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Single-nucleotide polymorphisms associated with performance traits in beef cattle grazing endophyte-infected tall fescue

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I am submitting herewith a thesis written by Bryan Christopher Bastin entitled "Single-nucleotide polymorphisms associated with performance traits in beef cattle grazing endophyte-infected tall fescue." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

Cheryl J. Kojima, Major Professor

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Dedication

This thesis is dedicated to two people without whom none of my research and education would have been possible. My wonderful wife Kelley Leigh Bastin, who encouraged me to pursue my graduate degree and worked full-time to support our family during the last two years, cannot be thanked enough. Her tireless efforts and encouragement were essential and I could not have achieved anything without her.

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Abstract

Tall fescue (*Lolium arundinaceum Schreb.*) is the most prevalent forage in the Midsouth United States due in part to the presence of the endophytic fungus *Neotyphodium coenophialum*. The fungus, while conferring hardiness to tall fescue, contributes to decreased production efficiency in cow-calf operations. A previous genome-wide association study was performed using the Illumina 50k bovine SNP chip. Twenty-four SNP were found to be associated ($P < 0.05$) with adjusted birth weight and adjusted 205-day weights of calves from 48 beef cows at Ames Plantation. The first objective was to validate each SNP by testing associations with several additional phenotypes. Custom Taqman genotyping assays (Applied Biosystems, Foster City, CA) were subsequently designed to genotype each SNP in beef cattle located at Tennessee Tech University (n = 654), to validate associations in a large, independent herd. The results yielded 15 associations that were significant ($P < 0.05$) with 6 phenotypes linked to those affected by fescue toxicosis. The second objective investigated the link between fescue toxicosis and the XK, Kell blood group complex subunit-related, member 4 (*XKR4*) gene. Serum prolactin concentrations were significant ($P = 0.0002$) for an intronic SNP within the gene, suggesting further investigation into the physiological function of the gene as well as providing a potential genetic marker for selection for cattle resistant to fescue toxicosis. The results of this study validate the majority of findings from the GWAS and provide effective initial steps towards utilizing genetic markers to improve the resistance to fescue toxicosis in cattle.

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List of Abbreviations

Ang-(1-7)	Angiotensin-(1-7)
BCS	Body Condition Score
bST	Bovine Somatotrophin
BW	Birth Weight
CD109	Cluster of Differentiation 109 gene
CPEB	Cytoplasmic Polyadenylation Element Binding Protein gene
CPR179	G Protein-coupled Receptor 179 gene
CV	Coefficient of Variation
dbSNP	Single Nucleotide Polymorphism database, NCBI
DNA	Deoxyribonucleic Acid
DRD2	Dopamine Receptor D2 gene
E+	Endophyte-infected tall fescue
E-	Endophyte-free tall fescue
FSH	Follicle Stimulating Hormone
FT	Fescue Toxicosis
G1SYN	Gamma-1-Syntrophin gene

GWAS	Genome-Wide Association Study
HCS	Hair Coat Score
LH	Luteinizing Hormone
LH-RH	Luteinizing Hormone-Releasing Hormone
MANEA	Glycoprotein Endo-α-1,2-Mannosidase gene
mRNA	Messenger Ribonucleic Acid
NOGO	Neurite Outgrowth Inhibitor gene
OT	Oxytocin
PEP	Prolyl Endopeptidase
PREP	Prolyl Endopeptidase gene
PRL	Prolactin
PRL	Prolactin gene
QTL	Quantitative Trait Locus
RAS	Renin-Angiotensin System
RHAG	Rh-Associated Glycoprotein gene
RTN4IP1	Reticulon-4-Interacting Protein 1, Mitochondrial Precursor gene
SNP	Single Nucleotide Polymorphism

SOCS7	Suppressor of Cytokine Signaling 7 gene
STAT3	Signal Transducer and Activator of Transcription 3 gene
STAT5	Signal Transducer and Activator of Transcription 5 gene
TGF-β	Transformation Growth Factor Beta
VGLUT	Vesicular Glutamate Transporter
WW	Weaning Weight
XKR4	XK, Kell Blood Group Complex Subunit-related Family, Member 4 gene

Introduction

Tall fescue (*Lolium arundinaceum Schreb.*) is the predominant forage in the Midsouth region of the United States known as the "fescue belt" (Fribourg *et al.* 1991). The widespread use of tall fescue for feeding grazing livestock is supported by the plant's easy establishment, resistance to pests and heavy grazing, and hardiness in conditions of extreme drought. These positive characteristics, however, are provided by the presence of an endophytic fungus (*N. coenophialum*) residing within the plant (Stuedemann & Hoveland 1988; Arechavaleta *et al.* 1989).

Animals grazing endophyte-infected tall fescue experience symptoms collectively known as tall fescue toxicosis (FT). Fescue toxicosis, a multi-system disorder, has been shown to cause decreased weight gain, increased heat retention, decreases in calving rate and conception (Paterson *et al.* 1995; Jones *et al.* 2003; Looper *et al.* 2010b), as well as a delayed onset of sexual maturity (Jones *et al.* 2003). Combined losses from fescue toxicosis represent an annual loss of approximately $600 million (Hoveland 1993) affected 20% of all beef cows in the United States (West & Waller 2007). In today's dollars, the economic losses likely exceed $1 billion annually in the combined cattle, horse, sheep and goat industries (Strickland *et al.* 2011).

Although fescue toxicosis has been extensively studied, little is known about the physiological mechanisms behind this disorder. Varying levels of demonstration and wide variety of presented symptoms make the identification and prevention of fescue toxicosis extremely difficult. Ergot alkaloids produced by the fescue endophyte are believed to be the primary causes of fescue toxicosis, so elimination

or reduction of these compounds represent one possible solution. The highest concentrations of ergot alkaloids are found in the seed head (Bacon 1995), so producers are encouraged to cut fescue early to mitigate the effects.

One possible method to combat fescue toxicosis may be through genetic methods. Genetic selection for lines resistant to fescue toxicosis can improve the performance of future generations grazing fescue, and the discovery of novel genetic markers can increase the effectiveness of selection.

Recently, we conducted a genome-wide association study (GWAS) that genotyped 48 Angus cows for over 50,000 individual single-nucleotide polymorphisms (SNP) to identify associations with two indicators of performance, adjusted 205-day weaning weights (WW) and adjusted birth weights (BW) (Campbell 2012). Twenty-four SNP had significant associations, and were the subject of our current research.

Our first objective in this study was to validate these SNP in a larger herd of cattle (n = 654) located at Tennessee Tech University with a wide-variety of phenotypes. Custom Taqman genotyping assays were designed and used to genotype every animal for each of the SNP in a high-throughput manner. Through validation of these SNP, we aim to include these SNP in a genotyping panel that could be used to make informed breeding decisions for cattle producers in the future.

Second, a candidate gene was chosen due its proximity to one of the SNP discovered in the association study. Research in human studies of neuropsychological disorders (Lavedan *et al.* 2009; Lantieri *et al.* 2010; Fijal *et al.* 2012) in humans found association with the XK, Kell blood group complex subunit-related, member-4 (*XKR4*) gene, while two studies (Lindholm-Perry *et al.* 2012; Porto Neto *et al.* 2012) in cattle yielded associations with performance traits usually affected by fescue toxicosis. We investigated three individual, intronic SNP within the *XKR4* gene for a link to the effects of fescue toxicosis. Elucidation of the physiological and endocrine mechanisms behind fescue toxicosis will not only yield useful genetic markers for selection purposes, but could also help us improve the health of herds across the Midsouth. These improvements can minimize the negative financial impact fescue toxicosis has on a large segment of the U.S. cattle population.

Literature Review

Tall Fescue

Tall fescue (*Lolium arundinaceum Schreb.*) is the most prevalent forage grass in the Midsouth United States (Stuedemann & Hoveland 1988), grazed by approximately 20% of all cattle in the United States (West & Waller 2007). Commonly referred to as the "fescue belt", (Fribourg *et al.* 1991; Paterson *et al.* 1995) the region covers over 14 million ha, including southern Illinois and Ohio, northern Mississippi and Georgia, eastern Oklahoma, and Virginia and the Carolinas. Kentucky-31, a strain of tall fescue released in 1941, is the most prevalent cultivar, and its hardiness led to the widespread use of tall fescue as a forage in the United States (Stuedemann & Hoveland 1988). Tall fescue displays many favorable characteristics, primarily due to the presence of an endophytic fungus (*N. coenophialum*) that resides within the forage.

Although concentrated in the seed head (Bacon 1995), the fescue endophyte resides in the entirety of the plant, sharing a symbiotic relationship with the grass. This relationship confers several advantageous characteristics that have allowed tall fescue to succeed as the primary forage in the southeastern United States. Endophyte-infected tall fescue (E+) shows a significant tolerance to drought as opposed to endophyte-free (E-) fescue; drought conditions caused the death of E- strains, while loss was limited to leaf tissue in E+ (Arechavaleta *et al.* 1989). This tolerance of drought conditions also confers advantages to the producer, as the need for management is lessened under external stressor conditions (Stuedemann & Hoveland 1988). Similarly, E+ fescue displays resistance to both grazing pressure

and herbivory by both livestock and insect species (Hill *et al.* 1991), further minimizing the need for intense management.

Fescue Toxicosis

The presence of the fungal endophyte is also indirectly responsible for the collection of symptoms arising in livestock species that graze endophyte-infected fescue, such as cattle, goats, sheep and horses. These symptoms are collectively referred to as fescue toxicosis (FT), and affect a wide variety of physiological systems. Fescue toxicosis represents a major source of economic loss in this region, approximately $600 million annually (Hoveland 1993). In today's dollars, the economic losses likely exceed $1 billion annually in the combined cattle, horse, sheep and goat industries (Strickland *et al.* 2011).

Fat necrosis and Fescue Foot are two issues attributed to E+ fescue. Fat necrosis manifests as degraded lipid deposits of varying size and shape located in the mesentery of the abdomen of animals grazing E+ fescue (Smith *et al.* 2004). Fescue foot is the most severe manifestation of fescue toxicosis, despite being least presented in grazing animals. Vasoconstriction-induced swelling and hyperemia occurs near the coronary band between hoof and dewclaw (Hemken *et al.* 1981b) and can result in gangrene of the extremities (Lyons *et al.* 1986) similar to symptoms seen in ergot poisoning (more commonly known as ergotism).

The primary and most widely observed concern (Hemken *et al.* 1984) is referred to as the "summer slump." Also known as fescue toxicosis, it is a syndrome

that affects animal performance, reproduction and calf performance. Cattle grazing E+ fescue display a decrease in daily gain and feed intake (Paterson *et al.* 1995), reduction in body condition score (BCS) (Looper *et al.* 2010b), reduction in reproductive rates, delayed onset of puberty (Jones *et al.* 2003), and an increase in hair coat score (Porter & Thompson 1992). Signs of fescue toxicosis were originally reported to manifest primarily in temperatures above 32° C, suggesting that the syndrome was related only to periods of high temperatures (Hemken *et al.* 1981a). However recent findings (Parish *et al.* 2003) demonstrate manifestation below 32° C as well, establishing fescue toxicosis as a problem not totally dependent on climate.

Ergot Alkaloids

The fungal endophyte produces ergot alkaloids that, when ingested, are the direct cause of the symptoms seen in fescue toxicosis. Ergovaline is the most prevalent ergot alkaloid (Yates *et al.* 1985b) found in tall fescue and is thought to be the primary cause of toxicosis. Other ergopeptines present in E+ fescue (such as ergonovine, ergotamine, ergocristine, ergocrypine and ergocornine) are thought to contribute to fescue toxicosis as well (Klotz *et al.* 2007; Foote *et al.* 2012).

Disruptions to normal blood flow in the animal are central to several symptoms presented by fescue toxicosis. Reduced blood flow to the core and periphery are hallmark signs seen in cattle grazing E+ tall fescue (Rhodes *et al.* 1991); this causes reduced heat dissipation (Rhodes *et al.* 1991) and an increase in energy expended to combat heat stress (Zanzalari *et al.* 1989; Aldrich *et al.* 1993). Ergovaline was shown to display constriction in bovine vasculature similar to

norepinephrine (Klotz *et al.* 2007), while other ergot alkaloids present in endophyte-infected fescue demonstrated relatively less vasoconstriction (Klotz *et al.* 2010).

Reproductive Symptoms of Fescue Toxicosis

Decreases in reproductive performance, much like most manifestations of fescue toxicosis, are observed during the high ambient temperature that accompany the summer months (Porter & Thompson 1992). Unfortunately, despite being studied extensively, the exact physiological mechanisms underlying this decrease in reproductive ability are still poorly understood. However, these symptoms are similar to those seen in cattle experiencing heat stress; therefore the link between fescue toxicosis and high temperatures supports research into this relationship.

Progesterone levels are decreased in heifers grazing endophyte-infected fescue; however, treatment with a dopamine antagonist returned progesterone concentrations to normal levels seen in those grazing endophyte-free fescue (Jones *et al.* 2003). These results suggest a preliminary link to mitigation of the negative effects of fescue toxicosis by restoration of normal progesterone levels. The mechanism of dopamine antagonism is similar to those discussed in relation to serum prolactin.

Economic loss due to decreased reproductive ability is significant in cattle grazing endophyte-infected tall fescue. Increased time to conception has been identified as a result of fescue toxicosis in ewes after exposure to a ram (Bond *et al.* 1988); this was attributed to embryonic mortality or delayed estrus. Similarly, a

10% increase in endophyte infestation resulted in a 3.5% decrease in conception rate (Schmidt *et al.* 1986). Reproductive losses associated with fescue toxicosis in cattle have also been observed including decreases in adjusted weaning weight, and increased pregnancy loss (Burke *et al.* 2001), and decreased circulating concentrations of progesterone (Jones *et al.* 2003) in cattle grazing endophyte-infected fescue compared to controls. Decreases in semen quality and other male bovine fertility characteristics have been reported in bulls (Schuenemann *et al.* 2005a; Schuenemann *et al.* 2005b). Scrotal circumference has a positive correlation with sperm production (Coulter & Foote 1976; Coulter & Keller 1982). Stowe *et al.* (2013) reported that cattle grazing endophyte-infected fescue for 126 days had marked decreases in scrotal circumference; the vasoconstrictive effect of ergot alkaloids on the testicular veins may induce increased cell death and decreased cell proliferation in the testes. These factors together may contribute to the loss in fertility demonstrated by the effects of fescue toxicosis.

Impaired function in luteal cells has been posited as a causative symptom of decreased conception rates and delayed onset of puberty (Ahmed *et al.* 1990). As a result, this luteal disturbance could lead to early follicular formation in cycling heifers, therefore decreasing the success rate of conception at insemination due to aged follicles (Burke *et al.* 2001).

Fescue Toxicosis and Prolactin

Arguably the most notable physiological impact of ergot alkaloids is their interaction with circulating prolactin (PRL) concentrations. Decreased serum

prolactin concentration has been consistently found to be an indicator of fescue toxicosis (Hoveland *et al.* 1983; Fribourg *et al.* 1991; Rice *et al.* 1997; Parish *et al.* 2003). Prolactin is secreted by the anterior pituitary gland, and is estimated to have over 300 distinct functions (Bole-Feysot *et al.* 1998), of which lactation in mammals is best known (Riddle *et al.* 1933).

Dopamine is the primary inhibitor of prolactin secretion (Lamberts & Macleod 1990); secretion of dopamine from the hypothalamus binds to dopamine D2 receptors to inhibit prolactin secretion from the lactotroph (Kebabian & Calne 1979). Ergovaline, a dopamine agonist, displays similar inhibitory effects at the dopamine D2 receptors, causing the decrease in prolactin that is displayed by fescue toxicosis (Lamberts & Macleod 1990). Treatment with domperidone, a dopamine antagonist, mitigated fatty-acid composition in plasma and follicular fluid and improves the effects of fescue toxicosis on oocyte maturation in cattle (Jones *et al.* 2003; Jones & King 2009). Pregnant mares fed endophyte-infected fescue were treated with domperidone gel foaled within 2 weeks of their estimated foaling date, had developed functional and lactating mammary glands at foaling, and adequate milk production for the first five days post-foaling compared to untreated controls grazing endophyte-infected fescue. (Cross *et al.* 2012).

Another symptom of fescue toxicosis is increased hair coat score (HCS), a scale from 1 to 5 describing the thickness of the hair coat in cattle. Prolactin, along with melatonin, is sensitive to photoperiod (Tucker & Ringer 1982). Prolactin levels are causally related to seasonal-associated changes in hair growth. As days grow

shorter in the winter, prolactin levels decrease as melatonin increases. Exposure to decreased photoperiods in minks was associated with a decrease in prolactin and induced growth of the thick winter coat; subsequent treatment with prolactin induced molting and growth of the summer coat (Allain *et al.* 1981). Treatment of red deer with bromocriptine, another dopamine agonist, delayed the seasonal increases in prolactin as well as hair growth (Curlewis *et al.* 1988). Therefore, as the long nights of winter transition to longer days (and increased light) in the summer, high levels of ergovaline consumed by cattle grazing endophyte-infested fescue inhibit prolactin increase, causing retention of the thick winter coat and subsequently, increases heat retention.

Genetic Resistance

The complex physiological nature of fescue toxicosis has made resolution of its adverse effects through a genetic approach difficult. Fescue toxicosis is a disorder that affects multiple systems and cannot be described as a binary trait like other disorders in which an individual is either affected or not. Genomic analysis of hepatic function in rats fed an endophyte-infected diet (Settivari *et al.* 2006) identified approximately 50 genes with altered expression, yet the link between the genetic pathways of these genes to the symptomatic presentation is minimally understood. However, identification of potential candidate genes such as these offers promising targets for further research.

The role of dopamine and dopamine receptors relating to fescue toxicosis has yielded a promising subject of genetic analysis. Dopamines inhibitory effect on

prolactin secretion mirrors the mechanism of ergovaline, a dopamine antagonist, (Yates *et al.* 1985a) to induce the symptoms typically associated with fescue toxicosis. Alternatively, dopamine antagonists have demonstrated ameliorative effects on animals fed endophyte-infected fescue. Ergovaline, prolactin and dopamine act on the dopamine D2 receptor, making the *DRD2* gene an attractive candidate gene. Steers grazing endophyte-infected fescue with the presence of the A allele at a novel single-nucleotide polymorphism in *DRD2* gene was associated with increased serum prolactin levels and decreased hair coat scores (Kojima *et al.* In press). Allele frequencies of the A allele differed in spring- and fall-calving cattle, suggesting possible indirect selection favoring the advantageous allele.

Mutations in the enhancer region of the prolactin gene (*PRL*) in cattle demonstrated alterations in calving rate and body condition score (Looper *et al.* 2010a) that reduced overall value and profitability, indicating the importance of the genetic component of prolactin in fescue toxicosis resistance. Upstream elements of the heat shock protein 70 gene contained multiple SNP; changes to these haplotypes were informative for associations to calving percentage (Rosenkrans *et al.* 2010).

Selection for genetic resistance to fescue toxicosis has proven to be effective, even when selecting based on phenotype, rather than genotype. Selection for post-weaning weight gain in eight generations of mice yielded a line that was resistant to the effects of endophyte-infected fescue (Hohenboken & Blodgett 1997). Investigation of progeny of two Hereford bulls (Gould & Hohenboken 1993) supported the role of selection in resistance to the effects of fescue toxicosis, with

one bull's progeny displaying higher feed intake and lower rectal temperatures. These various approaches to investigating the role of genetics offer support to the importance of genetic analysis of fescue toxicosis.

Summary

Tall fescue represents the primary forage for one fifth of the U.S. beef cattle population, and annual losses due to fescue toxicosis are estimated at approximately $1 billion. Substitution with alternative forages is not an economically feasible option, as few can match the advantages in management and survivability of endophyte-infected fescue. Not limited to a single physiological system, the impact of fescue toxicosis on performance, reproductive and carcass traits combined emphasizes the importance of continued research.

While most producers in the Midsouth "fescue belt" region rely on supplementation or management techniques to manage the effects of fescue toxicosis, the success of genetic selection for resistance may present the best opportunity to minimize loss. Our first objective was to validate twenty-four single nucleotide polymorphisms (SNP) discovered in a genome-wide association study in a relatively small group (n = 48) of Angus cows. By expanding our sample population to one of a larger size, diverse breed composition and varying levels of management, we hoped to validate these associations to merit inclusion in a marker-based genetic panel. Utilization of this panel to make informed breeding decisions should help producers across the mid-south improve the performance of cattle grazing tall fescue.

Recent studies have shown that treatment with dopamine antagonists such as domperidone can counteract the effects of ergovaline. Our second objective was to investigate the possible role of a candidate gene near one of the SNP identified in our association study. While little is reported in the literature about the XK, Kell blood group complex subunit-related, member 4 (*XKR4*) gene, it represented an ideal candidate due to its role in neurologic disorders relating to dopaminergic pathways, as well as performance traits in cattle that are affected by fescue toxicosis.

Chapter 1. Validation of single-nucleotide polymorphisms with phenotypic associations in beef cattle grazing endophyte-infected tall fescue

Abstract

Tall fescue (*Lolium arundinaceum Schreb.*) is the most prevalent forage in the Midsouth United States due in part to the presence of the endophytic fungus *Neotyphodium coenophialum*. The fungus, while conferring hardiness to tall fescue, contributes to decreased production efficiency in cow-calf operations. A genome-wide association study was previously performed using the Illumina 50k bovine SNP chip. Twenty-four SNP were found to be associated ($P < 0.05$) with adjusted birth weight and adjusted 205-day weights of calves from 48 beef cows at Ames Plantation (Campbell 2012). For the present study, custom Taqman genotyping assays (Applied Biosystems, Foster City, CA) were designed to genotype the significant SNP in beef cattle located at Tennessee Tech University (n = 654), to validate associations in a large, independent herd. Genotype-phenotype associations were tested using mixed models (SAS 9.3, Cary, NC) accounting for variability in calving season in calf-related traits, and least squares means compared with Fisher's least significant difference ($P < 0.05$). Eleven of the GWAS SNP were informative for such phenotypes as hair coat (scored 1-5), body condition (scored 1-9), weight per day of age (kg/d), adjusted 205-day weight (kg), and days to first calf. These data indicate that genetic polymorphisms found in a small herd may be informative and can be validated in a larger representative population in a cost-effective manner. Eight of the resulting SNP were located within or in proximity to candidate genes with functions that may be related to fescue toxicosis. Using these SNP for both candidate gene analysis as well as potential markers for

future selection purposes should prove to be a positive step forward in explaining and reducing loss associated with fescue toxicosis in cattle.

Introduction

Tall fescue (*Lolium arundinaceum Schreb.*) is the most prevalent forage grass in the Midsouth United States (Stuedemann and Hoveland, 1988) and cattle grazing this forage comprise approximately 20% of all beef cows raised in the United State (West and Waller, 2007). A cool-season perennial bunchgrass, tall fescue is easily established, drought and pest resistant and tolerant of grazing (Hill, *et al.* 1991). These and other positive characteristics are due mainly to the presence of an endophytic fungus (*N. coenophialum*) that resides within the grass. The endophyte produces many ergot alkaloids. One of the most important from a grazing animal standpoint is ergovaline (Yates, *et al.* 1985); ingestion of these serotonergic and dopaminergic agonists contribute to a variety of symptoms known collectively as tall fescue toxicosis. Reductions in weight gain, dry matter intake, reproduction rates, as well as delayed onset of puberty and peripheral vasoconstriction are observed in cattle affected by fescue toxicosis (Fribourg, *et al.* 1991).

Genetic selection for animals resistant to the effects of fescue toxicosis may be effective. Mice selected for genetic resistance to fescue toxicosis for eight generations yielded progeny with significant improvements in post-weaning growth (Hohenboken & Blodgett 1997). Two bulls in Missouri and Virginia were studied (Gould & Hohenboken 1993); the Missouri bull had a reputation for siring progeny with resistance to fescue toxicosis, the Virginia sire acting as a control. Fifteen progeny of both sires were fed endophyte-infected fescue feed, and the Missouri-

sired cattle demonstrated higher feed intake and lower rectal and surface body temperatures, indicating the possibility of a genetic contribution toward resistance to fescue toxicosis.

Genetic selection based on genetic markers across the entire genome has become a widely-used and highly-effective method of improving economic returns and performance in livestock across the world. The complex and varied nature of the symptoms of fescue toxicosis highlight the need to use a "wide-net" to search for associated genetic markers. Genome-wide analysis allows for increased insight into the impact of simple genetic variations on the complex conditions that arise from them (Hindorff *et al.* 2009).

The purpose of this experiment was to validate single-nucleotide polymorphisms (SNP) with associations to calf performance traits from cows in a genome-wide association study. Campbell (2012) analyzed the two groups of 24 each of low- and high-performing cows (performance linked number of calves and adjusted 205-day weaning weights) while grazing endophyte-infected tall fescue on Ames Plantation, a Research and Education Center located near Grand Junction, TN. All cattle were genotyped using a bovine 50k SNP chip (Illumina, San Diego, CA). Statistical analysis (SAS 9.3, Cary, NC) was performed using mixed-model analysis ($\alpha < 0.00001$). Twenty-four significant SNP were identified between adjusted birth weight (nine SNP total) and adjusted 205-day weaning weight (14 SNP total).

The current study included both an increased herd size (n = 654) as well as additional phenotypic indicators of fescue toxicosis. Our long-term goal is to utilize these SNP as part of an effective genetic marker panel that would enable cattle producers to make breeding decisions that lead to greater genetic improvement and production efficiency. We also aim to identify potential candidate genes that may reveal the effect of endophyte-infected tall fescue on the physiological mechanisms behind fescue toxicosis.

Materials and Methods

Custom Taqman genotyping assays (Applied Biosystems, Foster City, CA) were designed for each SNP (Table 1), and used to genotype a commercial mixed-breed (Angus, Simmental, Charolais) herd (n = 592) located at Tennessee Technological University in Cookeville, TN. Fluorescence of reporter dyes was quantified using a 7900HT Fast Real Time PCR System (Applied Biosystems, Carlsbad, CA). Half of the current herd, comprised of both cows and calves, was acquired two years ago and largely assembled through random purchases at public livestock sales in the area.

Forage samples were taken by randomly collecting grass tillers and noting the presence of tall fescue in every field to determine tall fescue coverage and endophyte infestation rate in July 2012. Endophyte infection analysis of fescue tillers (Agrinostics, Watkinsville, GA) revealed that all fields had > 80% endophyte infestation rate and tall fescue forage comprised the overwhelming species of all fields tested by manual sampling. Serum samples, hair samples for genotyping,

and other phenotypic data were collected during the month of June 2012, when effects of fescue toxicosis are often observed.

Adjusted 205-day weaning weight (WW) was determined using the formula below (Equation 1) described by the Beef Improvement Federation (BIF, 2010). Adjustments for the age of dam for weaning weight are shown in Table 2.

Equation 1

$$Adj\ 205\ WW = \frac{Actual\ WW - Birth\ Weight\ (BW)}{Age\ at\ Weaning\ in\ Days} \times 205 + BW + Dam\ Age\ Adj.$$

When phenotypic data such as weaning age, dam age or birth weight was missing, weight gained per day of age was used as a substitute for adjusted weaning-weight and analyzed separately. Body condition score (BCS) is an objective measure of overall condition scored 1-9, with 1 indicating an animal severely underweight and 9 severely overweight (Wagner *et al.* 1988).

Serum prolactin concentrations were derived using radioimmunoassay as previously described (Bernard *et al.* 1993), with intra- and inter-assay CV's of 6.6% and 10.3%, respectively.

Hair coat scores (1 = smoothest, 5 = roughest) (Tucker & Ringer 1982) were also recorded and DNA was isolated from hair follicles collected from tail hair using QuickExtract (Epicentre, Madison, WI).

All genotype-phenotype associations were tested using mixed model analysis of variance (SAS 9.3, Cary, NC) with genotype as a fixed effect. Associations were

declared significant at $\alpha = 0.05$. Variance between experimental units due to breed and field location was not significant, and both were not included in further analysis.

Results and Discussion

Fifteen associations were discovered within six phenotypes known to be affected by cattle grazing on endophyte-infected tall fescue. All results are displayed in Table 3. Phenotypes with significant genotype effects are described in Table 4.

Seven SNP were associated with hair coat score, including four on chromosome nine. This cluster of associated polymorphisms suggests the presence of a QTL functionally related to the mechanisms behind hair shedding and regrowth. Chromosome 9 also contained two SNP associated with serum prolactin concentration and days to first calf. One SNP located on chromosome 11 (BTA11) was significant for three unique phenotypes: body condition score, weight gained per day of age, and days to first calf. The SNP BTA19A also displayed multiple phenotypic associations (HCS, days to first calf). Polymorphisms on chromosomes 23 and 26 were also significant ($P = 0.047$ and $P = 0.01$ respectively) on adjusted 205-day weaning weight and hair coat score, respectively.

These SNP represent potential markers for breeding and selection. The proximity (or location within) to protein-encoding genes allows for the possibility that the genes function could be associated with the underlying mechanisms of

fescue toxicosis. Table 4 lists each SNP and their location relative to possible candidate genes.

SNP with Phenotypic Associations on Chromosome 9

The polymorphism rs42386739 (BTA9B) is located 5' (642,721 bp, genome assembly Bos_taurus_UMD_3.1) to the *CD109* gene. Cluster of Differentiation 109 (*CD109*) is part of the TGF-β system, and has been demonstrated to negatively regulate responses of human keratinocytes (Finnson *et al.* 2006). Keratinocytes are specialized cells found in the filaments of keratin, of which α-keratins comprise nails, claws, horns, and hair in mammals. Keratinocytes in the outer root sheath of hair proliferate at a high rate inward, possibly due to multipotent stem cells (Amoh *et al.* 2009), to produce the keratin needed for hair growth and shedding (Fuchs 2007). Hair coat score is often the most visible indicator of fescue toxicosis in cattle, as affected animals retain their thick winter coats into the summer months. Seasonal changes in serum prolactin are controlled by photoperiod, and thus, are responsible for hair coat shedding and regrowth in mammals (Tucker & Ringer 1982). Ergovaline inhibits the normal summer increase in prolactin concentration, causing retention of winter coats and therefore, an increase in hair coat score. Since the thick hair in the winter coat of cattle allow them to retain heat in the winter, it continues to serve this purpose in the high temperatures of the summer, causing animals to display multiple symptoms of heat stress (Olson *et al.* 2003). Another SNP, rs41658708 (BTA9G) was also located on chromosome 9 (33,138,797 bp from BTA9B), but was not located near any potential genes. However, the major

allele displayed an association with reduced serum prolactin concentration, which further supports the suggested link between *CD109* and increased hair coat scores in fescue toxicosis. It is possible that the negative regulation of TGF-β by *CD109* can inhibit the activity of keratinocytes to signal the shedding response of the thick winter coat, and presents an interesting link between this SNP and fescue toxicosis.

The SNP rs109060624 (BTA9C) is located within an intronic region (assembly Btau_4.6.1) in the Reticulon-4-interacting protein 1, mitochondrial precursor gene (*RTN4IP1*). The mitochondrial protein interacts with a member of the Reticulon-4 family, *NOGO,* which allows for axonal regeneration by promoting dendrite outgrowth on neurons (GrandPre *et al.* 2000). Ergot alkaloids have been shown to inhibit the function of vesicular glutamate transporters (VGLUT), which are located in neurons and facilitate glutamatergic neurotransmission across the terminal ends (Xue *et al.* 2011) of neurons. It is possible that the combined effects of shorter dendrite growth and decreased neurotransmitter function originating from these dendrites may possibly be linked to fescue toxicosis. If the inhibited function of *RTN4IP1* reduces dendrite growth, the decrease of neurotransmitter function due to ergot alkaloids may further enhance the negative effects of endophyte-infected tall fescue on cattle.

Two significant SNP located on chromosome 9 are close to genes with possible links to reproductive symptoms. The SNP rs110579143 (BTA9F) is located 3' (224,825 bp, assembly Bos_taurus_UMD_3.1) to the gene encoding the prolyl endopeptidase gene (*PREP*). Prolyl endopeptidase (PEP, also referred to as prolyl

oligopeptidase) has roles in the maturation and degradation of peptide hormones by cleaving C-terminal peptide bonds. Several of the hormones that utilize this enzyme are involved in reproduction, including luteinizing hormone-releasing hormone (LH-RH), angiotensin, and oxytocin (Walter *et al.* 1974). Ovulation in mammals is linked to a catalyzing surge of LH which increases protein synthesis in the theca and granulosa cells of the follicle (Reel & Gorski 1968). Studies (Yoshimura *et al.* 1996; Ferreira *et al.* 2007) have shown that the renin-angiotensin system (RAS) has an important role in the modulation of the ovulatory process. One component of this system is angiotensin II peptide, which increases following the LH surge (Acosta *et al.* 2000). Both angiotensin I and angiotensin II can be cleaved by PEP to form Angiotensin-(1-7) (Ang-(1-7)), which mediates steroidogenesis (Pepperell *et al.* 2006) in the corpus luteum. Tonellotto dos Santos *et al.* (2012) reported that differential expression of *PREP* in the theca and granulosa cells during the periovulatory period were associated with changes in enzymes and receptors that regulate ovulation in cattle. Fescue toxicosis increases the length of time until the date of birth of the first calf (Bagley *et al.* 1987). There was an association between days to first calf and SNP rs41622923 (BTA9H), which is located 2.55 Mb away from BTA9F (assembly Btau_4.6.1), which suggests another link with *PREP*. While the exact causative factor extending this period is as of yet unknown, these results support the finding of reduction in reproductive performances in cattle grazing endophyte-infected fescue.

Another SNP located on chromosome 9 (232,682 bp on 5' side, assembly Btau_4.6.1) near a candidate gene is rs43601136 (BTA9I), which encodes for glycoprotein endo-α-1,2-mannosidase (*MANEA*). This enzyme is produced in the Golgi body, and is responsible for catalyzing mannosyl cleavage, an important step in the synthesis of glycoproteins. A variety of reproductive hormones are glycoproteins, such as luteinizing hormone (LH), follicle-stimulating hormone (FSH), as well as many components of the zona pellucida, which surrounds the oocyte. As of yet, a specific link between *MANEA* and fescue toxicosis has not been suggested. However, as a catalyst in the synthesis of several hormones that are affected by fescue toxicosis, alterations in the *MANEA* gene could explain some of the effects of endophyte-infected fescue.

A SNP on Chromosome 19 is associated with Hair Coat Score (HCS) and Days to 1st Calf

As described, two of the most prominent phenotypes associated with fescue toxicosis in cattle are hair coat score and days to first calf. One significant SNP rs109259586 (BTA19A) is located extremely close to two genes. On the 5' side (566 bp) of the SNP, the G protein-coupled receptor 179 (*CPR179*) gene contains mutations that are directly responsible for autosomal-recessive night blindness (Audo *et al.* 2012). The second gene, however, is located 5,811 bp on the 3' side. This gene, suppressor of cytokine signaling 7 (*SOCS7*), has been demonstrated to be a regulator of signaling associated with prolactin, leptin, and bovine somatotrophin (bST), due to inhibition of the activation of STAT3/STAT5 (Martens *et al.* 2005).

Expression of this gene is also regulated by bovine somatotrophin and prolactin, so it plays a central and important role in the various physiological functions attributed to these hormones (Martens *et al.* 2004). Prolactin is central to fescue toxicosis response, as previously described, and growth traits such as body condition score (BCS) and adjusted 205-day weaning weights are affected by changes in concentrations of bST.

Additional Significant SNP

Three polymorphisms were located near or within genes with known functions that are not currently linked to fescue toxicosis. Associated with HCS in our study, rs41573652 (BTA26) is located in an intronic region of the gene encoding cytoplasmic polyadenylation element binding protein (*CPEB*), which is a RNA-binding protein that promotes poly-A tail elongation of mRNA (Hake & Richter 1994). Two additional SNP were found to be in association with adjusted 205-day weaning weight. rs42018396 (BTA14B) was located near the gamma-1-syntrophin (*G1SYN*) gene that produces a protein that binds to dystrophin, which when disrupted, is linked to muscular dystrophy in humans (Hoffman *et al.* 1987). The other SNP, rs41587222 (BTA23) is in proximity to the Rh-associated glycoprotein (*RHAG*) gene, which is linked to blood Rh-group antigen expression.

One result from this experiment was the significance of SNP BTA11 for three different phenotypes, BCS, weight gained per day of age, and days to first calf. This suggests that the SNP is in relatively close proximity to a possible quantitative trait locus (QTL) for each trait, or even a polymorphism located in a gene with altered

expression. This SNP offers a potential subject for future research that is not limited to fescue toxicosis.

Conclusion

This study validates our hypothesis that the polymorphisms, first discovered in a small sample population, can be informative for larger, more diverse populations. Selective genotyping through customized assays may be more efficient and cost-effective. The population in this study is representative of the average cow-calf operation in the Midsouth: diverse breed composition and varying intensity of management. The results from this study are an encouraging first step towards both the development of a multi-locus genotyping panel that can provide better selection accuracy, as well as the discovery of the physiological mechanisms of the symptoms of fescue toxicosis through candidate gene analysis.

Table 1. Location and assay information for SNP included in study

SNP	BTA	Position	Forward Primer Sequence	Reverse Primer Sequence	VIC Reporter Sequence	FAM Reporter Sequence
BTA9A	9	12042726	ACTCTGTCTCTGAGATTCGATTCAGT	CTAAGCTGAAATCTTTCATTCCCTAGCT	ATGCTGAAAACTCTGTCTCT	TGCTGAAAACTCTATCTCT
BTA9B	9	14194506	GGCACTTAGGGCATCCTTTCAG	GTGTTGGAAGAAGTTGTAAATATGGGAAA	CCTGTTTACCCTACCTTC	CCTGTTTACCTTACCTTC
BTA9C	9	43883136	CAGGTGGGCCCCCAAA	AGTGTCACTTAGGTGAAAACTAGTAGGA	CTGGGAAGCGAGTGTC	TGGGAAGCAAGTGTC
BTA9E	9	44075848	ACATGTGTATAAAACAGTGCAGTGTGA	GCTGCGTTCCAAGTCTCCTTT	TCTGGCTGTCTTTCC	TGGCCGTCTTTCC
BTA9F	9	44872698	GTCATCCCTCAAGGCAGATAAAGG	CAGACTATAATAAGGTCAGTGCAACGA	AGCTGGACCTGCAGAGC	AGCTGGACCTACAGAGC
BTA9G	9	47333303	CTTGTTTTGCTTCTGTTTGTTTTGGT	ACCAATTATAGACAGAGATTGTCAGAGTCA	TCTTGCTTTTGGTTTTT	TTGCTTTCGGTTTTT
BTA9H	9	47425511	ACCTAGCATGTCTGTACTGTGGAA	GCCTTGCTATTGATAGAGCCAACT	TCGACCAAAGTTTCTGC	CGACCAAAATTTCTGC
BTA9I	9	55496923	GCAGTTGTTAAAAACAATTTACCTTCTGTATTACT	TGTCCGCTGTCTTAGCTCAAG	TGGAGAAGGTGAACACGA	TGGAGAAGGTAAACACGA
BTA10	10	9900839	CCATGGGCCTGTCCTCATTAAAG	TGGCTGCGCCATGCT	CAGAAGGGTACGTACATGA	CAGAAGGGTACATACATGA
BTA11	11	16193493	CCAGGCCTCGTAGGAAGAG	CCGTGTGTCCTGCATAAGC	CCTGCTCGTGGCCAA	CCTGCTCATGGCCAA
BTA14B	14	22643306	TGGGAACAGAATTGTCACTCTTTAATTAGAAA	GTTCAAAAATCATTACTATTCTTCCTTGAGTTCC	CAAATTCACCGCCATACC	CAAATTCACCACCATACC
BTA17A	17	19975119	CTTAGCAGAAGCCGGCTTATCT	GCCTGGGTTAGAGATATTCTCAATTGAT	CAGCCCAAATTTATACTTATA	AGCCCAAATTTATGCTTATA
BTA17B	17	69005486	CATCAGAATCACCCAGCAGTCA	AGCTCGGAGATCAATGGCTTT	CTTGCCTCATTCTGGACTG	TTGCCTCATTCCGGACTG
BTA19A	19	39642202	GTCTGTACACACACCAATTATGGTTCT	CGTGCTGCTAAACTGGAATTTGAG	CCCTCGCCATCCCTA	CCCCTCGCTATCCCTA
BTA19B	19	60078948	GCGACCTGTGGCTGATTCA	CCCCAACTCCCTCTCACACT	TTGTCAGTCTGGCCCC	TCAGTCCGGCCCC
BTA23	23	22080127	CAGTAGCATAAATACCGGGAAGGAA	TGTCTAGTGACATTACACCACTCTTTATAGT	CATACACTGTGGTAAGTTA	CATACACTGTTGTAAGTTA
BTA26	26	13697600	GCAAAAATGTTGATAATCATTGAAGCTAAGTG	GACAAGATGGAAAGTCTGGAAACATTTATTTATC	CATAGTTCATTAAACAATTC	TAGTTCATTCAACAATTC
DRD2	15	404365	TCTGACCTTACCAGGAACAATCCA	CTGAAGCCAGGCTGAGTCT	ACCGGAATCACCC	ACCGGAGTCACCC
XKR4-1	14	2590669	TGCTCCTCCACAGACAATTCATATG	AGACGGGTGTTAGCACAGAAC	TCTCGCATTCTCC	TCTCGCGTTCTCC
XKR4-2	14	2667113	CGTCTTGAACAGGATTTCGGTTTTC	AGGCTACCAGATTTTTAGCACTAATGAAG	TGGTTGTAGCTAAAAGC	TGGTTGTAGCTGAAAGC
XKR4-3	14	2702753	GACGAGCCCACGTGGT	GCATCGCAGGCAGATTCTTTAC	TCCGAGCCGCCTGAG	ATCCGAGCCACCTGAG

Table 2. Standard age of dam adjustment factors (kg) for adjusted 205-day weaning weight[1]

Age of Dam at Calving (Years)	Weaning Weight Adjustment Factor	
	Male	Female
2	27.27	24.54
3	18.18	16.36
4	9.09	8.18
5-10	0.00	0.00
11 & older	9.09	8.18

[1]Beef Improvement Federation (2010)

Table 3. Analysis of variance results

SNP Name	Phenotype[1] (*P* value, α < 0.05)					
	Weight gained per day of age	Adjusted 205-day weaning weight	Serum prolactin concentration	Body condition score (BCS)	Hair coat score (HCS)	Days to first calf
BTA9A	0.1671	0.4268	0.4520	0.9117	0.9955	0.6108
BTA9B	0.1353	0.0574	0.3395	0.5954	0.0276	0.4853
BTA9C	0.0813	0.1593	0.0642	0.8136	<0.0001	0.9349
BTA9E	0.3138	0.2738	0.3370	0.2153	0.1380	0.2972
BTA9F	0.1027	0.0538	0.0795	0.8698	0.0089	0.9975
BTA9G	0.1076	0.5302	0.0006	0.3309	0.5323	0.9752
BTA9H	0.0982	0.7798	0.1049	0.3373	0.0597	0.0023
BTA9I	0.0914	0.4792	0.3786	0.5638	0.0447	0.3769
BTA10	0.2412	0.2163	0.6212	0.8108	0.0961	0.4592
BTA11	0.0014	0.9147	0.4313	0.0371	0.7889	0.0240
BTA14B	0.6075	0.0273	0.9200	0.6898	0.7924	0.8795

[1] SNP significant at α < 0.05 are shaded grey

Table 3 (continued)

	Phenotype[1] (*P* value, α < 0.05)					
SNP Name	Weight gained per day of age	Adjusted 205-day weaning weight	Serum prolactin concentration	Body condition score (BCS)	Hair coat score (HCS)	Days to first calf
BTA17B	0.5915	0.8949	0.1931	0.6794	0.4843	0.5617
BTA19A	0.0605	0.2973	0.1425	0.6795	0.0031	0.0010
BTA19B	0.6047	0.3635	0.9807	0.6447	0.2362	0.7814
BTA23	0.1756	0.0469	0.1150	0.8060	0.1856	0.1658
BTA26	0.6488	0.9967	0.0881	0.3241	0.0008	0.0504
DRD2	0.8098	0.9572	0.0912	0.4820	0.7857	0.6258
XKR4-1	0.5658	0.4536	0.0312	0.8348	0.0002	0.3102
XKR4-2	0.5925	0.5151	0.0023	0.8056	0.1528	0.3305
XKR4-3	0.5533	0.5677	0.0050	0.8579	0.1514	0.3422

[1] SNP significant at α < 0.05 are shaded grey

Table 4. Significant associations for GWAS-detected SNP

Phenotypic Association	SNP	P value	Genotype Means[1]		
			A	H	B
Hair coat score[2]	BTA9B	0.028	1.7^{B}	1.8^{A}	1.8^{AB}
	BTA9C	< 0.001	1.9^{A}	1.6^{B}	1.7^{B}
	BTA9F	0.009	1.8^{A}	1.7^{B}	1.8^{AB}
	BTA9I	0.045	1.7^{B}	1.7^{B}	1.9^{A}
	BTA19A	0.003	1.6^{B}	1.8^{A}	1.8^{A}
	BTA26	0.001	1.8^{A}	1.7^{B}	1.6^{B}
	XKR4-2	0.001	1.8^{B}	1.6^{C}	2.0^{A}
Body condition score[3]	BTA11	0.037	5.0^{A}	4.8^{B}	5.1^{A}
Serum prolactin concentration (ng/ml)[4]	BTA9G	0.011	$30.3^{B,5}$	35.9^{A}	
Weight gained per day of age (kg)[6]	BTA11	0.001	0.52^{A}	0.45^{B}	0.54^{A}
Adj. 205-d weight (kg)[7]	BTA14B	0.027	130^{A}	117^{B}	
	BTA23	0.047	129^{AB}	122^{B}	137^{A}
Days to 1st calf (days)[8]	BTA9H	0.002	749^{B}	1185^{A}	725^{B}
	BTA11	0.024	715^{B}	1073^{A}	781^{B}
	BTA19A	0.001	989^{A}	723^{B}	709^{B}

[1]Superscript letters within a row denote mean differences ($\alpha < 0.05$) by Fisher's LSD

[2]Scored 1 to 5 (1= slick summer coat, 5 = full winter coat) Tucker & Ringer (1982)

[3]Scored 1 to 9 (1= extremely thin, 9 = extremely obese) Wagner *et al.* (1988)

[4]Values log-transformed for normality

[5]Means back-transformed

[6]Recorded weight (kg) divided by days of age.

[7] See Equation 1. Beef Improvement Foundation (2010)

[8] Time in days from date of birth to calving date of first calf

Table 5. Candidate genes in proximity to selected SNP

Chromosome	SNP	Reference SNP ID[1]	In Gene?	Candidate Gene	Direction	Distance (bp) from gene
9	BTA9B	rs42386739	N	*CD109*[2]	3'	642,721
9	BTA9C	rs109060624	Y	*R4IP1*[3]	-	*Intronic*
9	BTA9F	rs110579143	N	*PREP*[2,3]	3'	224,825
9	BTA9I	rs43601136	N	*MANEA*[3]	5'	232,682
14	BTA14B	rs42018396	N	*G1SYN*[2,3]	5'	293,329
19	BTA19A	rs109259586	N	*SOSC7*[2,3]	3'	5,811
			N	*GPR179*[2,3]	5'	566
23	BTA23	rs41587222	N	*RHAG*[2,3]	3'	8,514
26	BTA26	rs41573652	Y	*CPEB3*[2,3]	-	*Intronic*

[1]Accession number, Single Nucleotide Polymorphism Database (dbSNP)

[2] NCBI bovine genome reference assembly Bos_taurus_UMD_3.1

[3] NCBI bovine genome reference assembly Btau_4.6.1

Chapter 2. A polymorphism in XKR-4 is significantly associated with serum prolactin concentration and could be a candidate gene for resistance for cattle grazing tall fescue

Disclosure

This chapter is revised based on a manuscript submitted by Bryan Bastin to the journal *Animal Genetics*:

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I served as the primary author of this manuscript, and contributed the majority of the conception, implementation and analysis of the project. Co-author contributions included assistance including, but not limited to, the following: editing of the text, assisting with or providing laboratory materials and methods, consultation regarding statistical methods utilized, and financial support.

Abstract

A single-nucleotide polymorphism within the XK, Kell blood group complex subunit-related family, member 4 (XKR4) gene located on BTA14 was significantly associated with serum prolactin concentrations in 592 cattle grazing endophyte-infected tall fescue (Lolium arundinaceum Schreb.). Depressed circulating serum prolactin concentrations are typically observed in cattle displaying symptoms associated with fescue toxicosis. Polymorphisms in this gene have been previously reported to be associated with rump fat thickness, residual feed intake, average daily feed intake, and average daily gain in cattle. Associations have also been reported between XKR4 genotype and effectiveness of the dopamine antagonist Iloperidone as a treatment of schizophrenia. Domperidone, a related dopamine antagonist, mediates effects of fescue toxicosis in livestock, including restoring depressed concentrations of prolactin. SNP rs42646708, one of three SNP located within the gene in linkage disequilibrium, was significantly ($P = 0.0002$) associated with serum prolactin concentrations, and explained 2.45% of the phenotypic variation. Effect of genotype at the SNP was tested across five breeds, with significant associations within both Angus ($P = 0.0275$) and Simmental ($P = 0.0224$) breeds. These results suggest XKR4 may play a role in mediating the negative effects of fescue toxicosis and could offer a marker for selection for resistance.

Introduction

Tall fescue (*Lolium arundinaceum Schreb.*) is the most prevalent forage grass in the Midsouth United States (Stuedemann & Hoveland 1988) and cattle grazing this forage comprise approximately 20% of all cattle raised in the United State (West & Waller 2007). A cool-season perennial bunchgrass, tall fescue is easily established, drought and pest resistant and tolerant of grazing (Hill *et al.* 1991). These and other positive characteristics are due mainly to the presence of an endophytic fungus (*N. coenophialum*) that resides within the grass. The endophyte produces ergot alkaloids such as ergovaline (Yates *et al.* 1985a); ingestion of these serotonergic and dopaminergic agonists contribute to a variety of symptoms known collectively as tall fescue toxicosis (FT). Reductions in weight gain, dry matter intake, reproduction rates, as well as delayed onset of puberty and peripheral vasoconstriction are observed in cattle affected by fescue toxicosis (Fribourg *et al.* 1991).

Decreased serum prolactin concentration in cattle has been shown to be a symptom of fescue toxicosis (Hoveland *et al.* 1983). Previous research identified an intronic single-nucleotide polymorphism (SNP) in the dopamine receptor D2 (*DRD2*) gene on bovine chromosome 15 that regulates prolactin secretion (Kojima *et al.* In press). The *DRD2* receptors are targeted by ergot alkaloids, causing a decrease in serum prolactin concentrations. Domperidone (a selective D2 antagonist) has been used in equine species to mediate the effects of fescue toxicosis (Cross *et al.* 2012).

The *XKR4* gene is sparsely represented in the literature; previous studies in humans show associations between XKR4 genotype and schizophrenia treatment efficiency using Iloperidone (Lavedan *et al.* 2009; Fijal *et al.* 2012). Iloperidone, a dopamine antagonist related to domperidone, is an antipsychotic used to treat the symptoms of schizophrenia. It acts as an antagonist to serotonin $5HT_{2A}$, dopamine D_2 and D_3, and serotonin $5HT_6$ receptors. Dopamine D2 antagonism increases prolactin secretion (Ben-Jonathan & Hnasko 2001); treatment with Iloperidone is associated with, among other symptoms, an elevation in prolactin (Jain 2000; Cutler *et al.* 2008).

Due to the similar physiological mode of action, *XKR4* was chosen as a candidate gene to explore a possible link to fescue toxicosis. To date, only two previous studies have been performed in cattle related to *XKR4,* one of which identified three SNP within the *XKR4* genes associated with subcutaneous rump fat thickness (Porto Neto *et al.* 2012). The other identified *XKR4* as a possible candidate gene due to associations with residual feed intake, average daily feed intake and average daily gain (Lindholm-Perry *et al.* 2012); reductions in these phenotypes are typically seen in cattle experiencing fescue toxicosis.

Materials and Methods

Custom Taqman assays were designed for three *XKR4* SNP (rs42646645, rs42646708 and rs41724387), and used to genotype a mixed-breed (Angus, Simmental, Charolais) herd (n = 592) located at Tennessee Technological University in Cookeville, TN. The herd comprises cows and calves, and over half of the current

herd was inherited two years ago; as well as a herd largely assembled through random purchases at public livestock sales in the area.

Forage samples were taken in every field to determine tall fescue coverage and endophyte infestation rate. Analysis (Agrinostics, Watkinsville, GA) revealed that all fields had > 80% endophyte infestation rate and tall fescue forage comprised the overwhelming species of all fields tested. Serum samples, hair samples for genotyping, and other phenotypic data were collected during the month of June 2012, when effects of fescue toxicosis can be observed.

Prolactin concentrations were derived using radioimmunoassay as previously described (Bernard *et al.* 1993), with intra- and inter-assay CV's of 6.6% and 10.3% respectively.

DNA was isolated from hair follicles using QuickExtract solution (Epicentre, Madison, WI), and genotyped using custom Taqman genotyping assays (Applied Biosystems, Carlsbad, CA) with primers and probes as described by Porto Neto *et al.* (2012).

Results and Discussion

Serum prolactin concentrations were log-transformed and analyzed using PROC MIXED in SAS 9.3 (Cary, NC) with genotype as the main effect. Additionally, analyses were performed within breed (Table 5). Linkage analysis performed with the *genetics* package in R resulted in $r^2 > 0.94$ and $D' = 1.00$; therefore, only rs42646708 results are presented, as similar results were found with

the other SNP. Overall, the minor A allele is advantageous in homozygous and heterozygous states (Table 5). Similar patterns were observed within Angus and Simmental, while Charolais showed no prolactin response. The prolactin reducing effect of fescue toxicosis appears to be mitigated at least in part by the presence of the minor A allele.

Additionally, a regression model of log(prolactin) on SNP genotype coded as the number of G (major) alleles present was fit (SAS Proc REG). The G allele effect of the *XKR4* SNP was -7.90 ± 2.25 ng/mL (results back-transformed), with $P = 0.0069$. The SNP effect accounted for 2.45% of the phenotypic variation.

These results suggest that the *XKR4* gene plays a role in the regulation of prolactin secretion in cattle and the SNP within the gene could be useful markers for selection for resistance to fescue toxicosis. The minor A allele plays an advantageous role in keeping normal concentrations of serum prolactin, especially within the Angus and Simmental breeds. The *XKR4* gene has been shown to have an effect on disorders in both the dopaminergic and adrenergic pathways, and these SNP suggest that further analysis of *XKR4* sequence and expression may elucidate the physiological and genetic basis of fescue toxicosis.

Table 6. SNP rs42646708 effects of XKR4 on serum prolactin concentrations by breed

Breed	n	*Freq*[1]	GG Mean[2]	AA Mean	AG Mean	*P-value*
Angus	210	0.171	$26.67^{B,3}$	39.37^{AB}	36.83^{A}	0.028
Simmental	158	0.358	31.97^{B}	49.96^{A}	44.57^{A}	0.022
Chiangus	116	0.220	27.70^{A}	23.43^{A}	29.33^{A}	0.780
Simangus	82	0.110	28.93^{A}	14.50^{A}	30.32^{A}	0.660
Charolais	26	0.231	48.26^{A}	31.68^{A}	41.19^{A}	0.760
All	592	0.224	28.68^{B}	41.44^{A}	37.22^{A}	0.0002

[1] Frequency of the minor allele (A)

[2] Back-transformed least squares means associated with genotype, ng/mL

[3] Superscript letters within a row denote mean differences ($\alpha < 0.05$) by Fisher's LSD

Conclusion

Tall fescue is the most prominent forage in the Midsouth U.S., and thus, fescue toxicosis is an extremely important topic of research. Fescue toxicosis has been widely studied, but the physiological mechanisms that underlie the disease are still poorly understood. Research into the ergot alkaloids produced by the fescue endophyte, have yielded some insight. Ergovaline is a serotonergic and dopaminergic agonist that is causative agent of the multitude of symptoms associated with fescue toxicosis. These symptoms, such as reduced serum prolactin concentration, increased hair coat score, decreased reproductive performance and reduced growth traits provide targets for research.

The most recent and promising method of combating fescue toxicosis is through the study of the genetic basis behind the physiological pathways thought to contribute to the disorder. An initial genome-wide association study yielded 24 single-nucleotide polymorphisms that were associated with two key traits, weaning weights and birth weights, in a group of 48 Angus cows. While this information would be useful to varying degrees, further validation in a larger, more diverse herd would allow for both useful insights into genes that contribute to the physiological pathways of fescue toxicosis, as well as markers for a specialized genetic panel that could be utilized to select for animals resistant to endophyte-infected fescue.

The first objective of the current project was validation of the 24 polymorphisms from the association study in a larger herd with a variety of phenotypes. Fifteen associations were significant for at least one of the target phenotypes, warranting inclusion in a genetic marker panel designed for selection

of animals resistant to the effects of fescue toxicosis. Of these fifteen SNP, nine were located within or in proximity with candidate genes that could elucidate the physiological mechanisms that cause toxicosis. Chromosome 9 yielded four possible candidate genes alone, each coding for proteins or enzymes that could be linked to fescue toxicosis. The *CD109* gene is a negative regulator of the TGF-β system which controls keratinocyte function; among other functions, these cells catalyze the cycle of growth, shedding and regrowth of mammalian hair, which presents a clear link to increased hair coat scores evident in affected animals. Ergot alkaloids have been shown to inhibit neurotransmitters in the brain, which suggested that an associated SNP's proximity to the *RTN4IP1* gene could shed light on this phenomenon. The protein interacts with another gene, *NOGO,* which promotes neuronal dendrite outgrowth – this growth allows for shorter gap junctions between neurons and increases the efficiency of neurotransmission.

The remaining SNP on chromosome 9 yielded the genes *PREP* and *MANEA,* which have roles in both the synthesis and regulation of glycoprotein hormones that are central to the mammalian reproductive system. Another SNP offered a potential candidate gene linked to reproductive performance. *SOSC7,* which modifies *STAT3/STAT5* regulation to inhibit prolactin and growth hormone signaling, also presents a strong link to fescue toxicosis symptoms. The remaining candidate genes (*G1SYN, GPR179, RHAG,* and *CPEB3)* did not have functions explicitly tied to symptoms of fescue toxicosis, but are still informative for physiological function.

The results of the first objective yielded possible candidate genes, an approach taken in the second objective. *XKR4* had little information known in the literature, with only possible links to function in both humans and bovine studies. In the analysis of serum prolactin concentrations associated with three SNP within the *XKR4* gene, the results presented strong evidence of *XKR4* playing a central role in prolactin secretion. The presence of the minor A allele at the representative polymorphism appears to mitigate the prolactin-reducing effect of fescue toxicosis, and accounted for 2.45% of the phenotypic variation. The protein encoded by the *XKR4* gene has no immediately-known function, but these results warrant further research as a causal link to fescue toxicosis.

Fescue toxicosis is a complex disorder with a wide variety of symptoms and potential causes. The results presented by these studies confirm that genetic research into this disease is a worthwhile endeavor, yielding both markers for genetic selection to increase performance in future herds, as well as a number of potential candidate genes that could shed some light of the physiological mechanisms that drive a disorder than has an economic cost of $1 billion per year. Utilization of these results allows for the possibility of a multitude of future experiments spanning a variety of disciplines of genetic research, and hopefully, brings fescue toxicosis one step closer to being a non-debilitating disorder in the future.

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Vita

Bryan Bastin was born on Little Rock Air Force Base, Arkansas to parents Shawn and Allison Bastin. Bryan grew up in a variety of locations, including North Dakota, Germany, Japan, and Florida before moving to Knoxville, Tennessee to attend the University of Tennessee in 2005. While pursuing his bachelor's degree in Animal Science, he met his wife Kelley Bastin (nee Beam) in 2007. Both completed their degrees in 2009, and were married the following summer of 2010. Following employment by the Animal Science department as an IT Assistant, Bryan began his graduate studies in 2011 towards a Master's degree in Animal Science with a concentration in genetics and bioinformatics. He plans to conclude his Master's degree in August 2013, followed a month later by the birth of his first child, daughter Lily Evelyn Bastin. He has also accepted a position at Genus P.I.C. (Pig Improvement Company) as a Project Manager in the Genetic Services division in Hendersonville, TN.