



5-2012

Improving Beef Cattle Performance on Tall Fescue

Brian Thomas Campbell
bcampb18@utk.edu

Recommended Citation

Campbell, Brian Thomas, "Improving Beef Cattle Performance on Tall Fescue." PhD diss., University of Tennessee, 2012.
https://trace.tennessee.edu/utk_graddiss/1277

This Dissertation is brought to you for free and open access by the Graduate School at Trace: Tennessee Research and Creative Exchange. It has been accepted for inclusion in Doctoral Dissertations by an authorized administrator of Trace: Tennessee Research and Creative Exchange. For more information, please contact trace@utk.edu.

To the Graduate Council:

I am submitting herewith a dissertation written by Brian Thomas Campbell entitled "Improving Beef Cattle Performance on Tall Fescue." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Animal Science.

John C. Waller, Major Professor

We have read this dissertation and recommend its acceptance:

F. David Kirkpatrick, Gina M. Pighetti, Gary E. Bates, Cheryl J. Kojima

Accepted for the Council:

Dixie L. Thompson

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

Improving Beef Cattle Performance on Tall Fescue

Dissertation

Presented for the

Doctor of Philosophy

Degree

The University of Tennessee, Knoxville

Brian Thomas Campbell

May 2012

Abstract

The overall goal of the studies described in this dissertation was to improve beef production of cows grazing endophyte infected tall fescue either through management practices or through identifying markers for genetic selection. Experiment 1 investigated differences in spring and fall calving herds grazing endophyte infected tall fescue. This study determined that managing for a fall calving beef herd is the more productive and efficient system. This is due to increased reproductive efficiency as well as traditionally greater market prices at the time of weaning. A spring calving system will have faster growing calves, but the increased weight of the calves is not enough to offset the added value of more calves produced in the fall calving herd. Experiment 2 was a study to validate a single nucleotide polymorphism (SNP) as a potential genetic marker found on the DRD2 gene. Steers with an AA genotype at this SNP have been shown to have greater prolactin levels and this study indicated that cows which have the AA genotype will have their first calf an average of 23 days earlier than cows with the GG genotype. Also when allelic frequency was examined it was shown that spring calving cows had a shift in allelic frequency away from Hardy-Weinberg equilibrium towards the A allele. Experiment 3 used a genome wide association study (GWAS) to confirm the presence of other SNPs that may be used as markers for resistance to tall fescue toxicosis in beef cattle. Twenty four SNPs were identified with nine SNPs associated with birth weight and 15 associated with weaning weight. Some of the SNPs are found within genes associated with production and carcass traits such as average daily gain, acid detergent fiber intake, marbling, and fat thickness. The

results of this study are very promising but more research needs to be completed.
The SNPs that have been identified need to be validated.

Table of Contents

Introduction	1
Literature Review	6
Tall Fescue	7
Fat Necrosis.....	8
Fescue Foot.....	9
Tall Fescue Toxicosis	10
Vasoconstriction.....	11
Serum Prolactin	12
Reproduction in Females	13
Reproduction in Males	15
Alleviation of Tall Fescue Toxicosis	16
Non-Infected Plants	17
Novel Endophytes.....	18
Overseeding	19
Dopamine Antagonists.....	20
Calving Season.....	20
Genetic Resistance.....	22
Genome Wide Association Study.....	25
Summary	27
A Comparison of Spring and Fall Calving Beef Herds Grazing Endophyte Infected Tall Fescue	30

Abstract.....	31
Introduction	33
Materials and Methods.....	34
Results and Discussion.....	37
Conclusions	46
Literature Cited	47
Appendix: Tables and Figures	50

**A Single Nucleotide Polymorphism in the Dopamine Receptor D2 Gene May
be Informative For Resistance to Fescue Toxicosis in Angus-Based Cattle ..** 60

Abstract.....	61
Introduction	63
Materials and Methods.....	64
Results and Discussion.....	69
Literature Cited	74
Appendix: Figures and Tables	77

**A Discovery of Genetic Markers for Resistance to Tall Fescue Toxicosis in
Angus-Based Cattle.....** 84

Abstract.....	85
Introduction	87
Materials and Methods.....	88
Results and Discussion.....	91
Conclusions	92
Literature cited	94

Appendix: Tables and Figures	96
Conclusions	100
Literature Cited	104
Vita	115

List of Tables

Table 1.1. Standard age of dam adjustment factors (kg) for adjusted birth weight and adjusted 205-day weaning weight.....	50
Table 1.2. Least squares means for calving interval, days to weaning and days to first calf	50
Table 1.3. Least squares means for birth weight, weaning weight, and average daily gain.....	51
Table 1.4. Sample budget for raising a replacement heifer from weaning to first calf	52
Table 2.1. Genotypic and allelic frequencies of cattle from different herds and calving seasons	77
Table 3.1. Standard age of dam adjustment factors (kg) for adjusted birth weight and adjusted 205-day weaning weight.....	96
Table 3.2. Significant single nucleotide polymorphisms by phenotype with corresponding location and <i>P</i> -value.....	97

List of Figures

Figure 1.1. Number of calves born per year in the spring and fall calving herds.....	53
Figure 1.2. Average age of cows in the herd in one year.....	54
Figure 1.3. Number of calves per cow in the spring and fall calving herds for the entire study	55
Figure 1.4. Average calving distribution for the Ames Plantation spring and fall calving herds.....	56
Figure 1.5. Percent of two year old cows culled from the herd per year	57
Figure 1.6. Average price of 227-272 kg steers sold through livestock auctions in the United States in July and October.....	58
Figure 1.7. Value of heifers, steers, and all calves sold for each herd.....	59
Figure 2.1. Location of the [A/G] SNP within the DRD2 gene	78
Figure 2.2. Agarose gel showing bands for different DRD2 genotypes	79
Figure 2.3. Serum prolactin concentrations by month and genotype for experiment 1	80
Figure 2.4. Hair coat scores by month and genotype for experiment 1	81
Figure 2.5. Serum prolactin concentrations by month and genotype for experiment 2	82
Figure 2.6. Hair coat scores by month and genotype for experiment 2.....	83

Introduction

The abundance of forage in the south eastern United States on land that is not suitable for crop production provides an ideal area for beef production. Traditionally beef production in this area of the U.S. is primarily cow-calf production. Most cows in the transition zone will be bred between April and July and will calve between December and March. Over 20% of the beef cows in the United States are raised in the humid transition zone, and the majority of the feed for the cows in this region will come from forages (West and Waller, 2007). Because most of the cattle grown in this area graze the cool-season forage tall fescue (*Lolium arundinaceum*), this area is known as the fescue belt.

The majority of tall fescue is infected with an endophytic fungus (*Neotyphodium coenophialum*). The endophyte relies on a symbiotic relationship with the plant for survival. Endophyte-infected (E+) tall fescue is easily established, resistant to drought stress, resistant to insects and nematodes, as well as the ability to withstand heavy grazing pressure (Stuedemann and Hoveland, 1988). These agronomic features are what led to the wide spread acceptance and establishment of Kentucky-31(KY-31) when it was released. However there are drawbacks to using this forage as a feed for livestock.

When cattle consume forage infected with this endophyte they develop the syndrome “tall fescue toxicosis”. Tall fescue toxicosis has been estimated to cause more than 600 million dollars in losses every year due to decreases in performance and reproduction (Hoveland, 1993). Cattle and other herbivores grazing E+ tall fescue have been shown to exhibit reduced weight gain, intake, reproductive rates, and levels of circulating prolactin. Also rectal temperatures and hair coat scores are

increased in cattle grazing E+. Even with the problems associated with cattle grazing this forage, its many agronomic features make it an attractive forage base for beef production.

Determining ways to make beef production on tall fescue more efficient and profitable has been investigated for many years. Many different management systems have been examined including drugs, supplemental feeds, forage systems, and feed additives to reduce the impact of the toxins. Most of the systems that have been investigated have either not worked or have been cost prohibitive. Many of the symptoms associated with livestock grazing E+ tall fescue are seen either exclusively or are more prominent when the endophyte is combined with elevated ambient temperatures. In order to minimize the combination of endophyte and high ambient temperature fall calving has been suggested, as this management system will reduce the endophyte level and temperature that cattle are exposed to during critical times in their biological cycle. Cows that calve in spring will be bred when temperatures are greater and the toxins are high in the plant. Cows that calve in fall will be bred when ambient temperatures are lower and toxin levels in forage are low. This suggests that a fall calving beef herd would be more efficient

Shortly after the release of KY-31 tall fescue it was noted that different cattle responded differently to the toxins and this has laid the basis for the belief that genetics play a role in animal responses. Cunningham (1948) reported that different cattle, even within the same herd would respond differently when grazing E+ tall fescue. Others have suggested the possibility of a genetic link. Hohenboken and Blodgett, (1997) reported that mice could be selected for either resistance or

susceptibility to the endophyte based on weight gain when fed a diet containing E+ seed. The ability to select for animals that were resistant to the effects of the endophyte would greatly increase the profitability of beef production in the humid transition zone of the United States.

Tall fescue toxicosis has been shown to impact many different biological systems in grazing livestock. This shows that the syndrome is affected by many genes. Looper et al., (2010) reported that different genotypes in the enhancer region of the prolactin gene have an impact on beef cow longevity when grazing E+ tall fescue. In another study performed at the University of Arkansas, Sales et al., (2011) reported that cows grazing E+ tall fescue with a CC genotype for a specific SNP in the coding region of cytochrome p450 had lower lifetime calving rates compared to those with a GG genotype. Due to the large number of genes that effect how an animal will respond to consuming tall fescue, a genome wide association study would be beneficial to examine large numbers of SNPs that are spread through the entire genome.

The objectives of these studies were to improve beef production from cattle grazing tall fescue through genetics and management. The first objective was to compare spring and fall calving management systems for profitability and efficiency. This study examined phenotypic indicators of increased production such as average daily gain, weight per day of age, number of calves per cow and calving interval. The second objective was to validate a SNP located on the DRD2 gene for the possibility of use as a genetic marker, comparing genotype with phenotypic measures of production. The third objective was to identify more single nucleotide

polymorphisms using the Illumina 50k SNP chip with high throughput analysis that may be used as genetic markers for resistance to tall fescue toxicosis, by comparing genotypes at over 50,000 SNPs with phenotypic data for either adjusted birth weight or adjusted 205 day weaning weight.

Literature Review

Tall Fescue

Tall Fescue (*Lolium arundinaceum*), a perennial bunchgrass, is the most prevalent forage grass in the humid transition zone of the United States, and the most prevalent strain of tall fescue is Kentucky 31 (Stuedemann and Hoveland, 1988). Tall fescue originated in Europe but became popular in the United States after the release of Kentucky-31 in 1943. Tall fescue is found in what is known as the fescue belt, covering over 14 million ha in the transition zone (Paterson et al., 1995). The transition zone is an area which encompasses southern Illinois and Ohio, south to northern Mississippi and Georgia, west to eastern Oklahoma, and east to the Piedmont of Virginia and the Carolinas (Fribourg et al., 1991). This unique environment supports both cool- and warm-season forage species. This is important because the transition zone is home to more than 20% of the beef cows in the US, with most of these grazing tall fescue for a majority of the year (West and Waller, 2007). It is a favorable forage for many reasons. It is easily established, and adaptable to a wide range of environments. It is tolerant of grazing pressure and herbivory by animals, insects, and nematodes (Hill et al., 1991). Tall fescue also has both a fall and spring growth season; the fall growth period is from mid-September to early December and the spring growth period starts in early March and ends in late June (Ball et al., 2007).

These agronomic features make tall fescue very desirable as forage, but most of the positive attributes associated with tall fescue are directly or indirectly linked to the presence of the endophytic fungus (*Neotyphodium coenophialum*). This endophytic fungus is found in all parts of the plant, including the stem and

leaves and is concentrated in the seedhead (Bacon, 1995). There are no spores or outward signs of the infection, and the fungus will complete its entire lifecycle between the cell walls of the plant (Bacon, 1995). Endophyte-infected tall fescue (E+) is very resistant to drought. In a study where E+ and endophyte-free (E-) plants were placed under drought stress, the E- plants died while the E+ plants experienced death of leaf tissue but the basal areas of the plants remained green and all plants survived (Arachevaleta et al., 1989). This ability to tolerate drought stress without loss of stand is beneficial because the E+ forage requires less management than many other types of forage because it can withstand external stressors, such as abuse, pests, over grazing, and drought (Stuedemann and Hoveland, 1988). Because tall fescue requires less maintenance and management than other types of forages it allows for the part-time management system used by a majority of beef producers in the fescue region. There are drawbacks to using tall fescue as forage for grazing livestock. When herbivores consume E+ tall fescue it induces a syndrome known as tall fescue toxicosis.

When consumed by livestock such as cattle, goats, sheep, and horses, *Neotyphodium C.* causes a disorder commonly referred to as “Tall Fescue Toxicosis”. Historically there have been three main issues associated with cattle consuming E+ tall fescue. These include: Fat Necrosis, Fescue Foot, and Summer Slump.

Fat Necrosis

Fat necrosis has been described as necrotic fat deposits in different shapes and sizes in the mesentery of the abdominal cavity of an animal that has been

grazing tall fescue (Smith et al., 2004). This has been reported in cattle, pigs, horses, and Eld's deer (Smith et al., 2004). Stuedemann et al., (1985) reported a link between cattle grazing tall fescue pastures that have been highly fertilized with nitrogen to an increase in the incidence of the necrotic fat deposits. There is also an association between low blood cholesterol concentration and fat necrosis in brood cows (Stuedemann et al., 1985). The necrotic fat in these deposits will contain greater levels of crude protein and ash with less ether-extractable material than non-necrotic fat (Stuedemann et al., 1985). The necrotic fat deposits can lead to death in some cases. Stuedemann et al., (1985) reported that cows died as a direct result of intestinal constriction by hard fat and others died as a result of fat which encompassed the omasum. However the role of the endophyte toxins and metabolites in fat necrosis is not fully understood (Bacon, 1995).

Fescue Foot

Fescue foot is the most severe form of fescue toxicosis, and it is also the least seen form. Fescue foot was first reported in New Zealand. When cattle were placed on tall fescue pastures, "within a fortnight" cattle became lame. This was generally seen first in the left hind foot and would sometimes be seen in the right hind foot (Cunningham, 1948). Hyperemia generally coupled with swelling occurs at the coronary band between the dewclaw and the hoof (Hemken et al., 1981). This sign of the toxicosis is generally seen in the late fall and winter but it has been reported at other times (Hemken et al., 1981). Fescue foot results in gangrene of the animal's extremities that closely resembles ergotism (Lyons et al., 1986). These

problems seem to occur in the colder areas of the fescue growing region and the winters in the southern region (Bacon, 1995).

Tall Fescue Toxicosis

Tall fescue toxicosis (commonly used to describe summer slump) has been shown to cause reduced weight gain and reduced daily feed intake (Paterson et al., 1995). Also it can cause decreased reproduction rates and can delay the onset of puberty (Jones et al., 2003). These signs are generally not seen until ambient temperatures exceed 32°C (Hemken et al., 1981). However others have reported signs of tall fescue toxicosis when temperatures are below 32°C (Parish et al., 2003).

Ergot alkaloids produced by the fungus are the cause of these problems in cattle. The primary cause of tall fescue toxicosis is ergovaline (Yates et al., 1985). Ergovaline is an ergopeptide and known dopamine agonist produced by the endophyte (Yates et al., 1985). These alkaloids are produced by the fungus in all parts of the plant (Lyons et al., 1986). The effects of fescue toxicosis have been shown to vary depending on how much toxin is ingested by the cattle and by the level of infestation within the field. Fribourg et al., (1991) reported that as the level of E+ tall fescue decreased from 80% to 3% the signs of fescue toxicosis decreased while the average daily gains of the cattle increased.

In a study comparing differing endophyte levels and stocking densities Gwinn et al. (1998) reported that as grazing pressure increased in a pasture there would be an increase in the endophyte infestation level. Eighteen pastures were used in this study with 0, 25, 60, or 80% endophyte infestation levels and high, medium or

low stocking densities. Changes in infestation level were analyzed and the largest changes to endophyte infestation level were in the 25 and 60% infested pastures with high stocking densities. This is due to increased pressure placed on the forage. The plants which contained the endophyte were able to withstand the stress placed upon them, and plants without the endophyte died.

Vasoconstriction

Fescue toxicosis is also linked to reduced blood flow to the periphery which mimics the symptoms that are commonly seen with animals experiencing heat stress. A high endophyte diet has been shown to reduce blood flow to both the core and the periphery reducing the animal's ability to dissipate heat (Rhodes et al., 1991). It has been reported that the toxicosis will reduce blood flow to the skin (Rhodes et al., 1991) reducing the evaporative cooling effects (Aldrich et al., 1993) while increasing the energy expenditure (Zanzalari et al., 1989). The reduced blood flow to the skin is caused by the effects of ergovaline on bovine vasculature. Several alkaloids are produced by E+ tall fescue. Lysergic acid is a weak constrictor of bovine vasculature and, due to the weak bonds formed between lysergic acid and vasculature tissue the ability for bioaccumulation to reach the levels of lysergic acid that would induce strong contractions, it is unlikely that this alkaloid plays a major role in tall fescue toxicosis (Klotz et al., 2006). When the impact of ergovaline was compared to norepinephrine, a potent vaso-constrictor in its ability to contract bovine vasculature norepinephrine contracted the vein for between 45 minutes to one hour while ergovaline caused a contraction that held a similar contraction of norepinephrine for 105 minutes (Klotz et al., 2007). Lysergic acid was also

examined and found to have lower contractile levels than did ergovaline. In a more recent study, examining other alkaloids produced by tall fescue Klotz et al. (2010) reported that Ergonovine, while being a potent vasoconstrictor, does not have the same contractile ability of norepinephrine. Ergocryptine, ergocristine and ergocornine were less potent constrictors than ergonovine (Klotz et al., 2010). Ergovaline is the primary cause of tall fescue toxicosis and while other alkaloids are produced their effect on the animal is minimal.

Collectively the decreased intake, reproduction, ADG, prolactin levels, and other problems will result in reduced ruminant productivity when grazing E+ tall fescue and this decrease in productivity will be compounded during the summer months when the ambient temperature is above 32°C. Even with the decrease in production that is seen when animals graze tall fescue the positive agronomic features of the plant make it popular as forage for beef production.

Serum Prolactin

A decrease in serum prolactin in beef cattle has been consistently used as a sign of fescue toxicosis in bulls, steers, heifers, and cows (Parish et al., 2003; Hoveland et al., 1983; Fribourg, 1991; Rice et al., 1997). Prolactin (PRL) is a protein hormone that is secreted by the anterior pituitary (Riddle et al., 1933). Lactation and development of the mammary gland are the primary roles of prolactin (Riddle et al., 1933). More recent reports have shown that PRL may be involved in as many as 300 other functions (Ben-Jonathan et al., 1996). Dopamine has been shown to be involved in the secretion of prolactin by inhibiting the release from the anterior pituitary. Ergocryptine a commercially available ergot alkaloid has been shown to

increase the production of dopamine in turn inhibiting the release of prolactin. This may explain how ergovaline is decreasing the levels of circulating prolactin.

Reproduction in Females

One of the classic signs of tall fescue toxicosis is a decrease in reproduction. Because the effects of tall fescue toxicosis are increased by high ambient temperature this reduction in reproduction is mainly seen during the summer months (Porter and Thompson, 1992). Reduced reproduction has been widely studied in beef cattle, and in mice but the exact cause of the decrease has not been determined.

A decrease in circulating prolactin may be one of the factors impacting reproduction of cattle, as well as causing an increase in hair coat score. Prolactin levels generally increase in the summer yet this increase in prolactin is not seen in cattle grazing tall fescue. Also decreases in PRL have been suggested as the primary cause of the changes in hair coat seen in animals grazing endophyte infected tall fescue (Porter and Thompson, 1992). Increased hair coats could act as insulation increasing the body temperature of the animal. An increase in body temperature could cause a decrease in reproduction by increasing the heat stress in the animal.

Jones et al., (2003) reported that when heifers grazing E+ tall fescue were treated with a D2 dopamine agonist, they had similar progesterone concentrations compared to heifers grazing E- tall fescue. The dopamine agonist was also able to maintain weight gain in heifers grazing E + tall fescue.

Ewes grazing E+ tall fescue have been shown to have an increased time to conception after exposure to a ram. This was attributed to delayed estrus or embryonic mortality because there was no change in weight gain, gestation length, or number of lambs born (Bond et al., 1988). Schmidt et al., (1986) reported a decrease in conception rate of 3.5% for every 10% increase in fungal infection in tall fescue. When the corpus luteum (CL) was analyzed, Ahmed et al., (1990) reported CL from cattle grazing E+ had fewer nuclei and an increased number of large luteal cells with increased diameter. This increased size was due to greater levels of cellularity with increases in the number of mitochondria, lipid droplets, and secretory granules (Ahmed et al., 1990). The impact of the endophyte on the CL may be a factor in the reduced reproductive rates in animals grazing E+ (Porter and Thompson., 1992). Rats fed E+ seed have also exhibit signs of tall fescue toxicosis (Zavos et al., 1986). When female rats were fed a diet containing 40% E+ seed they had an increase in the length of estrous cycles, and the estrous cycle was stopped in diestrous phases I and II. Those fed at 20% E+ seed continued to cycle but their estrous cycle was increased by 2.2 days. The rats with a lengthened estrous cycle also had reduced pregnancy rates (Varney et al., 1987).

In a study which investigated the effect of E+ on cycling heifers, Burke et al. (2001) reported impaired luteal function in the heifers grazing E+ when compared with those grazing E- tall fescue. The reduction in reproduction could be related to the follicles forming earlier in the estrous cycle. With earlier formation the follicles will be aged by the time of insemination reducing the likelihood of conception (Burke et al., 2001). The effects of tall fescue toxicosis mimic the signs of heat stress in

cattle. Heat stress in cattle has been shown to decrease reproduction on its own, so the combination of tall fescue toxicosis and heat stress is extremely detrimental to reproduction in beef cattle when breeding is taking place during the warmer months of the year (Burke et al., 2001). The impact of E+ tall fescue on reproduction in females has been greatly studied, but research on the impact of E+ tall fescue on the reproductive ability of males is lacking.

Reproduction in Males

Zavos et al., (1986), in a study examining rats reported that E+ tall fescue impacted not only the female but also the male. He reported reduced daily sperm production, decreased testicular parenchyma and epididymal weight in male rats. The impact on females is greater than it is in males even when fed the same diet, and the effect is even greater on breeding pairs when they are both consuming E+ tall fescue seed (Zavos et al., 1986).

The impact of tall fescue toxicosis has not been investigated to the same extent in bulls as it has been in cows and heifers. Schuenemann et al. (2005a) reported that when beef bulls were exposed to ergotamine tartrate, which mimics the effects of tall fescue toxicosis, there was a reduction in the potential of sperm to fertilize an oocyte invitro. When bulls grazed E+ tall fescue, scrotal temperatures were reduced but there were no differences in growth of scrotal circumference or concentrations of testosterone (Schuenemann et al., 2005b). In this study bulls grazing E+ and MaxQ tall fescue had semen collected and tested for sperm motility and morphology, then frozen for later analysis. There was no difference ($P > 0.05$) in motility or morphology when comparing semen from bulls grazing E+ and MaxQ

tall fescue (Schuenemann et al., 2005b). After freezing, the semen were thawed and used in *in vitro* fertilization. The embryos, fertilized with semen from bulls grazing E+ had decreased cleavage (Schuenemann et al., 2005b). The cleavage rate was decreased but the subsequent developmental competence of the embryos that did cleave was not effected (Schuenemann et al., 2005b).

In another study motility, morphology and fertilization characteristics of spermatozoa from bulls grazing either E+ or MaxQ tall fescue was examined. Gross motility of spermatozoa was not different immediately after collection (Harris et al., 2011). After the initial examination the semen was frozen for later use in *in vitro* fertilization. Immediately post thaw the gross motility of the spermatozoa was reduced in the semen from the bulls grazing E+ tall fescue (Harris et al., 2011). When this semen was evaluated for other fertilization characteristics there was decreased cleavage of presumptive zygotes and penetration of the sperm in *in vitro* fertilization (Harris et al., 2011). This suggests that the impact of the toxins produced in tall fescue toxicosis have a greater impact on spermatozoa beyond that of gross morphology (Harris et al., 2011).

Bulls grazing E+ tall fescue have normal semen when examined for gross motility and morphology but they have reduced ability to fertilize embryos. The exact cause of this reduction in fertilization is unknown and more research is needed to determine the causes and possible modes of correction.

Alleviation of Tall Fescue Toxicosis

In the pursuit of a method to alleviate tall fescue toxicosis, several experiments have been conducted to determine the most profitable method to

decrease the signs of the toxicosis associated with tall fescue. Researchers have removed the fungus from the plant, used dopamine to reduce the effects of ergovaline, as well as overseeding with clovers and other forages to dilute the effects of the toxins.

Non-Infected Plants

Planting non-infected tall fescue was the first method tried. However, removal of the endophyte caused plant longevity and hardiness to be greatly reduced (Read and Camp, 1986). When compared in a greenhouse, leaf blade thickness was reported to be 18% greater at 60 days and 25% greater at 160 days at low nitrogen levels in E+ plants as compared to E- plants, but at greater nitrogen levels this was not seen (Arechavaleta et al., 1989). At high nitrogen fertilization rates, the herbage mass of E+ fescue was greater than E- plants; also regrowth of E+ tall fescue plants was more rapid than that of E- plants (Arechavaleta et al., 1989). When compared with E+ fescue, (E-) resulted in improved average daily gains from 30 to 100% while maintaining normal reproduction as well as milk production (Hoveland, 1993). Under grazing conditions these reductions in persistence of E- tall fescue compared with E+ tall fescue were evident. Bouton et al. (2001) reported that E- tall fescue stands were greatly reduced when there was competition with bermudagrass along with grazing pressure. This may be due to the Ky-31 tall fescue not having genes for persistence that are not associated with the endophyte (Bouton et al., 2001). When selection for tall fescue was done, gene for persistence may not have been selected for because the endophyte provided the persistence. If this is the case when the endophyte is removed the

plants ability to persist is also completely removed.

Novel Endophytes

Based on the importance of endophyte presence for plant persistence, others have selected plants that contain an endophyte that produces low levels of ergovaline. The selection of plants that contain endophytes that produce no or low levels of ergovaline could have great benefits on cattle production (Agee and Hill, 1994). The endophytes contained in these plants are known as novel endophytes or nontoxic endophytes (NTE). When plants that contain novel endophytes are consumed, tall fescue toxicosis is not observed. Humphry et al. (2002) reported that dry matter disappearance was reduced and the degradable dry matter was also reduced for E+ tall fescue compare to NTE tall fescue. Because nutrient content of E+ and NTE tall fescue are similar, forages that are more digestible should lead to greater performance. Steers grazing NTE tall fescue had greater ADG than steers grazing E+ KY-31 tall fescue. Beck et al., (2008) reported ADG of 0.55 and 0.78 kg for steers grazing E+ and NTE tall fescue during fall and winter, respectively. During the spring they reported an ADG of 0.45 kg and 0.92 kg for cattle grazing E+ or NTE, respectively. This increase in gain should increase the profits of beef producers who chose to renovate E+ pastures and change to NTE pastures.

Economic analysis has indicated that when the infection rate in the pasture was above 74% then there was an economic benefit in renovation of the pastures using tall fescue that contains a novel endophyte (Zhuang et al., 2005). The stocking rate on the pastures has an impact on the profitability of replacing the pastures. In addition producers who are stocking at a relatively high rate may find it

more profitable to re-establish the pastures over those who have a lower stocking rate (Zhuang et al., 2005).

The drawback to establishing NTE pastures is the high establishment cost including time the land will be out of production and the effort that is required for renovation of E+ pastures. There is a loss of the use of the acres for one to two years which must be calculated in the cost of reestablishment. Also there are some pastures in the fescue belt that, due to topography, make renovation of the pastures impractical if not impossible.

Overseeding

Overseeding tall fescue pastures with clovers has been shown to help alleviate the effects of the toxicosis in the cattle grazing fescue, as well as increasing the digestibility of the forage. Lusby et al. (1990) reported steers that grazed a tall fescue/clover combination had greater gains in both the stocker phase and the feedlot phase than those steers that grazed only low or high endophyte E+ tall fescue. An added benefit of over seeding with legumes is that it will also reduce the need for nitrogen fertilizer in pastures. White clover (*Trifolium repens L.*) is the predominant legume seeded with tall fescue, but red clover (*Trifolium pratense L.*) is also used. A common claim is often made that the greater performance of animals consuming E+ tall fescue with the addition of clovers is attributable to dilution of the toxins. However, increased animal performance is found when animals consume E- tall fescue, orchardgrass, and bermudagrass pastures when clover is present (McLaren et al., 1983).

Dopamine Antagonists

Ergot alkaloids produced by the fungus in E+ tall fescue act as dopamine agonists which can cause depression in circulating serum prolactin (PRL) concentration in cattle consuming E+ tall fescue. The use of dopamine antagonists has increased the levels of circulating PRL in animals that are grazing E+ tall fescue (Lipham et al., 1989). This increase in PRL levels indicates that the toxicosis is reduced by the administration of dopamine. Domperidone, when given to cattle, has been reported to maintain normal ADG, as well as maintaining normal levels of circulating progesterone leading to the conclusion that treatment with dopamine can stop the effects of the toxicosis (Jones et al., 2003). Dopamine antagonists have been shown to be helpful in the reduction of tall fescue toxicosis not only in cattle but in horses as well. Domperidone has been reported to greatly reduce the effects of the toxicosis in horses without the side effects of other dopamine antagonists (Redmond et al., 1994).

Calving Season

Changing management so that critical times in the biological cycle of beef cows do not coincide with high concentrations of toxins have also been suggested. If beef producers are managing for a controlled breeding season they traditionally have either fall or spring calving cow herd. Fall calving cows generally calve September through early December. Spring calving cows generally calve January through early April. In Tennessee approximately 80% of beef calves are born in early spring. Spring calving, while popular in Tennessee has some disadvantages. The breeding season for spring calving cows will generally run from mid-March through June. This time frame is in line with an increase in ambient temperatures.

Cows that calve between January and March will be bred between April and June. During this breeding season cow will be grazing tall fescue and suffering from heat stress. The combination of tall fescue toxicosis and heat stress will reduce the conception rates of spring calving cows. In contrast, fall calving cows will have a breeding season from January to mid-March. These cows may be consuming E+ hay during this time, but they will not have the increased heat stress and should breed back easier than their spring calving counterparts. A study by Bagley et al., (1987) compared calving season and stocking rate. While not on tall fescue, this study showed no difference in calving interval, or reproduction rates between fall and spring calving cows, but it did show a greater weaning weight for fall born calves. Also they reported that the fall born calves had lower mortality rates than did the spring born calves.

A study at the University of Arkansas examining differences in post weaning performance between calves born in the spring and fall showed that there were differences ($P < 0.05$) between the seasons. In the two year study, the calves born in fall were heavier at weaning and had a greater adjusted weaning weight than did their spring born counter parts (Caldwell et al., 2009). The fall herd received more supplemental feed than the spring herd. The calving rate of the fall calving cows was also greater ($P < 0.05$) which would indicate that there is not as great of an effect of the endophyte on cows during the breeding season for the fall calving cows. The value of the calves born in the fall was also greater. This was due to both a greater calving rate which will increase the number of calves to be sold along with greater average market price at the time of weaning. This study indicated that a fall

calving season when cattle are grazing tall fescue would be the most profitable (Caldwell et al., 2009).

Genetic Resistance

With the problems and cost associated with pasture establishment of NTE and the failure of E- tall fescue to persist adequately there has been increased focus on finding animals that exhibit tolerance to the toxins. There have been reports since shortly after E+ tall fescue was released that some animals respond differently to the ergots. Cunningham (1948) reported that not all cattle were affected similarly, which has been confirmed by subsequent studies (Hohenboken and Blodgett, 1997; Looper et al., 2010). The first study which attempted to select for resistance reported that it was possible to select for genetic tolerance to tall fescue toxicosis. In this study the calves of bulls which had been selected for either resistance or susceptibility were tested for their resistance to tall fescue toxicosis (Lipsev et al., 1992). This study reported that, while the calves of both susceptible bulls and resistant bulls had an increase in rectal temperature when the ambient temperature reached 30°C, the calves of the susceptible bulls increased to greater levels than those from resistant sires when dietary ergovaline levels reached 200 ppm (Lipsev et al., 1992). Another study which compared two bulls identified a trend for calves from a bull with a reputation for producing calves resistant to E+ tall fescue to have greater intake and lower body temperatures than the control bull (Gould and Hohenboken, 1993). In this experiment the progeny of two bulls were used to determine if selection had produced animals resistant to tall fescue toxicosis. One bull raised in Missouri had the “commercial reputation” as producing

calves which were resistant to tall fescue toxicosis, and the other bull, raised in Virginia, had unknown resistance. The calves from these two bulls were fed in a multi-part controlled feeding study and had two periods in the study where they were fed a diet with E+ tall fescue seed which had a level of 3050 ppb ergovaline. The ability of the “resistant” bull’s calves to maintain body temperature may be a sign that selection is possible. Browning et al., (2004) reported that Hereford cattle were more susceptible to the ergots than Senepol cattle. Also Hereford cattle have been shown to be more susceptible than Brahman (Browning et al., 2004). These studies suggest that there could be a genotype by environment interaction. Both breeds (Senepol and Brahman) that were compared with Hereford are known to be heat tolerant, therefore the difference found could be the result of the heat tolerance and not tolerance to tall fescue toxicosis.

Mice have also been used as models for tall fescue toxicosis studies, and there have been lines of mice that have been selected for either resistance or susceptibility to tall fescue toxicosis. Hohenboken and Blodgett (1997) reported that for eight generations mice were selected for resistance or susceptibility based on their weight gain (post weaning) while consuming a diet containing E+ tall fescue seed. It was reported that selecting mice for resistance to tall fescue toxicosis was successful. The resistant line of mice did not have as great of a depression in growth and also had greater activities of two enzymes involved in detoxification reactions (Hohenboken and Blodgett, 1997).

Recently studies have explored the possibility of a genetic marker for selection of cattle resistance to tall fescue toxicosis. Looper et al., (2010) reported a

genetic marker for a gene in the enhancer region of the bovine prolactin gene.

Multiparous cows from a spring calving herd were used. These cows were purebred Angus, and Brahman, and their crossbred calves. The cows were genotyped and it was determined that the CC genotype is resistant and the TT genotype is susceptible to tall fescue toxicosis. They were also genotyped for a second SNP and the GG genotype was determined to be resistant and the AA was determined to be susceptible to tall fescue toxicosis. These cows were managed to achieve groups of low, moderate or high body condition scores. For the first SNP, cows with a CC genotype had increased calving rates ($P < 0.05$) over those animals with a homozygous recessive TT genotype. Results from the second SNP indicated that AG and GG genotype cows had earlier Julian calving dates than AA cows. This is important as Bourdon and Brinks (1983) suggested that if cows were managed for earlier Julian calving dates there would be an increase in the heritability of reproductive efficiency and profitability. This is due to calves that are born earlier in the calving season will be heavier at weaning, and the cows will have a longer period of time to rebreed and still calve within the defined calving season. In another study examining possible SNPs for resistance to tall fescue toxicosis, Rosenkrans et al. (2009) reported that there were two haplotypes within the heat shock protein 70 gene that increased the concentration of heat shock protein 70 when cattle grazed E+ tall fescue, and this interaction of haplotype and forage tended to be related to lifetime calving percentage.

Genome Wide Association Study

Simple traits controlled by only one or two genes have been instrumental in determining the actions of heredity, but most of the economically important traits in agriculture and important traits in medicine are complex or quantitative traits (Goddard and Hayes, 2009). Identifying the genes that control complex traits increase our knowledge of these traits, and would benefit animal agriculture through marker assisted selection. Recent advances in large panels of single nucleotide polymorphisms (SNP) in domesticated species has increased the interest in finding mutations that underlie the variation in quantitative traits through the use of genome wide association studies (Goddard and Hayes, 2009). Genome wide association studies (GWAS) have been used extensively starting in 2005 after the completion of the human genome project, and are continuing to be useful for increasing the understanding of disease resistance. The main goal of GWAS is to find biologic pathways for polygenic diseases and traits (Hirschhorn, 2009). Genome wide association studies have been able to identify pathways and genes that are known to be associated with different diseases, and have also been used to identify pathways and genes not thought to be associated with diseases (Hirschhorn, 2009). A GWAS compares SNPs from across the genome to a phenotype to determine what genes might be impacting that phenotype. The phenotype generally used is a disease state. Two groups, one that is impacted by the disease and one not, should be used. Differences are compared to see which SNPs are related to the disease state. Most common diseases are caused by many complex interactions between environmental factors and many genes (Wang et al., 2005). Because most common

diseases and disorders are impacted by many factors and genes a Genome Wide Association Study would be an ideal method to examine the disease.

The number of markers or SNPs required for GWAS is dependent upon the distance between the gene and the marker; this is referred to as linkage disequilibrium. Linkage disequilibrium is “the absence of linkage equilibrium so that the allele at one locus is correlated with the allele at another locus” (Goddard and Hayes, 2009). The closer the markers are to the gene the more closely the marker will be linked to the gene and the fewer markers you will need. If there is a large distance between the gene and the marker there is an increased chance that the marker will not be inherited with the gene.

There are generally two methods used for finding possible genetic markers. The first is a candidate gene study where the physiologic pathways that impact a disease are known. Only genes that control those pathways are investigated. In a GWAS the entire genome is scanned without focusing on one particular section of the genome. This is why GWAS are generally considered to be hypothesis free. In GWAS a phenotypic data is recorded for a sample of animals and then assayed for a genome-wide panel of markers. This is generally done with a SNP chip. For most GWAS the data are analyzed using a simple model that analyzes one SNP at a time to see the relationship between the disease state and the genotype at the SNP (Goddard and Hayes, 2009). Until recently cost was a prohibiting factor in completing GWAS but recently, several commercial SNP assays have been developed for different domestic species. Commercial Assays or SNP chips have

been developed for cattle, dogs, sheep, swine, and horses (Goddard and Hayes, 2009).

One problem with GWAS is the risk of false positives. Generally a 5% significance level is acceptable but with GWAS with 50,000 SNPs a 5% false discovery rate would result in 2,500 false positives. Another source of bias in the study that can lead to false discoveries is a mixture of samples that may be related to each other. In animal agriculture this is usually associated with breeds (Goddard and Hayes, 2009). Because of the relationships within breeds there may be a family that has an increased frequency of genetic markers which can lead to false positives, but this is easily adjusted for by including breed in the statistical model (Goddard and Hayes, 2009). Another issue with GWAS is that they can implicate genes whose functions are not known. This can be a problem currently but it will give ideas for future areas of research (Hirschhorn, 2009).

Summary

Tall fescue is the forage base for beef production in the humid mid-south transition zone and this area is home to approximately 20% of the U.S. beef herd. It has many agronomic features that make it an ideal forage for this area including drought and pest resistance. However there are drawbacks to using this forage for grazing livestock. Tall fescue toxicosis is the syndrome that is associated with animals grazing tall fescue infected with an endophyte. When animals consume E+ tall fescue, toxins are released which lead to the signs of tall fescue toxicosis, which include decreased circulating prolactin, intake, weight gain, and reproductive rates. There have been many attempts to reduce the impact of the endophyte but most of

these have either not worked or have not been adopted due to the high cost. Some of the methods that have been attempted are removing the endophyte, using tall fescue with endophytes which do not produce ergovaline, using drugs, and incorporating clovers and other forages to dilute the toxins.

Genetic resistance to tall fescue toxicosis has been suggested as a possible way to increase productivity of cattle grazing E+ tall fescue. There have been several studies using mice and cattle examining the possibility to select for animals that are more resistant to the toxins produced by E+ tall fescue. Recently there have been studies which identified possible genetic markers for resistance to tall fescue toxicosis. This could lead marker assisted selection for cattle that can consume E+ tall fescue and maintain performance. The current studies have only utilized candidate gene studies. These are limited to genes that are known to be related biological pathways impacted by the disease. A GWAS has the ability to examine large numbers of SNPs as potential markers. These SNPs are spread throughout the genome and are not restricted to known genes. This would be beneficial for tall fescue toxicosis because there are aspects of the disease that are unknown.

Our studies examined three possible ways to improve performance and productivity of beef cattle consuming E+ tall fescue that would be economical and easily implemented into current management strategies.

Objective 1 was to compare and contrast fall and spring calving herds consuming tall fescue. Fall calving beef herds should not be under the stress of tall fescue toxicosis during critical times of the year. Spring calving cows will have their

peak milk production coincide with the spring flush of forages. These differences in timing and biological cycles and performance of herds have not been examined when E+ tall fescue was the forage base for the cow herds.

Objective 2 was to validate a possible genetic marker for resistance to tall fescue toxicosis. This objective is broken down into 4 preliminary experiments. Experiment 1 examines serum prolactin concentrations and hair coat scores of steers grazing either E+ or Jesup MaxQ tall fescue. Experiment 2 examines serum prolactin concentrations and hair coat scores of steers grazing E+ tall fescue. Experiment 3 analyzed allelic and genotypic frequencies of cows grazing E+ tall fescue. This was conducted in Missouri and Tennessee and included both spring and fall calving cows. Experiment 4 examined production traits from spring and fall calving cows grazing E+ tall fescue in Tennessee.

Objective 3 was to identify possible genetic markers for resistance to tall fescue toxicosis through the use of the Illumina 50K Bovine SNP chip. This GWAS examined two groups of cows, one high performing and one low performing group. Performance was based on number of calves and adjusted 205-d weaning weight. The cows were from fall and spring calving herds and grazed E+ tall fescue for the majority of their lives.

**A Comparison of Spring and Fall Calving Beef Herds Grazing Endophyte
Infected Tall Fescue**

Abstract

Determining if a spring or fall calving season is the more efficient and profitable calving season for beef production has been debated heavily and researched slightly. In the mid-south transition zone tall fescue toxicosis plays a vital role in stress placed on cows and no research has been conducted comparing spring and fall calving seasons when cows are grazing tall fescue. Nineteen years of beef cow herd records were obtained from the Research and Education Center at Ames Plantation. The cow herds were under the same management and all cows were strictly culled for reproductive failure, age, and low performance of their calves. The cows primarily grazed tall fescue (*Lolium arundinaceum* Schreb.) with the wild-type endophyte (*Neotyphodium coenophialum*) that is known to cause the symptoms of tall fescue toxicosis. For the 19 years the total number of cows and calves were 478; 1534 and 474; 1727 for the spring and fall calving herds, respectively. Phenotypic parameters were days to first calf, calving interval, number of calves born, birth weight (BW), weaning weight of calves, adjusted 205-d weaning weight (205-d WW), average daily gain (ADG) from birth to weaning, and weight/day of age. The data were analyzed using a randomized block design. Cows in the spring calving herd averaged 27 days older at calving than the fall calving cows ($P < 0.0001$). The spring calving herd had a shorter calving interval ($P < 0.05$), and produced fewer calves per cow ($P < 0.05$). While calves born in the spring and fall herd had similar BW ($P = 0.751$), the calves born in the spring had a greater ADG and greater 205-d WW than those born in the fall. Even though the spring born calves gain faster and have greater 205-d WW the fall calving herd should increase

the income of the farm due to greater number of calves and lowered costs of replacement heifers.

Introduction

Beef production in Tennessee is primarily cow calf production and the vast majority of the cows calve in the early spring of the year. This spring calving season generally runs from January until the mid-April. The other popular controlled calving season is a fall calving season' starting in mid-September and ending in mid-November. There has been much debate and limited research to evaluate which calving season, spring or fall, is more advantageous. The timing of breeding and calving season may impact several components of the production system (May et al., 1999). Most research completed to date has focused mainly on the reproductive aspects of the cow herd. Several studies have shown that there is a shorter anestrus period for cows calving in the fall compared to those calving in the spring (Peters and Riley, 1982; King and Macleod, 1984). In a study that reviewed 15 years of data encompassing 1909 records of calves from Brahman x Hereford cows in Overton, TX, Gaertner et al., (1992) reported that cows that calve during the fall will wean heavier calves than those that calve during the winter or spring. In this study steer calves born from September 1st to December 15th had an average weaning weight of 305.1kg and those born from March 16th to May 31st weighed 207.1 kg at weaning ($P < 0.01$). Fall calving allows producers who market their calves at weaning to exploit traditionally greater seasonal market prices in June and July rather than October (Kreft et al., 1998). The objective of this study was to analyze the records of beef cows from Ames Plantation and determine which calving season was more productive and potentially more profitable.

Material and Methods

The beef herds at Ames Plantation are unique, in that the herds have been maintained under the same manager for the past 19 years. The herds are maintained as a true fall and a true spring calving herd in that cows are not switched between herds. The fall calving herd begins calving in mid-September and continued through mid-November. The spring calving herd begins calving in mid-February and continued through mid-April. Within each fall and spring calving herd there was a group of commercial and purebred Angus cows. This Angus herd is the third oldest pure bred Angus herd in the nation. Most bulls and replacement heifers are raised on the plantation, but bulls have been purchased to maintain genetic diversity in the herds. The commercial herd is primarily Angus genetics with minimal Simmental and Hereford influence. The bulls for the commercial herd are all purebred Angus. They are grazed primarily on tall fescue and supplemented with free choice mineral all year and are fed corn silage during the winter as needed. Cows are strictly culled due to failure to re-breed and for poor calf performance. Over the 19 years of this study the spring calving herd consisted of 478 cows and 1534 calves and the fall calving herd was 474 cows and 1727 calves. These records only include those cows which had a calf so percent calf crop was not calculated. These are the number of individual cows and their calves that were in the herds at some point during the entire study. The number of calves produced per year is shown in Figure 1.1. For this study the oldest cows were born in 1987 and had their first calves in 1990.

The herd records from Ames Plantation were obtained from hand written herd books and entered into an Excel® spreadsheet. The cow records include the cow's identification number, breed, calving herd, sire, dam, and date of birth. The records were also recorded on all of the calves produced by each cow; including calf number, date of birth, birth weight, weaning date, weaning weight and sire of calves.

These records were analyzed using the mixed model analysis of variance procedure in SAS version 9.2 (Carey, NC). Because it was thought that years within the study would differ, a randomized block design was utilized to control for variation of year. Treatments were either spring or fall calving herd. The level of significance was set at $P < 0.05$. Sire was also included in the model initially but was removed due to non-significance. This is most likely due to the same sires being used for both herds and the sires' similar genetic makeup.

Several different measures of productivity were used to make comparisons between the fall and spring calving herds. These include adjusted 205-d weaning weight, average daily gain, and weight per day of age. The number of days to first calving is the number of days from the dam's date of birth until her first calf is born. Weaning weight (WW) is a measure of the growth potential of the calf as well as a measure of the mothering ability of the dam. Because actual weaning weight is influenced by the age of the calf at weaning, sex of the calf, and age of the dam, adjusted 205-d weights is used as a comparison of calves and cows of different ages. Adjusted 205-d weaning weight was determined by following the formula shown below (BIF, 2010).

$$\text{Adj. 205-day wt.} = \frac{\text{Actual weaning wt.} - \text{Birth wt.}}{\text{Age at weaning in days}} \times 205 + \text{Birth wt.} + \text{Dam age adj.}$$

Average daily gain from birth to weaning = (WW-BW)/age at weaning.

Weight per day of age = WW/age at weaning.

Where:

Actual weaning weight = the weight of the calf on the day that it was weaned.

Birth weight = the weight of the calf on the day it was born shortly after birth.

Age at weaning = the number of days between the birth date of the calf and the date of weaning.

Adjustments for the age of the dam are shown in Table 1.

Value of the calves produced was determined by calculating the average weaning weight for steers and heifers from both herds. Steers in the spring herd averaged 241 kg while those in the fall herd averaged 235 kg. Heifers in both herds averaged 226 kg. Prices were obtained from the USDA Agricultural Market Service Livestock and Seed Division in Nashville (USDA-NASS). Price for steers and heifers was determined using the average price for steers and heifers for the month of weaning for the years 1995 to 2008. For steers the price used was the average price paid for steers weighing between 250 and 273 kg, and for heifers it was the average price paid for heifers weighing between 204 and 227 kg. The spring calving herd was weaned in October and the fall calving herd was weaned in June. Value

on a per cow basis was determined by adding the value of steers and heifers, for a particular herd and dividing that number by the number of cows in the herd. This measure allows for a comparison of gross income produce by each cow.

Results and Discussion

The most important part of beef production is the production of calves. Beef producers plan for their heifers to have their first calf at two years, about 730 days of age. In this study the number of days to the first calf was analyzed (Table 2). The fall calving herd heifers averaged 744 days of age at first calving and the spring herd heifers averaged 771 days at first calving ($P < 0.001$). This finding differs from other studies. Bagley et al., (1987) reported that cows in a fall calving herd were older at first calving than heifers in a spring calving herd. These differences are probably due to the forage the animals were grazing. In this study the cows were not grazing E+ tall fescue. Because heifers are still growing, and lactating after the birth of their first calf, a heifer that calves earlier will have more time to re-breed and still be in the calving season. Heifers that have their first calf earlier will also be the more productive cow and produce more kilograms of calf in their lifetime than heifers that have their first calf later (Lesmeister et al., 1973). Another study, which analyzed 92 cows for 15 years, reported that heifers calving at two years of age will, in their lifetime, produce more kilograms of calf at weaning than their counterparts calving at three years of age (Pinney et al., 1972). While the difference in days to first calf in this study is fairly short, approximately one month, the difference in number of calves per cow is supported by the findings of others.

Calving interval, another measure of reproductive success, is the number of days between births of subsequent calves from one cow (Table 2). The ideal calving interval is approximately one calf every 365 days. The shorter the time between calves, the more efficient the cows are over a lifetime of production. Wilson and Willis (1974) reported that cows that had shorter calving intervals would have more calves in their lifetime. Cows in the fall calving Ames Plantation herd had a longer calving interval than did the spring calving herd by ten days ($P = 0.0043$), but had more calves in their lifetime. A similar ten day longer calving interval was also reported by Rakestraw et al., (1986). This study examined differences in weight loss after calving and during breeding. Cows calving in the fall who maintained weight after calving did not experience the increase in calving interval (Rakestraw et al. 1986). Based on this research the longer calving interval of the fall calving herd may indicate that the fall calving cows need to have more nutrients supplemented to them after they calve. In contrast to what was reported by Peters and Riley (1982) that cows exposed to longer photoperiod during late pregnancy will begin cycling faster after parturition than those exposed to a shorter photoperiod. In our study the cattle exposed to longer photoperiod during late pregnancy had a longer calving interval. Factors other than photoperiod may also be impacting the calving interval.

The season of breeding was also reported to have more of an impact on anestrus period than either the type of cow (purebred or crossbred) or the energy content of the ration that was fed (King and Macleod, 1984). King and Macleod (1984) reported that after 60 days postpartum over 90% of fall calving cows had ovulated while only 56% of spring calving cows had ovulated regardless of cow type

or energy content of the diet. These studies both suggest that the fall calving cows should be cycling sooner and have a decreased calving interval when compared with the spring calving cows. In our study the fall calving cows had a longer calving interval than did the spring calving cows. This is likely due to lowered nutrient content of the diet shortly after calving. At this time nutrient requirements of cows are at their highest. Due to lower nutrient content of forages in the winter there is a nutrient deficiency and cows will take longer to begin cycling.

A longer calving interval could result in the fall calving herd having greater cull rates due to the cows not becoming pregnant within the breeding season. However the fall herd in this study had more calves per cow on average than did the spring herd ($P < 0.05$). Figure 1.2 shows the average age distribution of cows in the fall and spring calving herds in one year. The spring calving herd contained more 2 year old cows than did the fall herd. The fall herd averaged 3.6 calves per cow while the spring calving herd averaged 3.2 calves per cow for the entire study ($P < 0.05$). Figure 1.3 shows the number of calves that a cow had in her lifetime. The spring herd had more cows that only had one or two calves while the fall herd had more cows that had 7, 8, 9, and 10 calves. There are more cows in the spring herd that had fewer calves in their lifetime than the fall herd. Cows that have more calves will remain in the herd longer increasing the profits for that herd. The decrease in longevity of cows in the spring calving herd is most likely due to the increase in ambient temperature at the time of breeding and rebreeding for the spring calving herd. Breeding season for the spring herd is generally from mid-April through mid-July. It has been widely reported that when ambient temperatures are increased

during breeding there will be a reduction in the calving rate (Ulberg and Burfening, 1967; Cavestany et al., 1985; West et al., 2005). Ulberg and Burfening (1967) reported that for every 1°C increase in rectal temperature there was a 25% decrease in pregnancy rate. The spring calving cows in this study are also grazing E+ tall fescue which has been shown to reduce the calving rate as well (Porter and Thompson, 1992). Schuenemann et al., (2005) reported that when animals were supplemented with ergotamine tartrate to simulate tall fescue toxicosis the embryo development was retarded. If spring calving cows are not becoming pregnant early in the breeding season then the heat stress coupled with tall fescue toxicosis will lower the odds of them becoming pregnant, thus they are culled. This causes a shift in the distribution of calving in the spring herd to earlier in the calving season (Figure 1.4). A dummy regression was run on the slopes of the trend lines for the weeks 1-5, and 5-10 of the calving season. Slopes of the trendlines were 3.137 and 4.414, for the first half of the calving season for fall and spring calving herds respectively. There was no difference in the slope ($P > 0.05$) but there was a trend for the slope of the trendline for the spring calving herd to be greater than that for the fall calving herd. This suggests that the majority of the spring calving cows are calving early in the calving season indicating that they became pregnant early in the breeding season, and if they do not become pregnant early in the breeding season it decreases the chances that they will eventually become pregnant.

When comparing calving rates of the two herds the economic impact of having to replace more cows in the spring herd must be considered. Increased rate of replacement affects both income and expenses (Mark and Rasby, 2004). In an

economic simulation study, Mark and Rasby (2004) evaluated different replacement rates for cows and the impact on income of beef operations. In herds that have greater cull rates of cows their income from heifer calf sales will decrease and income from cull cow sales will increase, there may also be a difference in the income of steer calves due to heifers weaning lighter weight calves than mature cows (Mark and Rasby, 2004). Also the nutrition costs will also be increased for the heifers, since they are growing and require greater quality feed and forage due to increased nutrient demand. Mark and Rasby, (2004) reported that the difference in a 10 and 30% replacement rate in a cow herd has an economic loss associated with the greater replacement rate of 92.86 dollars per head. This would indicate that with a 30% replacement rate in a 100 cow herd there is a loss of \$9,286 due to having to replace more cows (Mark and Rasby, 2004). Because two year old cows that have just had their first calf have the highest nutrient requirements of any animal in a beef herd they are at the greatest risk of being culled from the herd due to reduced conception rate. The increased nutrient requirements are due to nutrient demands for growth as well as for lactation. Cull rates of two year old cows were compared between the two herds (Figure 1.5). Two year old cows in the spring calving herd were culled at a greater rate than those in the fall calving herd ($P = 0.026$). In the spring calving herd 30% of two year old cows were culled after having their first calf while only 16% of fall calving two year olds were culled. This is probably due to the added stress placed on spring calving cows, during breeding from heat and tall fescue toxicosis. On average cows stayed in the spring herd for 3 years and in the fall herd for 4 years ($P < 0.05$). This lower replacement rate would increase the

profitability of the fall calving herd. In 2002 there was a large spike in the number of 2 year olds in the spring calving herd. This is probably due to climatic conditions. In 1998, 1999, and 2000 there was a drought in Tennessee. During drought conditions there is an increase in heat stress coupled with lower forage quantities which would increase the stress on cattle and reduce reproductive rates in cows as well as replacement heifers. In 2001 the drought ended and greater numbers of heifers were able to become pregnant which caused the increase in 2-year old cows in 2002. A similar increase in numbers of 2-year old cows was seen in 2007 and a similar pattern of drought was seen in 2005 and increased rainfall in 2006. The changes in rainfall help to explain major variations in numbers of replacement heifers seen in certain years.

Calves are sold on a weight basis so cows that produce heavier weight calves will increase the gross income for the beef producer. There was no difference found in the BW of the calves between the spring and fall calving herds (Table 3). This is similar to results reported by Bagley et al., (1987) who also found no difference when comparing the BW of calves born in the spring or fall. In this study there was no difference in the BW of calves born in the spring or fall ($P = 0.751$). Birth weights for the spring and fall herd were 33.25 and 33.18 kg, respectively. Similar BW between the two herds is probably related to similar genetics and management. Both of the Ames Plantation herds were under the same management protocol, grazed similar pastures, were often bred to the same bulls, and were given the same nutritional supplements.

Most calves in the humid mid-south transition zone of the U.S. are sold at weaning, so the price paid for the calves is based on their weaning weight. The weaning weight (Table 3) of the calves born in the spring and the fall were similar ($P = 0.1138$). But the adjusted 205-d weaning weights (Table 3) of the spring and fall herd were different ($P < 0.05$). Adjusted 205-d weaning weights for the spring and fall herd were 250.6 and 234.1 kg respectively. Using 205-d WW allows the comparison of cows that are different ages and calves that were weaned at different ages. Differences in adjusted 205-d weights are due to the spring herd having greater numbers of young cows and calves born in the fall which are older at weaning. The fall and spring calving herds averaged 257 days and 239 days from birth to weaning respectively (Table 2). The longer time from birth to weaning for the fall herd ($P < 0.001$) allows them to have similar weaning weights even though the ADG for the fall herd was lower ($P = 0.0096$). If the calves born in the fall did not have the added 18 days to grow they would be about 16 kg lighter than the calves born in the spring. Our findings differ from others comparing weaning weight for spring and fall calving herds. Gaertner et al., (1992) reported that calves born in the fall and winter will have greater weaning weights than those born in the spring. The calves in our study were similar in BW and WW but the calves born in the fall grew at a lower rate and had a longer time to weaning. The difference in ADG between the fall and spring herd was 0.04 kg per day ($P < 0.05$). Adjusted 205-d weaning weights were used to compare the herds for production adjusting for age of dam and age of the calves while the actual weaning weight was used for economic calculations.

Cow/calf producers have several options for marketing calves at weaning: direct sale or retained ownership through the stocker for finishing phase to increase profits (Reisenauer Leesburg et al., 2007). Most calves in the southeastern United States are marketed at weaning. Value of calves the calves sold is determined by the weight of the animals and the price paid. Prices of calves are generally greater in June than it is in October (Figure 1.6). The steers from the fall herd and spring herd had a total value of \$435,351 and \$352,911, respectively. The heifers from the fall and spring herd had a total value of \$373,406 and \$313,138, respectively (Figure 1.7) over the entire study. When the value of calves was examined on a yearly basis the steers from the fall and spring herd averaged \$22,913 and \$18,574, respectively. The heifers from the fall and spring herd averaged \$19,653 and \$16,481 per year, respectively. The difference in value of the calves born in the fall and spring herd was \$142,708 or \$7,511 per year. This price difference was due in part to the greater price that is generally paid for calves in June. Because the southeast has predominately spring calving cows, during October the market is generally saturated with calves weighing between 227 and 272 kg, driving the price down (Figure 1.6). Cow/calf producers who maintain a spring calving herd have the ability to retain their calves through the winter in a stocker phase and market them in the spring. This production practice would allow producers to sell heavier weight animals along with the exploiting the traditionally greater markets of early spring and increase gross income. In the Ames Plantation herds the fall calving cows had more calves per cow than did the spring calving herd (Figure 1.3). Therefore, more calves to market in June than in October added to the total value of the fall calving herd.

The fall herd also did not have to replace as many females as did the spring herd and was able to market more heifers than the spring calving herd. The cost of raising a replacement heifer from birth to calving is different for every farm. There are many factors that can change the cost such as nutrition, management, interest, and veterinary costs. Dhuyvetter et al., (2012) estimated costs of producing replacement heifers and included both explicit costs as well as implicit costs such as opportunity cost, operator labor, and owned feed (Table 4). The cost of raising replacement heifers can be quite high (Dhuyvetter et al., 2012). The cost of replacement heifers was greater for the spring herd. Due to greater cull rates there would be fewer heifers to market as they would have to be retained to replace cows which did not calve.

In order to standardize a value per cow to best assess the differences in each herd, the total value for steers and heifers sold, in each herd was divided by the number of cows in each herd. This gives us a value of cows on a per head basis allowing the comparison of the value of cows in the herds, even though there are different numbers of cows in each herd. The average value of a cow in the fall and spring calving herd was \$1,702 and \$1,393 respectively. Thus these values indicate that on average a fall calving cow will gross \$308/cow more for Ames Plantation over the lifetime of these herds. The number of cows in each herd was similar over the time period of this study but the fall calving herd produced a more income for the farm. This is due to the fall calving herd producing more calves/cow, selling calves at greater prices, and not having to replace as many cows.

Conclusions

While the spring calving herd is the most traditional, it is not the best time for calving in the mid-south region of the U.S. This study indicates that a fall calving season would allow for the cows to stay in the herd longer and produce more calves which would result in more income for the producers. There are tradeoffs between having a spring calving herd or a fall calving herd. The spring calving herd will have calves that have a greater ADG from birth to weaning and will have greater WW when calves are weaned at a standard age. Savings from not having to replace cows as often, having greater calving rates, and more calves to market with the fall calving herd should outweigh the benefits of the spring calving herd making the fall herd more desirable.

Literature Cited

- Bagley, C. P., J. C. Carpenter Jr., J. I. Feazel, F. G. Hembry, D. C. Huffman, and K. L. Koonce. 1987. Influence of calving season and stocking rate on beef cow-calf productivity. *J. Anim. Sci.* 64:687-694.
- Beef Improvement Federation. 2010. BIF Guidelines for uniform beef improvement programs (9th Ed.). Raleigh, NC.
- Cavestany, D., A. B. El-Wishy, and R. H. Foote. 1985. Effect of season and high environmental temperature on fertility of Holstein cattle. *J Dairy Sci.* 68:1471-1478.
- Dhuyvetter, K.C., M. Langemeier, and S. Johnson. 2012. Raising beef replacement heifers. Kansas State University: Farm Management Guide. Accessed Mar. 1, 2012. <http://www.ksre.ksu.edu/library/agec2/MF2566.pdf>.
- Gaertner, S. J., F. M. Rouquette Jr., C. R. Long, and J. W. Turner. 1992. Influence of calving season and stocking rate on birth weight and weaning weight of Simmental-sired calves from Brahman-Hereford F1 dams. *J. Anim. Sci.* 70:2296-2303.
- King, G. J. ,and G. K. Macleod. 1984. Reproductive function in beef cows calving in the spring or fall. *Anim. Reprod. Sci.* 6:255-266.
- Kreft, B., J. Kreft, R. Cargo, D. Schmidt. 1998. Fall calving in North Dakota. North Dakota State University Central Grasslands Research Extension Center. Accessed Feb. 15, 2012. <http://ag.ndsu.nodak.edu/streeter/98report/fall98.htm>.

- Lesmeister, J. L., P. J. Burfening, and R. L. Blackwell. 1973. Date of first calving in beef cows and subsequent calf production. *J. Anim. Sci.* 36:1-6.
- Mark, D. R., and R. J. Rasby. 2004. Effects of replacement rate on cow herd budget. Faculty Publication: Agricultural Economics. Paper 59. Accessed Feb. 12, 2012. <http://digitalcommons.unl.edu/ageconfacpub/59>.
- Peters, A. R., and C. M. Riley. 1982. Milk progesterone profiles and factors affecting post-partum ovarian activity in beef cows. *Anim. Prod.* 34:145-153.
- Pinney, D. O., D. F. Stephens, and L. S. Pope. 1972. Lifetime effects of winter supplemental feed level and age at parturition on range beef cows. *J. Anim. Sci.* 34:1067-1074.
- May, G. J., L. W. Van Tassell, J. W. Waggoner, and M. A. Smith. 1999. Relative costs and feeding strategies associated with winter/spring calving. *J. Range Manage.* 52:560-568.
- Rakestraw, J., Lusby, K. S., Wettemann, R. P., and Wagner, J. J. 1986. Postpartum weight and body condition loss and performance of fall-calving cows. *Theriogenology.* 26:461-473.
- Reisenauer Leesburg, V. L., M. W. Tess, and D. Griffith. 2007. Evaluation of calving seasons and marketing strategies in Northern Great Plains beef enterprises. I. Cow-calf systems. *J. Anim. Sci.* 85:2314-2321.
- Schuenemann, G.M., M. E. Hockett, J. L. Edwards, N. R. Rohrbach, K. F. Breuel, and F. N. Schrick. 2005. Embryo development and survival in beef cattle administered ergotamine tartrate to simulate fescue toxicosis. *Reprod. Biol.* 2:137-150.

Ulberg, L. C. and P. J. Burfening. 1967. Embryo death resulting from adverse environment on spermatozoa or ova. *J. Anim. Sci.* 26:571-577.

U.S. Department of Agriculture, Agricultural Marketing- Livestock and Seed Division
1995-2009 *Monthly Averages (for each year)*, Nashville, TN.

West, J. W. 2003. Effects of heat stress on production in cattle. *J. Dairy Sci.* 86:2131–2144.

Wilson, A., and M. B. Willis. 1974. Comparative reproductive performance of Brahman and Santa Gertrudis cattle in a hot humid environment 2. Factors affecting calving interval. *Anim. Prod.* 18:43-48.

Table 1.1. Standard age of dam adjustment factors (kg) for adjusted birth weight and adjusted 205-day weaning weight¹

Age of Dam (Years) ²	Birth Weight Adjustment Factor	Weaning Weight Adjustment Factor	
		Male	Female
2	3.64	27.27	24.54
3	2.27	18.18	16.36
4	0.90	9.09	8.18
5-10	0.00	0.00	0.00
11 & Older	1.36	9.09	8.18

¹Beef Improvement Federation (2010)

²Age of dam at calving

Table 1.2. Least squares means for calving interval, days to weaning and days to first calf^{1,2}

Treatment	Days to First Calf ³	Calving Interval ⁴
Fall Calving	744 ^b	400 ^a
Spring Calving	771 ^a	390 ^b

¹All measurements were done in days

²Means within a column with no common letter differ, $P < 0.05$

³Days to first calf = Number of days from the birth of a cow to the date of birth of her first calf

⁴Calving interval = Number of days between birth dates of one cows consecutive calves

Table 1.3. Least squares means for birth weight, weaning weight, and average daily gain^{1,2}

Treatment	BW ³	WW ⁴	ADJ 205 ⁵	ADG ⁶
Fall Calving	33.25 ^a	236.67 ^a	234.10 ^b	0.79 ^a
Spring Calving	33.18 ^a	232.37 ^a	250.61 ^a	0.83 ^b

¹All measurements are expressed in kilograms

²Means within a column with no common letter differ, $P < 0.05$

³BW = Birth weight

⁴WW = Weaning weight

⁵ADJ 205 = Adjusted 205 day weaning weight

⁶ADG = Average daily gain

Table 1.4. Sample budget for raising a replacement heifer from weaning to first calf¹.

Input	Cost
Opportunity cost of the heifer (250 kg)	\$730.84
Feed Costs	\$498.23
Labor (5 hrs @ \$10.00/hr)	\$50.00
Veterinary, drugs, supplies	\$25.50
Marketing costs	\$12.00
Breeding costs	\$33.63
Utilities, fuel, oil	\$33.39
Facility and equipment repairs	\$35.76
Miscellaneous	\$17.84
Depreciation on facilities and equipment	\$10.09
Interest on facilities and equipment	\$5.49
Insurance and taxes on facilities and equipment	\$0.49
Interest on Heifer calf and ½ operating costs @ 6.5%	\$97.40
Professional fees (legal, accounting, etc.)	\$4.96
Total	\$1555.62

¹Adapted from “Raising beef replacement heifers” Dhuyvetter et al., (2011)

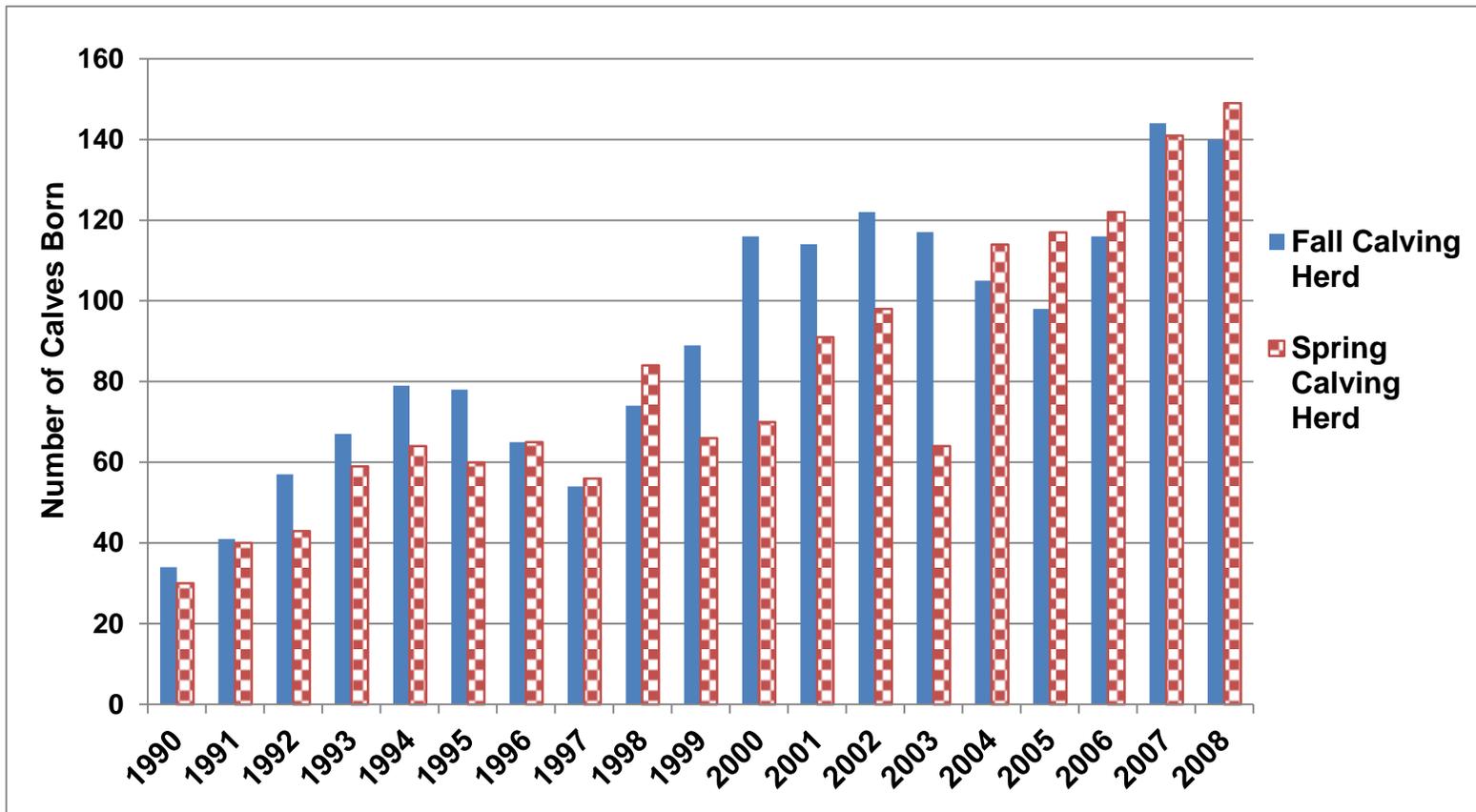


Figure 1.1. Number of calves born per year in spring and fall calving herds.

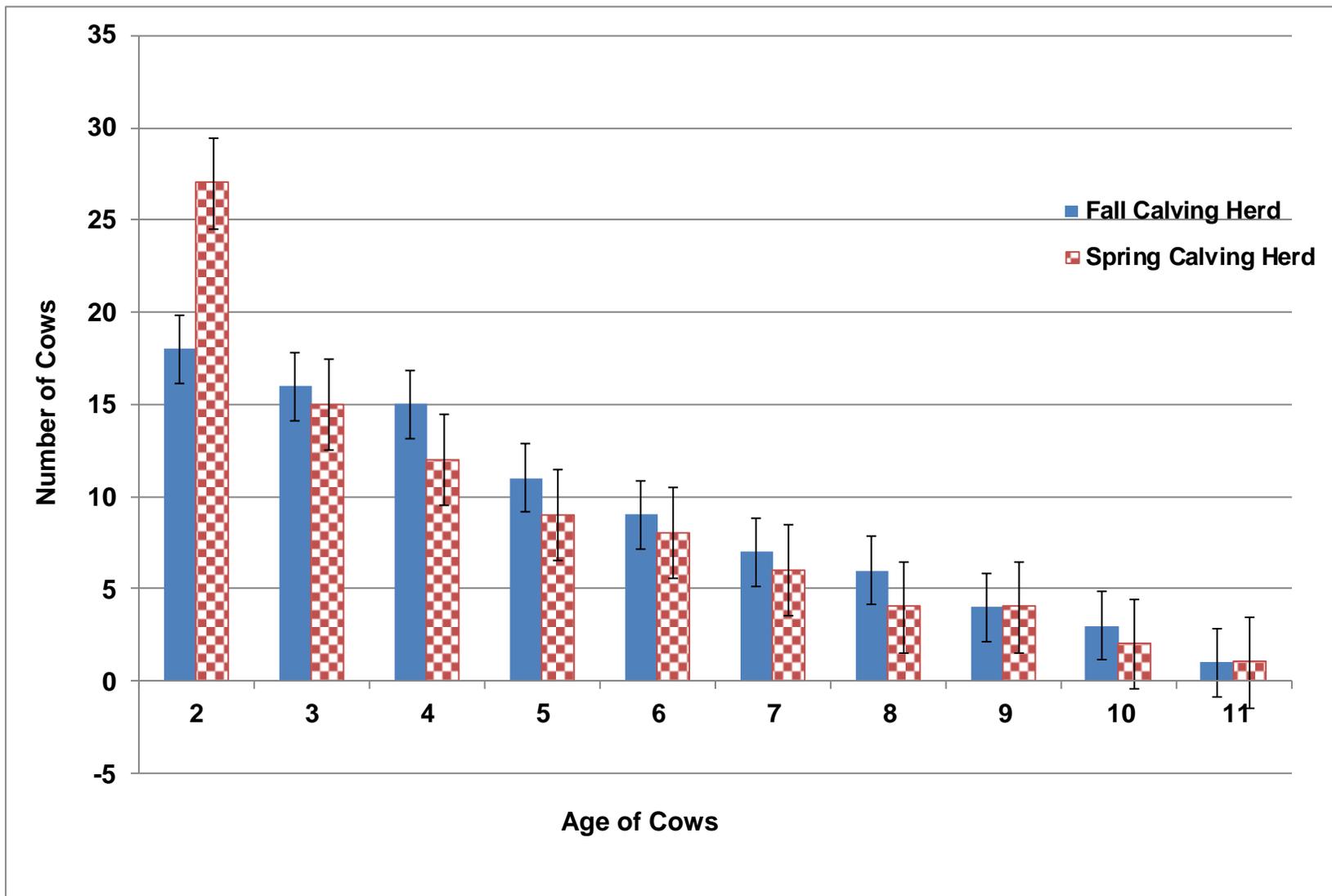


Figure 1.2. Average age of cows in the herd.

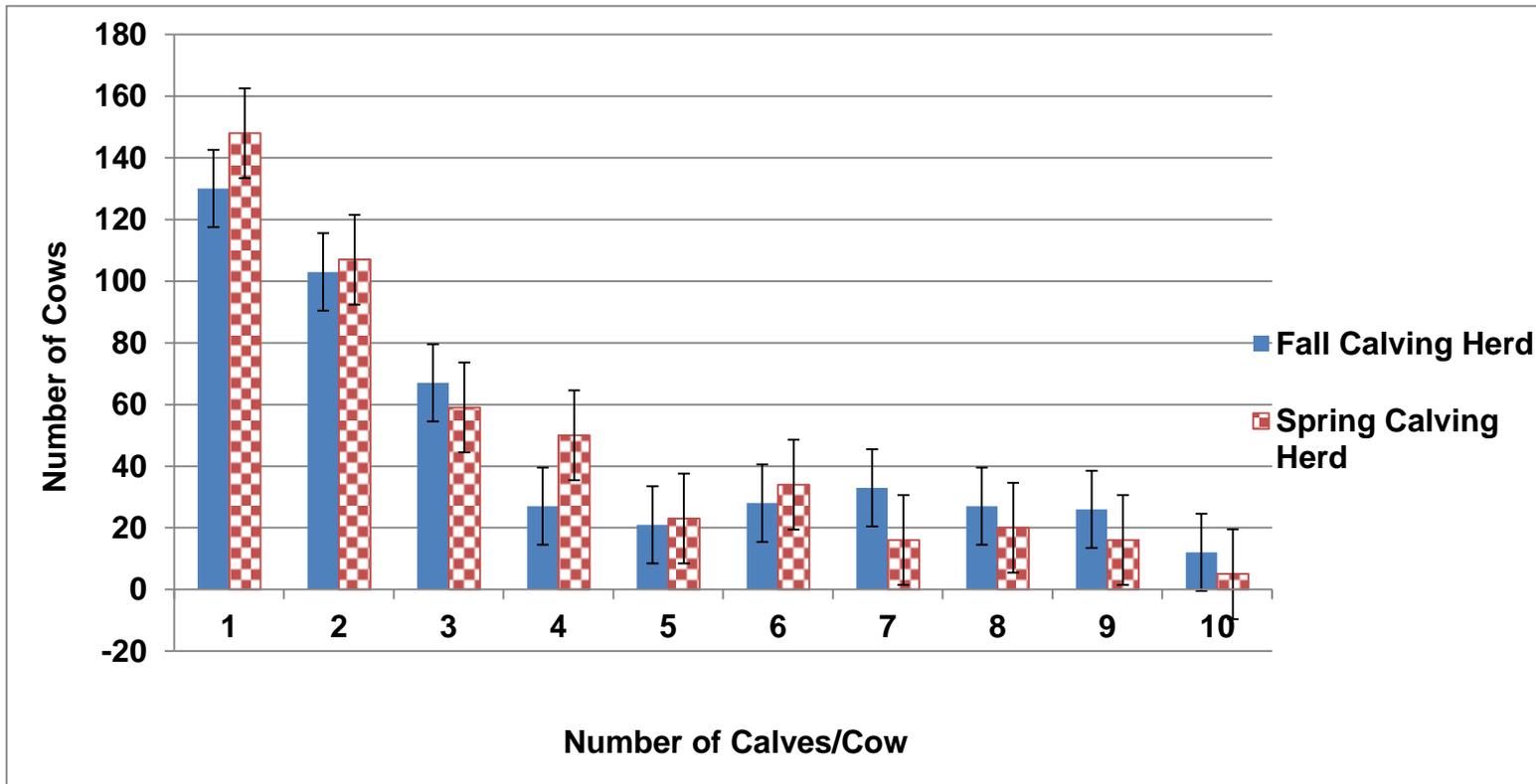


Figure 1.3. Number of calves per cow in the spring and fall calving herds for the entire study.

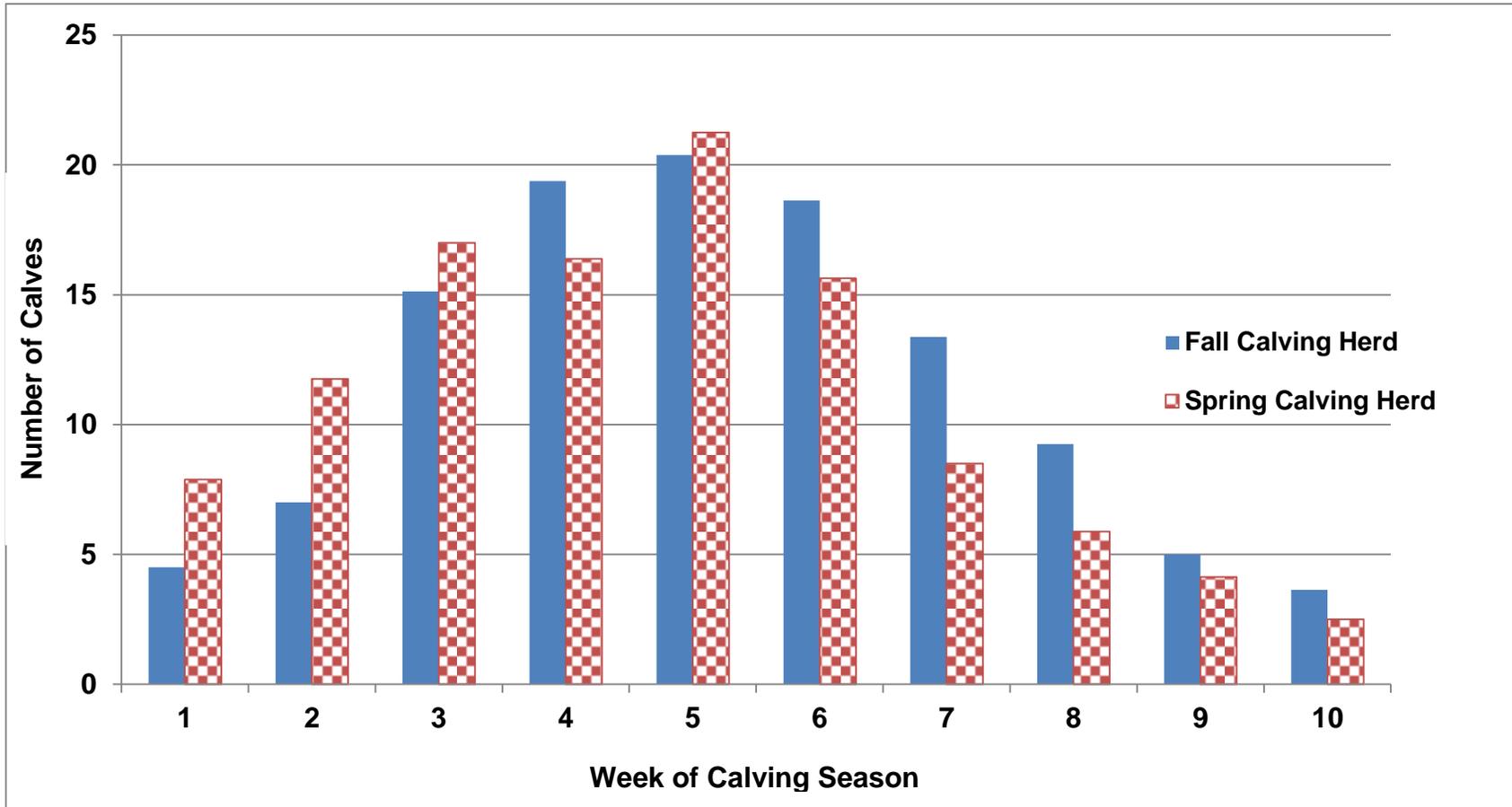


Figure 1.4. Average calving distribution for the Ames Plantation spring and fall calving herds.

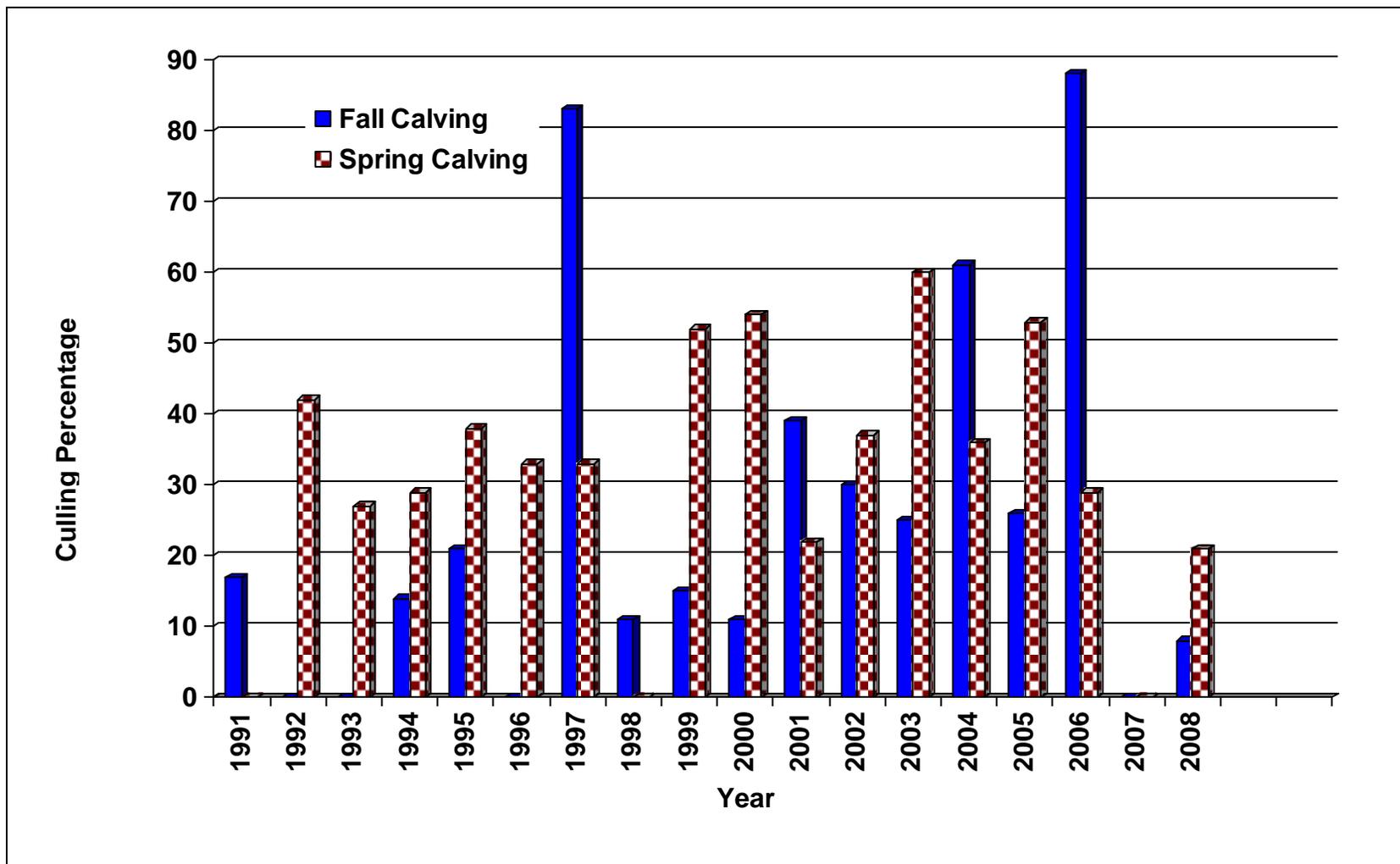


Figure 1.5. Percent of two year old cows culled from the herd per year.

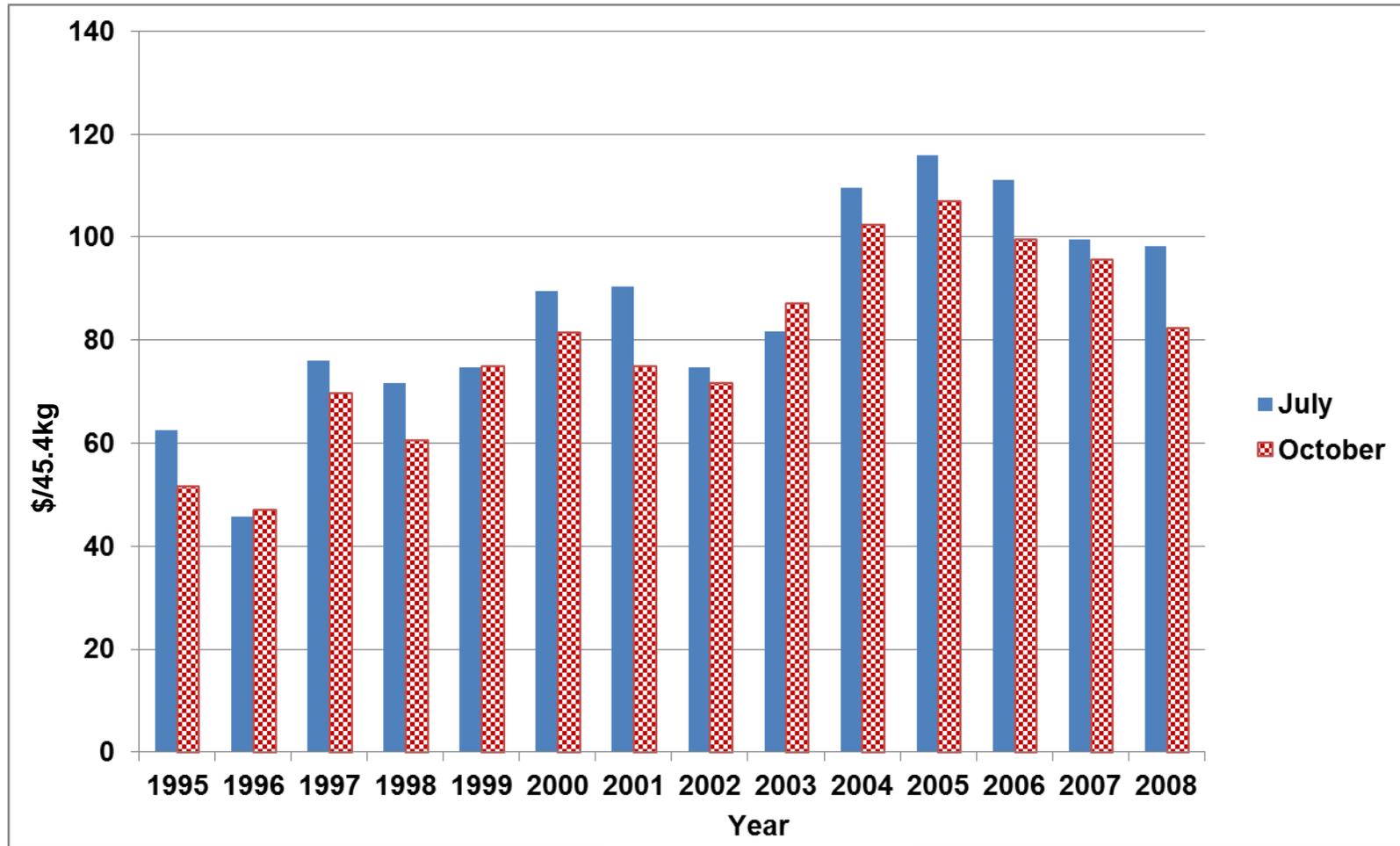


Figure 1.6. Average price of 227-272 kg steers sold through livestock auctions in the United States in July and October.

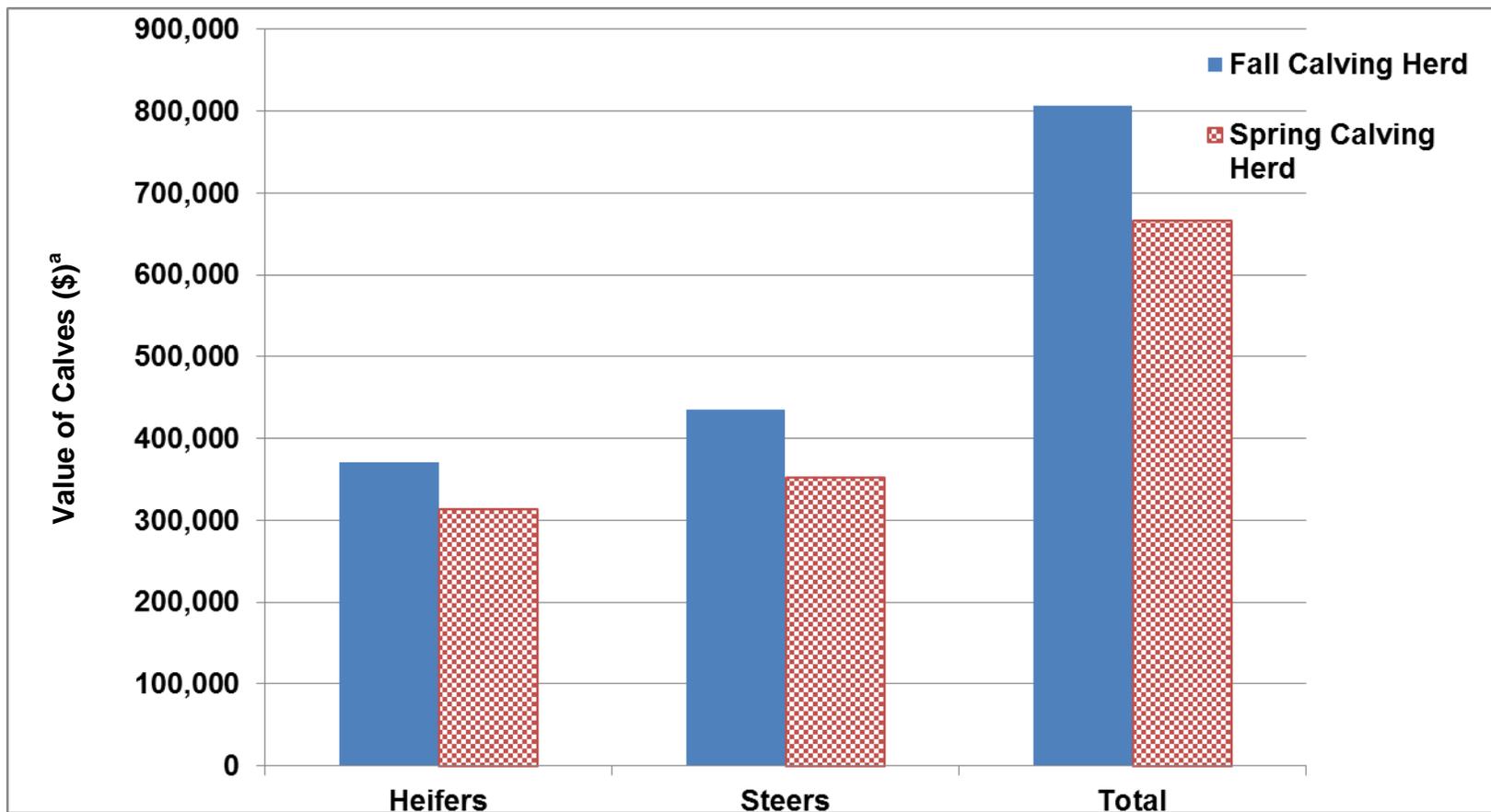


Figure 1.7. Value of heifers, steers and all calves sold at weaning for each herd

^aValue of the animals was calculated by taking the average price paid for steers and heifers from 1995-2008 at the time of weaning and multiplying by the total number of steers and heifers.

**Validation of Single Nucleotide Polymorphism on DRD2 Gene in Angus Based
Cattle**

Abstract

Tall fescue (*Lolium arundinaceum* Schreb.) pastures are widespread in the Southeastern United States. Typically, the tall fescue in these pastures is infected with an ergot-alkaloid producing strain of the endophytic fungus *Neotyphodium coenophialum*. The toxic alkaloids in the forage of these pastures are responsible for tall fescue toxicosis (FT), a syndrome which may include reduced animal feed intake, weight gain, circulating prolactin concentrations, fertility and thermoregulation, while increasing vasoconstriction and thickness of the summer hair coat. Prolactin concentrations are known to be controlled by dopamine and the drug domperidone, a known dopamine antagonist has been shown to negate the effects of tall fescue toxicosis. An intronic SNP was discovered within the dopamine receptor D2 gene (DRD2) where a guanine/adenine substitution existed, creating two alleles, G and A. Forty-two Angus-based steers were grazed on 'Kentucky 31' tall fescue containing a toxic form of the endophyte (endophyte-infected fescue or E+; n = 21), or 'Jesup MaxQ' tall fescue containing a non-toxic strain of endophyte (non-toxic endophyte-infected fescue or NTE; n = 21). Homozygous GG steers grazing E+ had decreased serum prolactin concentrations in May compared to AA steers ($P < 0.05$). Effect of genotype was then assessed in 53 Angus-based steers that grazed on E+ tall fescue. Genotype was associated with serum prolactin concentrations ($P = 0.004$) and hair coat score ($P = 0.01$) such that GG animals had decreased prolactin and increased hair coat scores relative to AA animals. As many herds in Tennessee and Missouri are spring-calving and therefore more prone to the effects of FT, we hypothesized that non-intentional

selection for the advantageous allele was occurring in spring-calving herds (SP) but not in fall-calving herds (FA). The “A” allele was more prevalent ($P = 0.016$) in SP animals ($n = 269$) relative to FA animals ($n = 357$), and genotypic frequencies differed ($P = 0.026$) between these populations such that the frequency of the AA genotype was greater (26.02% vs. 20.73%) and the frequency of the GG genotype was decreased (22.30% vs. 32.21%) in SP relative to FA. These data suggest that the advantageous allele is more prevalent in herds more affected by FT (e.g. spring-calving Angus cattle grazed on tall fescue), perhaps a byproduct of selection for longevity, fertility, and growth in cow-calf herds affected by FT. The DRD2 SNP may have use in the selection of animals resistant to fescue toxicosis.

Introduction

Tall fescue (*Lolium arundinaceum* Schreb.), a cool-season perennial bunch grass, is the most prevalent forage in the Southeastern United States (Stuedemann and Hoveland, 1988). Tall fescue is easily established, adaptable to a wide range of environments, and is tolerant of grazing (Hill et al., 1991). Most positive attributes associated with tall fescue are directly or indirectly linked to the presence of an endophytic fungus (*N. coenophialum*); however, ingestion of the ergot-like alkaloids produced by endemic strains of the endophyte causes a disorder in grazing animals known as tall fescue toxicosis (FT). Ergovaline, an ergopeptide and a known dopamine agonist produced by the endophyte, has been implicated as the primary cause of the toxicosis (Yates et al., 1985, Klotz et al., 2007; 2008). Reduced weight gain, dry matter intake, reproduction rates, and delayed onset of puberty are observed in FT-affected cattle (Fribourg et al., 1991). Additionally, animals suffering from fescue toxicosis have reduced blood flow to both the core and periphery, thereby reducing the animal's ability to dissipate heat (Rhodes et al., 1991; Jones et al., 2003).

Decreased serum prolactin concentrations in beef cattle have been consistently noted as a sign of fescue toxicosis (Hoveland et al., 1983; Fribourg et al., 1991; Rice et al., 1997; Parish et al., 2003). The dopamine receptor D2 (DRD2) gene found on bovine chromosome 15 plays a role in prolactin secretion (Civelli et al., 1993). The link between DRD2 and prolactin levels makes this gene a good candidate for containing a polymorphism to serve as a marker for resistance to the disease. A single-nucleotide polymorphism (SNP) was discovered at position 534 within the DRD2 gene where a

guanine/adenine substitution exists. Two experiments were performed to assess the informativeness of this SNP as a marker for resistance to fescue toxicosis. A third experiment examined allele frequencies in spring vs. fall-calving herds grazed on tall fescue in MO and TN.

Materials and Methods

Animals

All procedures involving animals were reviewed, and approved by the University of Tennessee Institutional Animal Care and Use Committee. The original SNP discovery and Exp. 1 were performed using predominantly Angus steers (n = 42) at the East Tennessee Research and Education Center, located near Knoxville, TN. Experiment 2 used predominantly Angus steers (n = 53) from both the East Tennessee Research and Education Center and the Highland Rim Research and Education Center, located near Springfield, TN. Experiment 3 utilized herds from both TN and MO. Predominantly Angus cows, heifers and steer calves from spring- and fall-calving herds (n = 383) were at the Research and Education Center at Ames Plantation (Grand Junction, TN). Cows from spring- and fall-calving herds at the Forage Systems Research Center near Linneus, MO (approximately $\frac{3}{4}$ Angus and $\frac{1}{4}$ Gelbvieh; n = 243) were also used. Table 1 characterizes the collections of animals used for each experiment with respect to breed composition and allelic and genotypic frequency at the DRD2 SNP.

Identification of the DRD2 SNP

Genomic DNA was isolated from 5 – 10 tail hair follicles of 42 Angus steers using Quicextract (Epicentre, Cambridge, UK). A genomic amplification was then performed

for the isolated DNA samples using the GenomiPhi V2 DNA amplification kit (GE Healthcare, Piscataway, NJ) followed by an ethanol precipitation and resuspension in 50 µL of water. The polymerase chain reaction (PCR) was utilized to amplify a 794 base pair (bp) portion of the DRD2 gene. Sequences of the primers used were 5'-TATAGCCCCATTCCTGCTTC-3' and 5'-CGTGTGTTGTAGAGCATGGGC-3'. Cycling conditions were 2 min at 94°C; 35 cycles at 30 sec at 94°C; 30 sec at 58°C; 30 sec at 68°C; followed by 10 min at 68°C and held until further processing at 4°C. The total reaction volume was 20 µL. Direct sequencing of the PCR product revealed an intronic A/G SNP which created a Tfi I restriction site (5'-GAWTC-3') with the "A" allele.

Genotyping

Genomic DNA was isolated and the DRD2 segment was amplified from tail hair follicles as described above. Following PCR, 5 µL of amplified product was subjected to a 2 h digestion reaction at 65°C with 2.5 units Tfi I (USB Biolabs, Boston, MA) in a total reaction volume of 20 µL. Half of the reaction volume was used in agarose gel electrophoresis against a DNA size ladder (Promega, Madison, WI) and genotypes were called based on fragment size.

Experiment 1

Forty-two Angus-based steers of unknown parentage housed at the East Tennessee Research and Education Center were weaned in early autumn and grazed on 'Kentucky 31' tall fescue containing a toxic form of the endophyte (endophyte-infected fescue or E+; n = 21), or 'Jesup MaxQ' tall fescue containing a non-toxic variety of endophyte (NTE; n = 21). Blood was collected via jugular venipuncture monthly in

April, May, and June. Blood was centrifuged for 15 min at 10,000 x g and serum was stored frozen (-20°C) for assaying prolactin as described by Bernard et al. (1993). The intra- and inter-assay coefficients of variation were 10.0 and 8.4%, respectively. In June, hair coat scores as described by Saker et al., (2001), with a score of 1 indicating a slick smooth hair coat and 5 indicating rough dead hair covering most of the body, were also recorded, and a sample of 5 to 10 tail hairs containing follicles was collected from each steer for genomic DNA extraction and DRD2 genotyping as described above. The genotypes were then compared with the collected phenotypic data and genotype-phenotype associations were tested.

Experiment 2

Effect of genotype was then assessed in 53 Angus-based steers at the Highland Rim Research and Education Center and at the East Tennessee Research and Education Center (Blount Unit). All steers were weaned in the fall and placed onto E+ tall fescue pastures. Steers were combined from the two locations in order to increase the numbers of animals in the study. All steers were born and raised on the research and education centers and were Angus based but of unknown parentage. Blood was collected via jugular venipuncture monthly in April, May, and June and processed for analysis of serum prolactin concentrations as described above. The intra- and inter-assay coefficients of variation were 10.0 and 8.4%, respectively. In June, hair coat scores (1 = smoothest, 5 = roughest) (Saker et al., 2001) were also recorded, and a sample of 5 – 10 tail hairs containing follicles was collected from each steer for genomic DNA extraction and DRD2 genotyping as described above. The genotypes were then

compared with the collected phenotypic data and genotype-phenotype associations were tested.

Experiment 3

Most herds in Tennessee and the southeastern United States are spring-calving and, therefore, more prone to the effects of FT. We hypothesized that non-intentional selection for the advantageous allele was occurring in these spring-calving herds but not in fall-calving herds. Herds calving in the fall are impacted by FT but not to the same extent as spring-calving herds due to the timing of the breeding season. Genotypes were obtained from 248 and 135 animals (cows and heifer calves) from spring and fall-calving TN herds, respectively, and 109 and 134 cows from spring and autumn-calving MO herds, respectively (all available animals from each herd were genotyped). For the TN herds, a total of 43 sires contributed an average of 3.0 and 2.6 cows to the spring- and fall-calving herds, respectively. Fifteen of the sires were used in both herds. Based on cow genotype, 11 sires were confirmed heterozygotes (“AG”), 8 sires had at least one “A” allele, and 14 had at least one “G” allele. The genotypes of the remaining sires could not be determined with the cow data available. For the MO herds, a total of 44 sires contributed an average of 2.9 and 2.5 cows to the spring- and fall-calving herds, respectively. Twenty-three of the sires sired cows in both herds. Based on cow genotype, 8 sires were heterozygotes, 15 sires had at least one “A” allele, and 13 sires had at least one “G” allele. The genotypes of the remaining sires could not be determined with the cow data available.

Experiment 4

The effect of DRD2 genotype on performance was evaluated in a subset of the spring- and fall-calving TN herds used in Experiment 3. Cows (n=234) that were raised and maintained on E+ tall fescue were genotyped. Performance traits included days to first calf, calving interval, adj. 205-d weaning weight, average daily gain, and weight per day of age.

Statistical analysis

Exp. 1: Serum prolactin concentration data were log transformed and analyzed by mixed model analysis of variance (SAS, Cary, NC) as a complete randomized design with autoregressive correlation among months. Main effects included the genotype, (AA, AG, or GG), the endophyte (NTE or E+), and the repeated measure “month” (April, May, and June). Hair coat scores were not transformed and analyzed without repeated measures: the main effects included genotype and endophyte. Exp. 2: Data were analyzed as described above. Main effects included site, genotype and the repeated measure “month”. There was no effect of endophyte as all animals in this experiment grazed E+ pastures. For both experiments, least squares means were compared using Fisher’s protected least significant difference. Significance was set as $P < 0.05$; meaningful *post-hoc* comparisons were also noted. For ease of viewing, all figures depict raw means and SEM. Exp. 3: Allelic and genotypic frequencies were tested for Hardy-Weinberg equilibrium and compared across various populations using an online chi-square test (Preacher, 2001). Exp. 4: Data were analyzed as described for Experiments 1 and 2. Main effects included genotype and calving season (spring or

fall). Least squares means were compared using Fisher's protected least significant difference. Significance was set as $P < 0.05$.

Results and Discussions

Identification of the DRD2 SNP

Figure 2.1 shows the location of the SNP within the DRD2 amplicon and representative results of RFLP analysis with Tfi I. The A/G substitution causing the SNP occurs at position 404365 relative to the *Bos taurus* chromosome 15 genomic contig NW_001493347.2 and lies within the third intron of the DRD2 gene. Digestion of the 793 bp amplicon with Tfi I yields the following products based on genotype: AA) 532 and 261 bp, AG) 793, 532 and 261 bp, and GG) 793 bp only (Figure 2.2).

Experiment 1

While genotype had no significant effect on serum prolactin (Figure 2.3) in steers grazing NTE, homozygous GG steers grazing E+ had decreased serum prolactin concentrations in May compared to AA steers ($P = 0.02$). Because tall fescue cultivars with novel endophytes (such as Jesup MaxQ) do not produce ergovaline (Gunter and Beck, 2004), cattle that consume this forage do not experience the decrease in serum prolactin and other signs that are associated with FT. The greater prolactin levels in AA steers grazing E+ tall fescue in May (when daily high temperatures average over 25°C in TN) indicates that this genotype provides a benefit by reducing the impact of the endophyte. This may be due to a lowered receptor affinity for ergovaline, which would decrease the agonistic effects. If the SNP is in linkage disequilibrium it may still be impacting the gene even though there will be no direct effect. In June there was no

difference in serum prolactin based on genotype. The combination of high temperatures in June (when the daily high temperature averages approximately 30°C and can often reach 32°C) and ergot-like alkaloids in the diet may have overwhelmed any advantage the genotype may have offered.

Prolactin is involved with the shedding of winter coats in animals. This is generally seen as a function of increased day length, which causes an increase in prolactin. Because cattle grazing E+ tall fescue do not have the seasonal increase in prolactin, they do not shed the winter hair coat; hence, a rough and retained hair coat is a core symptom of tall fescue toxicosis. This failure of cattle grazing E+ tall fescue to shed their winter hair coats has been reported by many others (Hoveland et al., 1983; Saker et al., 2001). Steers that had the AG genotype had lower hair coat scores when compared to those with the GG genotype ($P = 0.04$), but no overall effect of genotype in our model was detected (Figure 2.4). Lower hair coat scores should allow these cattle to better dissipate heat, which should reduce heat stress during the humid summers of the mid-south. Olson et al. (2003) reported that cattle with slick hair coats had lower rectal temperatures than did cattle with long hair coats. Animals that shed their winter hair coats are less impacted by the hot, humid summers found in the mid-south transition zone and are more productive. When the cattle cannot shed their winter hair coat it increases the heat retained in the body; combined with a decrease in blood flow to the periphery, the increase in internal body temperature creates more heat stress than noted in contemporaries who are in the same environment but not exposed to the E+ pastures.

While there was no overall effect of genotype on hair coat score, the difference between AG and GG genotypes in steers grazing E+ pastures in this preliminary study, coupled with the observed differences in serum prolactin concentrations, warranted a second, larger experiment focusing on steers grazing E+ pastures only.

Experiment 2

Steers grazing E+ pastures with the AA genotype had greater serum prolactin concentrations than did GG steers in April and May, ($P = 0.02$ and $P = 0.002$, respectively), but not in June ($P > 0.05$, Figure 2.5). Fescue toxicosis has been shown to be temperature dependent. As the ambient temperature increases, the effects of the toxicosis are greater; in June the ambient temperature probably overwhelmed the benefit of genotype. Serum prolactin concentrations were numerically depressed in all genotypes in June.

An effect of genotype on hair coat score was observed ($P_{\text{genotype}} = 0.02$) such that animals with the GG genotype had increased hair coat scores when compared to both AA and AG animals (Figure 2.6). This observation also supports a beneficial effect of the A allele in cattle grazing E+ pastures.

All steers in experiment 1 and 2 were spring born calves and were mainly of Angus descent. In both groups of steers, the A allele and AA or AG genotypes were more frequent than the G allele or GG genotype. The animals used in Experiment 1 had a genotypic frequency of 0.33 for the AA genotype and a frequency of 0.22 for the GG genotype, with an allelic frequency for the A allele of 0.56. The steers used in Experiment 2 displayed a similar profile with genotypic frequencies of 0.38 and 0.26 for

AA and GG genotypes respectively, and an allelic frequency of .56 for the A allele. It appears that some sort of selective pressure has been inadvertently applied in these herds, favoring the retention of the A allele. As many spring-calving herds in the fescue belt actively cull poor performers, this shift supports the efficacy of this SNP as a possible marker for resistance to tall fescue toxicosis. Fall-calving herds, while still selecting for productivity, may not be selecting for the A allele because they are not as affected by FT. Dams in a fall-calving herd would not be exposed to high endophyte load in early spring (while nursing fall-born calves and/or during re-breeding), whereas dams of spring-born calves would have to deal with greater endophyte load while lactating and breeding in April-June. Based on this supposition, a hypothesis was formed that allelic and genotypic frequencies would favor the A allele in spring-calving but not fall-calving herds. To test this hypothesis we examined two large operations in TN and MO that each managed separate spring-calving and fall-calving herds.

Experiment 3

As the TN and MO herds were similar in profile within calving season, genotypic and allelic frequencies were determined for combined groups for each season and were found to differ between spring- and fall-calving groups ($P = 0.03$ and 0.02 for genotypic and allelic frequencies, respectively). The calving seasons were fall and spring calving with fall cows calving between September and November and spring cows calving between mid-January and mid-April. The frequency of the A allele in the combined spring-calving herds was 0.56 as compared to a frequency of 0.48 in the combined fall-calving herd. The AA and AG genotypes were more numerous in spring-calving herds

(0.26 vs. 0.21 and 0.52 vs. 0.47, spring and fall respectively), and fewer GG animals were found in the spring-calving herds (0.22 vs. 0.32, spring and fall respectively). These data support the hypothesis that a prevalence of the A allele can be observed in spring-calving herds and may have arisen due to specific selective pressure to perform well during exposure to FT.

Experiment 4

Genotype at the DRD2 SNP was informative for days to first calving ($P_{(\text{genotype})} = 0.05$) in both spring- and fall-calving herds ($P_0 = 0.72$) such that cows with AA genotypes calved over a full estrous cycle earlier than did cows with GG genotypes (733.57 ± 7.6 d vs. 757.96 ± 7.1 d, respectively; $P < 0.06$).

Almost 20% of the U.S. beef herd is raised on tall fescue (West and Waller, 2007) and the endophyte and the toxicosis associated with it causes significant economic loss to the beef industry. This SNP has shown promise for its use as a marker to identify cattle that are resistant to the effects of endophyte-derived alkaloids. Further research is needed to validate this marker and to search for other markers that may be of more significance. This search should be expanded to other genes whose products are involved in the physiological response to the endophyte, such as those within dopaminergic, adrenergic and serotonergic signaling pathways. A comprehensive (multi-locus) genetic test could be very helpful in selecting for replacement animals; this is especially so when selecting bulls, as currently the majority of bulls used for artificial insemination are not raised on tall fescue.

Literature Cited

- Beef Improvement Federation. 2010. Guidelines for Uniform Beef Improvement Programs (9th Ed.). Raleigh, NC.
- Bernard, J. K., A. B. Chestnut, B. H. Erickson, and F. M. Kelly. 1993. Effects of prepartum consumption of endophyte-infected tall fescue on serum prolactin and subsequent milk production of Holstein cows. *J. Dairy Sci.* 76:1928–1933.
- Blackshaw, J. K., and A. W. Blackshaw. 1994. Heat stress in cattle and the effect of shade on production and behavior: A review. *Aust. J. Exp. Agric.* 34:285-295.
- Civelli, O., J. R. Bunzow, and D. K. Grandy. 1993. Molecular diversity of the dopamine receptors. *Annu. Rev. Pharmacol. Toxicol.* 33:281-307.
- Fribourg, H. A., K. D. Gwinn, A. B. Chestnut, J. W. Oliver, and J. C. Waller. 1991. Tall fescue and the fungal endophyte. *Tenn. Farm Home Sci.* 160:10-29.
- Gunter, S.A., and P.A. Beck. 2004. Novel endophyte-infected tall fescue for growing beef cattle. *J. Anim. Sci.* 82 (E. Suppl.):E75-E82.
- Hill, N. S., D. P. Belesky, and W. C. Stringer. 1991. Competitiveness of tall fescue as influenced by *Acremonium coenophialum*. *Crop Science.* 31:185-190.
- Hoveland, C. S., S. P. Schmidt, C. C. King, J. W. Odom, E. M. Clark, J. A. McGuire, L. A. Smith, H. W. Grimes, and J. L. Holliman. 1983. Steer performance and association of *Acremonium coenophialum* fungal endophyte on tall fescue pasture. *Agronomy Journal.* 75: 821-824.

- Jones, K.L, S. S. King, D. Cazac, and D. L. Cross. 2003. Domperidone can ameliorate deleterious reproductive effects and reduced weight gains associated with fescue toxicosis in heifers. *J. Anim. Sci.* 81:2568-2574.
- Klotz, J. L., L. P. ., Bush, D. L. Smith, W. D. Shafer, L. L. Smith, B. C. Arrington, and J. R. Strickland. 2007. Ergovaline-induced vasoconstriction in an isolated bovine lateral saphenous vein bioassay. *J. Anim. Sci.* 85:2330-2336.
- Klotz, J. L., B. H. Kirch, G. E. Aiken, L. P. Bush, and J. R. Strickland. 2008. Effects of selected combinations of tall fescue alkaloids on the vasoconstrictive capacity of fescue-naive bovine lateral saphenous veins. *J. Anim. Sci.* 86:1021-1028.
- Olson, T. A., C. Lucena, C. C. Chase Jr., and A. C. Hammond. 2003. Evidence of a major gene influencing hair length and heat tolerance in *Bos taurus* cattle. *J. Anim. Sci.* 81:80–90.
- Parish, J. A., M. A. McCann, R. H. Watson, N. N. Paiva, C. S. Hoveland, A. H. Parks, B. L. Upchurch, N. S. Hill, and J. H. Bouton. 2003. Use of nonergot alkaloid-producing endophytes for alleviating tall fescue toxicosis in stocker cattle. *J. Anim. Sci.* 81: 2856-2868.
- Preacher, K. J. 2001 Calculation for the chi-square test: An interactive calculation tool for Chi-square tests of goodness of fit and independence [Computer software]. Available from <http://www.quantpsy.org>.
- Rhodes, M. T. , J. A. Paterson, M. S. Kerley, H. E. Garner, and M. H. Laughlin. 1991. Reduced blood flow to peripheral and core body tissues in sheep and cattle induced by endophyte-infected tall fescue. *J. Anim. Sci.* 69:2033-2043.

- Rice, R. L., D. J. Blodgett, G. G. Schurig, W. S. Swecker, J. P. Fontenot, V. G. Allen, and R. M. Akers. 1997. Evaluation of humoral immune responses in cattle grazing endophyte-infected or endophyte-free fescue. *Vet. Immunol. Immunopathol.* 59:285-291.
- Saker, K. E., V. G. Allen, J. P. Fontenot, C. P. Bagley, R. L. Ivy, R. R. Evans, and D. B. Wester. 2001. Tasco-Forage: II. Monocyte immune cell response and performance of beef steers grazing tall fescue treated with a seaweed extract. *J. Anim. Sci.* 79:1022–1031.
- Schuenemann, G.M., M. E. Hockett, J. L. Edwards, N. R. Rohrbach, K. F. Breuel, and F. N. Schrick. 2005. Embryo development and survival in beef cattle administered ergotamine tartrate to simulate fescue toxicosis. *Reprod Biol.* 2005 Jul;5(2):137-50.
- Stuedemann, J. A., and C. S. Hoveland. 1988. Fescue endophyte: History and impact on animal agriculture. *J. Prod. Agr.* 1:39-44.
- West, C. P., and J. C. Waller. 2007. Humid transition zone. In R.F. Barnes et al. (eds) *Forages: The science of grassland agriculture*. Vol. 2, 6th ed. Blackwell Pub. Prof., Ames, IA.
- Yates, S. G, R. D. Plattner, and G. B. Garner. 1985. Detection of ergopeptine alkaloids in endophyte-infected, toxic KY-31 tall fescue by mass spectrometry/mass spectrometry. *J. Agric. Food Chem.* 33:719-722.

Table 2.1.

Genotypic and allelic frequencies of cattle from different herds and calving seasons.

Herd ⁴	Calving Season	n	Genotypic Frequency			Allelic Frequency		HWE ¹	<i>P</i>	
			AA	AG	GG	A	G		Genotypic Frequency ²	Allelic Frequency ²
Experiment 1 Angus Steers	Spring	42	0.33	0.45	0.22	0.56	0.45			
Experiment 2 Angus Steers	Spring	53	0.38	0.36	0.26	0.56	0.44			
MO Angus x Gelbveigh Cows	Fall	109	0.18	0.49	0.33	0.43	0.57	0.99		
	Spring	134	0.27	0.50	0.23	0.52	0.48	0.99	0.19	0.05
TN Angus Cows and Calves	Fall	248	0.22	0.46	0.32	0.45	0.55	0.61		
	Spring	135	0.25	0.53	0.21	0.52	0.48	0.73	0.13	0.08
Combined MO and TN ³	Fall	357	0.21	0.47	0.32	0.44	0.56	0.68		
	Spring	269	0.26	0.52	0.22	0.52	0.48	0.85	0.03	0.02

¹Chi-square goodness-of-fit test against allelic frequencies at Hardy-Weinberg Equilibrium.

²Chi-square two-tailed test between frequencies of fall- and spring-calving herds within TN, MO, or both sites combined.

³Genotypic and allelic frequencies between TN and MO sites within fall- and spring-calving herds did not differ ($P = 0.85$ and $P = 0.93$, respectively).

CTTGAGCGGGGCCTTCAGGTTGGCCCGGAAAGCCCGGCTGCTGCGCTTGGTGTT
 GACGCGCTTGCGCCGCTGCGGAGGACGATGTAGATCTTGATGTAGACCAGCAG
 GGTGACGATGAAGGGCACGTAGAAGGAGACGATGGAGGAGTAGACCACGAAGGC
 GGGGTTGGCGATGATGCACTCGTTCTGGTCTGGGGAGGGAAGAAGGAAAGCCCT
 GGGGGTGGGCGCGTCAGCGAGGCCCCCCCACCTGGGGCCACAGTGTCTGCCTG
 CCACCAAGGAGCAATGTTTACAGAGAGTACGGCTCGATGCCTAAGGTGGCCTGGA
 AGCCTTAGATGTAAACACGCAAATCAGAGTATTA AAAACAACCATGAACAAAAAGCT
 GCTTCCAAGTGTTCATTAATAACCCCTCGTTGCTTCGTCATTGGTTTTGTCATCC
 ATGAGCCCACAAGTGGAGCCACGTTTTTTTTGGAAAAATAATGGGAGAAAACAAT
 TTTTAAAAAGTAGCTTATTATTTTAGCTTTTCCTGTGTGTTGTGGGGGGAGATCTGA
 CACCTTTTAATTTTAGGAACAAGACCACTTGGTCCTCTTGCTGAACTCAACCTGTG
 TTCCACTGGCTTTCCACGTTCCCTGTCAGATCTAGCAGATGACTGTGCTGTCTCCC
 AGGGCCTTTCCAGATTAATTCAACCATG**TATAGCCCCATTCCCTGCTTC**TTGAAATTC
 CCATGACTCTTAGATTTTTAACCACATGCTTTTGAGTCTTCCTGTGTCTGTCCAGT
 AATGGTCTCAAAGGCACGCTGGCATTATGTGCAAGGCATTATGTGAGGCATTATG
 AGCCCCCAGAGGCTCCACATGGCGGCTGTGCCTTCTCTGAGATGCTCCTGACTG
 CCTCACACAGGACCCGGTCACGATGGGCTAGCCTCTTATTTTTTTCTACCTTTTCTG
 CTCCAGGTGGCTCAGGCCTGGCCTGAGTTTGGCCAGTCCTTGGGGCTGAAGCTTC
 ATTATCAGGGGTGATGGAGGCCAGCAGCCTCCCACCCTGGGTGCACACACACA
 GACAGGCACAGACATGCAGTCTGCCAGGCTGTCTGGTTGGCTCCAATTTAATAAA
 GTTGAAAATCACCAGGCATCCTCCTGAAACATTATCCCCATTCTCACCCAACACC
 ATGTGTCTCCAAGCCATTTTTCTCTCTGACCTTACCAGGAACAATCCAGGAGACC
GA[A/G]TCACCCTGACCCAGGAGACTCAGCCTGGCTTCAGTCTAAGGTGGTGAA
 CAGGCTCTTGGTGGGCCACCACCCATGTCCACGCCAGGGTTCAGCTCCCTCAGG
 GGCCAGCTGAGCCCCAGGAGGACAGAGGCAGGACTCACCTGTGTTGTTGAGTCC
 GAAGAGCATCGGGCAGGAGATGGTGAAGGACAGGACCCAGACGATGGCGATCAT
 GACCGTGACCCGCGCTTGGAGCTGTAAC**CGTGTGTTGTAGAGCATGGGC**ATGGC
 CACGGCTGTGTAC

Figure 2.1. Location of the [A/G] SNP within the DRD2 gene. The SNP is shown relative to gi|269932427:2865059-2876976 Bos taurus breed Hereford chromosome 15 genomic scaffold, Bos_taurus_UMD_3.1, whole genome shotgun sequence nucleotides 3944-5446 (exons 4 and 5 of bovine DRD2). Exon sequence is shaded grey, primer

sequences are bold and underlined, and the Tfi I restriction site containing the intronic SNP is bolded and italicized.

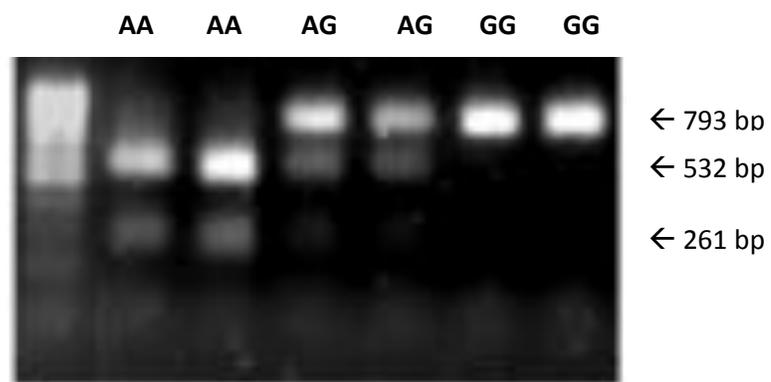


Figure 2.2. Agarose gel showing bands for different DRD2 genotypes.

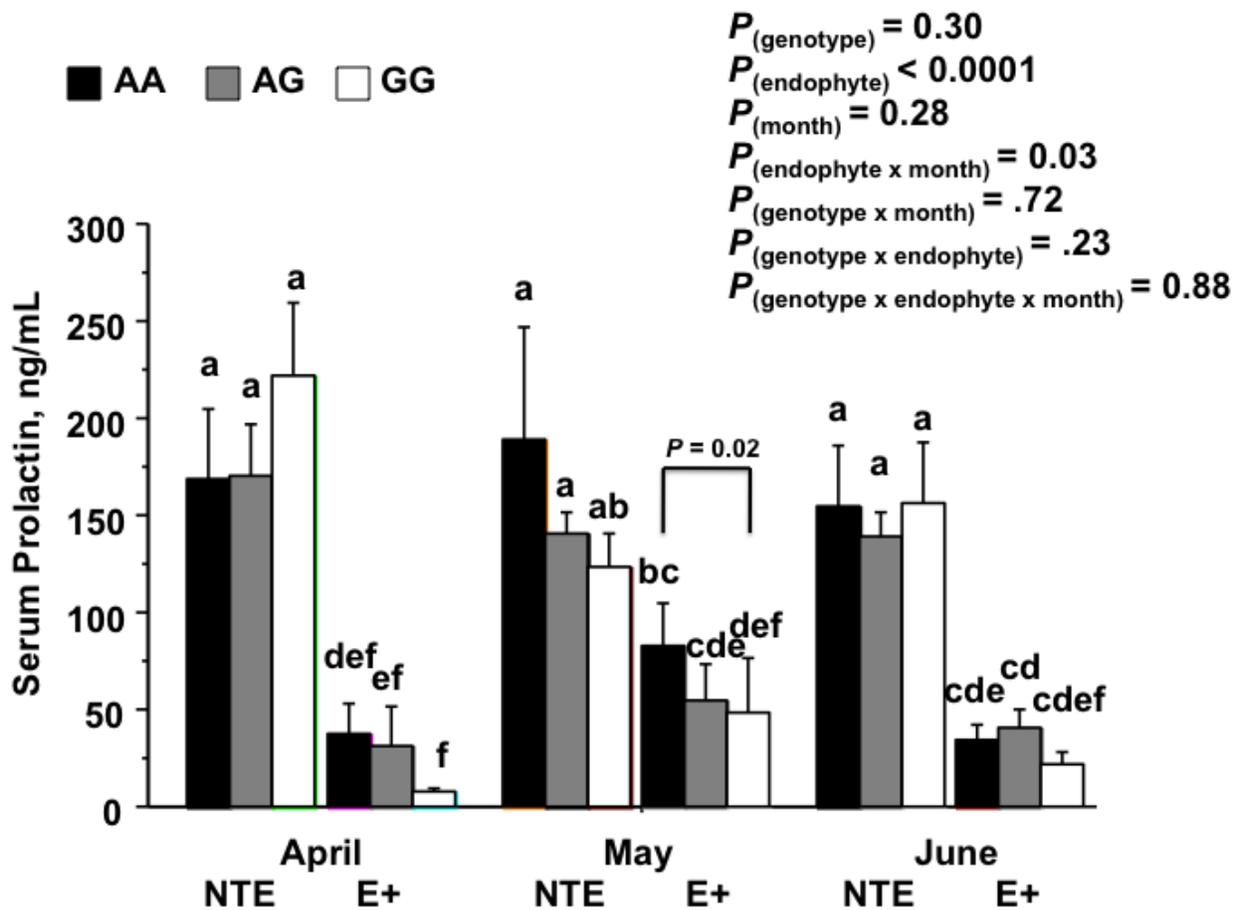


Figure 2.3. Serum prolactin concentrations by month and genotype for Experiment 1. Forty-two Angus-based spring-calved steers were weaned in early autumn and grazed on ‘Kentucky 31’ tall fescue containing a toxic form of the endophyte (E+; n = 21), or ‘Jesup MaxQ’ tall fescue containing a non-toxic variety of endophyte (NTE; n = 21). AA, AG, GG represent the genotypes produced by the SNP in the DRD2 gene. Raw means with SE are shown; means without common letters differ ($P < 0.05$). A comparison between AA and GG genotypes in May is also shown.

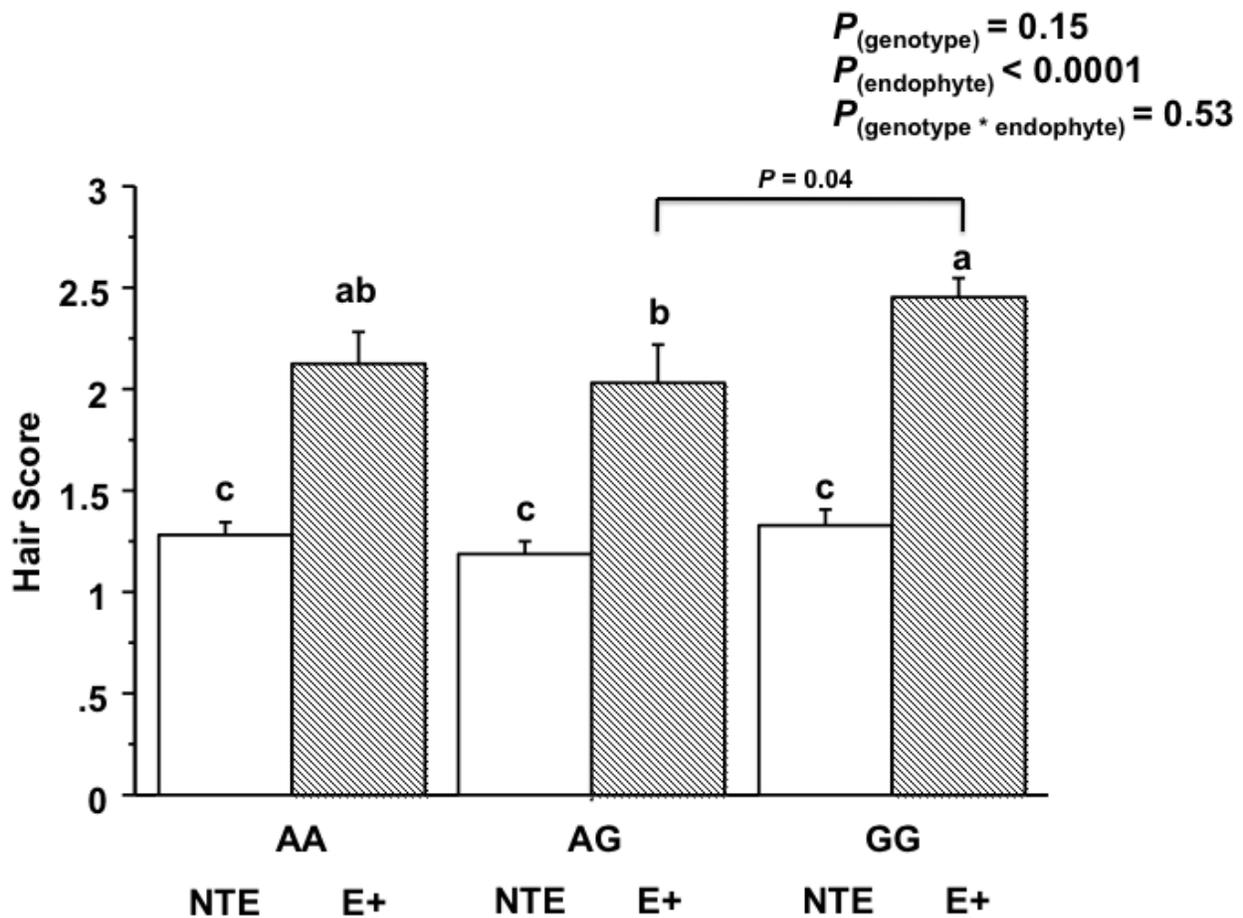


Figure 2.4. Hair coat scores by month and genotype for Experiment 1. Forty-two Angus-based spring-calved steers were weaned in early autumn and grazed on ‘Kentucky 31’ tall fescue containing a toxic form of the endophyte (E+; n = 21), or ‘Jesup MaxQ’ tall fescue containing a non-toxic variety of endophyte (NTE; n = 21). AA, AG, GG represent the genotypes produced by the SNP in the DRD2 gene. Raw means with SE are shown; means with different letters differ ($P < 0.05$). A comparison of AG and GG genotypes on E+ pasture is also shown.

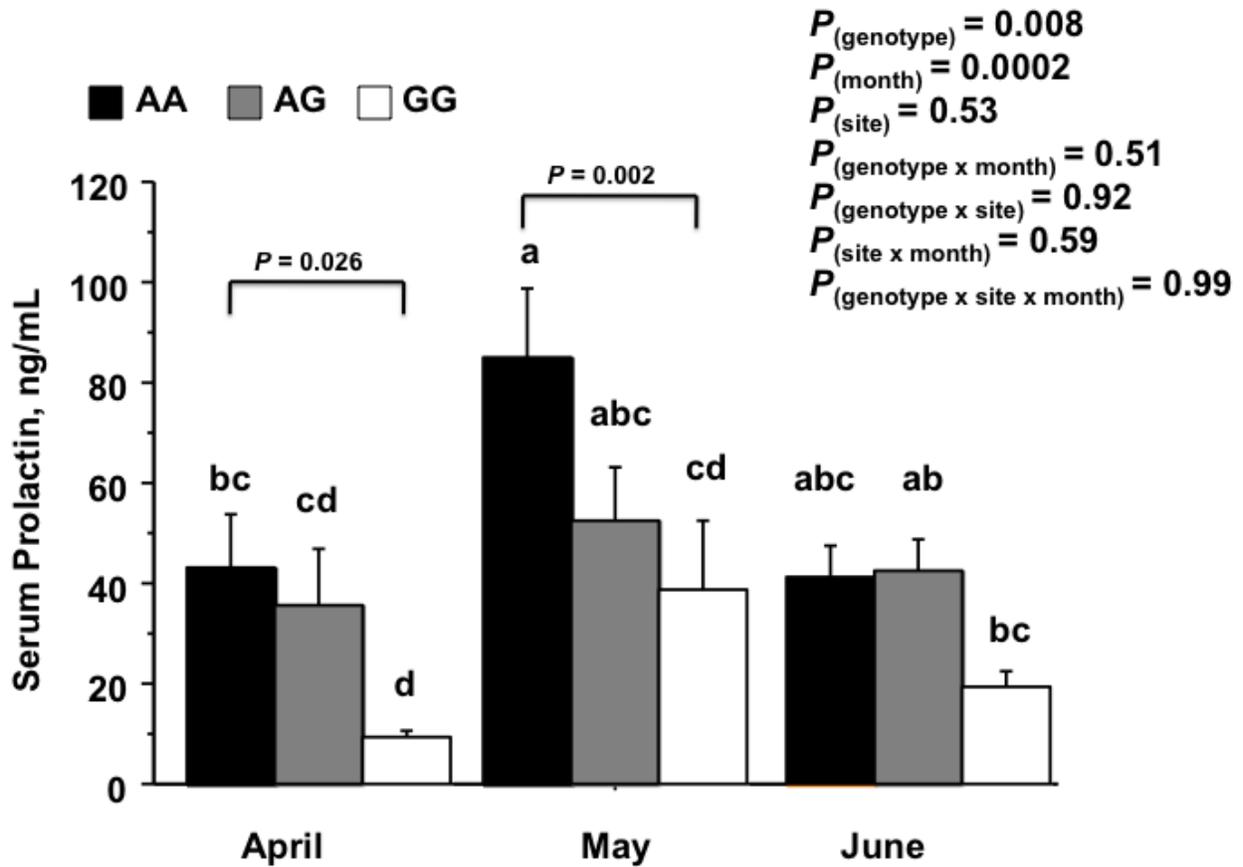


Figure 2.5 Serum prolactin levels by month and genotype for Experiment 2. Fifty-three Angus-based spring-born steers at two sites were weaned in the fall and placed onto E+ tall fescue pastures. Raw means with SE are shown; means with different letters differ ($P < 0.05$). Comparisons between AA and GG genotypes within April and May are also shown.

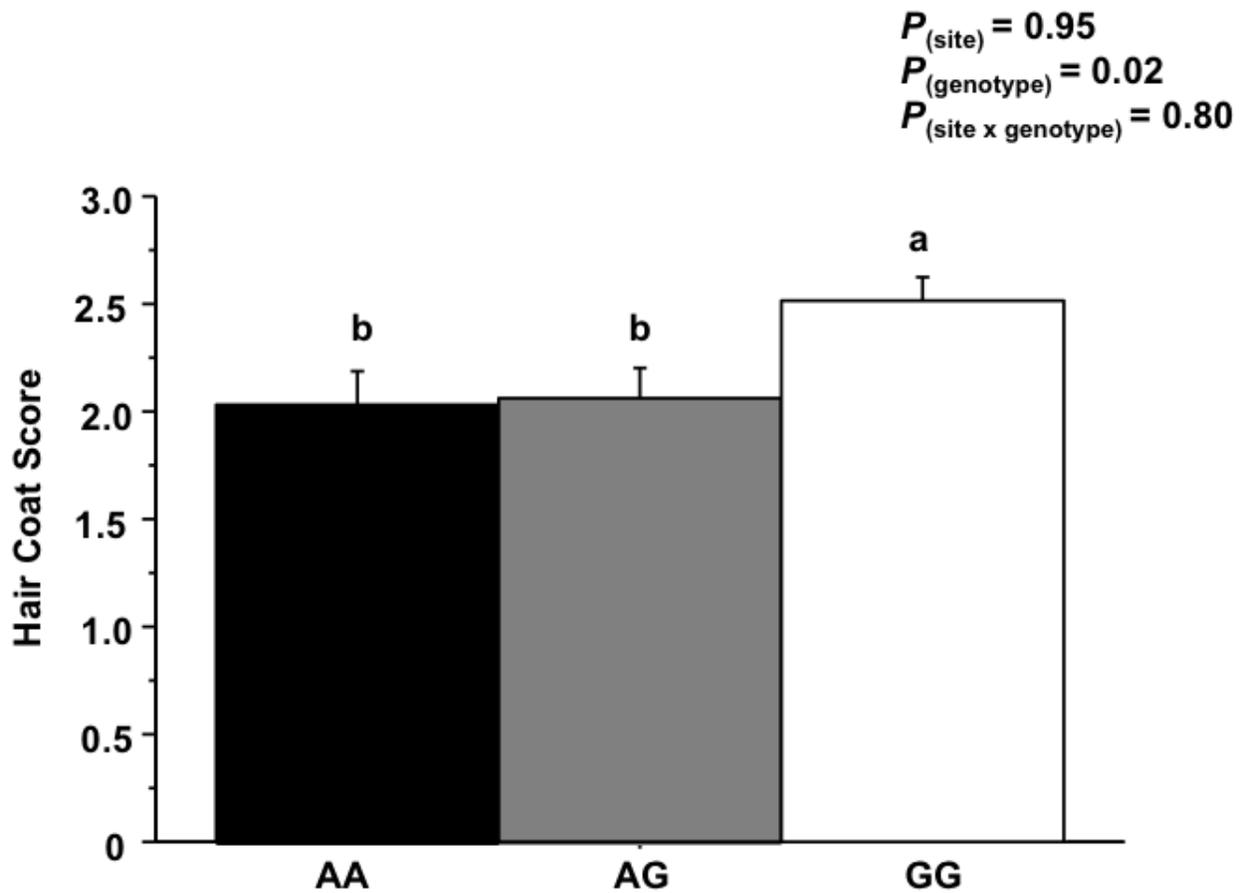


Figure 2.6. Hair coat scores by month and genotype for Experiment 2. Fifty-three Angus-based spring-born steers at two sites were weaned in the fall and placed onto E+ tall fescue pastures. Raw means with SE are shown; means with different letters differ ($P < 0.05$).

Discovery of Genetic Markers for Resistance to Tall Fescue Toxicosis

Abstract

Tall fescue toxicosis causes an estimated 600 million dollars in losses to the U.S. beef industry every year (Hoveland et al., 1993). The losses seen are due to an endophytic fungus (*Neotyphodium coenophialum*) that lives in between the cell walls of the plant. When tall fescue containing this endophyte (E+) is consumed beef cattle will exhibit signs of tall fescue toxicosis. Many methods have been used to reduce the deleterious effects of the endophyte but there is still no silver bullet. The ability to select for animals that will perform more efficiently on endophyte-infected tall fescue would be an efficient method to reduce the economic impact on the more than 20% of the U.S. beef herd who consume E+ tall fescue. Recently researchers have investigated single nucleotide polymorphisms (SNP) as possible markers in candidate gene studies. However this type of study is limited to those genes that are known to be influenced by tall fescue toxicosis. Tall fescue toxicosis is known to impact many different biological systems in cattle. These systems are controlled by many genes therefore to investigate many genes at once would be very beneficial when addressing the problem of tall fescue toxicosis. Genome wide association studies use genetic markers that are spread throughout the genome to identify areas of the genome that are associated with phenotypic traits. This method allows researchers to identify SNPs that are involved with genes not known to be associated with a disease. A high throughput method was utilized to identify SNPs that were possible genetic markers for resistance to tall fescue toxicosis in beef cattle. Forty eight cows from the University of Tennessee Research and Education

Center at Ames Plantation were selected, from the total herd, based on their reproductive performance and the growth of their calves while grazing E+ pastures. Genetic analysis was performed using the Illumina bovine 50K SNP chip on hair samples taken from the tail switch of cows. Genotypes were compared to the adjusted birth weight (ABW) and adjusted 205-d weaning weight (205-d WW) of the cow's calves. Nine SNPs were significantly associated ($P < 0.00001$) with ABW and 15 with 205-d WW. The significant SNPs were then mapped using a bovine genome browser. Some of these SNPs may be beneficial for use in marker assisted selection, but they will need to be validated.

Introduction

Tall fescue toxicosis is the most prevalent and costly syndrome, caused by a grass toxin, to the beef industry in the United States (Roberts and Andrae, 2004). It is estimated that this syndrome costs beef producers over 600 million dollars annually in lost revenue (Hoveland, 1993); more recent data suggests that this may be underestimated (Allen and Segarra, 2001). This economic loss is from the reduction in reproduction and animal performance caused by the toxins consumed when cattle graze tall fescue (*Lolium arundinaceum*) infected with the endophytic fungus *Neotyphodium coenophialum*.

Many methods have been explored to decrease the impact of tall fescue toxicosis on beef cattle and other grazing herbivore. Most methods that have been investigated were not very successful. Successful methods were generally expensive to implement and therefore had low rates of application. Most recently there have been investigations to determine if there is a genetic component to tall fescue toxicosis. Some animals appear to be more susceptible to the toxins than others. The earliest reference to differences in cattle performance on E+ tall fescue was Cunningham (1948) who reported that different cattle, even with in the same herd, will respond differently to grazing E+ tall fescue. Looper et al. (2010) reported differences in SNPs in the enhancer region of the bovine prolactin gene have an impact on profitability traits of cattle.

Genome wide association studies (GWAS) are a relatively new method, of genetic testing that use single nucleotide polymorphisms (SNP). In GWAS, cattle

with differing performance records are compared to many SNPs and an analysis is conducted to determine if there is a relationship between a SNP and the variation in performance in the animal. There is increasing evidence that GWAS is an extremely powerful method to identify genes that are involved in diseases (Wellcome Trust Case Control Consortium, 2007). By using a GWAS, there is the ability to find genes that are involved in the disease even though there is no known relationship between the gene and the disease. This is useful in determining the genes that impact diseases and other complex traits that are polygenic.

Materials and Methods

Cattle from the University of Tennessee Research and Education Center at Ames Plantation herd (Grand Junction, TN), were selected for analysis using the Illumina SNP 50 bovine SNP chip. These cattle represent both fall and spring calving animals and were under the same management for the past 20 years. Cattle primarily grazed endophyte-infected (E+) tall fescue and during the winter they were supplemented with corn silage as needed. Free choice beef minerals were provided all year. Both herds were under strict culling protocols with reproductive failure as primary cause for removal from the herd. Cows were also culled for low performance of their calves.

A subset of 48 cows were selected from the current herd ($n = 234$) of cows to create two groups of cows, a high performing group ($n = 24$) and a low performing group ($n = 24$). Cows selected for the high performing group have consecutively had at least three calves and these calves had the highest adjusted 205-d weaning

weight (205-d WW). Cows selected for the low performing group had the lowest 205-d WW of the entire herd, regardless of the number of calves they have produced. Adjusted 205-d weaning weights were chosen as the method of selection due to adjustments for age of dam and sex of calf to obtain an unbiased comparison regardless of those variables. Adjusted 205-d weaning weights were determined using the formula described by the Beef Improvement Federation (BIF, 2010). Adjustment factors are shown in Table 1.

$$\text{Adj. 205-day wt.} = \frac{\text{Actual weaning wt.} - \text{Birth wt.}}{\text{Age at weaning in days}} \times 205 + \text{Birth wt.} + \text{Dam age adj.}$$

Adjusted 205-d WW was determined using the formula above and adjusted BW was determined using the formula described by the beef improvement federation (BIF, 2010). Adjustments for the age of dam for birth weight and weaning weight are shown in Table 1. The equation used to determine adjusted birth weight (ABW) was (Adjusted Birth Weight = Birth Weight + Age-of-Dam Adjustment).

The preparation and hybridization of the 50k SNP chips were completed by an external service provider (GeneSeek, Lincoln, NE). A mixed model ANOVA tested for mean differences in genotypes, homozygous for the most abundant allele (A), homozygous for the least abundant allele (B), and heterozygous (H). Response variables were 205-d WW and ABW of the calves accounting for variation in the age of the cows. ANOVA was used to adjust for variation in calving season of the cows.

Significance was declared for false discovery rate (FDR) protected P -values less than 0.00001. This analysis resulted in 24 SNPs with genotype differences in 205-d WW and ABW.

Single nucleotide polymorphisms that were identified as significantly related to either ABW or adj. 205-d WW ($P < 0.00001$) were then analyzed using a chi-square contingency table in Proc-FREQ in SAS 9.2. This analysis shows the differences in allelic frequency between the high and low performing groups, for the significant SNPs. A chi-square table was created for each SNP. This analysis identified cattle that were homozygous for the most prevalent allele as an “A”, cattle that were homozygous for the least prevalent allele “B” and cattle with a heterozygous genotype as “H”. The contingency table provides the difference in the frequency of genotypes in the high and low performing group for each significant SNP.

Significant SNPs were also investigated in a genome browser and their locations were mapped on the bovine genome. Mapping was completed using the bovine genome build 6.2 and the UMD 3.1 assembly. The reference SNP (RS) number was determined and the genetic sequence was blasted to the bovine genome. An RS number is a unique identifier that is assigned to each SNP once it is identified in the NCBI SNP database. A 99% identity was determined and the closest gene to the SNP was determined (Table 2). The implicated genes were then investigated to find possible physiological associations with the effects of tall fescue toxicosis.

Results and Discussion

Tall fescue toxicosis is the most prevalent grass toxin impacting the beef industry in the United States (Roberts and Andrae, 2004). This syndrome occurs when livestock graze tall fescue infected with an endophytic fungus. The endophyte, *Neotyphodium coenophialum* when ingested by herbivores causes reduced intake, weight gain, circulating prolactin levels, birth weight, milk production, and reproductive rates (Stuedemann and Hoveland, 1988). This reduction in milk production and weight gain will lead directly to lower weaning weights. The toxins consumed when animals graze E+ tall fescue also have a constrictive effect on vasculature which will decrease the blood flow to the periphery (Klotz et al., 2006). The reduction in blood flow decreases the ability of the animal to cool its body through evaporation leading to increased core body temperatures. Therefore cattle consuming E+ tall fescue will also exhibit an increase in rectal temperatures and hair coat scores (Aiken et al., 2001). Because tall fescue toxicosis impacts so many biological systems it is believed that it is polygenic, therefore methods to identify possible markers for resistance to tall fescue toxicosis should examine many genes.

A genetic link to tall fescue toxicosis has been suggested for many years. In recent studies the ability to select for animals which are tolerant to tall fescue toxicosis has been reported (Lipsev et al, 1992; Hohenboken and Blodgett, 1997). Lipsey et al. (1992) reported that calves from a resistant bull had lower rectal temperatures when grazing tall fescue compared to calves from a susceptible bull. In a study using mice, Hohenboken and Blodgett (1997) were able to select for

animals that did not have reduced average daily gain when fed a diet containing E+ tall fescue seed. More recently Looper et al. (2010) reported discovering and validating two SNPs that were linked to increased cow longevity when grazing E+ tall fescue. This early genetic research indicates that there is a strong genetic link between the syndrome and animal performance, but it is controlled by many genes.

The GWAS identified several SNPs that were significant ($P < 0.00001$). Fifteen SNPs were significant for 205-d WW and nine were significant for ABW (Table 2). Some of these SNPs were close to each other in the genome and many were on the same chromosome. Several of these SNPs were also located within genes. On chromosome 9 there are 4 SNPs that are located within an area of less than one million base pairs. This is a good indication that there is something in that area that is related to tall fescue toxicosis.

More research is needed to continue this study and begin to validate these SNPs. The next step in this research will be to genotype large numbers of cattle to determine if different genotypes at these SNPs are related to differences in production traits. The genotypes will be compared to a variety of production data that is important to efficiency in beef production. Some of the SNPs may be in linkage disequilibrium with causative SNPs within genes. More research will be necessary to determine the relationship between the SNPs and tall fescue toxicosis.

. Conclusions

The results from the SNP chip study are very promising, but more research is needed to determine if our findings can be used in selecting animals for resistance

to tall fescue toxicosis. Additionally many of these SNPs are located outside of genes, and the genes with which they are related are not known. All SNPs will need to be validated to determine if they will be useful as a genetic marker and if they are useful the link between the SNP and tall fescue toxicosis will need to be determined.

Literature Cited

- Aiken, G. E., E. L. Piper, and C. R. Miesner. 2001. Influence of protein supplementation and implant status on alleviating fescue toxicosis. *J. Anim. Sci.* 79:827-832.
- Beef Improvement Federation. 2010. BIF Guidelines for Uniform Beef Improvement Programs (9th Ed.). Raleigh, NC.
- Cunningham, I. J. 1948. Tall fescue grass is poison for cattle. *N. Z. J. Agric.* 77:519.
- Flori, L., S. Fritz, F. Jaffrezic, M. Boussaha, I. Gut, S. Heath, J. Foulley, and M. Gautier. 2009. The genome response to artificial selection: A case study in dairy cattle. *PLoS ONE* 4(8): e6595. doi:10.1371/journal.pone.0006595.
- Hohenboken, W. D., and D. J. Blodgett. 1997. Growth and physiological responses to toxicosis in lines of mice selected for resistance or susceptibility to endophyte-infected tall fescue in the diet. *J. Anima. Sci.* 75:2165-2173.
- Hoveland, C. S. 1993. Importance and economic significance of the *Acremonium* endophytes to performance of animals and grass plant. *Agri., Ecosys. and Environ.* 44:3-12.
- Klotz, J. L., L. P. Bush, D. L. Smith, W. D. Shafer, L. L. Smith, A. C. Vevoda, A. M. Craig, B. C. Arrington, and J. R. Strickland. 2006. Assessment of vasoconstrictive potential of D-lysergic acid using an isolated bovine later saphenous vein bioassay. *J. Anim. Sci.* 84:3167-3175.

- Lavedan, C., L. Licamele, S. Volpi, J. Hamilton, C. Heaton, K. Mack, R. Lannan, A. Thompson, C. D. Wolfgang, and M. H. Polymeropoulos. 2008. Association of the *NPAS3* gene and five other loci with response to the antipsychotic iloperidone identified in a whole genome association study. *Mol. Psychiatry* 14:804-819.
- Lipsey, R. J., D. W. Vogt, G. B. Garner, L. L. Miles, and C. N. Cornell. 1992. Rectal temperature changes of heat and endophyte stressed calves produced by tolerant or susceptible sires. *J. Anim. Sci.* 70(Suppl. 1):188 (Abstr.).
- Looper, M. L., R. W. Rorie, C. N. Person, T. D. Lester, D. M. Hallford, G. E. Aiken, C. A. Roberts, G. E. Rottinghaus, and D. F. Rosenkrans Jr. 2008. Influence of toxic endophyte-infected fescue on sperm characteristics and endocrine factors of yearling Brahman-influenced bulls. *J. Anim. Sci.* 87:1184-1191.
- Looper, M. L., S. G. Black, S. T. Reiter, R. Okimoto, Z. B. Johnson, M. A. Brown, and C. F. Rosenkrans Jr. 2010. Identification of polymorphisms in the enhancer region of the bovine prolactin gene and association with profitability traits of beef cattle. *Prof. Anim. Sci.* 26:103-108.
- Roberts, C. A., and J. A. Andrae. 2004. Tall fescue toxicosis and management. Available at www.plantmanagementnetwork.org/cm/ Crop Manage. doi:10.1094/CM-2004-0427-01-MG.
- Wellcome Trust Case Control Consortium. 2007. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature.* 447:661-78.

Table 3.1. Standard age of dam adjustment factors (kg) for adjusted birth weight and adjusted 205-day weaning weight¹

Age of Dam (Years) ²	Birth Weight Adjustment Factor	Weaning Weight Adjustment Factor	
		Male	Female
2	3.64	27.27	24.54
3	2.27	18.18	16.36
4	0.90	9.09	8.18
5-10	0.00	0.00	0.00
11 & Older	1.36	9.09	8.18

¹Beef Improvement Federation (2010)

²Age of dam at calving

Table 3.2. Significant Single Nucleotide Polymorphisms by phenotype with corresponding location and P-value.

SNP ID	BTA²	20 Base Pair Region	Phenotype¹	P-Value
ARS-BFGL-NGS-72844	1	135692578-135692598	BW	3.89797E-05
Hapmap58210-rs29015574	9	12042716-12042736	WW	9.97E-06
BTB-01233147	9	14194496-14194516	WW	6.51231E-05
ARS-BFGL-NGS-38561	9	43883126-43883146	BW	2.30E-06
BTA-97536	9	43969692-43969712	BW	7.04896E-05
Hapmap57331-rs29009884	9	44075838-44075858	BW	2.13521E-05
ARS-BFGL-NGS-77863	9	44872688-44872708	BW	3.02388E-05
Hapmap51064-BTA-83646	9	47333293-47333313	BW	1.95337E-05
Hapmap40497-BTA-121906	9	47425501-47425521	BW	1.91E-06
BTB-00393397	9	55496913-55496933	BW	0.000080749

Table 3.2

SNP ID	BTA²	20 Base Pair Region	Phenotype¹	P-Value
ARS-BFGL-NGS-101697	10	9900829-9900849	WW	8.09244E-05
Hapmap29803-BTA-126260	11	16193483-16193503	WW	4.46639E-05
Hapmap57415-rs29022496	14	22300854-22300874	WW	2.30105E-05
BTB-01252321	14	22643296-22643316	WW	2.30105E-05
BTB-01252375	14	22677640-22677660	WW	2.30105E-05
BTB-01337014	14	23721703-23721723	WW	2.30105E-05
BTB-01417864	14	24220060-24220080	WW	2.30105E-05
Hapmap44352-BTA-16069	17	19975109-19975129	WW	2.30105E-05
ARS-BFGL-NGS-57507	17	69005476-69005496	WW	4.55026E-05

Table 3.2

SNP ID	BTA²	20 Base Pair Region	Phenotype¹	P-Value
ARS-BFGL-BAC-32323	19	60078938-60078958	WW	7.76901E-05
BTA-50697-no-rs	20	46346406-46346426	BW	2.18381E-05
Hapmap39064-BTA-55787	23	22080117-22080137	WW	2.30105E-05
Hapmap38926-BTA-94783	26	13697590-13697610	WW	7.57951E-05

¹BW = Adjusted birth weight; WW = Adjusted 205d Weaning weigh

²BTA = *Bos Taurus* Chromosome

Conclusions

Tall fescue toxicosis is the most costly grass induced syndrome impacting the beef industry in the United States, costing upwards of 600 million dollars every year. This is caused by animals consuming plants infected with an endophytic fungus. Tall fescue is the base forage for approximately 20% of the U.S. beef herd. There have been many different approaches to reduce or eliminate the effects of this syndrome. To date these methods have either, not worked, been very expensive to implement, have not been practical solutions, or a combination of these. Reducing the impact of tall fescue toxicosis will greatly improve beef production leading to more profit for the beef industry in the mid-south transition zone.

Management of beef herds so that critical times in the biological cycle of the cows lifetime do not coincide with times of high ambient temperature and high endophyte concentrations in the diet of the cow herd is a beneficial method of reducing the impact of the endophyte. This method is not only practical but economical as well and should not put extra financial burdens on beef producers. Our first experiment comparing spring and fall calving beef herds consuming tall fescue showed that managing cattle so that they will calve in the fall will increase the reproductive efficiency. This is probably due to a decrease in heat stress and tall fescue toxicosis during the time of breeding. Also if producers continue to market their calves at weaning, calves born in the fall will be sold in historically higher markets which would increase the gross income of the operation. These benefits

combine to make the fall calving season an attractive option for beef producers in the mid-south.

Another option to reduce the impact of tall fescue toxicosis is through genetic selection of animals that are resistant to the toxins produced by the endophyte. Since shortly after tall fescue was released in 1941 there have been reports of animals responding differently to the toxins. Our candidate gene study examined the effect of an intronic SNP on the DRD2 gene where an adenine/guanine substitution exists. In preliminary studies it was shown that animals with an AA genotype at this SNP have increased prolactin levels and decreased hair coat scores suggesting that the impact of the toxins is reduced. Experiment 3 of this study showed shifts in Hardy Weinberg equilibrium towards the AA genotype in spring calving herds from Tennessee and Missouri. When examining 234 cows from Tennessee it was discovered that cattle with the AA genotype will calve an average of 23 days earlier than their GG counterparts. There are many reports which suggest that earlier calving will lead to increased lifetime productivity which makes this an important finding.

Tall fescue toxicosis is a polygenic trait; because of this a GWAS was completed utilizing the Illumina bovine 50K snp chip. This study identified 24 SNPs that may be related to tall fescue toxicosis. Some of these SNPs are located in genes with known functions that will require more research to determine the link to tall fescue toxicosis. Other SNPs are located in parts of the bovine genome that have not been well defined. This data is preliminary and validation studies will be

needed to identify which of these genes will be useful as genetic markers for future selection of beef cattle.

These studies are very promising in identifying methods that can reduce the effect of tall fescue toxicosis. Management changes with minimal changes in input costs have the ability to increase the profitability of beef production. Changing from a spring to a fall calving season would accomplish this and would also decrease the impact of heat stress on beef cows raised in the mid-south. The ability to select for animals resistant to tall fescue toxicosis would greatly increase the profitability of beef production in the mid-south allowing for greater production on the current forage base. Most of the bulls used for artificial insemination are not raised on tall fescue and a genetic marker for resistance would be beneficial to the beef producers in the tall fescue belt allowing them to select for the most productive sires.

In conclusion this research provides a basis for increasing the profitability of beef production on tall fescue. First we examined two management styles comparing the benefits and problems with each calving season. Then we examined a possible genetic marker for resistance to tall fescue toxicosis. Finally we completed a GWAS searching for the possibility of more markers for resistance to tall fescue toxicosis in beef cattle. This study revealed 24 potential markers which will have to be examined in later studies. These three studies have the possibility of greatly improving beef production in the humid mid-south transition zone.

Literature Cited

- Agee, C. S., and N. S. Hill. 1994. Ergovaline variability in *Acremonium*-infected tall fescue due to environment and plant genotype. *Crop Science* 34:221-226.
- Ahmed, N. M., S. P. Schmidt, J. R. Arbona, D. N. Marple, D. I. Bransby, R. L. Carson, D. A. Coleman, and C. H. Rahe. 1990. Corpus luteum function in heifers grazing endophyte-free and endophyte-infected Kentucky-31 tall fescue. *J. Anim. Sci.* 68(Suppl. 1):468 (Abstr.).
- Aldrich, C. G., M. T. Rhodes, J. L. Miner, M. S. Kerley, and J. A. Paterson. 1993. The effects of endophyte-infected tall fescue consumption and use of a dopamine antagonist on intake, digestibility, body temperature, and blood constituents in sheep. *J. Anim. Sci.* 71:158-163.
- Arechavaleta, M., C. W. Bacon, C. S. Hoveland, and D. E. Radcliffe. 1989. Effect of the tall fescue endophyte on plant response to environmental stress. *Agron. J.* 81:83-90.
- Bacon, C. W. 1995. Toxic endophyte-infected tall fescue and range grasses: Historic perspectives. *J. Anim. Sci.* 73:861-870.
- Bagley, C. P., J. C. Carpenter Jr., J. I. Feazel, F. G. Hembry, D. C. Huffman, and K. L. Koonce. 1987. Influence of Calving season and stocking rate on beef cow-calf productivity. *J. Anim. Sci.* 64:687-694.
- Ball, D. M., C. S. Hoveland, and G. D. Lacefield. 2007. *Southern Forages*. Potash and Phosphate Inst. and the Foundation for Agronomic Res., Norcross, GA.

- Beck, P. A., C. B. Stewart, J. M. Phillips, K. B. Watkins, and S. A. Gunter. 2007. Effect of species of cool-season annual grass interseeded into bermudagrass sod on the performance of growing calves. *J. Anim. Sci.* 85:536-544.
- Beck, P.A., S. A. Gunter, K. S. Lusby, C. P. West, K. B. Watkins, and D. S. Hubbell. 2008. Animal performance and economic comparison of novel and toxic endophyte tall fescues to cool-season annuals. *J. Anim. Sci.* 86:2043–2055.
- Ben-Jonathan, N., J.L. Mershon, D. L. Allen, and R. W. Steinmetz. 1996. Extrapituitary prolactin: distribution, regulation, functions, and clinical aspects. *Endocr. Rev.* 17:639-669.
- Bond J., G. P. Lynch, D. J. Bolt, H. W. Hawk, G. Jackson Jr., and R. J. Wall. 1988. Reproductive performance and lamb weight gains for ewes grazing fungus-infected tall fescue. *Nutr. Rep. Intt.* 37:1099-1115.
- Bourdon, R. M., and J. S. Brinks. 1983. Calving rate versus calving interval as a reproductive measure in beef cattle. *J. Anim. Sci.* 57:1412-1417.
- Bouton, J. H., R. N. Gates, and C. S. Hoveland. 2001. Selection for persistence of endophyte-free Kentucky 31 tall fescue. *Crop Sci.* 41:1026-1028.
- Browning Jr., R. 2004. Effects of endophyte-infected tall fescue on indicators of thermal status and growth in Hereford and Senepol steers. *J. Anim. Sci.* 82: 634-643.
- Burke, J. M., D. E. Spiers, F. N. Kojima, G. A. Perry, B. E. Salfen, S. L. Wood, D. J. Patterson, M. F. Smith, M. C. Lucy, N. G. Jackson, and E. L. Piper. 2001.

- Interaction of endophyte-infected fescue and heat stress on ovarian function in the beef heifer. *Biol. Reprod.* 65:260-268.
- Caldwell, J. D., K. P. Coffey, W. K. Coblenz, J. A. Jennings, D. S. Hubbell III, D. L. Kreider, M. L. Looper, and C. F. Rosenkrans Jr. 2009. Performance by fall-calving cows grazing tall fescue pastures with different proportions stockpiled. Online. *Forage and Grazinglands* doi:10.1094/FG-2009-0312-01-RS.
- Cunningham, I. J. 1948. Tall fescue grass is poison for cattle. *N. Z. J. Agric.* 77:519.
- Flori, L., S. Fritz, F. Jaffrezic, M. Boussaha, I. Gut, S. Heath, J. Foulley, and M. Gautier. 2009. The genome response to artificial selection: A case study in dairy cattle. *PLoS ONE* 4(8): e6595. doi:10.1371/journal.pone.0006595.
- Fribourg, H. A., C. S. Hoveland, and K. D. Gwinn. 1991. Tall fescue and the fungal endophyte: A review of current knowledge. *Tenn. Farm Home Sci.* 160:30-37.
- Gould, L. S., and W. D. Hohenboken. 1993. Differences between progeny of beef sires in susceptibility to fescue toxicosis. *J. Anim. Sci.* 71:3025-3032.
- Goddard M. E., and B. J. Hayes. 2009. Mapping genes for complex traits in domestic animals and their use in breeding programmes. *Nat. Rev. Genet.* 10:381-391.
- Gwinn, K. D., H. A. Fribourg, J. C. Waller, A. M. Saxton, and M. C. Smith. 1998. Changes in *Neotyphodium coenophialum* infestation levels in tall fescue pastures due to different grazing pressures. *Crop Sci.* 38:201-204.

- Harris, J. P., J. L. Edwards, L. A. Rispoli, R. R. Payton, N. R. Rohrbach, T. M. Prado, J. C. Waller, A. M. Saxton, and F. N. Schrick. 2011. Fertilization characteristics of spermatozoa from bulls grazing tall fescue pastures. *Reprod. Fertil. Dev.* 24:202-203 (Abstr.).
- Hemken, R. W., J. A. Boling, L. S. Bull, R. H. Hatton, R. C. Buckner, and L. P. Bush. 1981. Interaction of environmental temperature and anti-quality factors on the severity of summer fescue toxicosis. *J. Anim. Sci.* 52:710-714.
- Hill N. S., D. P. Belesky, and W. C. Stringer. 1991. Competitiveness of tall fescue as Influenced by *Acremonium coenophialum*. *Crop Sci.* 31:185-190.
- Hirschhorn J. N., 2009. Genome wide association studies illuminating biologic pathways. *N. Engl. J. Med.* 360:1699-1701.
- Hohenboken, W. D., and D. J. Blodgett. 1997. Growth and physiological responses to toxicosis in lines of mice selected for resistance or susceptibility to endophyte-infected tall fescue in the diet. *J. Anim. Sci.* 75:2165-2173.
- Hoveland, C. S., S. P. Schmidt, C. C. King, Jr., J. W. Odum, E. M. Clark, J. A. McGuire, L. A. Smith, H. W. Grimes, and J. L. Holliman. 1983. Steer performance and association of *Acremonium coenophialum* fungal endophyte on tall fescue pasture. *Agron. J.* 75:821-824.
- Hoveland, C. S. 1993. Importance and economic significance of the *Acremonium* endophytes to performance of animals and grass plant. *Agri., Ecosys. and Environ.* 44:3-12.

- Humphry, J. B., K. P. Coffey, J. L. Moyer, F. K. Brazle, and L. W. Lomas. 2002. Intake, digestion, and digestive characteristics of *Neotyphodium coenophialum*-infected and uninfected fescue by heifers offered hay diets supplemented with *Aspergillus oryzae* fermentation extract or laidlomycin propionate. *J. Anim. Sci.* 80:225-234.
- Jones K. L., S. S. King, K. E. Griswold, D. Cazac, and D. L. Cross. 2003. Domperidone can ameliorate deleterious reproductive effects and reduced weight gain associated with fescue toxicosis in heifers. *J. Anim. Sci.* 81:2568-2574.
- Klotz, J. L., L. P. Bush, D. L. Smith, W. D. Shafer, L. L. Smith, A. C. Vevoda, A. M. Craig, B. C. Arrington, and J. R. Strickland. 2006. Assessment of vasoconstrictive potential of D-lysergic acid using an isolated bovine lateral saphenous vein bioassay. *J. Anim. Sci.* 84:3167–3175.
- Klotz, J. L., L. P. Bush, D. L. Smith, W. D. Shafer, L. L. Smith, B. C. Arrington, and J. R. Strickland. 2007. Ergovaline-induced vasoconstriction in an isolated bovine lateral saphenous vein bioassay. *J. Anim. Sci.* 85:2330-2336.
- Klotz, J. L., B. H. Kirch, G. E. Aiken, L. P. Bush, and J. R. Strickland. 2010. Contractile response of fescue-naive bovine lateral saphenous veins to increasing concentrations of tall fescue alkaloids. *J. Anim. Sci.* 88:408–415.
- Lamberts, S. W., and R. M. Macleod. 1990. Regulation of prolactin secretion at the level of the lactotroph. *Physiol. Rev.* 70:279-318.

- Lipham, L. B., F. N. Thompson, J. A. Stuedemann, and J. L. Sartin. 1989. Effects of metoclopramide on steers grazing endophyte-infected fescue. *J. Anim. Sci.* 67: 1090-1097.
- Lipsev, R. J., D. W. Vogt, G. B. Garner, L. L. Miles, and C. N. Cornell. 1992. Rectal temperature changes of heat and endophyte stressed calves produced by tolerant or susceptible sires. *J. Anim. Sci.* 70(Suppl. 1):188 (Abstr.).
- Looper, M. L., S. G. Black, S. T. Reiter, R. Okimoto, Z. B. Johnson, M. A. Brown, and C. F. Rosenkrans Jr. 2010. Identification of polymorphisms in the enhancer region of the bovine prolactin gene and association with profitability traits of beef cattle. *Prof. Anim. Sci.* 26:103-108.
- Lusby, K. S., W. E. McMurphy, C. A. Strasia, S. C. Smith, and S. H. Muntz. 1990. Effects of fescue endophyte and interseeded clovers on subsequent finishing performance of steers. *J. Prod. Agric.* 3:103-105.
- Lyons, P. C., R. D. Plattner, and C. W. Bacon. 1986. Occurrence of peptide and clavine ergot alkaloids in tall fescue grass. *Science* 232:487-489.
- McLaren, J. B., R. J. Carlisle, H. A. Fribourg, and J. M. Bryan. 1983. Bermudagrass, tall fescue, and orchardgrass pasture combinations with clover or N fertilization for grazing steers. I. Forage growth and consumption, and animal performance. *Agron. J.* 75:587-592.
- Parish, J. A., M. A. McCann, R. H. Watson, N. N. Paiva, C. S. Hoveland, A. H. Parks, B. L. Upchurch, N. S. Hill, and J. H. Bouton. 2003. Use of non-ergot

- alkaloid-producing endophytes for alleviating tall fescue toxicosis in stocker cattle. *J. Anim. Sci.* 81:2856-2868.
- Paterson, J. A., M. S. Kerley, B. Larson, J. Forcherio, and M. Samford. 1995. The effects of fescue toxicosis on beef cattle productivity. *J. Anim. Sci.* 73:889-898.
- Porter, J. K., and F. N. Thompson Jr., 1992. Effects of fescue toxicosis on reproduction in livestock. *J. Anim. Sci.* 70:1594-1603.
- Read, J. C., and B. J. Camp. 1986. The effect of fungal endophyte *Acremonium coenophialum* in tall fescue on animal performance, toxicity, and stand maintenance. *Agronomy Journal* 78:848-850.
- Redmond, L. M., D. L. Cross, J. R. Strickland, and S. W. Kennedy. 1994. Efficacy of domperidone and sulpiride for fescue toxicosis in horses. *Amer. J. Vet. Res.* 55:722-729.
- Rhodes, M. T., J. A. Paterson, M. S. Kerley, H. E. Garner, and M. H. Laughlin. 1991. Reduced blood flow to peripheral and core body tissues in sheep and cattle induced by endophyte-infected tall fescue. *J. Anim. Sci.* 69:2033-2043.
- Rice, R. L., D. J. Blodgett, G. G. Schurig, W. S. Swecker, J. P. Fontenot, V. G. Allen, and R. M. Akers. 1997. Evaluation of humoral immune responses in cattle grazing endophyte infected or endophyte free fescue. *Vet. Immun.* 59:285-291.

- Riddle, O., R.W. Bates, and S.W. Dykshorn. 1933. The preparation, identification and assay of prolactin-a hormone of the anterior pituitary. *Am. J. Physiol.* 105:191-216.
- Rosenkrans Jr., C., M. Roe, M. Brown, Z. Johnson, H. Brown, and M. Looper. 2009. Association of Single Nucleotide Polymorphisms Within the Bovine Heat Shock Protein 70 Gene and Calving Rates. *Reprod. Fertil. Dev.* 22:280-281 (Abstr.).
- Sales, M. A., M. J. Larson, S. T. Reiter, A. H. Brown Jr., M. A. Brown, M. L. Looper, K. P. Coffey, and C. F. Rosenkrans Jr. 2011. Effects of bovine cytochrome P450 single nucleotide polymorphism, forage type and body condition on production traits in cattle. *J. Anim. Physiol. Anim. Nutr.* doi: 10.1111/j.1439-0396.2011.01176.x
- Schmidt, S. P., D. A. Danilson, J. A. Holliman, H. W. Grimes, and W. B. Webster. 1986. Fescue fungus suppresses growth and reproduction in replacement beef heifers. *Alabama Agric. Exp. Sta. Highlights Agric. Res.* 33:150-151.
- Schuenemann, G. M., J. L. Edwards, M. D. Davis, H. E. Blackmon, F. N. Scenna, N. R. Rohrbach, A. M. Saxton, H. S. Adair, F. M. Hopkins, J. C. Waller, and F. N. Schrick. 2005a. Effects of administration of ergotamine tartrate on fertility of yearling beef bulls. *Therio.* 63:1407-1418.
- Schuenemann, G. M., J. L. Edwards, F. M. Hopkins, N. R. Rohrbach, H. S. Adair, F. N. Scenna, J. C. Waller, J. W. Oliver, A. M. Saxton, F. N. Schrick. 2005b.

- Fertility aspects in yearling beef bulls grazing endophyte infected tall fescue pastures. *Reprod. Fertil. Dev.* 17:479-486.
- Smith, G. W., D. S. Rotstein, and C. F. Brownie. 2004. Abdominal fat necrosis in a pygmy goat associated with fescue toxicosis. *J. Vet. Diagn. Invest.* 16:356-359.
- Stuedemann, J. A., T. S. Rumsey, J. Bond, S. R. Wilkinson, L. P. Bush, D. J. Williams, and A. B. Caudle. 1985. Association of blood cholesterol with occurrence of fat necrosis in cows and tall fescue summer toxicosis in steers. *Am. J. Vet. Res.* 9:1990-1995.
- Stuedemann, J. A., and C. S. Hoveland. 1988. Fescue endophyte history and impact on animal agriculture. *J. Prod. Agric.* 1:39-44.
- Varney D. R., M. Ndefru, S. L. Jones, R. Newsome, M. R. Siegel, and P. M. Zavos. 1987. The effect of feeding endophyte infected tall fescue seed on reproductive-performance in female rats. *Comp. Physiol. Biochem.* 87:171-175.
- Wang, W. Y. S., B. J. Barratt, D. G. Clayton, and J. A. Todd. 2005. Genome-wide association studies: theoretical and practical concerns. *Nature. Rev. Genet.*
- West, C.P. and J. C. Waller. 2007. Humid transition zone. In R.F. Barnes et al. (eds) *Forages: The science of grassland agriculture*. Vol. 2, 6th ed. Blackwell Pub. Prof., Ames, IA.

- Yates, S. G., R. D. Plattner, and G. B. Garner. 1985. Detection of ergopeptine alkaloids in endophyte infected, toxic Ky-31 tall fescue by mass spectrometry. *J. Agri. Food Chem.* 33:719-722.
- Zanzalari, K. P., R. N. Heitmann, J. B. McLaren, and H. A. Fribourg. 1989. Effects of endophyte-infected fescue and cimetidine on respiration rates, rectal temperatures and hepatic mixed function oxidase activity as measured by hepatic antipyrine metabolism in sheep. *J. Anim. Sci.* 67:3370-3378.
- Zavos, P. M., B. Salin, J. A. Jackson, Jr., D. R. Varney, M. R. Segel, and R. W. Hemken. 1986. Effects of feeding tall fescue seed infected by endophytic fungus (*Acremoniurn Coenophialum*) on reproductive performance in male rats. *Theriogenology.* 25:281-290.
- Zhuang, J., M. A. Marchant, C. L. Schardl, and C. M. Butler. 2005. Economic analysis of replacing endophyte-infected with endophyte free tall fescue pastures. *Agron. J.* 97:711-716.

VITA

Brian Campbell was born in Fort Knox, KY in December of 1983. He was raised in Virginia. Brian graduated from Colonial Heights High School in 2002. He then began his academic career at Texas A&M-Commerce. He graduated from Texas A&M-Commerce in December of 2005 with a bachelor's degree in Animal Science. Brian began his graduate career at The University of Tennessee in August of 2006, with a major in Animal Science. In August of 2008 Brian Completed his master's degree and his thesis was entitled "Tall Fescue Based Forage Systems Supplemented with Winter Annuals for Stocker Cattle". In the fall of 2008 Brian began his Ph.D. program at The University of Tennessee with a major in Animal Science.