8-2016

Association of genotype at DRD2 with growth and reproductive performance traits in bulls grazing toxic endophyte-infected tall fescue

Kaysie Jennings
University of Tennessee, Knoxville, kjennin5@vols.utk.edu

To the Graduate Council:

I am submitting herewith a thesis written by Kaysie Jennings entitled "Association of genotype at DRD2 with growth and reproductive performance traits in bulls grazing toxic 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

We have read this thesis and recommend its acceptance:

John T. Mulliniks, Gary E. Bates

Accepted for the Council:
Dixie L. Thompson

Vice Provost and Dean of the Graduate School

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

Association of genotype at DRD2 with growth and reproductive performance traits in bulls grazing toxic endophyte-infected tall fescue

A Thesis Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville

Kaysie Jennings
August 2016

DEDICATION

This thesis is dedicated to my family for their unwavering love and support. However, there are a few individuals that I would like to especially recognize, people who have supported me through this endeavor understanding that in doing so they were sacrificing their time with me, my grandparents.

My Pa and my Nanny, Ray and Jean Jennings, have always provided overwhelming support. They instilled within me at a young age a work ethic, a faith, and a glowing definition of love through the example they have set for me over the years. I can never thank them enough for their guidance over the years and their continuous love and support.

My Granny, Benny Kay McCasslin, and my Grandfather, "W.C." McCasslin helped me to discover my passion for animal agriculture at a young age and have loved me unconditionally. Some of my fondest memories are from summers spent on their farm. The days spent working tirelessly on their farm are the times I remember most and are the fundamental reason why I chose to pursue a degree in Animal Science.

ACKNOWLEDGEMENTS

There are a number of people that have contributed to my success throughout my graduate education that I cannot begin to thank enough for their assistance, instruction, advice, and support. First, my major professor, Dr. Cheryl Kojima, who has been my mentor since sophomore year of my undergraduate career when she became my undergraduate advisor. Over the past five years, she has advised me through coursework, listened to problems regardless of whether they were school related or not, and provided invaluable advice regarding academia as well as life. Dr. Kojima has played a huge role in my life, and I cannot thank her enough for her kindness, her guidance, and her friendship. Above all else, I must thank her for refusing to write a letter of recommendation for me as a part of my veterinary school application. This is just one of the many examples of a time where Dr. Kojima understood me better than I knew myself at a given moment. She knew that I was meant to be a graduate student, and she knew that deep down I wanted to go to graduate school as well. Thank you for not only granting me this opportunity to pursue a graduate education, but thank you for also helping me to find my voice, encouraging me to make mistakes, and teaching me that it is okay to deviate from plan A if you have a change in heart.

My committee members have provided advice, helped me to gain experience, and introduced me to tools that have aided immensely as I have worked towards completing my graduate studies at the University of Tennessee. Dr. Gary Bates met with me on multiple occasions to talk about tall fescue, which helped substantially as I conducted my graduate research. In addition, Dr. Bates taught me how to collect tiller samples and

provided advice on numerous occasions. Dr. Travis Mulliniks (aka T-Mully) provided me with advice on numerous occasions that aided in my graduate studies. Dr. Mulliniks also allowed for me to go to the farm with his graduate students on several occasions to gain more experience and took me to my first conference along with his graduate students. I can never thank Dr. Bates and Dr. Mulliniks enough for their advice, their assistance, and their encouragement as I have worked towards completing my graduate studies.

In addition to my committee members, there are a number of individuals who have played a significant role in my graduate education. Dr. Scott Pratt at Clemson University provided me with the phenotypic data recorded on the bulls from his studies over the past five years and aided in the completion of my graduate project through providing advice and answering questions. Without Dr. Pratt, his graduate student, Callie Burnett, and his team at Clemson University, this project would not have been possible. Dr. Arnold Saxton aided in statistical analysis for my graduate studies and provided advice on numerous occasions. It was an honor to learn under an individual that is as successful and as highly respected in his field as Dr. Saxton. Bryan Bastin fostered my love for genetics as he served as the graduate teaching assistant for the Animal Breeding and Genetics course that first sparked my interest in genetics. Bryan has always been available for genetics discussions and has provided advice on numerous occasions about research as well as life. I never imagined that the many genetics conversations I had with him as a sophomore undergraduate student at the University of Tennessee would take me this far. Bryan has always pushed me to be my best by giving me a benchmark to reach for as I try to keep up with his success.

A number of individuals from the Department of Animal Science have positively impacted me throughout my time at the University of Tennessee. Leszek Wojakiewicz helped me to acclimate to the research side of graduate school. He was always patient, and he taught me a number of laboratory techniques. Eddie Jarboe was always there regardless of whether I needed help getting in touch with someone or just needed someone to talk to momentarily about something unrelated to graduate school. His warm smile and compassion for everyone that he came in contact with was truly contagious, and I am blessed that I was able to know and work with him. Dr. Neal Schrick talked with me on numerous occasions about beef cattle production, fescue toxicosis, and physiological concepts pertaining to my project, which not only aided in the completion of my project but also helped to further spark my interest in beef cattle genetics.

Lastly, I must thank my fellow graduate students, who have assisted me over the past two years. Sarah Howard, thank you for your friendship over the past five years. I am so grateful that we were able to take on the challenge of graduate school together, and I cannot wait to hear all about your success post-graduate school. Sierra Lockwood, thank you for taking me under your wing when I started graduate school and being what I would consider a lifelong friend. Sarah Edwards, Jeremy Hobbs, and Sydney Reese, thank you for your friendship as well as your advice, your assistance, and allowing me to go with you to the farm to gain experience. To the rest of my fellow graduate students, thank you for your friendship, your advice, your assistance, and your support. I am blessed to have known each one of you and look forward to hearing stories of your successes in the future.

ABSTRACT

Fescue toxicosis can negatively impact growth and fertility of beef cattle grazing tall fescue infected with an endophytic fungus. The fungus produces ergot alkaloids that contribute to the hardiness of the plant, but can be detrimental to the animal if consumed. A single nucleotide polymorphism (SNP) in the dopamine receptor D2 gene (DRD2) has been associated with serum prolactin concentrations and hair coat scores in cattle grazing toxic endophyte-infected (TE) tall fescue; cattle with the GG genotype had decreased serum prolactin concentrations and increased hair coat scores. Separately, heifers with the AA genotype calved sooner than AG or GG heifers. The objective of this study was to examine the DRD2 SNP for genotype-phenotype associations in bulls grazing either TE tall fescue or non-toxic endophyte-infected (NTE) tall fescue. Bulls remained on their respective pasture treatments from February through mid-June. Statistical analysis focused solely on the months of April and May. Urine samples, semen samples, body weights (BW), scrotal circumferences (SC), and body condition scores were collected. Bulls were genotyped using a bovine Taqman genotyping assay (Applied Biosystems, Foster City, CA). Genotype at DRD2 was associated with weight gain such that GG bulls gained less weight than AA or AG bulls regardless of pasture type (P=0.0055). Bulls on TE tall fescue gained more weight than bulls on NTE tall fescue (P=0.0433). Genotype at DRD2 was associated with SC such that AA and GG bulls had a greater SC than AG bulls (P=0.0033). A trend was also observed between scrotal circumference and treatment (P=0.0768) with bulls grazing NTE tall fescue tending to have a smaller scrotal circumference than bulls grazing TE tall fescue. Urine ergot alkaloid concentrations were greater for bulls on TE tall fescue (P<0.001) and were also associated with DRD2 genotype; concentrations were almost two-fold greater in GG bulls than AA or AG bulls (P=0.0046). A genotype*treatment interaction was observed such that the GG bulls grazing TE tall fescue exhibited the greatest urine ergot alkaloid concentrations (P=0.0018). These findings further support the utilization of DRD2 genotype as a selection tool to enhance the performance of beef cattle grazing TE tall fescue.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1. INTRODUCTION

Tall fescue (*Lolium arundinaceum Schreb*.) is cool-season perennial bunchgrass in the mid-south region of the United States, a region more commonly referred to as the "fescue belt" (Stuedemann and Hoveland, 1988). In this region, tall fescue is the most prevalent forage grazed by livestock. Tall fescue is widely utilized due to ease of establishment, pest resistance, and drought resistance (Stuedemann and Hoveland, 1988; Arachevaleta et al., 1989). The hardiness of tall fescue is due to the presence of an endophytic fungus (*Epichloe coenophialum*) between the cell walls of the plant (Stuedemann and Hoveland, 1988; Bacon, 1995). However, while the toxic fescue endophyte provides a number of benefits to livestock producers, it can negatively impact the growth, reproduction, and performance of livestock that respond negatively to the ingestion of ergot alkaloids produced by the toxic endophyte (Stuedemann and Hoveland, 1988).

The negative performance observed in livestock grazing toxic endophyte-infected (TE) tall fescue is recognized as fescue toxicosis (Yates et al., 1985). Symptoms of fescue toxicosis include decreased average daily gain, decreased feed intake, and decreased body condition score (Looper et al., 2010). In addition, an increase in hair coat score has been observed (Porter and Thompson, 1992). Reproductive implications of fescue toxicosis include a delayed onset of puberty as well as shorter estrous cycles in heifers (Jones et al., 2003). Fescue toxicosis negatively impacts livestock performance and productivity, resulting in estimated economic losses in excess of $1 billion annually across the cattle, horse, sheep, and goat industries (Hoveland, 1993).

There are a number of proposed solutions to address the negative impact of TE tall fescue on livestock animal production. One such solution is to cut tall fescue early in the reproductive period, when the highest concentrations of ergot alkaloids are in the seeds at the top of the plant. Another method to minimalize the effects of TE tall fescue on livestock performance is to dilute pastures with clover or another legume (McLaren et al., 1983). Non-toxic endophyte infected (NTE) strains of tall fescue pose little threat to livestock that graze it (Gunter and Beck, 2004). These suggested management practices focus primarily on the plant side of the fescue toxicosis syndrome and only lessen the negative impact of the tall fescue forage on livestock that graze it.

One alternative method to combat the negative effects that TE tall fescue is through genetic selection of livestock. Genetic selection of cattle resistant to TE tall fescue and dissemination of those genetics across herds in the impacted region would vastly improve livestock performance in future generations. Recent studies have indicated a marker associated with TE tall fescue resistance. A single nucleotide polymorphism (SNP) contained in the dopamine D2 receptor gene (DRD2) has been reported to be associated with serum prolactin concentrations, body condition scores, and hair coat scores, all of which have been established as symptoms of fescue toxicosis (Campbell et al., 2014). Further validation may prove this SNP as a useful marker for selection of cattle resistant to the negative effects of grazing TE tall fescue (Campbell et al., 2014).

A study was initiated to assess association of DRD2 genotype with performance data collected from mature Angus bulls (n=25) located at Simpson Research and

Education Center (Pendleton, SC), a facility of Clemson University. The objective of this study was to characterize the association of genotype at DRD2 with weight gain, scrotal circumference, and urine ergot alkaloid concentrations in bulls grazing TE tall fescue. All of the Angus bulls on study at Simpson Research and Education Center were genotyped at DRD2 using a custom bovine Taqman genotyping assay (Applied Biosystems, Foster City, CA). In addition, phenotypic data was recorded on these bulls every four weeks throughout the duration of the study, which occurred from late February until mid-June. Phenotypic data included body weights, scrotal circumferences, urine ergot alkaloid concentrations, body condition scores, and semen samples for semen analysis. Additional evidence of genetic influence on these traits will support the potential of this SNP as a selection tool for producers to select for cattle that are resistant to TE tall fescue.

CHAPTER 2. LITERATURE REVIEW

Tall Fescue

Tall fescue (*Lolium arundinceum Schreb.*) is a cool-season perennial bunchgrass that is abundant in the temperate region of the southeastern United States. In this region of the United States, tall fescue is the primary forage grazed by livestock (Stuedemann and Hoveland, 1988). Tall fescue is suspected to have entered the United States as a contaminant in a different type of grass seed imported from Europe several decades ago. In 1941, the most common cultivar of tall fescue was discovered on a hillside in Kentucky and was aptly named ‘Kentucky 31’ (Fergus, 1972), which has become the most prevalent cultivar of tall fescue (Stuedemann and Hoveland, 1988).

Tall fescue is widely utilized due to the positive characteristics of the forage, which are attributed to the plant through the presence of an endophytic fungus (*Epichloe coenophiala*) (Stuedemann and Hoveland, 1988).The endophyte is contained within the entirety of the fescue plant and is most highly concentrated within the seedhead of the plant (Bacon, 1995). Furthermore, there are no outward signs of expression to detect in determining whether a pasture contains the endophytic fungus. The fungus undergoes its lifecycle in its entirety between the cell walls of the plant (Bacon, 1995). A symbiotic relationship exists between the endophyte and the plant, which allows for the endophyte to reside and thrive within the plant and confer a number of advantageous characteristics, which have contributed to the establishment of tall fescue as the primary forage (Arachevaleta et al., 1989). It has been estimated that over half of the tall fescue in the United States contains endophyte (Hoveland, 1993).

There are both toxic endophyte infected (TE) and non-toxic endophyte infected (NTE) strains of tall fescue available in the United States, with TE tall fescue exhibiting a greater tolerance to drought as compared to NTE strains of tall fescue (Stuedemann and Hoveland, 1988). In addition, TE tall fescue exhibits resistance to grazing pressure as well as herbivory by livestock and insect species, and the forage is easily established and has good seed production (Stuedemann and Hoveland, 1988). All of these attributes of TE tall fescue provide benefits to producers who spend less time performing intensive maintenance on these pastures as a result of the hardiness of the forage (Stuedemann and Hoveland, 1988).

Ergot Alkaloids

The toxic endophytic fungus contained within tall fescue produces ergot alkaloids, which when ingested are the direct cause of a number of symptoms that are identified collectively as fescue toxicosis. The toxic fescue endophyte produces a number of ergot alkaloids in differing concentrations (Yates et al., 1985; Lyons et al., 1986). As a result, each animal that is impacted by ergot alkaloids responds differently depending on the concentrations of each ergot alkaloid ingested among other physiological factors (Craig et al., 2015).

Fescue Toxicosis

While the toxic fescue endophyte provides a number of benefits to producers, it is also responsible for a collection of symptoms in livestock that graze TE tall fescue. These symptoms are collectively identified as fescue toxicosis (Hoveland, 1993). Fescue toxicosis impacts a number of physiological systems due to the ability of ergot alkaloids to interact with a number of different receptor types throughout the body (Berde and Stürmer, 1978). The overall implications are vast economic losses estimated to be in excess of $1 billion dollars annually in the cattle, horse, sheep, and goat industries combined as a result of poor animal performance due to fescue toxicosis (Strickland et al., 2011).

Reduction in blood transport throughout the body of the animal is the key cause to a number of symptoms exhibited when experiencing fescue toxicosis (Rhodes et al., 1991). The reduction in blood flow to the core and peripheral regions of the body results in heat stress conditions due to a reduction in heat dissipation (Rhodes et al., 1991). The heat stress condition may impact other physiological systems thus exacerbating the distress caused by the toxic substances from TE tall fescue (Hemken et al., 1979; Hurley et al., 1980; Hemken et al., 1981; Hemken et al., 1984).

Fescue toxicosis can manifest itself in a number of ways. Two issues associated with grazing TE tall fescue are fat necrosis and fescue foot (Stuedemann and Hoveland, 1988). Fat necrosis is the phenomena in which degraded lipid deposits appear in the mesentery of the abdominal region of animals grazing TE tall fescue (Smith et al., 2004). The development of such adipose deposits around the intestines can lead to issues pertaining to digestion as well as calving (Bush et al., 1979). The most severe manifestation of fescue toxicosis is fescue foot. Fescue foot occurs due to vasoconstriction and hyperemia near the coronary band that lies between the hoof and dewclaw in the limb (Hemken et al., 1981). Vasoconstriction and hyperemia in this region of the limb can result in gangrene of the extremities. This same phenomena can also occur with the tail, resulting in the sloughing off of the tail in extreme cases. Fescue foot is most commonly observed in the fall and winter months. However, incidences of fescue foot have been observed at other times in the year (Hemken et al., 1981). Despite being the most severe, fescue foot is the least common of any manifestation of fescue toxicosis in livestock animals that graze TE tall fescue (Lyons et al., 1986).

The primary of all concerns associated with livestock grazing TE tall fescue is commonly referred to as the "summer slump", a term often used interchangeably with fescue toxicosis (Stuedemann and Hoveland, 1988). Fescue toxicosis negatively impacts animal performance in addition to affecting reproductive performance and the performance of offspring (Paterson et al., 1995). Cattle grazing TE tall fescue exhibited decreases in daily gain, feed intake, and a reduction in body condition score (Looper et al., 2010). Furthermore, cattle grazing TE tall fescue displayed reduced reproductive rates, delayed onset of puberty (Jones et al., 2003), and an increase in hair coat score (Porter and Thompson, 1992). Signs of fescue toxicosis were initially reported to occur at temperatures exceeding 32 ℃ (Hemken et al., 1981). However, recent findings have reported manifestations of symptoms of fescue toxicosis at temperatures below 32 ℃, indicating that the presentation of symptoms of fescue toxicosis is not necessarily mitigated by climate (Parish et al., 2003).

Fescue Toxicosis and Prolactin

A depression in serum prolactin concentrations has been noted to be one of the key physiological indicators of fescue toxicosis (Hoveland et al., 1983; Fribourg et al., 1991; Rice et al., 1997; Parish et al., 2003). The interaction that takes place between the ergot alkaloids ingested by animals grazing TE tall fescue and circulating prolactin results in altered serum prolactin concentrations (Ben-Jonathan and Hnasko, 2001).

Serum prolactin is secreted by the anterior pituitary gland and is best known for its role in mammalian lactation. However, prolactin is known to serve over 300 physiological functions, including playing a role in the shedding of winter hair coats in bovine species (Ben-Jonathan and Hnasko, 2001).

Prolactin secretion is negatively regulated by dopamine, a neurotransmitter secreted from the hypothalamus (Lamberts and Macleod, 1990). Upon secretion from the hypothalamus, dopamine binds to dopamine D2 receptors, which inhibits secretion of prolactin from lactotrophs of the anterior pituitary (Kebabian and Calne, 1979). Ergovaline, an ergot alkaloid that is believed to be one of the key causative ergot alkaloids in fescue toxicosis, is recognized as a dopamine agonist; therefore, when ergovaline binds to dopamine D2 receptors, it causes the decline in serum prolactin concentrations commonly displayed in animals with fescue toxicosis (Lamberts and Macleod, 1990). Treatment with a dopamine agonist such as domperidone has been shown to negate the impact that fescue toxicosis has on oocyte maturation in cattle (Jones et al., 2003; Jones and King, 2009). Pregnant mares that were fed TE tall fescue and administered domperidone gel foaled within two weeks of their anticipated foaling date, had developed functional and lactating mammary glands at foaling, and produced adequate levels of milk for the first five days following foaling compared to control mares grazing TE tall fescue that were not administered domperidone gel (Cross et al., 2012).

Increased hair coat score (HCS) is another symptom of fescue toxicosis and is impacted by serum prolactin concentration. A hair coat score is an assigned score to describe the thickness of hair coats in cattle in which a score of 1 is assigned to cattle who have completely shed their winter coat, while a score of 5 is assigned to cattle that have a full hair coat that has not shed. Levels of serum prolactin are related to seasonal changes in hair growth, so as days in the winter grow shorter, prolactin levels decrease inversely with melatonin levels (Tucker and Ringer, 1982). A study evaluating hair coats in minks found that as photoperiod decreased, serum prolactin concentrations decreased and induced growth of a thick winter coat. Upon treatment with prolactin, shedding of the winter coat occurred as well as growth of a summer coat (Allain et al., 1981). As winter transitions to summer and the hours of daylight each day increases, the high levels of ergovaline ingested by cattle grazing TE tall fescue inhibit the increase of prolactin levels. This results in the retention of the animal's thick winter coat thus contributing to the heat stress conditions associated with fescue toxicosis (Bastin, 2013).

Fescue Toxicosis and Weight Gain

One commonly observed sign of fescue toxicosis in livestock is a decrease in weight gain and daily feed intake (Paterson et al., 1995). The decline in animal performance on TE tall fescue puzzled researchers and producers for a number of years because well maintained tall fescue possessed superior forage quality and should allow for good animal performance (Bush and Buckner, 1973). However, despite the dense nutritional value of tall fescue, cattle were still not gaining weight as efficiently as cattle grazing orchard grass (Stuedemann and Hoveland, 1988). In addition, Petritz et al. (1980) reported that weight gain of calves grazing TE tall fescue was lower as compared to calves on tall fescue-clover.

A decrease in weight gain and in feed intake can occur to greater or lesser degrees depending on the amount of ergot alkaloids present in the forage being grazed by cattle (Fribourg et al., 1991). If there are greater concentrations of ergot alkaloids in tall fescue, symptoms of fescue toxicosis are known to be more severe, including the symptomatic decrease in weight gain and feed intake (Fribourg et al., 1991). Fribourg et al. (1991) reported that as the TE tall fescue infestation rate in a pasture decreased from 80% to 3%, signs of fescue toxicosis decreased and average daily gains of the cattle increased.

Fescue Toxicosis and Female Reproduction

Reproductive performance of cattle grazing TE tall fescue exhibits a decline primarily in the warmer months of the year, similar to other manifestations of the fescue toxicosis symptoms (Porter and Thompson, 1992). However, the physiological phenomena underlying the decline in reproductive performance of livestock grazing TE tall fescue are not fully characterized.

Implications of grazing TE tall fescue on female livestock have been more clearly characterized through prior research. Heifers grazing TE tall fescue have been found to exhibit decreased progesterone levels (Jones et al., 2003). Treatment with a dopamine antagonist countered the negative effects of the TE tall fescue, returning the progesterone levels back to a normal range (Jones et al., 2003). Browning et al. (1997) linked fescue toxicosis to reduced luteinizing hormone concentrations, a hormone that plays a key role in ovulation as well as the formation of the corpus luteum that is necessary to maintain pregnancy.

In addition to the impact of fescue toxicosis on female hormone concentrations, Bond et al. (1988) indicated that ewes grazing TE tall fescue exhibited increased time to conception after exposure to a ram. It was found that this increase in time to conception was generally attributed to delayed estrus or to embryonic mortality (Bond et al., 1988). For every 10% increase in toxic endophyte infestation of forage being grazed, conception rates decreased by 3.5%, conveying the lapse and reproductive efficiency on TE tall fescue (Schmidt et al., 1986).

Fescue Toxicosis and Male Reproduction

Much less is understood about the implications of grazing TE tall fescue on reproductive performance of male livestock. Multiple studies reported that bulls grazing TE tall fescue exhibited decreased scrotal circumference (Jones et al., 2004; Stowe et al., 2013). Other studies, however, reported that scrotal circumferences did not differ between TE tall fescue and NTE tall fescue. (Schuenemann et al., 2005a; Schuenemann et al., 2005b). A positive correlation between sperm production and scrotal circumference has been observed, indicating that as scrotal circumference decreases, bull fertility may also decrease (Coulter and Foote, 1976; Coulter and Keller, 1982). Based on the conflicting findings from previous studies in regards to scrotal circumference of bulls fed TE tall fescue, it may be inaccurate to attribute any declines in fertility of bulls grazing TE tall fescue to a decline in scrotal circumference (Coulter and Foote, 1976; Coulter and Keller, 1982; Schuenemann et al., 2005a; Schuenemann et al., 2005b).

Semen quality has been an area of interest in assessing the impact of fescue toxicosis on male reproduction. Grazing TE tall fescue did not impact the percentage of normal sperm or percentage of primary or secondary sperm abnormalities in yearling bulls grazing TE tall fescue for roughly 224 days (Schuenemann et al., 2005a; Schuenemann et al., 2005b). A study by Pratt et al. (2015b) assessed semen samples collected from bulls and reported that percentage of normal sperm cells decreased by day 84 of grazing TE tall fescue. Despite the decrease in percentage of normal sperm cells, the percentages were still greater than the 70% normal range necessary to pass a breeding soundness exam (Pratt et al., 2015b). The conflicting findings between studies in regards to the impact of grazing TE tall fescue on semen quality could be due to varying ergot alkaloid concentrations of forage or other environmental factors such as ambient temperature (Pratt et al., 2015b). Therefore, further studies are needed to characterize the effect of TE tall fescue on sperm.

The impact of freezing semen for artificial insemination is a common practice in beef production that cannot be overlooked when examining semen efficacy. Thawed semen has been found to be impacted by grazing TE tall fescue (Pratt et al., 2015b). While fresh samples collected from bulls on TE tall fescue and non-toxic endophyte-infected (NTE) tall fescue did not differ via computational semen quality analysis, differences did exist during post-thaw analysis. Semen quality analysis of post-thaw semen revealed a decrease in sperm motility, motile sperm concentration, progressive motile sperm concentration, total motile sperm, and total progressive motile sperm from bulls grazing TE tall fescue (Pratt et al., 2015b). However, the ability of sperm to fertilize an oocyte post-thaw has yet to be evaluated.

The ability of semen samples to achieve fertilization is also an important attribute to evaluate. Oocytes fertilized from in vitro fertilization with semen from bulls fed TE tall fescue exhibited reduced cleavage rates compared to those fertilized by semen from bulls grazing NTE tall fescue (Schuenemann et al., 2005a; Schuenemann et al., 2005b). The percentage of cleaved embryos that continued to develop did not differ across forage types (Schuenemann et al., 2005a; Schuenemann et al., 2005b). Super-structural damage occurs and is undetected during a breeding soundness exam or other semen quality analysis, leading to untimely cleavage of oocytes fertilized by semen from bulls fed TE tall fescue (Schuenemann et al., 2005a; Schuenemann et al., 2005b). The concept of super-structural damage that is undetected using gross morphological diagnostics is further supported by the prior observation that sperm can remain motile while having lost their ability to maintain fertilization capacity (Austin, 1975). While a decrease in sperm efficiency is evident, it is also clear that the integrity of sperm in question was not altogether compromised due to further development to the blastocyst stage beyond cleavage (Schuenemann et al., 2005a; Schuenemann et al., 2005b).

One of the primary concerns of the impact of TE tall fescue on male reproductive physiology is the fact that bulls are passing breeding soundness exams regardless of whether they are fed TE tall fescue or not (Stowe et al., 2013). If TE tall fescue is further confirmed to impact bull fertility, then the breeding soundness examination protocols that are common today may need to become more sensitive to identify the super-structural damage (Schuenemann et al., 2005a).

Evaluation of the collective effect of reproductive efficiency when mating sires grazing TE tall fescue to dams grazing TE tall fescue cannot be discounted as a study in mice indicated that such matings may have a collaborative impact on pregnancy (Ross et al., 2004). Ross et al. (2004) reported that the matings of males from lines that were selected for susceptibility to a toxic endophyte-infected diet to females that were also selected for susceptibility to a toxic endophyte-infected diet resulted in litter sizes being reduced by 0.5 on average. Conversely, matings of males from lines selected for resistance to a toxic endophyte-infected diet to females also selected for resistance to a toxic endophyte-infected diet resulted in litter sizes being increased by 1.0 on average. This indicates a collaborative impact on reproduction by both male and female lines that are susceptibile to a toxic endophyte-infected diet.

The physiology behind the implications of grazing TE tall fescue on male reproduction continues to be elucidated. Prolactin receptors (PRLR) were found to be present in the bovine testis and the epididymis of bulls (Pratt et al., 2015a) and in golden hamsters, rats, humans, red deer, and sheep (Klemcke et al., 1983; Guillaumot et al., 1984; Bouhdiba et al., 1989; Ouhtit et al., 1993; Clarke et al., 1995; Guillaumot et al., 1996; Lincoln et al., 2001; Hair et al., 2002). Furthermore, presence of PRLR in sperm cells as well as differentiating germ cells was noted in bulls (Pratt et al., 2015a); and in other species such as rats and humans (Hondo et al., 1995; Hair et al., 2002). Pratt et al. (2015a) indicated that the presence of PRLR in various regions of the male bovine reproductive tract may link fescue toxicosis to male reproductive performance due to the known relationship between prolactin concentrations and fescue toxicosis. Modulation of prolactin by ergot alkaloids through the dopaminergic receptors could contribute to altered reproductive function in bulls.

Dopamine D2 Receptor Gene

There are five different subtype receptors that the neurotransmitter dopamine activates. These five receptor subtypes are identified as D_1-D_5 accordingly. Of the dopamine receptor subtypes, the D_1 and D_5 receptors are similar, commonly being identified as D_1-like. Likewise, the D_2, D_3, and D_4 receptors are similar and therefore identified as D_2-like. All of the dopamine receptors, regardless of whether they are classified as D_1-like or D_2-like, are part of the G protein-coupled receptor family (Seeman and Van Tol, 1994). These receptors have an expansive yet heterogeneous distribution in the brain (Boyson et al., 1986).

The dopamine D_2 receptor gene (DRD2) has showed promise as a potential marker for resistance to TE tall fescue due to the inhibitory role that dopamine is known to play in prolactin secretion. The inhibitory effect of dopamine on prolactin secretion results in decreased circulating prolactin concentrations similar to fescue toxicosis. Moreover, ergovaline, a prominent ergot alkaloid produced by the toxic endophyte, is a known dopamine agonist (Yates et al., 1985). Administration of dopamine antagonists, such as domperidone, have been known to counteract the negative effects of TE tall fescue in horses as well as cattle (Cross et al., 1995; Jones et al., 2003). Redmond et al. (1992) found that administration of domperidone to pregnant mares grazing TE tall fescue increased serum prolactin and progesterone levels in addition to nearly eliminating symptoms of fescue toxicosis. In addition, non-pregnant heifers fed a toxic endophyte-infected diet gained less weight than non-pregnant heifers on an endophyte-free diet. However, when domperidone was administered to non-pregnant heifers on a toxic endophyte-infected diet the effects of the endophyte were overturned (Jones et al., 2003). Jones et al. (2003) also reported that non-pregnant heifers on a toxic endophyte-infected diet exhibited shorter estrous cycles and lower mid-cycle progesterone concentrations than non-pregnant heifers fed a toxic endophyte-infected diet that were administered domperidone. Due to ergovaline, prolactin, and dopamine acting on dopaminergic D2 receptors, DRD2 has been identified as a potential candidate gene in selection for resistance to the negative effects of grazing TE tall fescue (Campbell et al., 2014).

Within DRD2 at region 404, 365 on bovine chromosome 15 there is an intronic A/G single nucleotide polymorphism (SNP) that has been associated with performance of cattle grazing toxic endophyte-infected tall fescue (Campbell et al., 2014). At this region of DRD2, cattle may exhibit one of three genotypes: AA, AG, or GG.

Previous research has shown that there is no genotype effect on serum prolactin concentrations in steers grazing NTE tall fescue (Campbell et al., 2014). Steers with a GG genotype exhibited a reduction in serum prolactin concentrations and increased hair coat scores in April and May as compared to steers with an AA genotype when grazing toxic endophyte-infected tall fescue (Campbell et al., 2014). These results indicate that the A allele suppresses the effects of the toxic endophyte on cattle that are grazing toxic endophyte-infected tall fescue, and that animals with a GG genotype tend to exhibit decreased performance for a number of traits. These findings support the DRD2 SNP as a valuable marker that may prove useful in the selection of cattle that are resistant to fescue toxicosis.

Cytochrome P450

Cytochrome P450 is one of many enzyme groups that plays a role in xenobiotic metabolism and is responsible for a number of physiological functions (Hari Kumar and Kuttan, 2006). It exists primarily in the liver, but it is also found to lesser extents in the intestines, lungs, and kidneys (Krishna and Klotz, 1994). One of the most significant roles of the cytochrome P450 enzyme family is its role in hepatic metabolism of drugs, toxins, and other foreign compounds (Pollock, 1994). A study in rats by Moochhala et al. (1989) established that cytochrome P450 is responsible for the metabolism of ergot alkaloids such as bromocriptine and ergotamine, among other structurally similar ergot alkaloids. In addition, ergot alkaloids inhibit cytochrome P450 activity in humans, thus inhibiting their own metabolism or clearance out of the body (Althaus et al., 2000). The inhibition of ergot alkaloid clearance due to inhibition of cytochrome P450 has been found to result in bioaccumulation of ergot alkaloids in the body, thus exacerbating the symptoms of fescue toxicosis in cattle (Klotz, 2015).

Cytochrome P450 activity is negatively regulated through the binding of dopamine receptors (Moochhala et al., 1989), specifically by D2 dopamine receptors (Althaus et al., 2000). A review by Kvernmo et al. (2006) summarized studies that evaluated a number of ergot-derived dopamine agonists, non-ergot derived dopamine agonists, and their respective pharmokinetic properties as they pertain to Parkinson’s disease and restless legs syndrome in humans. One of the ergot-derived dopamine agonists discussed by Kvernmo et al. (2006) is bromocriptine. Bromocriptine inhibits cytochrome P450 3A4 specifically, which is known to be abundant in the liver and intestines. Through binding of bromocriptine to dopaminergic D2 receptors, inhibition is believed to occur such that concentrations of the substrates of cytochrome P450 3A4 increase within the plasma. However, bromocriptine is also metabolized by cytochrome P450 3A4, therefore inhibiting its own metabolism. This inhibition creates a scenario of increased bromocriptine activity through decreased clearance, resulting in bioaccumulation of bromocriptine (Wynalda and Wienkers, 1997).

Specific cytochrome P450 enzymes are believed to play a greater role in hepatic metabolism of ergot alkaloids in cattle than other members of the cytochrome P450 enzyme family. Wright and Paine (1994) reported that cytochrome P450 1-3 are the most fundamental enzymes of the cytochrome P450 family in terms of xenobiotic metabolism. However, which members of the expansive cytochrome P450 enzyme family are playing fundamental roles in metabolism of ergot alkaloids in beef cattle remains to be elucidated.

While the enzyme system responsible for the metabolism of ergot alkaloids produced by the fungal endophyte in TE tall fescue has not been fully explained in beef animals, studies have indicated that cytochrome P450 3A is responsible for metabolism of structurally similar ergot alkaloids in both cell cultures and in vivo studies of man, mice, rats, rhesus monkeys, and dogs (Maurer et al., 1983). Moubarak and Rosenkrans (2000) investigated the role of the cytochrome P450 3A superfamily of enzymes in metabolism of ergot alkaloids by beef liver microsomes. Results from this study indicated that the presence of cytochrome P450 3A activity in beef steers and clearly showed that cytochrome P450 3A played a role in metabolism of the ergot alkaloids that the liver microsomes were exposed to (Moubarak and Rosenkrans, 2000). While it appears that cytochrome P450 3A may play the most significant role in ergot alkaloid metabolism in beef cattle, cytochrome P450 is an expansive enzyme family. Therefore, there may be other enzymes within the cytochrome P450 enzyme system that are playing a role in metabolism of ergot alkaloids to a lesser degree. Cytochrome P450 activity is modulated in part by dopaminergic signaling through dopamine D2 receptors, and alteration in the receptor function through validation in gene sequence may have a significant impact on cytochrome P450's ability to metabolize ergot alkaloids (Althaus et al., 2000).

Ergot Alkaloid Metabolism

While it is understood that there are a number of ergot alkaloids produced by the toxic fescue endophyte, how these ergot alkaloids move throughout the system remains unclear (Klotz, 2015). Ayers et al. (2009) established that ergot alkaloids are able to be absorbed across the rumen in cattle. However, Westendorf et al. (1993) showed that of the ergot alkaloids ingested by sheep, approximately 50 to 60 % are recovered in the abomasal contents. Moyer et al. (1993) indicated that ergovaline is not stable in the rumen environment and is transported across gastric tissues in lesser amounts than lysergic acid and lysergic acid amides (Hill et al., 2001). As a follow-up study, Hill (2005) further investigated lysergic acid and proposed it as a potential toxin resulting in fescue toxicosis. The proposition of lysergic acid as a causative agent of fescue toxicosis was supported by De Lorme et al. (2007) when they proposed that ergopeptine ergot alkaloids such as ergovaline are metabolized in the rumen into lysergic acid prior to absorption. These findings suggest that a portion of the ergot alkaloids ingested are either undergoing microbial degradation or absorption in the reticulorumen (Foote et al., 2011).

In addition to the lack in clarity as to which ergot alkaloids are playing the most critical role in fescue toxicosis, there is still much to understand about how the ergot alkaloids ingested are metabolized and ultimately excreted. This would not only be determined by whether absorption or some form of microbial degradation is taking place, but also by the molecular weights and polarity of the materials that are ultimately absorbed (Smith, 1966). If a particular toxin ingested has a molecular weight greater than or equal to approximately 300 g/mol, then it is going to enter the biliary system through the liver and ultimately be excreted in the feces. If a substance is below a molecular weight of 300 g/mol, then it is small enough to be excreted in the urine (Slaytor and Wright, 1962). The molecular weights of lysergic acid and ergovaline are 268.32 g/mol and 533.62 g/mol respectively, so while lysergic acid is small enough to be excreted in the urine, ergovaline would be excreted in the feces. Therefore in recovering the ergot alkaloids ingested, different types of samples would need to be collected in addition to examining the pathways and metabolism of each ergot alkaloid.

The exact pathway of each of the ergot alkaloids ingested when grazing TE tall fescue remains to be elucidated. This is further complicated by findings indicating that ingestion of each ergot alkaloid does not always equal its excretion (Klotz, 2015). Multiple studies assessing ingestion and excretion of ergovaline and lysergic acid, two of the toxins believed to play a primary role in the negative performance of cattle susceptible to fescue toxicosis, have indicated that while more ergovaline was consumed than lysergic acid, more lysergic acid was recovered from excreta than ergovaline in all three of the species examined. Meanwhile, only 35% to 55% of the ergovaline ingested was found in excreta in horses, sheep, and cattle (Schultz et al., 2006; De Lorme et al., 2007; Merrill et al., 2007). In another study, ergovaline and ergovalinine were found to be excreted in the feces, but while 47% to 61% of ergovaline and ergovalinine were recovered in the abomosal contents, only 5% to 6% was recovered in the feces of dairy cattle (Westendorf et al., 1993). This suggests that ergovaline and other ergopeptides are metabolized or are being absorbed and retained within the body, accounting for only a small portion of the ergot alkaloids being recovered.

Summary

Fescue toxicosis has negatively impacted beef cattle performance in the southeastern region of the United States. Researchers have made strides in characterizing the performance of beef cattle exposed to TE tall fescue for a number of traits. In addition, scientists have established methods to improve performance on TE tall fescue. There is no concrete solution at this time to eliminate negative performance on TE tall fescue and ultimately improve profitability for beef producers that rely on TE tall fescue as their primary forage. However, studies in recent years have indicated that genetic selection for better performance on TE tall fescue may be a viable solution to an issue that is decades old.

A SNP on the DRD2 gene has been found to be associated with a number of traits also characterized as symptoms of fescue toxicosis. Results from previous studies indicate the potential to utilize the SNP on the DRD2 gene to select for better performance on TE tall fescue. Furthermore, recent findings associated with the cytochrome P450 family of enzymes and hepatic metabolism of ergot alkaloids may indicate that the DRD2 genotype alters hepatic clearance of ergot alkaloids. However, there is much to be learned about the DRD2 SNP in question prior to establishing it as a useful selection tool for producers to select cattle that exhibit resistance to the negative effects of TE tall fescue. The objective of this study was to further characterize the association of genotype at the DRD2 locus with growth and performance traits in bulls grazing TE tall fescue. Through examining DRD2 genotype association with more growth and performance traits in animals grazing TE tall fescue, we hope to provide further evidence that the DRD2 genotype has the potential to be utilized through marker assisted selection to select cattle that will perform well on TE tall fescue. Utilization of marker assisted selection looking at this specific DRD2 single nucleotide polymorphism should prove beneficial to producers across the mid-south region of the United States as they aim to improve beef cattle performance and efficiency on TE tall fescue.

CHAPTER 3. GENOTYPE AT DRD2 IS ASSOCIATED WITH ERGOT ALKALOID CONCENTRATIONS IN URINE SAMPLES

Abstract

Fescue toxicosis is a syndrome estimated to result in economic losses in excess of $1 billion dollars across various livestock industries in the mid-south region of the United States. Studies have indicated that the identification of genetic markers may allow for selection of cattle that are resistant to fescue toxicosis. A single nucleotide polymorphism in the dopamine receptor D2 gene (DRD2) has been found to be associated with serum prolactin concentrations as well as hair coat scores in cattle grazing toxic endophyte-infected (TE) tall fescue. In this study, DRD2 genotype was associated with weight gain (P=0.0055) such that GG bulls gained less weight than either AA or AG bulls. Bulls on TE tall fescue gained more weight than bulls on NTE tall fescue (P=0.0433). Genotype at DRD2 was associated with SC such that AA and GG bulls had a greater SC than AG bulls (P=0.0033). A trend was also observed between scrotal circumference and treatment (P=0.0768) with bulls grazing NTE tall fescue tending to have a smaller scrotal circumference than bulls grazing TE tall fescue. Additionally, DRD2 genotype was associated with ergot alkaloid concentrations in the urine (P=0.0046) such that bulls with a GG genotype exhibited higher urine ergot alkaloid concentrations than either AA or AG bulls. A treatment (TE/NTE) by DRD2 genotype interaction effect was observed (P=0.0018), indicating that GG bulls grazing TE exhibited greater urine ergot alkaloid concentrations than either AA or AG bulls of either treatment group. These results support the potential of DRD2 as a tool for selection for better performance in beef cattle grazing TE tall fescue.

Introduction

Tall fescue (*Lolium Arundinaceum schreb*.) is a cool season perennial bunch grass known for its tolerance to pests and drought as well as its ease of establishment (Bacon, 1995). The hardiness of the forage is attributed to the presence of an endophytic fungus (*Epichloe coenophialum*) within the forage (Stuedemann and Hoveland, 1988). The endophyte within the plant excretes ergot alkaloids (Lyons et al., 1986). These ergot alkaloids, recognized as both dopaminergic and serotonergic agonists, are the causative source for a number of symptoms identified collectively as fescue toxicosis (Fribourg et al., 1991).

Previous studies have found that an intronic single nucleotide polymorphism (SNP) at the dopamine D2 receptor gene (DRD2) located on chromosome 15 within the bovine genome regulates serum prolactin secretion, which is a key indicator of fescue toxicosis (Civelli et al., 1993; Campbell et al., 2014). In addition, it has been reported that genotype at the DRD2 SNP is associated with hair coat scores (Campbell et al., 2014). However, the effects of DRD2 genotype on performance at a number of other traits remain unclear and should be characterized before promoting DRD2 genotype as a potential tool to select cattle for better performance on toxic endophyte-infected tall fescue.

Materials and Methods

All procedures in this study involving animals were reviewed and approved by the Institutional Care and Use Committee at Clemson University. Angus bulls maintained at Simpson Research and Education Center (Pendleton, SC) were utilized in this study (n=25). In order to be eligible for inclusion in this study, bulls had to be approximately a year of age, pass a breeding soundness exam, and weigh at least 454kg.

Bulls were placed on a treatment of either KY-31 pasture (n=13), tall fescue infected with toxic endophyte, or Texoma pasture (n=12), tall fescue infected with non-toxic endophyte. Bulls remained on their assigned treatment from February until mid-June of 2014, being rotated to a second pasture of the same treatment in order to ensure that adequate forage was available to the bulls throughout the duration of the study.

Every 28 days body weights, body condition scores, and hair coat scores were recorded for each bull. Body condition scores were noted for each bull such that a score of 1 indicated an emaciated bull and a score of 9 indicated an obese bull (Wagner et al., 1988). Hair coat scores were recorded for each bull such that a score of 1 indicated a fully shed hair coat and a score of 5 indicated that the bull still had a full hair coat that had not been shed (Belew et al., 1989).

Semen samples were collected from each bull every four weeks by restraining each bull in a standard animal handling chute and electroejaculating each bull using a Pulsator IV electroejaculator (Agtech, Manhattan, KS) on the programmed collection mode. Volume of each ejaculate was recorded at the time of collection, and a computerized sperm quality analyzer (SQA-Vb; A-Tech, Los Angeles, CA) was used to estimate sperm cell concentration, percent motility, percent progressive motility, motile sperm concentration, progressive motile sperm concentration, sperm velocity, total sperm number, total motile sperm concentration, and total progressive motile sperm concentration for each collected sample as described by Stowe et al. (2013). From the semen samples collected, a sperm pellet was obtained for each bull, frozen, and shipped on dry ice to the University of Tennessee (Knoxville, TN) for genotyping.

Every four weeks urine samples were collected from each bull by placing a container on an extended pole under each bull as they urinated while being worked through a standard animal handling chute. These samples were analyzed using the phytoscreen urine ergot alkaloid kit (Agrinostics, LTC Co., Watkinsville, GA), an enzyme-linked immunosorbent assay (ELISA) test for ergot alkaloids. The ELISA test works by detecting the presence of the lysergic ring structure that is common among ergot alkaloids, so the ELISA test is non-specific and does not differentiate between ergovaline and lysergic acid.

Sperm pellet DNA was extracted from semen samples utilizing the protocol provided from QuickExtract solution (Epicentre, Madison, WI) for genotyping at the DRD2 single nucleotide polymorphism (SNP) (rs41749780). Genotypes were declared from the extracted DNA via a custom Taqman genotyping assay as well as QuantStudio 6 software (Applied Biosystems, Foster City, CA). Once the bulls on study were genotyped, the DRD2 genotypes were then checked for associations with the phenotypic measures that were collected every four weeks for the duration of the study. However, the focus of this study was placed on the data collected in the months of April and May. Emphasis was placed on those months because those are the months in which symptoms of fescue toxicosis can be readily observed without concern for heat stress resulting from following summer months influencing any phenotypic measures collected.

The distribution of genotypes at DRD2 across the two pasture treatment groups is shown in Table 1. Since bulls were genotyped after the study was conducted, there is an uneven distribution of genotypes across the TE tall fescue and NTE tall fescue treatments.

Table 1. Distribution of DRD2 genotype across treatments for bulls on study.

Treatment	**Frequency of DRD2 Genotype**		
	AA	AG	GG
TE	8	3	2
NTE	5	2	5

Statistical analysis was conducted using SAS 9.4 (SAS Institute, Cary, NC). A mixed model analysis of variance was performed with main effects of treatment (TE or NTE tall fescue pasture), DRD2 genotype, and the interactions between these main effects included in the analysis. Significance was noted such that $P \leq 0.05$ and trends noted such that $0.05 < P \leq 0.1$.

Results and Discussion

Results reported are only those that showed statistical significance or trends upon data analysis. Results from the semen quality analysis are not featured due to lack of statistical power and will need to be evaluated for associations with DRD2 genotype in a future study. A summary of all results analyzed for this study can be found in Table 2, located in the appendix.

While all bulls on study, regardless of treatment, gained weight during the 28 day time period from April to May, there was not a statistically significant relationship between the treatment by genotype interaction and weight gained from April to May (P=0.2132) (Figure 1). However, GG genotype bulls gained less than half as much weight as the AA or AG bulls regardless of TE or NTE treatment (P=0.0055). Bulls on TE tall fescue gained more weight from April to May than bulls on NTE tall fescue (P=0.0433).

The interaction between DRD2 genotype and pasture treatment exhibited no significant relationship with weight gain from April to May, which may be in part due to the uneven distribution of genotypes across treatment groups in this study. In addition, the small sample size in this study may also play a role in these results. Therefore, another study should be performed with bulls being sorted onto pastures according to their genotype at the DRD2 locus in order to further examine whether there is a relationship between weight gained during the 28 day period from April to May and the interaction between genotype at DRD2 and pasture treatment.

The results for weight gain in the 28 day period from April to May by DRD2 genotype alone coincide with results from previous studies in which it has been found that cattle with the GG genotype at the DRD2 locus have exhibited a decreased performance for a number of traits including their body weights (Campbell et al., 2014). However, Campbell et al. (2014) investigated body weights in steers and heifers rather than bulls for association with DRD2 genotype. This current study and Campbell et al.

(2014) suggest that cattle with the GG genotype exhibit a slower growth rate than cattle with either the AA or AG genotype.

Bulls on TE tall fescue gained more weight than bulls on NTE tall fescue, which could be due to the uneven distribution of genotypes across pasture treatments in this study. Most of the bulls on TE had an AA genotype at the DRD2 locus, which Campbell et al. (2014) found to be the genotype that gains the most weight when looking at steers and heifers. Another study should be performed that sorts bulls onto pasture treatments evenly by DRD2 genotype with a larger sample size in order to determine if bulls on TE tall fescue gain more weight in the 28 day period from April to May than bulls on NTE tall fescue.

Scrotal circumference was associated with DRD2 genotype (P=0.0033) such that bulls with the AG genotype had a smaller scrotal circumference than bulls with either an AA or GG genotype at DRD2. A trend was also observed between scrotal circumference and treatment (P=0.0768) with bulls grazing NTE tall fescue tending to have a smaller scrotal circumference than bulls grazing TE tall fescue. However, the interaction of DRD2 genotype and scrotal circumference was not found to be statistically significant (P=0.3752) (Figure 2).

Due to the small sample size and uneven distribution of genotypes within this study across treatments, it is difficult to interpret the relationships between DRD2 genotype and scrotal circumference. Furthermore, the relationship between pasture type and scrotal circumference should be described further through additional research. Future studies should be conducted with larger sample sizes and an even distribution of DRD2 genotypes across pasture types in order to determine if associations of scrotal circumference with DRD2 genotype, treatment, and the interaction between DRD2 genotype and pasture type exist.

Bulls grazing TE tall fescue exhibited elevated concentrations of ergot alkaloids in urine samples collected. An association of DRD2 genotype with ergot alkaloid concentrations in urine was observed (P=0.0046). An association of pasture type (TE/NTE) with ergot alkaloid concentrations in urine was also observed (P<0.0001). Furthermore, an interaction between pasture type and genotype at the DRD2 locus was associated with ergot alkaloid concentrations in urine (P=0.0018) (Figure 3). Bulls with a GG genotype grazing TE tall fescue exhibited a nearly two-fold greater ergot alkaloid concentration in urine samples collected than AA or AG bulls on TE tall fescue. As expected, concentrations were low regardless of genotype in bulls grazing NTE tall fescue.

Since the DRD2 gene controls dopaminergic D2 receptor function, the GG genotype at the DRD2 SNP could result in altered function of D2 receptors. Dopamine D2 receptors regulate cytochrome P450 activity, a family of enzymes that serve a number of functions (Wójcikowski et al., 2008). In addition, it has been discovered that cytochrome P450 regulates metabolism of a variety of substances that enter the liver, including ergot alkaloids (Moochhala et al., 1989; Pollock, 1994). Furthermore, cytochrome P450 is known to also be inhibited by ergot alkaloids or the binding of a dopamine agonist at the dopamine D2 receptors (Rosenkrans Jr and Ezell, 2015). As cytochrome P450 activity decreases, so does metabolism in the liver, resulting in bioaccumulation of substances that have yet to be metabolized (Klotz et al., 2009).

Given the relationship of cytochrome P450 with the dopaminergic pathway as well as hepatic metabolism, we speculate that DRD2 function may be modulated by genotype such that the G allele has an increased binding affinity for ergot alkaloids. This increased binding of ergot alkaloids, which are known dopamine agonists (Wójcikowski et al., 2008), to D2 receptors would result in decreased cytochrome P450 activity. Decreased cytochrome P450 activity would then result in bioaccumulation of ergot alkaloids due to a reduction in metabolism. This, in turn, results in an elevated concentration of ergot alkaloids being excreted in the urine, which has been found to be the common source of excretion for peptide-free ergoline alkaloids in cattle and sheep (Stuedemann et al., 1998; Gooneratne et al., 2011).

These results further indicate that the GG genotype at the DRD2 SNP is associated with exacerbated symptoms of fescue toxicosis. In addition, results suggest that there is a potential link between genotype at DRD2 and metabolic processes in the liver by way of dopamine D2 receptor modulation of cytochrome P450 activity, which could be elucidated through further analysis. These findings affirm the potential of the DRD2 single nucleotide polymorphism as a potential tool for selection of cattle that perform better on toxic endophyte-infected tall fescue.

CHAPTER 4. CONCLUSION

Fescue toxicosis has been impacting the mid-south region of the United States for decades, causing losses in excess of $1 billion dollars annually across multiple livestock industries. While solutions to combat the negative impact of toxic endophyte-infected tall fescue on the livestock industry have been suggested, many of the proposed solutions involve diluting fescue pastures or planting another forage in place of tall fescue, which would compromise the hardiness of the pasture and require more intensive maintenance and grazing management by producers. Fescue toxicosis negatively impacts multiple physiological systems and impacts the livestock industry at multiple stages of production, indicating the necessity for further research and alternative solutions to combat the negative implications of fescue toxicosis.

While producers currently rely on management practices and supplementation to manage the prevalence of fescue toxicosis within their herds, genetic selection for resistance to fescue toxicosis may revolutionize the way that producers combat the negative effects of toxic endophyte-infected tall fescue in order to minimize economic losses. Previous studies have indicated that DRD2 could be the key to establishing a genetic marker to select for resistance to fescue toxicosis. The objective of this study was to characterize the association of genotype at DRD2 with growth and performance traits in bulls grazing TE tall fescue in order to further validate DRD2 as a potential selection tool. Results indicated that DRD2 is still a strong candidate for selection of cattle resistant to fescue toxicosis. These results also indicated exciting findings that should be examined in future studies in order to not only learn more about the potential of DRD2, but also to further elucidate the physiology behind the fescue toxicosis syndrome.

REFERENCES

Allain, D., L. Martinet, and J. Rougeot. 1981. Effects of melatonin implants on changes in the coat, plasma prolactin and testis cycle in the mink (*mustlea vison*). INRA Services Publications, Versailles, France.

Althaus, M., A. Retzow, J. Castell, M.-J. Gómez-Lechón, Z. Amalou, T. Rose, and K. Appel. 2000. In vitro identification of the cytochrome p450 isoform responsible for the metabolism of a-dihydroergocryptine. Xenobiotica 30: 1033-1045.

Arachevaleta, M., C. Bacon, C. Hoveland, and D. Radcliffe. 1989. Effect of the tall fescue endophyte on plant response to environmental stress. Agronomy Journal 81: 83-90.

Austin, C. 1975. Sperm fertility viability and persistence in the female tract. Journal of Reproduction and Fertility 22: 75-89.

Ayers, A. W., N. Hill, G. Rottinghaus, J. Stuedemann, F. Thompson, P. Purinton, D. Seman, D. Dawe, A. Parks, and D. Ensley. 2009. Ruminal metabolism and transport of tall fescue ergot alkaloids. Crop science 49: 2309-2316.

Bacon, C. W. 1995. Toxic endophyte-infected tall fescue and range grasses: Historic perspectives. Journal of animal science 73: 861-870.

Bastin, B. C. 2013. Single-nucleotide polymorphisms associated with performance traits in beef cattle grazing endophyte-infected tall fescue.

Belew, B., C. Peters, K. Moore, R. Lipsey, and A. Decker. 1989. Relationship between cow symptoms of fescue toxicosis and calf weaning weight. , University of Missouri, Columbia.

Ben-Jonathan, N., and R. Hnasko. 2001. Dopamine as a prolactin (prl) inhibitor. Endocrine reviews 22: 724-763.

Berde, B., and E. Stürmer. 1978. Introduction to the pharmacology of ergot alkaloids and related compounds as a basis of their therapeutic application Ergot alkaloids and related compounds. p 1-28. Springer.

Bond, J., G. Lynch, D. Bolt, H. Hawk, C. Jackson Jr, and R. Wall. 1988. Reproductive performance and lamb weight gains for ewes grazing fungus-infected tall fescue. Nutrition reports international.

Bouhdiba, M., B. Leroy-Martin, J. P. Peyrat, P. S. Pol, J. Djiane, and J. Leonardelli. 1989. Immunohistochemical detection of prolactin and its receptors in human testis/immunhistochemische bestimmung von prolaktin und seinen rezeptoren im menschlichen hoden. Andrologia 21: 223-228.

Boyson, S. J., P. Mcgonigle, and P. B. Molinoff. 1986. Quantitative autoradiographic localization of the d1 and d2 subtypes of dopamine receptors in rat brain. The Journal of neuroscience 6: 3177-3188.

Browning, R., F. Thompson, J. Sartin, and M. Leite-Browning. 1997. Plasma concentrations of prolactin, growth hormone, and luteinizing hormone in steers administered ergotamine or ergonovine. Journal of animal science 75: 796-802.

Bush, L., J. Boling, and S. Yates. 1979. Animal disorders. In: R. C. Buckner and L. P. Bush (eds.) Tall fescue. Agronomy monograph. p 247-292. American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, Madison, WI.

Bush, L., and R. Buckner. 1973. Tall fescue toxicity. Anti-Quality Components of Forages: 99-112.

Campbell, B., C. Kojima, T. Cooper, B. Bastin, L. Wojakiewicz, R. Kallenbach, F. Schrick, and J. Waller. 2014. A single nucleotide polymorphism in the dopamine receptor d2 gene may be informative for resistance to fescue toxicosis in angus-based cattle. Animal biotechnology 25: 1-12.

Civelli, O., J. R. Bunzow, and D. K. Grandy. 1993. Molecular diversity of the dopamine receptors. Annual review of pharmacology and toxicology 33: 281-307.

Clarke, L., M. Edery, A. Loudon, V. Randall, M. Postel-Vinay, P. Kelly, and H. Jabbour. 1995. Expression of the prolactin receptor gene during the breeding and non-breeding seasons in red deer (cervus elaphus): Evidence for the expression of two forms in the testis. Journal of endocrinology 146: 313-321.

Coulter, G., and R. Foote. 1976. Relationship of testicular weight to age and scrotal circumference of holstein bulls. Journal of dairy science 59: 730-732.

Coulter, G., and D. Keller. 1982. Scrotal circumference of young beef bulls: Relationship to paired testes weight, effect of breed, and predictability. Canadian Journal of Animal Science 62: 133-139.

Craig, A. M., J. L. Klotz, and J. M. Duringer. 2015. Cases of ergotism in livestock and associated ergot alkaloid concentrations in feed. Frontiers in chemistry 3.

Cross, D., L. Redmond, and J. Strickland. 1995. Equine fescue toxicosis: Signs and solutions. Journal of animal science 73: 899-908.

Cross, D., C. Reinemeyer, J. Prado, R. Donnell, K. Bond, H. Farr, and S. Longhofer. 2012. Efficacy of domperidone gel in an induced model of fescue toxicosis in periparturient mares. Theriogenology 78: 1361-1370.

De Lorme, M., S. Lodge-Ivey, and A. Craig. 2007. Physiological and digestive effects of-infected tall fescue fed to lambs. Journal of animal science 85: 1199-1206.

Fergus, E. 1972. A short history of kentucky 31 fescue. In: Proc. 29th Southern Pasture Forage Crop Improvement Conference. p 136-139.

Foote, A., D. Harmon, J. Strickland, L. Bush, and J. Klotz. 2011. Effect of ergot alkaloids on contractility of bovine right ruminal artery and vein. Journal of animal science 89: 2944-2949.

Fribourg, H., A. Chestnut, R. Thompson, J. Mclaren, R. Carlisle, K. Gwinn, M. Dixon, and M. Smith. 1991. Steer performance in fescue-clover pastures with different levels of endophyte infestation. Agronomy Journal 83: 777-781.

Gooneratne, S., M. Scannell, M. Wellby, and L. Fletcher. 2011. Changes in concentrations of lysergol in urine and prolactin in plasma, rectal temperature and respiration rate in sheep selected for resistance or susceptibility to ryegrass staggers and fed ergovaline. New Zealand veterinary journal 59: 233-238.

Guillaumot, P., I. Sabbagh, J. Bertrand, and H. Cohen. 1984. Prolactin receptors in the hypoprolactinemic male rat (ipl nude rat): Measurement in testis and induction in liver. Journal of steroid biochemistry 20: 347-351.

Guillaumot, P., E. Tabone, and M. Benahmed. 1996. Sertoli cells as potential targets of prolactin action in the testis. Molecular and cellular endocrinology 122: 199-206.

Gunter, S., and P. Beck. 2004. Novel endophyte-infected tall fescue for growing beef cattle. Journal of animal science 82: E75-E82.

Hair, W., O. Gubbay, H. Jabbour, and G. Lincoln. 2002. Prolactin receptor expression in human testis and accessory tissues: Localization and function. Molecular human reproduction 8: 606-611.

Hari Kumar, K. B., and R. Kuttan. 2006. Inhibition of drug metabolizing enzymes (cytochrome p450) in vitro as well as in vivo by phyllanthus amarus schum & thonn. Biological and Pharmaceutical Bulletin 29: 1310-1313.

Hemken, R., J. Boling, L. Bull, R. Hatton, R. Buckner, and L. Bush. 1981. Interaction of environmental temperature and anti-quality factors on the severity of summer fescue toxicosis. Journal of animal science 52: 710-714.

Hemken, R., L. Bull, J. Boling, E. Kane, L. Bush, and R. Buckner. 1979. Summer fescue toxicosis in lactating dairy cows and sheep fed experimental strains of ryegrass-tall fescue hybrids. Journal of animal science 49: 641-646.

Hemken, R., J. Jackson, and J. Boling. 1984. Toxic factors in tall fescue. Journal of animal science 58: 1011-1016.

Hill, N., F. Thompson, J. Stuedemann, G. Rottinghaus, H. Ju, D. Dawe, and E. Hiatt. 2001. Ergot alkaloid transport across ruminant gastric tissues. Journal of animal science 79: 542-549.

Hill, N. S. 2005. Absorption of ergot alkaloids in the ruminant Neotyphodium in cool-season grasses. p 271-290. Blackwell Publishing.

Hondo, E., M. Kurohmaru, S. Sakai, K. Ogawa, and Y. Hayashi. 1995. Prolactin receptor expression in rat spermatogenic cells. Biology of reproduction 52: 1284-1290.

Hoveland, C., S. Schmidt, C. King, J. Odom, E. Clark, J. Mcguire, L. Smith, H. Grimes, and J. Holliman. 1983. Steer performance and association of acremonium coenophialum fungal endophyte on tall fescue pasture. Agronomy Journal 75: 821-824.

Hoveland, C. S. 1993. Importance and economic significance of the acremonium endophytes to performance of animals and grass plant. Agriculture, ecosystems & environment 44: 3-12.

Hurley, W., E. Convey, K. Leung, L. Edgerton, and R. Hemken. 1980. Bovine prolactin, tsh, t and t concentrations as affected by tall fescue summer toxicosis and temperature. Journal of animal science 51: 374-379.

Jones, K., and S. King. 2009. Effect of domperidone supplementation of fescue-fed heifers on plasma and follicular fluid fatty acid composition and oocyte quality. Journal of animal science 87: 2227-2238.

Jones, K., S. King, K. Griswold, D. Cazac, and D. Cross. 2003. Domperidone can ameliorate deleterious reproductive effects and reduced weight gain associated with fescue toxicosis in heifers. Journal of animal science 81: 2568-2574.

Jones, K., C. Mccleary, S. King, G. Apgar, and K. Griswold. 2004. Consumption of toxic fescue impairs bull reproductive parameters. The Professional Animal Scientist 20: 437-442.

Kebabian, J. W., and D. B. Calne. 1979. Multiple receptors for dopamine. Nature 277: 93-96.

Klemcke, H. G., A. Bartke, and K. T. Borer. 1983. Testicular prolactin receptors and serum growth hormone in golden hamsters: Effects of photoperiod and time of day. Biology of reproduction 29: 605-614.

Klotz, J. 2015. Physiologic effects of ergot alkaloids: What happens when excretion does not equal consumption. Journal of animal science 93.

Klotz, J., B. Kirch, G. Aiken, L. Bush, and J. Strickland. 2009. Bioaccumulation of ergovaline in bovine lateral saphenous veins in vitro. Journal of animal science 87: 2437-2447.

Krishna, D. R., and U. Klotz. 1994. Extrahepatic metabolism of drugs in humans. Clinical pharmacokinetics 26: 144-160.

Kvernmo, T., S. Härtter, and E. Burger. 2006. A review of the receptor-binding and pharmacokinetic properties of dopamine agonists. Clinical therapeutics 28: 1065-1078.

Lamberts, S., and R. Macleod. 1990. Regulation of prolactin secretion at the level of the lactotroph. Physiological Reviews 70: 279-318.

Lincoln, G. A., J. Townsend, and H. N. Jabbour. 2001. Prolactin actions in the sheep testis: A test of the priming hypothesis. Biology of reproduction 65: 936-943.

Looper, M., S. Reiter, B. Williamson, M. Sales, D. Hallford, and C. Rosenkrans. 2010. Effects of body condition on measures of intramuscular and rump fat, endocrine factors, and calving rate of beef cows grazing common bermudagrass or endophyte-infected tall fescue. Journal of animal science 88: 4133-4141.

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.

Maurer, G., E. Schreier, S. Delaborde, R. Nufer, and A. Shukla. 1983. Fate and disposition of bromocriptine in animals and man. Ii: Absorption, elimination and metabolism. European journal of drug metabolism and pharmacokinetics 8: 51-62.

Mclaren, J., R. Carlisle, H. Fribourg, and J. Bryan. 1983. Bermudagrass, tall fescue, and orchardgrass pasture combinations with clover or n fertilization for grazing steers. I. Forage growth and consumption, and animal performance1, 2. Agronomy Journal 75: 587-592.

Merrill, M., D. Bohnert, D. Harmon, A. Craig, and F. Schrick. 2007. The ability of a yeast-derived cell wall preparation to minimize the toxic effects of high-ergot alkaloid tall fescue straw in beef cattle. Journal of animal science 85: 2596-2605.

Moochhala, S., E. Lee, G. Hu, O. Koh, and G. Becket. 1989. Effects of bromocriptine on hepatic cytochrome p-450 monooxygenase system. The Japanese Journal of Pharmacology 49: 285-291.

Moubarak, A., and C. Rosenkrans. 2000. Hepatic metabolism of ergot alkaloids in beef cattle by cytochrome p450. Biochemical and biophysical research communications 274: 746-749.

Moyer, J. L., N. S. Hill, S. A. Martin, and C. S. Agee. 1993. Degradation of ergoline alkaloids during in vitro ruminal digestion of tall fescue forage. Crop science 33: 264-266.

Ouhtit, A., G. Morel, and P. Kelly. 1993. Visualization of gene expression of short and long forms of prolactin receptor in rat reproductive tissues. Biology of reproduction 49: 528-536.

Parish, J., M. Mccann, R. Watson, N. Paiva, C. Hoveland, A. Parks, B. Upchurch, N. Hill, and J. Bouton. 2003. Use of nonergot alkaloid-producing endophytes for alleviating tall fescue toxicosis in stocker cattle. Journal of animal science 81: 2856-2868.

Paterson, J., C. Forcherio, B. Larson, M. Samford, and M. Kerley. 1995. The effects of fescue toxicosis on beef cattle productivity. Journal of animal science 73: 889-898.

Petritz, D., V. Lechtenberg, and W. Smith. 1980. Performance and economic returns of beef cows and calves grazing grass-legume herbage. Agronomy Journal 72: 581-584.

Pollock, B. G. 1994. Recent developments in drug metabolism of relevance to psychiatrists. Harvard Review of Psychiatry 2: 204-213.

Porter, J., and F. Thompson. 1992. Effects of fescue toxicosis on reproduction in livestock. Journal of animal science 70: 1594-1603.

Pratt, S., S. Calcatera, H. Stowe, M. Dimmick, F. Schrick, S. Duckett, and J. Andrae. 2015a. Identification of bovine prolactin in seminal fluid, and expression and localization of the prolactin receptor and prolactin-inducible protein in the testis and epididymis of bulls exposed to ergot alkaloids. Theriogenology 83: 662-669.

Pratt, S., H. Stowe, B. K. Whitlock, L. Strickland, M. Miller, S. Calcatera, M. Dimmick, G. Aiken, F. Schrick, and N. Long. 2015b. Bulls grazing kentucky 31 tall fescue exhibit impaired growth, semen quality, and decreased semen freezing potential. Theriogenology 83: 408-414.

Redmond, L., D. Cross, J. Strickland, and S. Kennedy. 1992. Efficacy of domperidone and sulpiride as treatments for equine fescue toxicosis in gravid mares grazing endophyte-infected tall fescue. Journal of animal science 70: 2.

Rhodes, M., J. Paterson, M. Kerley, H. Garner, and M. Laughlin. 1991. Reduced blood flow to peripheral and core body tissues in sheep and cattle induced by endophyte-infected tall fescue. Journal of animal science 69: 2033-2043.

Rice, R., D. Blodgett, G. Schurig, W. Swecker, J. Fontenot, V. Allen, and R. Akers. 1997. Evaluation of humoral immune responses in cattle grazing endophyte-infected or endophyte-free fescue. Veterinary immunology and immunopathology 59: 285-291.

Rosenkrans Jr, C. F., and N. S. Ezell. 2015. Relationships among ergot alkaloids, cytochrome p450 activity, and beef steer growth. Frontiers in chemistry 3.

Ross, M. K., W. D. Hohenboken, R. G. Saacke, and L. A. Kuehn. 2004. Effects of feeding endophyte-infected fescue seed on reproductive traits of male mice divergently selected for resistance or susceptibility to fescue toxicosis. Theriogenology 61: 651-662.

Schmidt, S., D. Danilson, J. Holliman, H. Grimes, and W. Webster. 1986. Fescue fungus suppresses growth and reproduction in replacement beef heifers. Highlights of agricultural research-Alabama Agricultural Experiment Station (USA).

Schuenemann, G., J. Edwards, F. Hopkins, N. Rohrbach, H. Adair, F. Scenna, J. Waller, J. Oliver, A. Saxton, and F. Schrick. 2005a. Fertility aspects in yearling beef bulls grazing endophyte-infected tall fescue pastures. Reproduction, Fertility and Development 17: 479-486.

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, and J. C. Waller. 2005b. Effects of administration of ergotamine tartrate on fertility of yearling beef bulls. Theriogenology 63: 1407-1418.

Schultz, C., S. Lodge-Ivey, L. Bush, A. Craig, and J. Strickland. 2006. Effects of initial and extended exposure to an endophyte-infected tall fescue seed diet on faecal and urinary excretion of ergovaline and lysergic acid in mature geldings. New Zealand veterinary journal 54: 178-184.

Seeman, P., and H. H. Van Tol. 1994. Dopamine receptor pharmacology. Trends in pharmacological sciences 15: 264-270.

Slaytor, M., and S. Wright. 1962. The metabolites of ergometrine and lysergic acid diethylamide in rat bile. Journal of Medicinal Chemistry 5: 483-491.

Smith, G. W., D. S. Rotstein, and C. F. Brownie. 2004. Abdominal fat necrosis in a pygmy goat associated with fescue toxicosis. Journal of Veterinary Diagnostic Investigation 16: 356-359.

Smith, R. L. 1966. The biliary excretion and enterohepatic circulation of drugs and other organic compounds Fortschritte der arzneimittelforschung\ progress in drug research\ progrès des recherches pharmaceutiques. p 299-360. Springer.

Stowe, H., M. Miller, M. Burns, S. Calcatera, J. Andrae, G. Aiken, F. Schrick, T. Cushing, W. Bridges, and S. Pratt. 2013. Effects of fescue toxicosis on bull growth, semen characteristics, and breeding soundness evaluation. Journal of animal science 91: 3686-3692.

Strickland, J., M. Looper, J. Matthews, C. Rosenkrans, M. Flythe, and K. Brown. 2011. Board-invited review: St. Anthony's fire in livestock: Causes, mechanisms, and potential solutions. Journal of animal science 89: 1603-1626.

Stuedemann, J., N. Hill, F. Thompson, R. Fayrer-Hosken, W. Hay, D. Dawe, D. Seman, and S. Martin. 1998. Urinary and biliary excretion of ergot alkaloids from steers that grazed endophyte-infected tall fescue. Journal of animal science 76: 2146-2154.

Stuedemann, J. A., and C. S. Hoveland. 1988. Fescue endophyte: History and impact on animal agriculture. Journal of Production Agriculture 1: 39-44.

Tucker, H. A., and R. K. Ringer. 1982. Controlled photoperiodic environments for food animals. Science 216: 1381-1386.

Wagner, J., K. Lusby, J. Oltjen, J. Rakestraw, R. Wettemann, and L. Walters. 1988. Carcass composition in mature hereford cows: Estimation and effect on daily metabolizable energy requirement during winter. Journal of animal science 66: 603-612.

Westendorf, M., G. Mitchell, R. Tucker, L. Bush, R. Petroski, and R. Powell. 1993. In vitro and in vivo ruminal and physiological responses to endophyte-infected tall fescue. Journal of dairy science 76: 555-563.

Wójcikowski, J., K. Gołembiowska, and W. A. Daniel. 2008. Regulation of liver cytochrome p450 by activation of brain dopaminergic system: Physiological and pharmacological implications. Biochemical pharmacology 76: 258-267.

Wright, M. C., and A. J. Paine. 1994. Induction of the cytochrome p450 3a subfamily in rat liver correlates with the binding of inducers to a microsomal protein. Biochemical and biophysical research communications 201: 973-979.

Wynalda, M. A., and L. C. Wienkers. 1997. Assessment of potential interactions between dopamine receptor agonists and various human cytochrome p450 enzymes using a simple in vitro inhibition screen. Drug metabolism and disposition 25: 1211-1214.

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. Journal of agricultural and food chemistry 33: 719-722.

APPENDIX

Table 2. Summary of total results excluding semen analysis data.

	TE			NTE			P-value
Phenotype	**AA**	**AG**	**GG**	**AA**	**AG**	**GG**	
Weight gain (kg)	$54.6429 \pm 6.4827_{A}$	$56.6667 \pm 9.0924_{A}$	$8.7500 \pm 12.1280_{B}$	$29.0000 \pm 7.6704_{B}$	$27.5000 \pm 12.1280_{AB}$	$12.5000 \pm 7.6704_{B}$	0.2132
Body condition score	$5.3929 \pm 0.1064_{A}$	$5.4167 \pm 0.1626_{A}$	$5.7500 \pm 0.1991_{A}$	$5.4000 \pm 0.1259_{A}$	$5.6250 \pm 0.1991_{A}$	$5.3500 \pm 0.1259_{A}$	0.5682
Scrotal circumference (cm)	$36.9643 \pm 0.6387_{A}$	$35.0000 \pm 0.9757_{AB}$	$36.8250 \pm 1.1950_{A}$	$36.3700 \pm 0.7558_{A}$	$31.9500 \pm 1.1950_{B}$	$36.2700 \pm 0.7558_{A}$	0.3752
Urine ergot alkaloid conc. (ng/mL)	$69.7400 \pm 10.1433_{B}$	$77.7000 \pm 14.3449_{B}$	$155.6500 \pm 16.0380_{A}$	$21.6800 \pm 10.1433_{C}$	$10.2750 \pm 16.0380_{C}$	$14.8778 \pm 10.6920_{C}$	0.0018
Serum prolactin conc. (ng/mL)	$14.0514 \pm 19.7532_{C}$	$13.2753 \pm 24.1926_{C}$	$9.9230 \pm 29.6298_{C}$	$64.6832 \pm 18.7395_{BC}$	$165.0200 \pm 29.6298_{A}$	$91.5900 \pm 18.7395_{B}$	0.1123
Seminal fluid prolactin conc. (ng/mL)	$16.5265 \pm 2.2891_{B}$	$21.4055 \pm 3.6194_{AB}$	$19.2419 \pm 4.1793_{AB}$	$16.9924 \pm 2.7360_{AB}$	$9.3029 \pm 5.1186_{B}$	$24.5198 \pm 2.7360_{A}$	0.1082

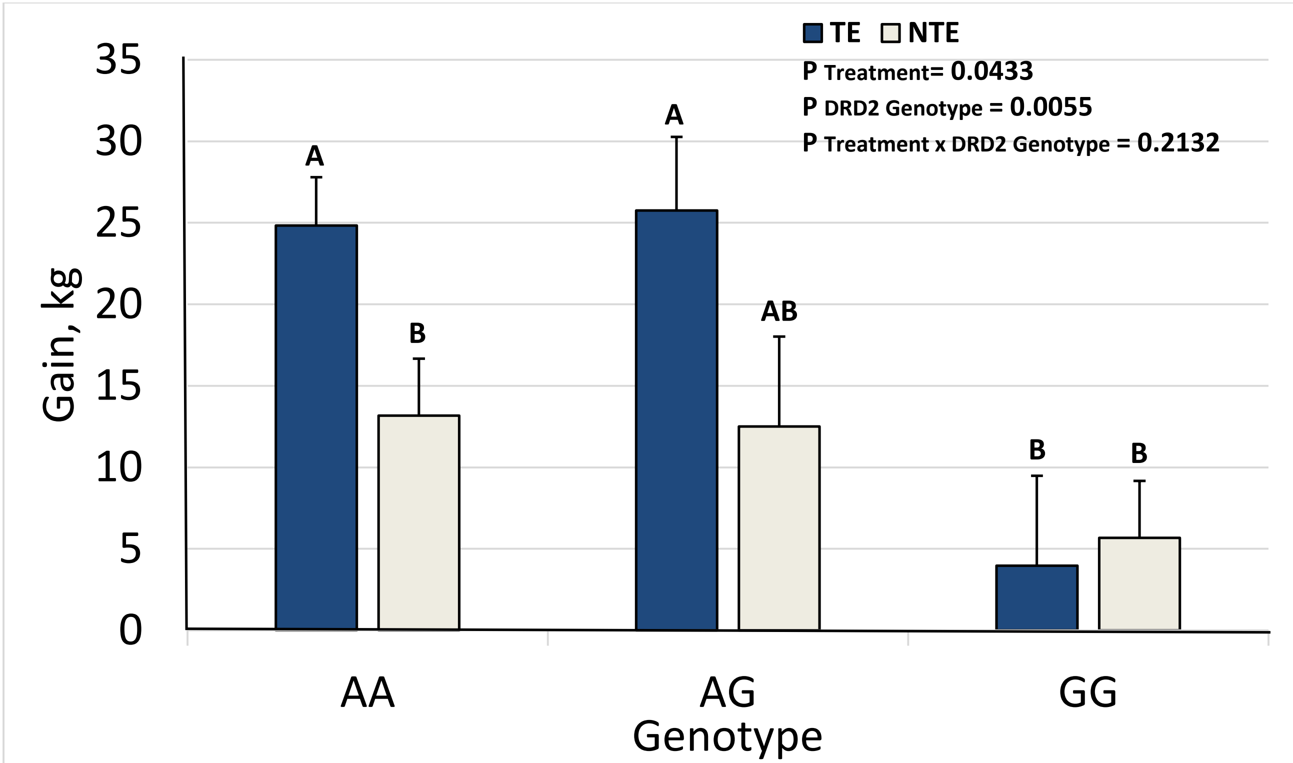

Figure 1. Weight gained from April to May according to pasture treatment and DRD2 genotype.

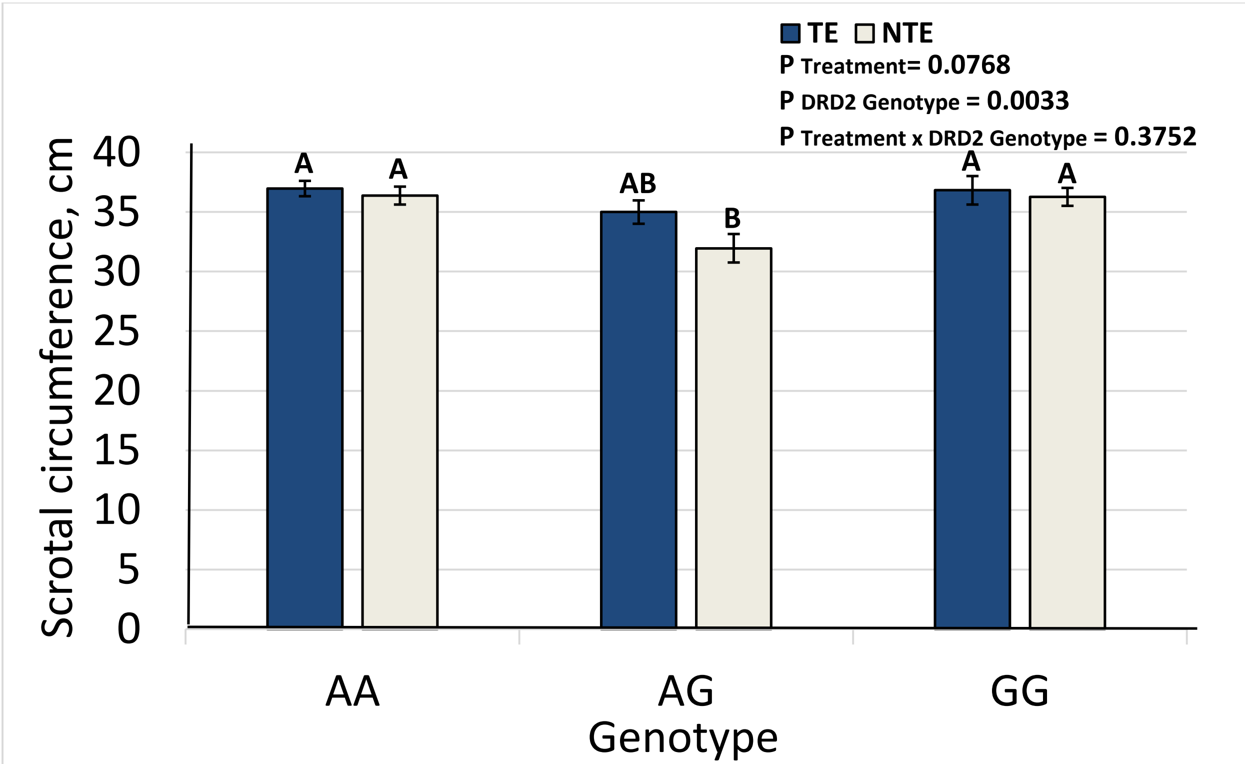

Figure 2. Scrotal circumference of bulls by DRD2 genotype by treatment.

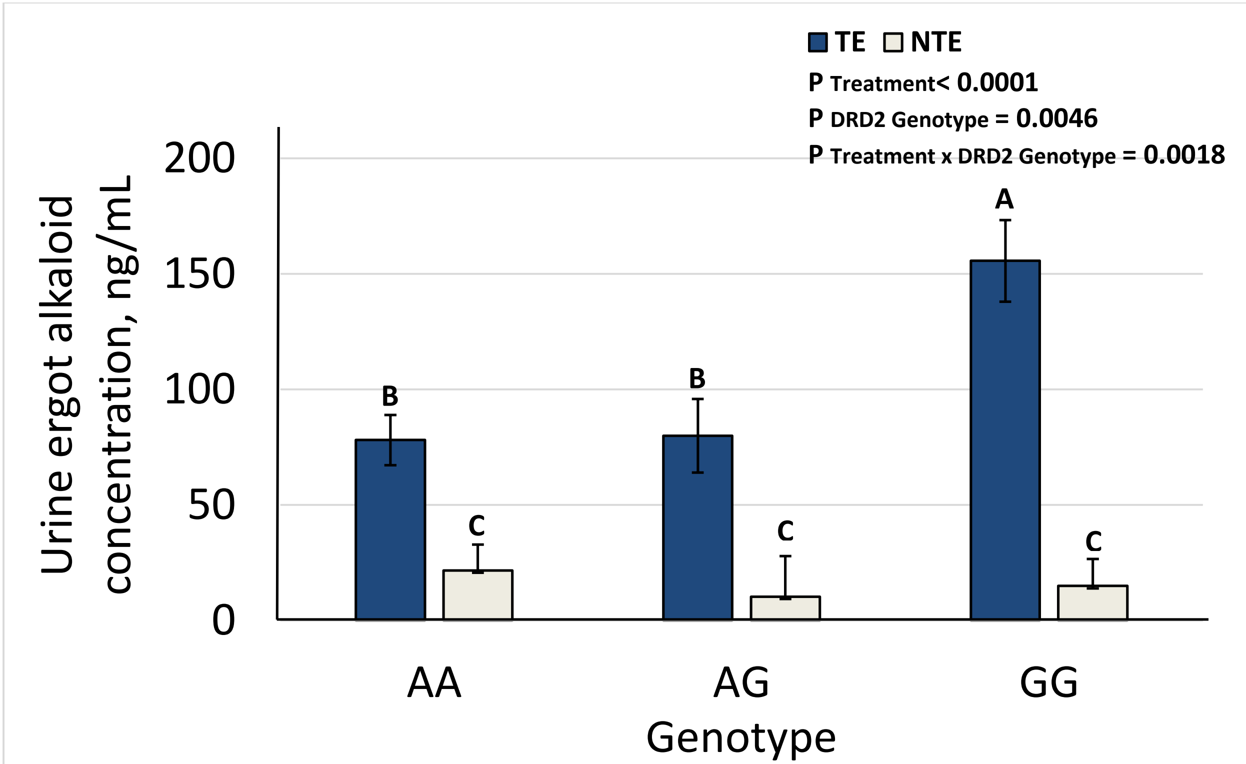

Figure 3. Urine ergot alkaloid concentrations for each DRD2 genotype by treatment.

VITA

Kaysie Jennings was born in Murfreesboro, Tennessee to parents Philip and Karen Jennings. She was raised in Lebanon, Tennessee as well as Watertown, Tennessee, where she graduated from Watertown High School in 2010. In August 2010, she moved to Knoxville, Tennessee, where she pursued a Bachelor of Science Degree in Animal Science at the University of Tennessee. Upon completion of her undergraduate career, Kaysie stayed at the University of Tennessee to work towards obtaining a Master of Science Degree in Animal Science with a concentration in animal breeding and genetics. Kaysie plans to conclude her Master's degree in July 2016. Upon completion of her degree program at the University of Tennessee, Kaysie will move to Fort Collins, Colorado to begin a Doctor of Philosophy Degree at Colorado State University in animal breeding and genetics.