and 76 beats/min for the E+ group. Increased HR has been observed in animals experiencing fescue toxicosis (Bond et al., 1984; Walls and Jacobsen, 1970). Without the influence of ergot alkaloids, Walls and Jacobsen (1970) found increased HR in a heat stressed environment versus a thermoneutral setting (76 beats/min in 34.8°C and 59.3 beats/min in 15°C). Consuming E+ tall fescue extracts, cattle had increased HR in 15°C (69 beats/min vs. 61 beats/min) (Walls and Jacobsen, 1970). In contrast, McLeay et al. (2002) and Aiken et al. (2007) observed a temporary decrease in HR with exposure to ergot alkaloids followed by a gradual increase. Eisemann et al. (2014) found a decrease in HR in animals consuming E+ fescue seed under thermoneutral and, to a greater extent, heat-stress conditions. However, HR did not decrease over the 14 day trial period (Eisemann et al., 2014). A decrease in HR was observed over 21 days in both treatment groups based on weekly measurements before gradually increasing again (80 beats/min to 65 betas/min and 80 beats/min to 69 beats/min for E- and E+, respectively, from 12 May to 2 June) (Figure 8.2). During the first 21 days, barn temperatures ranged from 13°C - 31°C and average THI was 69. HR of the E+ group remained above that of the E- group, however the HR between the two treatments was not significantly different (*P*=0.1). Average daily high barn temperature was 27°C and overall average THI was 72. Although the barn temperature exceeded the thermoneutral housing threshold of 21°C (Eisemann et al., 2014), the average THI did not exceed the threshold of 75 for thermal stress-limiting measures (Hahn, 1999). Thus, the barn environment may not have been extreme enough to elicit physiological responses associated with heat stress. However, the addition of the ergot alkaloids was expected to reduce the abilibty of those heifers to tolerate the hot and humid conditions. It is suspected that either a higher dose of ergot alkaloids or more extreme ambient conditions may have been necessary in order to induce symptoms associated with fescue toxicosis, such as increased HR.

Systolic blood pressure (BP), diastolic BP, and systolic diastolic differential (S-D) did not differ between treatments (*P*>0.05), however there was an observed increase in both systolic and diastolic measurements during the first week of ergot alkaloid exposure in the E+ group before dropping below the E- group for the rest of the trial (Figures 9.2 and 10.2). While the E- group also experienced a rise in BP during the first week, the decline was more gradual and not to the extent of the E+ group. Animals consuming E+ fescue seed have previously shown reduced BP as compared to animals consuming E- fescue seed in thermoneutral conditions (Eisemann et al., 2014), however studies involving heat stress have reported increased blood pressure due to dietary ergot alkaloids (Browning and Leite-Browning, 1997; Browning 2000; Eisemann et al., 2014). Increased blood flow to the peripheral tissues in order to release body heat, in addition to vasoconstriction due to a high dosage of ergot alkaloids, results in an increase in BP. This suggests that either the ambient temperatures or ergot alkaloid concentrations in this study were not high enough to stimulate physiological changes associated with thermal stress. As stated above, the barn environment likely provided an environment to cause some heat stress in the cattle, and the ergot alkaloid concentration in the E+ TMR was high enough to cause fescue toxicity symptoms (Aldrich et al., 1993). However, it is also possible that sampling technique had an effect on BP and heart rate. The current study took measurements on animals restrained in a squeeze chute while holding the tails straight out to reduce movement of the cuffs. Eisemann et al. (2014) minimized intervention by letting the tails drop with the cuffs on, the animals were not restrained, and there were no more than two people in the barn at any given time. Perhaps more extreme environmental conditions or a sampling environment with less human intervention could have produced greater differences in the current data set.

Body temperature. Ambient temperatures of above 25°C have been shown to elevate core body temperatures of feedlot cattle (Scharf et al., 2011). Under the influence of E+ tall fescue, cattle have rectal temperatures elevated between 0.4 and 1.2°C (Hemken et al., 1981; Hannah et al., 1990; Burke et al., 2006; Burke et al., 2001; Parish et al., 2003; Walls and Jacobson, 1970). Barn temperatures and ambient temperatures rose above the critical 25°C threshold in this study (Figures 1.2 and 2.2). However, the addition of ergot alkaloids did not affect the rectal temperatures of the heifers in the E+ group (P>0.05). Over the course of the study, rectal temperatures averaged 39.2°C for both E- and E+ groups. This is slightly higher than the normal temperature for cattle (38.6°C) and is likely due to the high ambient temperatures. Although the concentration of ergot alkaloids in the present study should have been high enough based on previous studies (Aldrich et al., 1993; Eisemann et al., 2014), they may not have been high enough to increase rectal temperatures. Additionally, the heifers were housed in a covered barn and were not influenced by sunlight, thereby reducing the possibility of solar heat-induced stress.

Skin surface temperatures were recorded with a thermal imaging camera that measured the low, high, and average skin temperatures in the specified area. There was no difference in skin surface temperatures between treatment groups (P>0.05). Average skin surface temperatures were 36.0°C and 36.2°C and high temperatures were 37.6 and 37.7°C for E- and E+, respectively. There was a sample time by treatment interaction on sample date 4, where the E+ group had increased high skin surface temperatures (P<0.05; 35.5°C and 36.2°C for E- and E+, respectively). Eisemann et al. (2014) fed steers diets containing E+ or E- fescue seed (approximately 285 ppb ergovaline for E+) and recorded skin surface temperatures using similar methodology. The steers consuming E+ fescue seed had higher skin surface temperatures than those on the E- diet, suggesting that vasodilation occurred in order to transfer a greater amount of core body heat to the skin surface. The maximum ambient temperature was 32.3°C and hair coats were clipped in order to minimize the effect of hair coat on study parameters. In the current study, the maximum barn and ambient temperatures were 34.7°C and 36.6°C, respectively. Because both temperatures and dietary ergot alkaloid concentrations were higher than those in Eisemann et al. (2014), the differing results may have been due to sampling location. In the current study, each pen of heifers walk/ran down an uncovered lane and stood in the sun prior to entering the scale before the squeeze chute. Although each heifer had approximately 10 minutes of shade while on the scale, this sun exposure may have produced different skin surface temperatures than in Eisemann et al. (2014), whose animals remained in a shaded barn throughout the trial.

Caudal vein and artery areas. There was a treatment effect ($P<0.05$) as well as a treatment by sample date interaction effect ($P<0.05$) on caudal artery area (Figure 12.2). The average artery areas were 10 mm^2 and 9 mm^2 for E- and E+, respectively. This reduction in artery area in the E+ group is likely due to the capacity of ergot alkaloids to bind to biogenic amine receptors in the muscles responsible for contractions (Dyer, 1993; Oliver et al., 1998; Liang et al., 1998). Because arterial blood vessels are surrounded by smooth muscle, contractions stimulated by the ergot alkaloids could result in vasoconstriction. Irregular muscle contractions have been found in sheep influenced by ergot alkaloids (McLeay and Smith, 2006). There was no difference in caudal vein area between the two treatment groups ($P>0.05$, Figure 13.2). Venus blood vessel contractions were not expected, for they are not under the influence of muscular contractions.

Uterine and ovarian artery and vein areas. A similar pattern was seen in the blood vessels of the reproductive organs as in the caudal veins and arteries in the heifers. There was a treatment x day interaction between treatments on the uterine artery area (*P*<0.05). Uterine artery area increased from 16 mm^2 to 80 mm^2 and from 20 mm^2 to 109 mm^2 for E+ and E-, respectively, from the end of June to mid July (Figure 14.2). In contrast to the caudal vein results, however, there was a decreased uterine vein area in the E+ treatment group in mid July (*P*<0.05, Figure 15.2). The ovarian artery area followed the same pattern as the uterine artery area (Figure 16.2). There was a decreased ovarian artery area with the addition of E+ fescue seed in mid July (*P*<0.05, 15 mm^2 and 12 mm^2 for E+, 19 mm^2 and 16 mm^2 for E-). The ovarian vein area results follow a similar pattern to the other measurements, however the E+ group had greater vessel area in early July (*P*<0.05; Figure 17.2). Vasoconstriction of the blood vessels leading to and from the reproductive organs is detrimental to the reproductive health of livestock. The capacity of ergot alkaloids to reduce blood flow to the reproductive organs through vasoconstriction and thickening of the intimal layer of small peripheral vessels seen in this trial as well as other studies (Julien et al., 1974; Williams et al., 1975; Garner and Cornell, 1978) can inhibit the delivery of critical nutrients to the organs as well as prevent sufficient reproductive hormones from reaching the circulatory and central nervous systems, negatively impacting reproductive fitness.

Hair coat and shedding. Both hair coat scores and hair shedding scores were higher for the E+ group (*P*<0.05, Figures 18.2 and 19.2). This supports previous studies documenting a rough hair coat on cattle consuming E+ tall fescue (Bond et al., 1984; Hemken et al., 1979; Steen et al., 1979). The mechanism for this physiological effect is not understood, however retained hair coats in cattle consuming E+ tall fescue exacerbate heat stress during the summertime, thereby worsening the severity of “summer slump” (Bond et al., 1984).

Progesterone and Corpus Luteum. A reduced concentration of circulating reproductive hormones has been observed in animals experiencing fescue toxicosis (Jones et al., 2003; Seals et al., 2005). Progesterone was analyzed on 4 dates in order to determine the percentage of heifers experiencing estrous cycling (30 June, 7 July, 14 July, and 18 July). Progesterone concentrations greater than 1 ng/ml on consecutive dates indicated cyclicity. There was no difference in progesterone concentrations between treatment groups (P>0.05), and all heifers were determined to be cycling by the 18 July sample date. Either a higher concentration of ergot alkaloids in the TMR or increased ambient temperatures may have been needed in order to elicit a reduction in serum progesterone concentrations as seen in previous studies.

Summary

Fescue toxicosis is a major concern of the livestock industry due to its adverse health and production effects, including reduced intake and weight gain, disrupted circulating reproductive hormones, and reduced tolerance to heat stress, amongst others. This study aimed to exhibit fescue toxicity symptoms in beef heifers naïve to KY-Tall Fescue by feeding ground fescue seed in a total mixed ration. Average daily gain was lower for the E+ group (0.8 kg/d and 1.0 kg/d for E+ and E-, respectively), and body condition scores tended to be higher for the E- group. Hair coat and hair shedding scores were also higher in the E+ treated animals, indicating rough hair coats often seen in animals under the influence of endophyte-infected fescue. Heart rate, rectal temperature, respiration rate, and blood pressure did not differ between the two groups. There was no treatment effect on skin surface temperature, however there was a sample time x treatment interaction at a few points in the study, where the E+ group had higher skin surface temperatures. Vasoconstriction was observed through measuring caudal blood vessel areas. Caudal artery area was smaller for the E+ group (10 mm^2 and 9 mm^2 for E- and E+, respectively), however caudal vein area did not differ between treatments. This supports previous findings suggesting that ergot alkaloids stimulate involuntary muscle contraction, thereby influencing the smooth muscles surrounding arterial blood vessels and reducing blood flow to the peripheral tissues. In order to detect the occurrence of vasoconstriction to the reproductive organs, uterine and ovarian blood vessel areas were measured. There was a treatment effect on ovarian artery area (13 mm^2 and 11 mm^2 for E- and E+, respectively). Additionally, there was treatment x sample time interactions on all reproductive blood vessel areas, with E- group areas being larger than the E+ group in late July. Based on these results, vasoconstriction to the reproductive organs may be partly responsible for the disrupted circulating reproductive hormones in cattle consuming E+ tall fescue, contributing to reduced reproductive performance. Because there was a lack of certain physiological responses seen in similar studies, factors besides the treatments and ambient conditions were likely involved in the presence and severity of fescue toxicosis in the current study. These could include housing conditions, animal handling and sampling methods, individual animal tolerance to the toxins, or classes of ergot alkaloids fed. Further research is needed in order to pinpoint the particular interactions between study parameters and responses in order to explain why there is so much variation between studies investigating fescue toxicosis.

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Table 1.2: Total mixed ration composition, DM basis

TMR Item	
Corn silage (%DM)	65.0
Fescue seed (%DM)	10.0
Concentrate (%DM)	25.0
Concentrate Item	
Corn (%DM)	15.2
Soybean meal (%DM)	8.3
Limestone (%DM)	1.0
TM salt (%DM)	0.5
Rumensin®[1] 90 (g/kg)	1.8
Vitamin ADE (g/kg)	5.4

TMR fed at 4.6% of body weight
[1] Elanco, Indianapolis, IN

Table 2.2: Alkaloid content of tall fescue seed

Seed type[1]	E-	E+
Ergosine (ppb)	0	1,430
Ergotamine (ppb)	0	1,405
Ergocornine (ppb)	0	960
Ergocryptine (ppb)	0	1,940
Ergocristine (ppb)	0	665
Ergovaline (ppb)	45	3,910
Total (ppb)	45	10,310

Seed was analyzed through University of Missouri Veterinary Medical Diagnostic Laboratory (Columbia, MO) using HPLC (Rottinghaus et al., 1993)
[1] E-: Non-infected fescue seed (Southern States KY-31); E+: Endophyte-infected fescue seed (Pennington KY-31)
Lots of seed were sub-sampled and analyzed in late April prior to initiation of trial

Table 3.2: Nutritive value of total mixed ration

Date		May, June		July	
Item	Concentrate[1]	E- TMR[2]	E+ TMR	E- TMR	E+ TMR
DM (%)	91.38	43.63	43.7	45.68	43.45
CP (%DM)	22.8	10.2	10.98	9.73	9.35
NDF (%DM)	7.79	31.01	30.97	28.52	31.74
ADF (%DM)	4.06	19.64	18.11	16.71	17.59
TDN[3] (%DM)	79.46	77.98	79.38	80.66	79.85
Fat (%DM)	2.87	3.16	2.89	3.11	2.91
Ash (%DM)	8.56	4.23	4.14	3.75	3.72
Ca (%DM)	1.82	0.52	0.45	0.43	0.41
P (%DM)	0.42	0.27	0.3	0.27	0.28
S (%DM)	0.24	0.14	0.14	0.13	0.13
Mg (%DM)	0.18	0.18	0.18	0.18	0.17
Na (%DM)	0.44	0.12	0.11	0.10	0.08
K (%DM)	1.05	0.90	0.90	0.81	0.78
Cu (ppm)	55	21	18	17	15
Fe (ppm)	156	380	375	447	426
Mn (ppm)	49	48	48	48	46
Zn (ppm)	93	46	44	40	36

[1] Concentrate composition (DM basis): 15.2% corn, 8.3% soybean meal, 1% limestone, 0.5% TM salt, 1.8 g/kg Rumensin® (Elanco, Indianapolis, IN) 90, 5.4 g/kg vitamin ADE

[2] TMR composition (DM basis): 65% corn silage, 25% concentrate, 10% fescue seed

[3] TDN = 92.5135 – (0.7965 x ADF)

Table 4.2: Nutritive value of fescue seed

Date	May, June		July	
Item	E-	E+	E-	E+
DM (%)	91.54	91.92	91.44	91.52
CP (%DM)	14.52	14.63	14.68	13.78
NDF (%DM)	27.76	31.46	28.38	29.08
ADF (%DM)	13.42	14.24	12.84	13.04
TDN[1] (%DM)	81.82	81.17	82.29	82.13
Fat (%DM)	1.37	1.76	1.93	1.23
Ash (%DM)	4.79	5.00	4.58	4.72
Ca (%DM)	0.23	0.24	0.21	0.21
P (%DM)	0.35	0.37	0.41	0.38
S (%DM)	0.20	0.20	0.20	0.19
Mg (%DM)	0.16	0.20	0.19	0.17
Na (%DM)	0.01	0.00	0.01	0.01
K (%DM)	0.64	0.52	0.47	0.54
Cu (ppm)	6	7	6	5
Fe (ppm)	155	76	78	85
Mn (ppm)	78	61	53	93
Zn (ppm)	33	34	33	30

[1] TDN = 92.5135 – (0.7965 x ADF)

Table 5.2: Intake and average daily gain of heifers consuming TMR with ground fescue seed of varying ergot alkaloid concentrations

Item	E+	E-	SEM	*P*-val
Intake ($kg*d^{-1}$)	18.1	18.5	1.5	0.178
ADG ($kg*hd*d^{-1}$)	0.81	1.05	0.04	0.001*

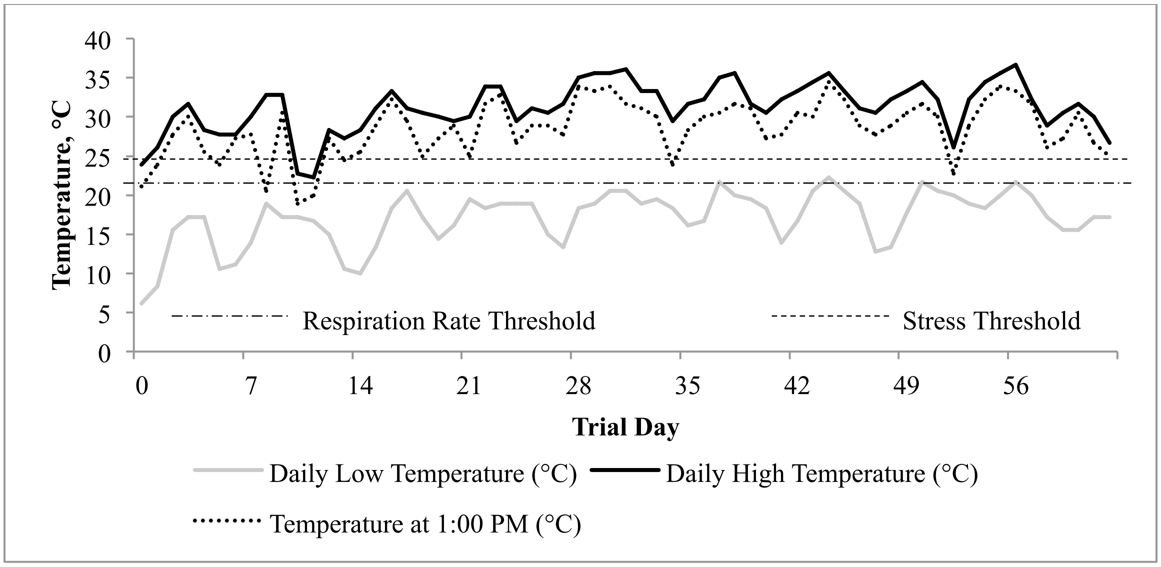

Figure 1.2: Daily ambient temperatures from May through July
21°C defined as the critical temperature to raise respiration rate due to an increase in ambient temperature (Hahn, 1999). 25°C defined as the ambient temperature needed to cause a reduction in feed intake in order to prevent metabolic heat production (Hahn et al., 1992).

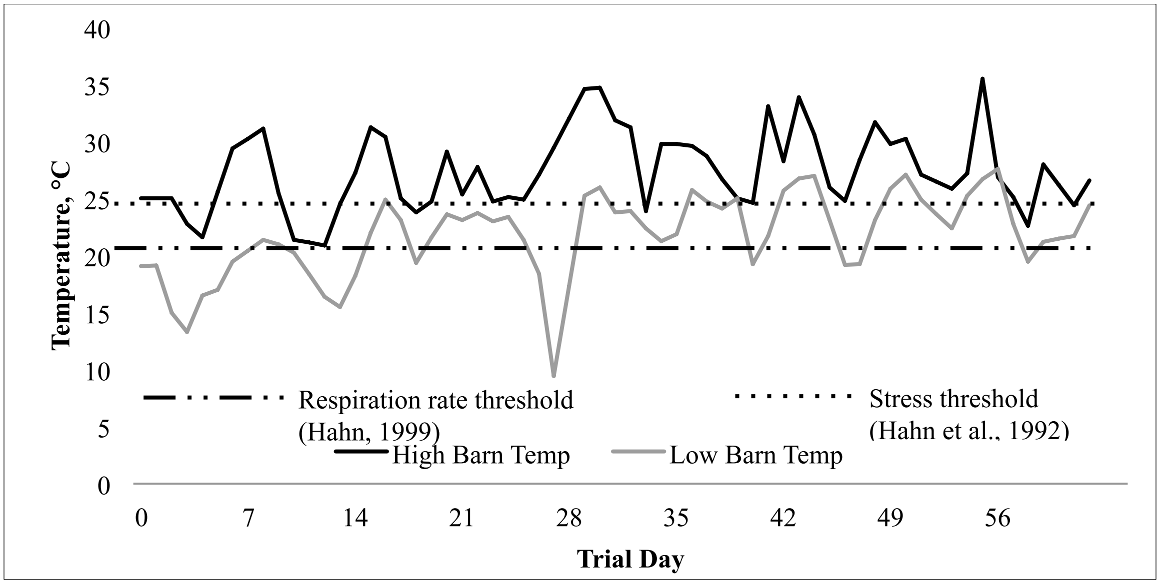

Figure 2.2: Daily barn temperatures from May through July
21°C defined as the critical temperature to raise respiration rate due to an increase in ambient temperature (Hahn, 1999) and as the upper critical temperature for thermoneutral housing (Eisemann et al., 2014). 25°C defined as the ambient temperature needed to cause a reduction in feed intake in order to prevent metabolic heat production (Hahn et al., 1992).

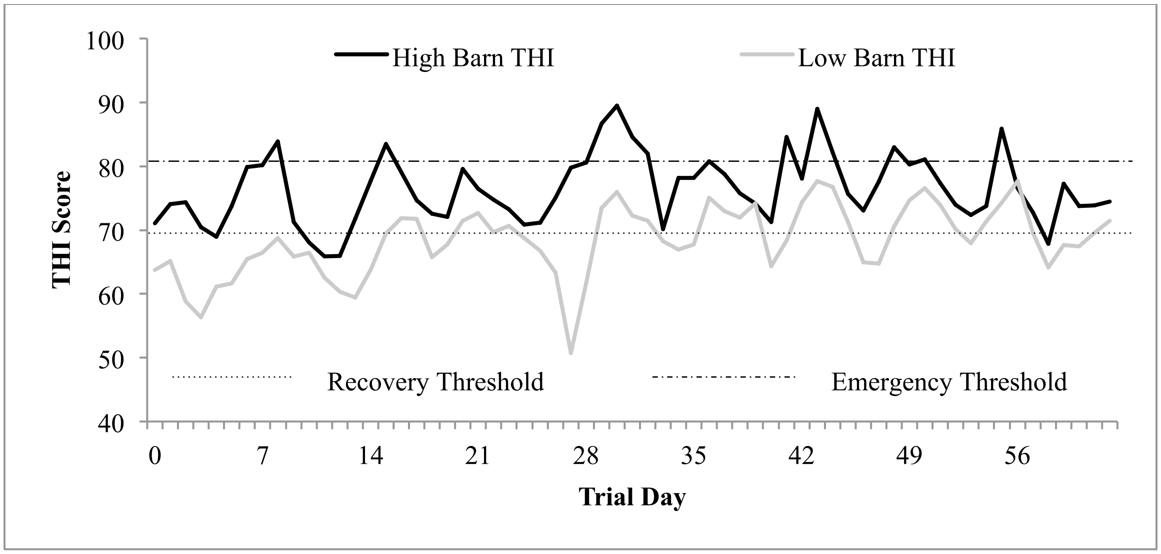

Figure 3.2: Barn temperature humidity index from May through July
THI = Tdb – [0.55-(0.55 x RH / 100) x (Tdb – 58); Tdb = dry bulb temperature, °F, and RH = relative humidity. Recovery (non-life threatening) and emergency (high-risk) thresholds as described by Hahn (1999).

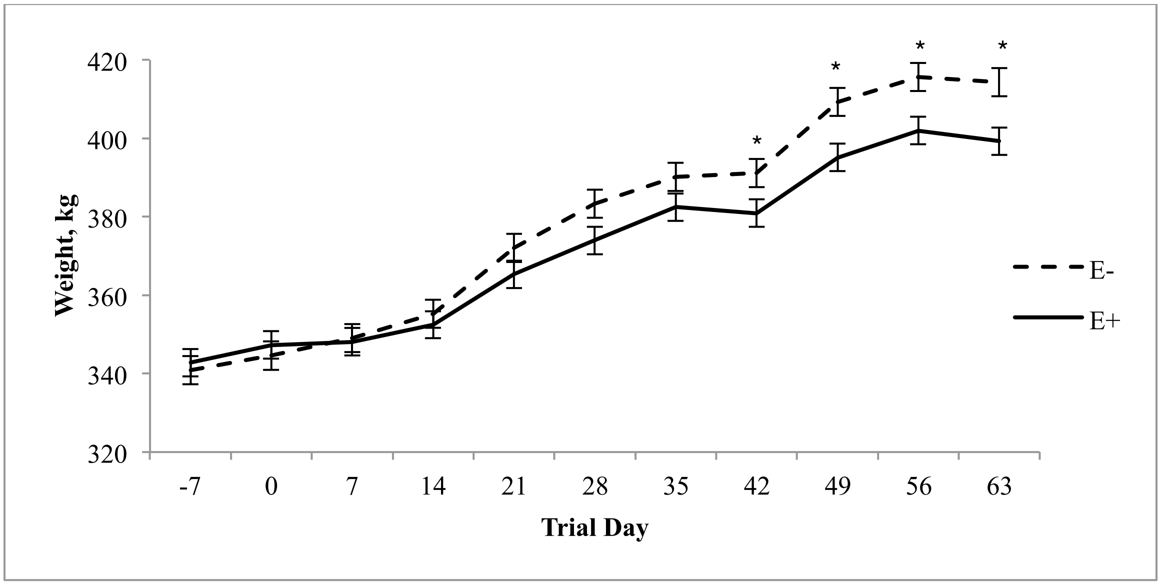

Figure 4.2: Weight of heifers consuming TMR with ground fescue seed from May through July.

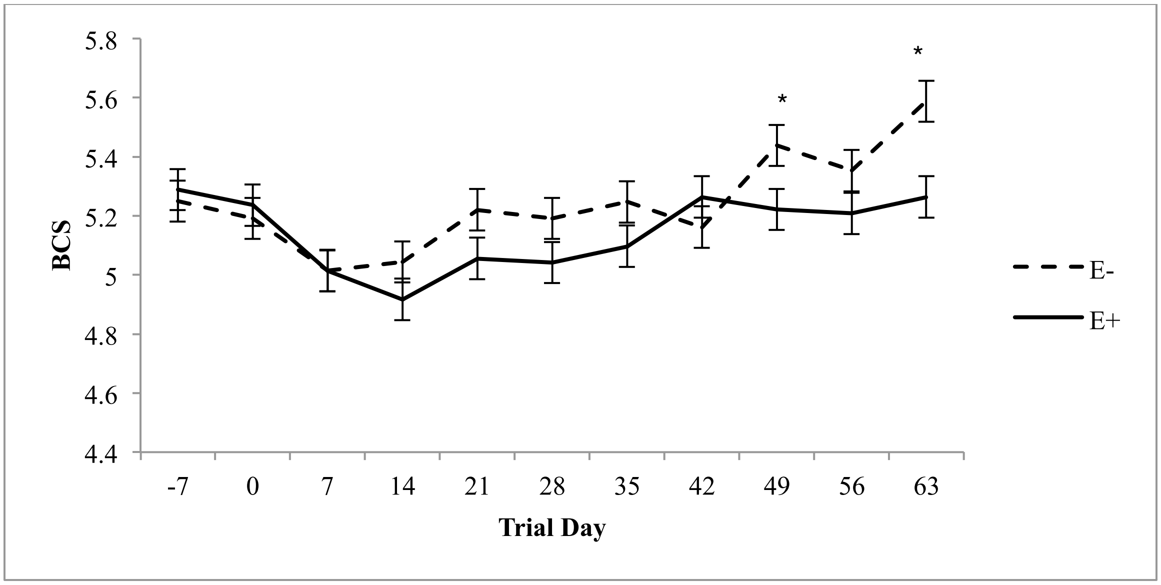

Figure 5.2: Body condition score of heifers consuming TMR with ground fescue seed from May through July.

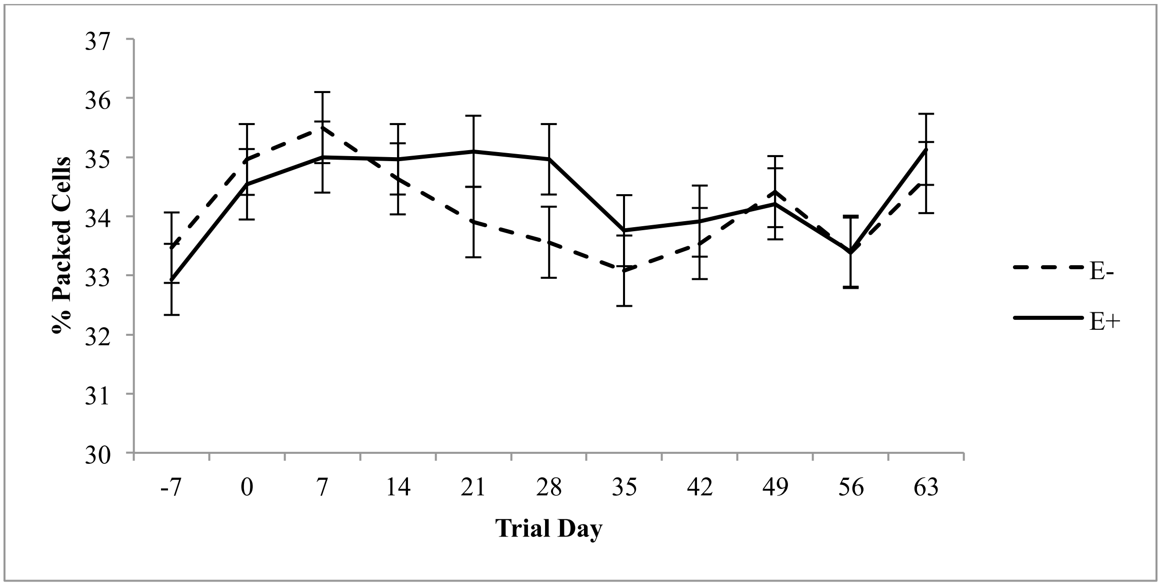

Figure 6.2: Hematocrit values of heifers consuming TMR with ground fescue seed from May through July.

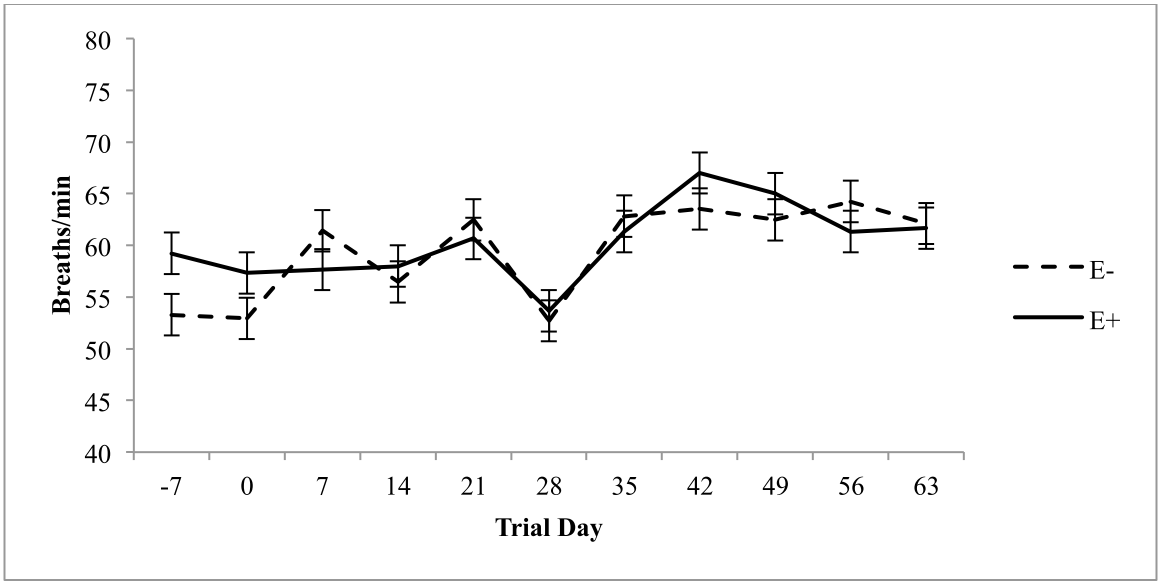

Figure 7.2: Respiration rate of heifers consuming TMR with ground fescue seed from May through July.

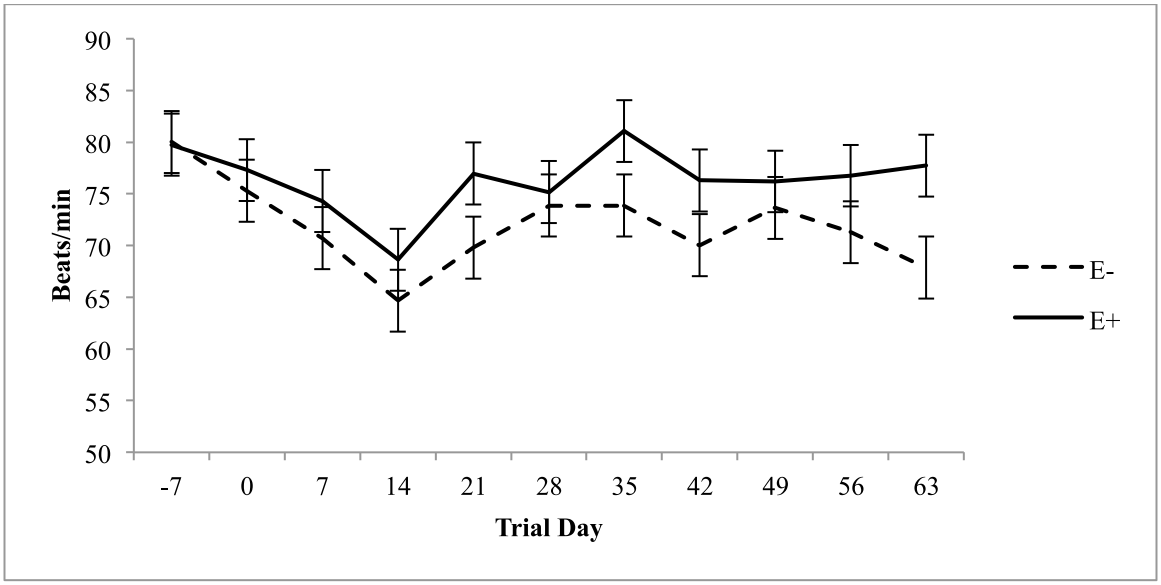

Figure 8.2: Heart rate of heifers consuming TMR with ground fescue seed from May through July.

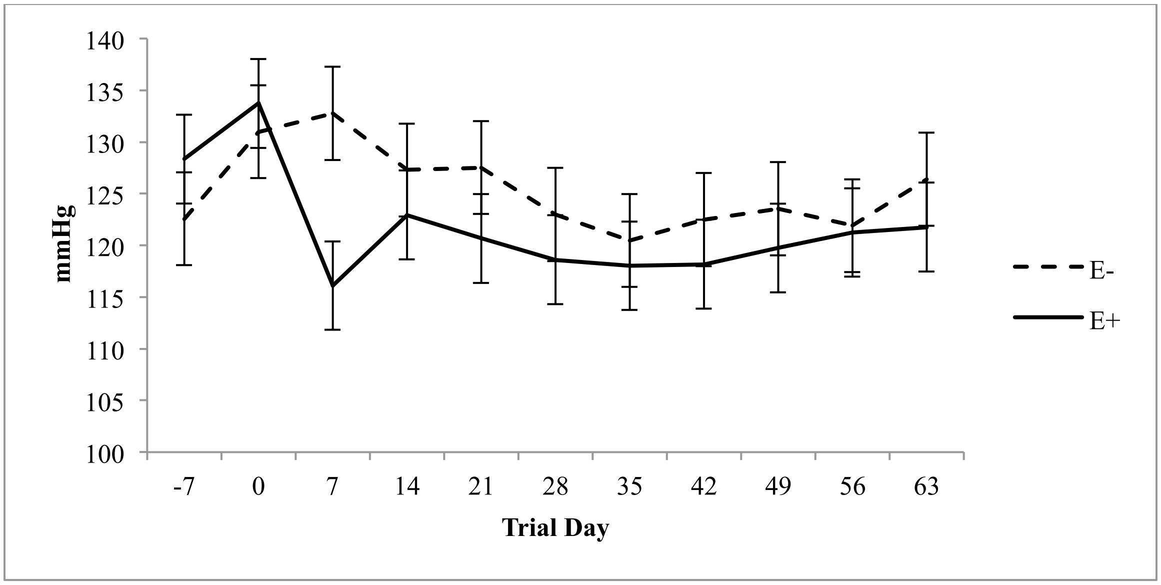

Figure 9.2: Systolic blood pressure of heifers consuming TMR with ground fescue seed from May through July.

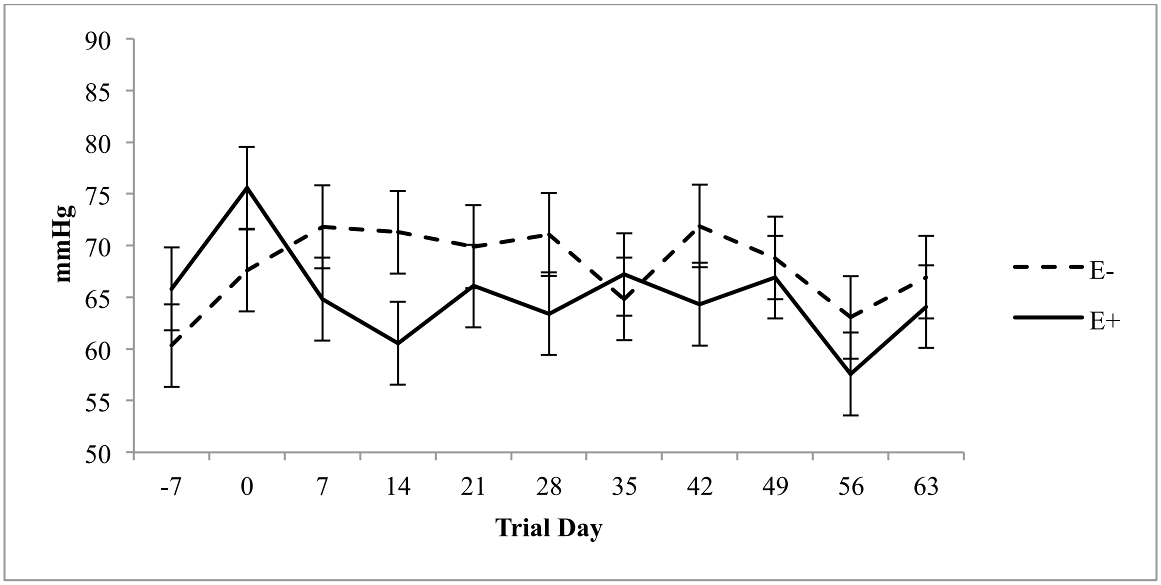

Figure 10.2: Diastolic blood pressure of heifers consuming TMR with ground fescue seed from May through July.

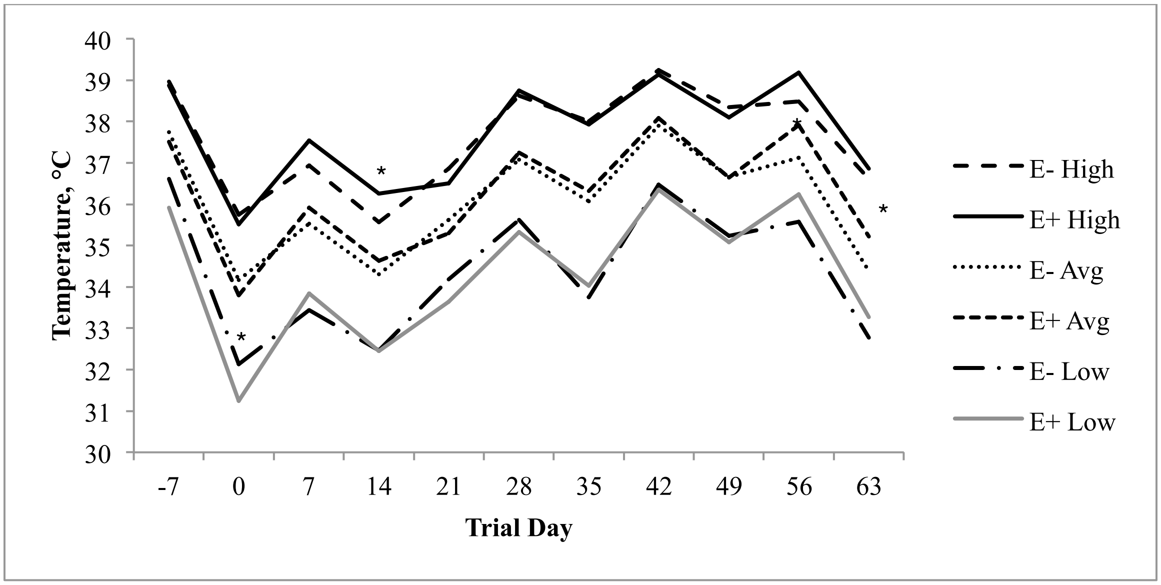

Figure 11.2: Skin surface temperature of heifers consuming TMR with ground fescue seed from May through July.

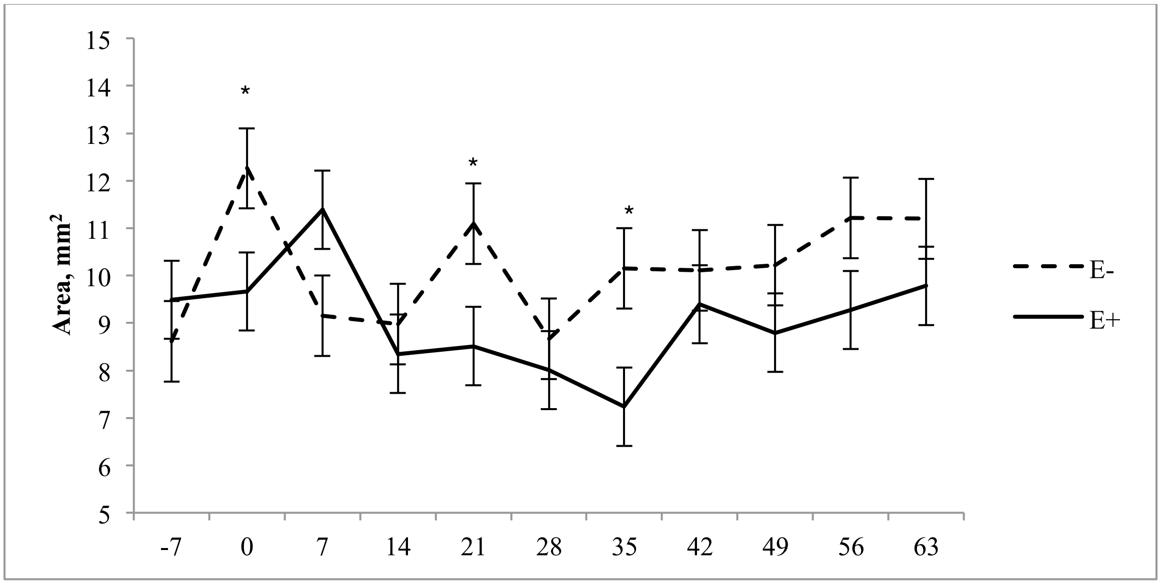

Figure 12.2: Caudal artery area of heifers consuming TMR with ground fescue seed from May through July.

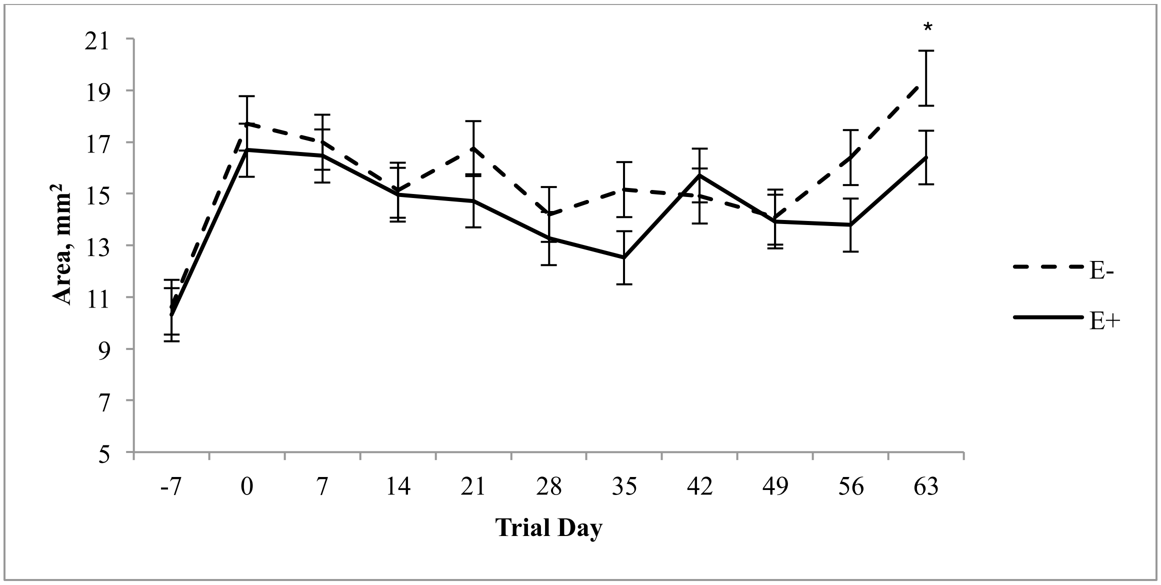

Figure 13.2: Caudal vein area of heifers consuming TMR with ground fescue seed from May through July.

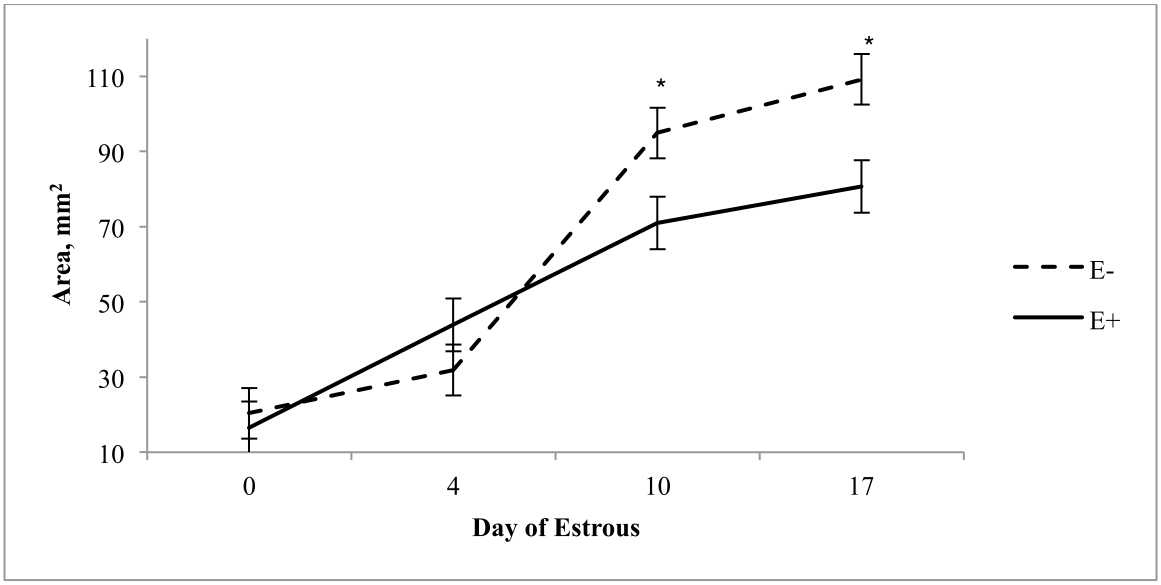

Figure 14.2: Uterine artery area of heifers consuming TMR with ground fescue seed.

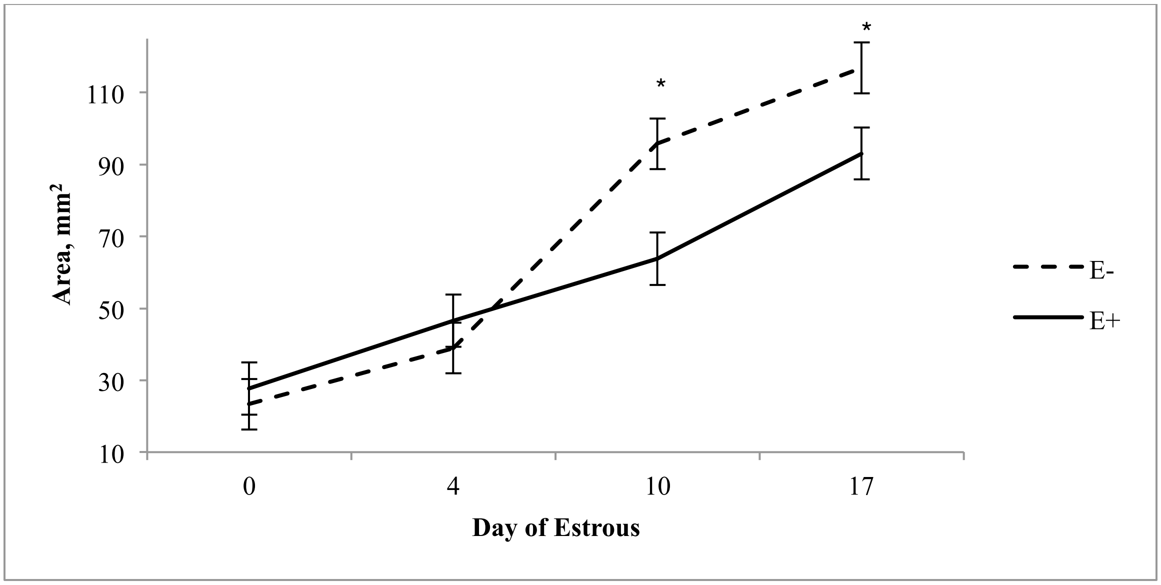

Figure 15.2: Uterine vein area of heifers consuming TMR with ground fescue seed.

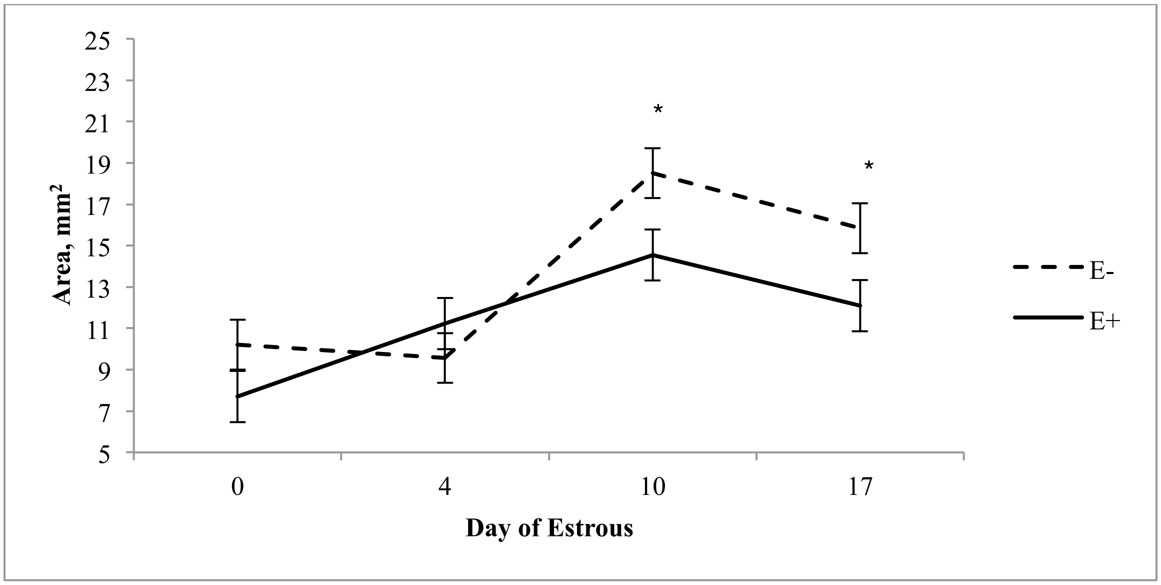

Figure 16.2: Ovarian artery area of heifers consuming TMR with ground fescue seed.

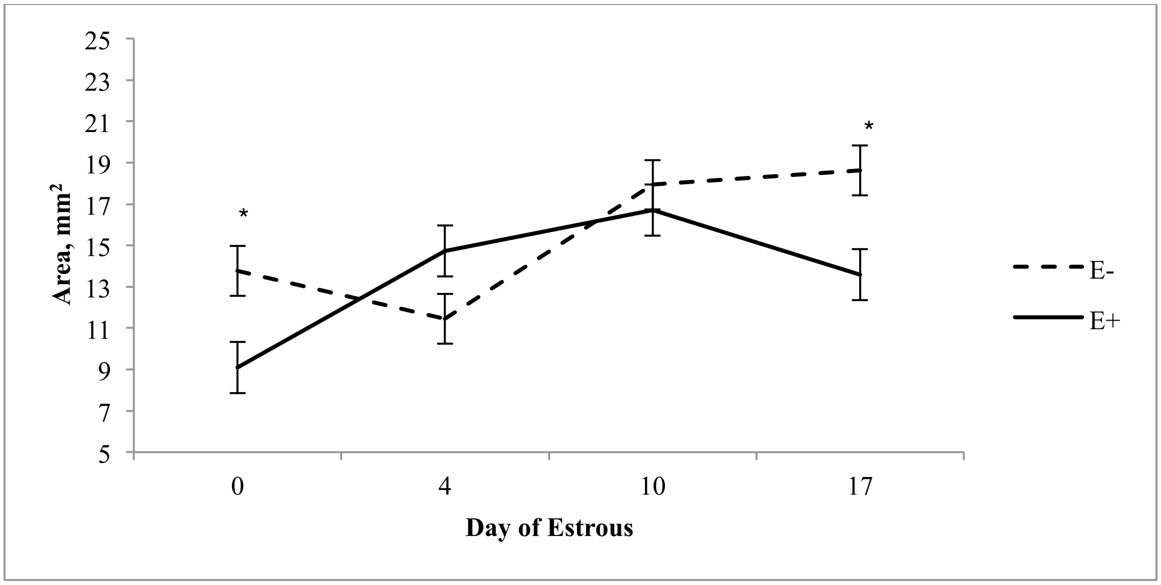

Figure 17.2: Ovarian vein area of heifers consuming TMR with ground fescue seed.

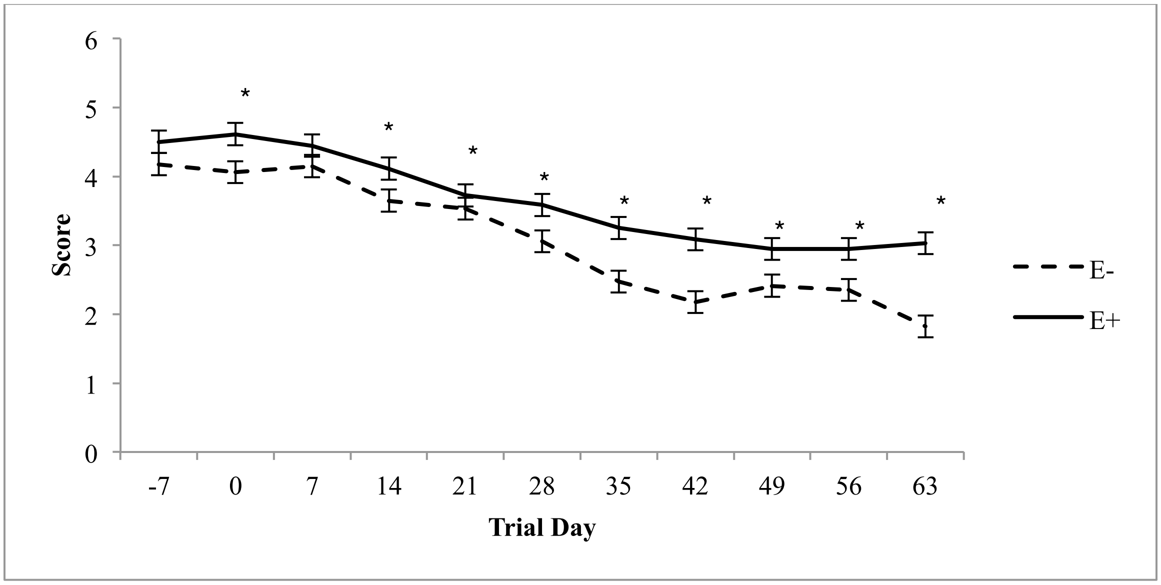

Figure 18.2: Hair coat scores of heifers consuming TMR with ground fescue seed from May through July.

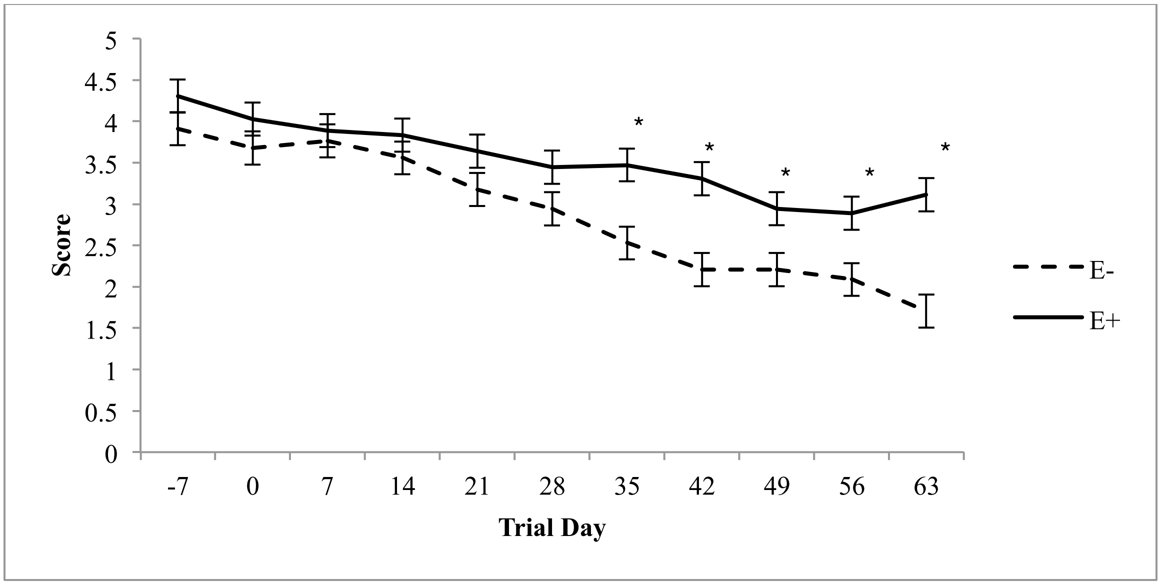

Figure 19.2: Hair shedding scores of heifers consuming TMR with ground fescue seed from May through July.