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# Association of DRD2 Single Nucleotide Polymorphism and Fescue Toxicosis in Ruminants

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ASSOCIATION OF DRD2 SINGLE NUCLEOTIDE POLYMORPHISM  
AND FESCUE TOXICOSIS IN RUMINANTS

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A Dissertation  
Presented to  
the Graduate School of  
Clemson University

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In Partial Fulfillment  
of the Requirements for the Degree  
Doctor of Philosophy  
Animal Science

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by  
Sarah Adams Wilbanks  
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## ABSTRACT

Fescue Toxicosis is a prevalent problem in the Southeastern, United States causing an estimated loss of one billion dollars per year to the livestock industry (Roberts & Andrae, 2010). There has been much research aimed at understanding the grass itself, the endophyte and the ergot alkaloids it produces, as well as its relationship and impacts on consuming animals. Many mitigation plans have been established and have been shown to control the amount of alkaloids subsequent animals are ingesting; however, research is at the tipping point of needing a genetic approach to fescue toxicosis.

This research looked into the bovine genome to apply the previously identified Single Nucleotide Polymorphism (SNP) found by Campbell et al. (2014) in the Dopamine (DRD2) gene (rs41749780) to a cow/ calf production setting in order to determine if genotype provided advantages for cows (N=74) consuming toxic fescue during late gestation and the effects on their calves until weaning. Furthermore, the Ovine genome was screened for SNP's in the DRD2 gene in sheep (N= 57, N=61;, N=22) in a series of experiments and associations of the SNP and grazing toxic tall fescue were analyzed.

Since fescue toxicosis can affect all ruminant species, the ovine genome was screened for possible SNP sites in the DRD2 gene, and one SNP (g270a) was located and identified. The objectives of this study presented in Chapter 2, were 1) identify a SNP in the DRD2 gene, and 2) determine if there are any genotypic associations with fescue toxicosis. Results indicated ewes of a particular genotype (G|G) had heavier fetal weights and were able to manipulate circulating hormonal levels during pregnancy. This study

warranted further investigation, presented in Chapter 3, concluding a genotypic difference in lamb weight and growth with no associations with the dietary treatment of the ewe through gestation. Therefore, a follow up study was designed to further determine if there is an outperforming genotype when ewes were on the same plain of nutrition in the production setting. Conclusions can be made that the G|G genotyped ewes had no production benefits at birth ( $P=.180$ ) but lambs tended to be heavier by weaning ( $P=.059$ ). These lambs tended to have greater average daily gain (ADG) ( $P=0.079$ ) when compared to lambs from A|A ewes. It appears the G|G genotype has production advantage for lambs from birth to weaning.

Furthermore, Chapter 4 applies SNP rs41749780 to cow/calf pairs during late gestation to weaning consuming E- or E+ forages. Seventy-four Angus-based cows were genotyped for the DRD2 SNP with the objective of investigating the effects of dopamine 2 receptor (DRD2) single nucleotide polymorphism (SNP; rs41749780) and fescue treatment during the last trimester on cow and calf performance. Dam weight, serum prolactin, body condition scores (BCS) and hair coat scores (HCS) were collected every 30 d during the last trimester of gestation. Cows with G|G genotype had greater ( $P=0.012$ ) amount of rump fat deposition compared to A|A cows. Cows with DRD2 genotype of G|G tended to have lower ( $P = 0.07$ ) prolactin levels at d 0 and 90 of grazing. Adjusted calf birth and weaning weights did not differ ( $P>0.23$ ) by fescue pasture type or cow genotype. Milk production was estimated using weigh-suckle-weigh at d 30 post-calving. Fescue pasture type and cow genotype did not alter ( $P > 0.40$ ) milk production.

In evaluation of calf sire, there tended to be an interaction ( $P = 0.07$ ) between fescue pasture type and cow genotype in calves that were sired by natural service.

Results concluded grazing toxic tall fescue during late gestation reduced dam BW, rump fat deposition, and serum prolactin levels but did not alter adjusted birth or weaning weights of all calves born. However when evaluated by calf sire, adjusted birth and weaning weight for natural service sired calves showed that response to grazing E+ fescue during late gestation depended on cow DRD2 genotype.

The collection of these studies show the potential for a production advantage for G|G genotypes in sheep flocks. In cattle, it appears there may be a genotypic advantage for calves born to dams of A|A genotypes, however due to a variety of other influences a second year study is warranted in order to develop a deeper understanding.

## **DEDICATION**

I would like to dedicate this work to my animals, who without the love of animals in the first place, I would have never embarked on this journey.

## **ACKNOWLEDGMENTS**

I would like to acknowledge all of the family and friends that have encouraged me to follow my dreams. Especially, my husband, Ryan, who has pushed me to keep going when things seemed impossible. You have set an example of work ethic that inspires me daily. To my mom and dad, who initially instilled in me the love for animals and the importance of education, thank you. Also, to Dr. Susan Duckett, without you, I would have never had this opportunity. Thank you for being so patient and understanding. I will never forget your kindness. To the other members of my committee, Dr. Andrae, Dr. Strickland, and Dr. Dobbins for their unwavering support and guidance as a coworker, student, and mentor at Clemson.

## TABLE OF CONTENTS

	Page
TITLE PAGE.....	i
ABSTRACT .....	ii
DEDICATION.....	iv
ACKNOWLEDGMENTS .....	v
LIST OF TABLES.....	viii
LIST OF FIGURES.....	x
CHAPTER	
<b>I.    LITERATURE REVIEW .....</b>	<b>1</b>
History.....	1
Causative Agents.....	4
Effects of Fescue Toxicosis .....	6
Alkaloid Digestion and Absorption.....	12
Identified Management Plans .....	16
Cattle Genome .....	26
Dopamine Neurotransmitter .....	28
Fescue Toxicosis in Other Species.....	31
Literature Cited.....	34
<b>II.    DOES GENOTYPE PLAY A ROLE IN THE RESISTANCE TO           FESCUE TOXICOSIS IN OVINE? .....</b>	<b>48</b>
Abstract.....	48
Introduction.....	50
Materials and Methods .....	52
Results .....	56
Discussion.....	58
Literature Cited.....	61



<b>IV. THE EFFECTS OF MATERNAL DRD2 SNP G270A ON SUBSEQUENT LAMB WEIGHT AND GROWTH CONSUMING TOXIC FESCUE.....</b>	<b>73</b>
Abstract.....	73
Introduction.....	75
Materials and Methods .....	76
Results .....	81
Discussion.....	83
Literature Cited.....	86
<b>V. EFFECTS OF MATERNAL GENOTYPE AND FESCUE PASTURE TYPE GRAZED DURING THE LAST TRIMESTER ON COW/CALF PRODUCTION .....</b>	<b>99</b>
Abstract.....	99
Introduction.....	101
Materials and Methods .....	102
Results and Discussion.....	106
Literature Cited .....	114
<b>VI. CONCLUSIONS AND IMPLICATIONS.....</b>	<b>126</b>
<b>APPENDICES.....</b>	<b>129</b>
A. Timeline of Fescue Toxicosis Research.....	129
B. Literature Review .....	138

## LIST OF TABLES

Table		Page
2.1	SNP g270a's location in the DRD2 Gene.....	65
2.2	Primers for the DRD2 gene that were used for Identifying the SNP.....	66
4.1	Total ergot alkaloid levels for each pasture replicate and hay .....	121
4.2	Effects of dam DRD2 genotype and fescue pasture type on BW, prolactin, BCS and HCS during LATE gestation.....	122
4.3	Effects of dietary fescue treatment and DRD2 genotype on adjusted calf birth weight and weaning weight of calves.....	123

## LIST OF FIGURES

Figure	Page
2.1 Allelic frequencies between A A, A G, and G G genotypes for the Suffolk ewe flock.....	67
2.2 Prolactin concentrations in ewes with different DRD2 genotype (A A, A G, G G) fed endophyte-infected (E+) or endophyte-free (E-) tall fescue seed during d 35 to d 85 (MID) gestation and/or d 86 to 133 (LATE) gestation.....	68
2.3 Cortisol, triiodothyronine (T3), thyroxine (T4), and IGF-I concentrations by ewe DRD2 genotype.....	70
2.4 Fetal lamb weight at d 133 of gestation in ewes with different DRD2 genotypes (A A, A G, G G) fed endophyte- infected (E+) or endophyte-free (E-) tall fescue seed during d 35 to 85 (MID) gestation and/or d 86 to 133 (LATE) gestation.....	72
3.1 Genotypic distribution of A A, A G, and G G genotypes for the Suffolk Ewe flock in experiment 1.....	88
3.2 Genotypic distribution of A A, A G, and G G genotypes for the Suffolk Ewe flock in experiment 2.....	89
3.3 Lamb birth and weaning weight by maternal genotype (A A, A G or G G) in experiment 1.....	90
3.4 Birth weight of lambs by dietary treatment (E+ or E-) or ewes fed during d 35-85 (MID) or d 86- 133 (LATE) gestation in experiment 1.....	91
3.5 Lamb ADG from birth to weaning by maternal genotype (A A, A G or G G) in experiment 1.....	92
3.6 Average Daily Gain (ADG) of lambs at the time of weaning from ewes consuming endophyte infected (E+) or endophyte free (E-) seed during d 35-85 (MID) or d 86- 133 (LATE) gestation in experiment 1.....	93
3.7 Lamb birth weights from maternal genotype (A A or G G) in Experiment 2.....	94

List of Figures (Continued)

Figure	Page
3.8 Lamb weaning weights by maternal genotype (A A or G G) in Experiment 2 .....	95
3.9 Lamb ADG by maternal genotype (A A o G G) from birth to weaning for experiment 2.....	96
3.10 Ewe prolactin concentrations in ewes consuming endophyte free (E-) or endophyte infected (E+) tall fescue seed by genotype (A A, A G, or G G) during d 35-85 (MID) or d 86- 133 (LATE) gestation in experiment 1 .....	97
4.1 Genotypic distribution of mature Angus cows (n = 225) at Simpson Farm.....	119
4.2 A timeline of events including genotyping the Simpson herd to the weaning of subsequent calves .....	120
4.3 Fescue type by maternal DRD2 genotype on birth and weaning weights for natural service calves only .....	124

## CHAPTER ONE

### LITERATURE REVIEW

Egotism has affected people directly or indirectly since as far back as 98-55 BC. Through consumption of rye bread in France, thousands of people fell victim to the toxic effects of ergots. Epidemics broke out in the middle ages with victims suffering gangrene, and central nervous system problems resulting in the death of over 40,000 people (Barger, 1931). The problem was not the rye bread itself, but a spore producing fungi, *Claviceps purpurea* that infected rye seed. When ingested, it elicited severe toxic effects on humans.

Certain fungi such as *C. Purpurea* (external spore-producing fungi) and *Epichloe coenphialum* (endophytic fungi that grow within a plant-without sporulation) produce mycotoxins called ergot alkaloids. As history progressed, we discovered that not only human consumption of claviceps was detrimental, but consumption of *E. coenphialum* would have large negative impacts on livestock.

Tall fescue, *Lolium arundinaceum*, originated from Europe, and was then established in eastern Kentucky by shipments of infected meadow fescue seed. Found in 1890, variety Kentucky 31 (KY-31) was growing in the mountainous regions of eastern Kentucky. This variety was not officially discovered and named until E.N. Fergus a professor at the University of Kentucky saw it in 1931 and obtained seed for trials. He was impressed with how the grass stayed green all winter. Grass trials in Kentucky and Virginia denoted superior growth, height, competitive ability, and drought tolerance compared to meadow fescue (Garman, 2000).

By the late 19<sup>th</sup> century, tall fescue was described as being “an exceedingly valuable grass for mowing or pasture” (Lamson-Scriber, 1896). Tall fescue, in a sense, took over the Southeast by storm. It was robust; surviving drought, heat stress, disease and heavy grazing pressures. However, during the 1960’s-1970’s producers began reporting problems they were observing in their herds, such as rotting of hooves, poor hair coats, and difficulty calving, among others.

In 1977, researchers at USDA Russell Research Center in Athens, GA reported a fungus inside the grass that was suspected to be associated with what we now know as fescue toxicosis in grazing animals. To confirm this, a grazing experiment conducted by Hoveland et al. (1983) grazed steers on tall fescue with low and high endophyte infections. They confirmed that ergot alkaloids produced by the endophyte were the cause of fescue toxicosis. Gains on tall fescue were substantially lower than on orchardgrass; calf gains and conception rates were substantially lower on tall fescue than on tall fescue-clover (Petritz et al., 1980).

By the time fescue toxicosis was identified, tall fescue occupied close to 15 million ha in the United States and was deemed the most important pasture grass in the US (Buckner et al., 1979). Researchers then began trying to devise ways to reduce the effects of the endophyte. Early focus was to create varieties without the toxic endophytes.

Through the 1980’s producers renovated their pastures and replanted endophyte free varieties resulting in full stand failure within three years (Andrae & Roberts, 2007). Notable varieties developed include The University of Georgia’s “Georgia 5” and “Jesup” endophyte free varieties.

It was then determined how important the symbiotic relationship of the infecting fungi is to the plant's hardiness traits. Several grazing studies then focused on the alkaloid concentrations during the growing season. One study in Georgia showed ergopeptine alkaloid concentrations increased in late spring to coincide with anthesis, decreased in the summer as plants enter dormancy, and increased again to maximum concentration in early autumn at the initiation of fall regrowth (Belesky et al., 1988). Later, a similar study was completed in Missouri (1991) with similar results.

Research was also conducted to determine what part of the plant was the most toxic to grazing animals. A study completed by Rottinhaus et al. (1991) developed a HPLC method for alkaloid (ergovaline) detection in live plant tissues. Also, they reported higher ergovaline concentrations in leaf blade, sheaths and stems with fertilization. These combined advancements in the understanding of the plant lead scientists and extension personnel to develop and distribute new management plans for producers.

Eventually, novel fescue varieties were developed, which contained endophytes, but not those that produced the ergot alkaloids that are toxic to livestock. These varieties have been slow to be adopted by producers due to costs associated with a total renovation. Currently, management strategies including rotational grazing, overseeding with legumes, supplementation, stockpiling, and pasture management are proven ways to control the amount of alkaloid ingested throughout the most detrimental times of year for a cow/calf operation.

## CAUSATIVE AGENTS

Fescue toxicosis stemmed from a parasitic fungus, *Lolium arundinaceum*. The fungi infects tall fescue by invading the plant's intracellular spaces. This parasitic relationship is not all bad, however, *Lolium arundinaceum* lives its entire life within the plant where it feeds, gains protection, and replicates itself through the seed head. This fungus is commonly referred to as "the endophyte," because it grows inside ("endo") the plant ("phyte") (Roberts & Andrae, 2010). The endophyte does not harm the plant itself, it actually defends the plant from consumption by grazing animals and pests. When looking at this plant, a person would never know that it was infected with a fungus as there are no observable characteristics by the naked eye.

Due to the endophyte infection, tall fescue has harnessed the title of the hardiest cool season grass in the Southeast. It is drought tolerant, due to greater root depth and volume that increase water uptake over time, resulting in better survival under moisture stress. This variety also has increased seed germination rate, seedling vigor, tiller growth rate, seed production, and mineral uptake (Ball et al., 2007). Tall fescue also handles heavy and close grazing pressures well. Tolerances of tall fescue are attributed to rapid regrowth capacity, their underground storage organs, and their ability to grow new tillers following defoliation (Karban & Baldwin, 2007).

The life cycle of the endophyte is simple; the fungus moves from the germinating seed into the seedling and colonizes mainly in leaf sheaths, meristems and internodes of elongating stems, but not roots (Christensen & Voisey, 2009). Alkaloids contain a tetracyclic ergoline ring structure and can be classified into one of three classes



including: lolines, peramine, and ergot alkaloids. Much debate has occurred in the last thirty years of research on ergot alkaloids, as to which of the alkaloids are the most toxic. The alkaloids that poison livestock are in the ergot alkaloid class particularly lysergic acid or its derivatives (Strickland et al., 2011).

After the discovery of the symbiosis of the fungus and plant and its effects on the animal, researchers needed a way to identify ergot alkaloid concentrations in stands. Yates et al. (1985) used mass spectrometry to identify significant levels of ergopeptine alkaloids in crude extracts of tall fescue from pastures producing clinical signs of fescue foot in cattle. Research completed by Lyons et al (1986) isolated ergot alkaloids from endophyte free plants and concluded ergovaline was deduced to be the one with most responsibility for animal toxicosis (Lane, 1999). Ergovaline, however is not the only alkaloid that plays a role, the other peptides have been shown, in lesser amounts to elicit vasoconstrictive actions as well. Other research has attempted to conclude where and what form the alkaloids are deposited or excreted and is discussed further in this review.

## **EFFECTS OF FESCUE TOXICOSIS**

### *General Symptoms*

Toxicity symptoms vary in affected animals. Symptoms can include gangrene (ear, tail tips, or teats), elevated rectal temperature, excessive salivation, increased respiration rate, lameness, loss of hooves, loss of tail switch, poor thrift, reduced body weight gain, reduced grazing time, reduced milk production, reduced serum prolactin, rough hair coat, and swelling in the fetlock or hoof (Strickland, 2011). Ruminants are not

the only animal that suffer ill effects, mare horses often experience dystocia, and retained placentas which results in death of foals and sometimes the mare.

Research looking into the effects of fescue toxicosis on animals was proceeded in humans, who were ingesting claviceps on infected rye. The diagnosis in humans was called St. Anthony's Fire, and was described as a feeling of a person's limbs being on fire. This feeling was caused by constriction of blood flow to peripheral tissues. This sensation is very similar to the occurrence when cattle or sheep ingest endophyte infected tall fescue. It is well documented ergot alkaloids cause blood flow to peripheral tissues to be decreased, resulting in the symptoms stated above (Klotz, 2015).

#### *Performance Reduction*

In cattle, fescue toxicosis reduces cow performance. Commonly referred to as "summer slump", especially in the South, is a commonly observed problem of fescue toxicosis. Cows experiencing fescue toxcosis have poor gains, difficulty shedding winter coats, lower milk production and a reduced conception rate. Failure to shed their winter coat is especially tough on the animals in the summer months. Due to the vasoconstrictive actions, cattle cannot thermoregulate and dissipate heat leading to increases in body temperature, suppressed appetites, and lack overall performance to their non-toxic counterparts.

The fungal infection of tall fescue benefits the grass but causes a decrease in intake and is likely evolved as a strategy to prevent other grazing by cattle and other livestock (Klotz, 2015). Reduced gains have been reported across numerous studies evaluating cattle performance on tall fescue (Hoveland et al., 1983, Boling, 1985,

Schmidt et al., 1986). There are many possibilities for a reduction in gains, however, reduced gains are most often attributed to the reduction in intake. It has been documented by Bond et al. (1984) that grazing time was reduced on high endophyte infected pastures when compared to pastures with low endophyte levels. Other studies have shown cattle gaining 30 to 100 % less consuming endophyte infected tall fescue compared to consumption of an endophyte-free tall fescue (Paterson et al., 1995). An early theory to explain reduced gains/intake identified gut fill limiting voluntary intake due to restrictive flow of the contents through the digestive track (Allen, 1996).

Much research has been completed to try to understand the association of ergot alkaloids and decreased intake, but inconsistencies in the work are present and no clear picture is presented. In a series of studies from 2012-2015, researchers aimed to determine the association and quantity of ergot alkaloids with reduced gains. Koontz et al. (2013) found that the consumption of ergot alkaloids causes decreased metabolism. A series of experiments by Foote et al., (2013) and Koontz et al., (2015) concluded a general decrease in digestive function determining the consumption of ergot alkaloids did not negatively impact the overall energy balance of digestion of feed but caused a decreased blood flow and passage rate in the stomachs of steers.

For cattle experiencing summer slump, animals have a reduced ability to dissipate body heat, and blood flow to peripheral (rib skin), core-body (duodenum colon), and brain (cerebellum) tissues was decreased (Rhodes et al. 1991). Patterson et al. (1995) found steers grazing E+ during periods of temperature stress steers consumed less forage than those grazing E-. Due to these causes, dopamine antagonist therapy initially

developed to treat fescue toxicosis in gravid mares, has been used in beef cattle (Jones, 2008). Results are positive, but the costs associated with the treatment are not practical.

Fat necrosis is another symptom of fescue toxicosis, and has been shown in aged cows who have long-term grazed toxic fescue (Studemann et al., 2015). Fat necrosis is generally found in the abdominal cavity, and cannot be detected without necropsy. This hard fat deposit puts pressure on the digestive and reproductive tracts, causing compression on the gastrointestinal and urinary tracts leading to the obstruction of viscous organs. Fat necrosis tends to be more prevalent in cattle who are grazing toxic fescue with high levels of applied nitrogen or chicken litter (Williams et al., 1969; Studemann et al., 1975).

The most dramatic visible symptom occurring on cattle grazing tall fescue is “fescue foot” a gangrenous condition of feet and/or tails (Bush and Buckner, 1973, Bush et al., 1979). Fescue foot is generally observed in cattle grazing tall fescue in cool seasons of the year, namely winter, and therefore seems to be associated with lower ambient temperature (Garner and Cornell, 1978). The lower ambient temperature coupled with ergot alkaloids induces vasoconstriction in order to conserve body heat (Curtis, 1983), resulting in a dramatic loss of blood flow to the extremities.

According to Strickland et al. (2011), little is known about the specific cellular mechanisms involved in impaired production of animals displaying signs of fescue toxicosis. The biological activity of the ergot alkaloids in animal systems is largely due to the structural similarities of the ergoline ring structure to the biogenic amines; serotonin, dopamine, norepinephrine, and epinephrine (Berde, 1980; Weber, 1980). These alkaloids

are found to affect many psychological traits the neurotransmitters elicit including appetite, cardiovascular function, muscle contraction, body temperature regulation, and endocrine activity.

Dopamine receptors have been proven to bind the ergo-peptide, Ergovaline. Dopamine is transported to the anterior pituitary via the hypothalamic portal system (Guyton, 1986). It's interaction with the dopamine 2 receptor elicits an inhibitory action on prolactin secretion.

### *Effects on Calving*

The effects of fescue toxicosis are widespread and affect individual animals differently. Differing amounts of alkaloids consumed and the variance between animals determines the extent of impacts in each animal. In cattle, conception rates and milk production are often reduced (Paterson et al., 1995). Furthermore, difficult calving and loss of calf body weight are often observed. Cattle production in the southeastern U.S. is typically a cow-calf operation where 70% of calves produced in the region are sold at weaning (McBride and Mathews, 2011)

This is also the primary location of tall fescue, making it the most cultivated pasture grass in the U.S. with more than 35 million acres.

Research has shown that maternal processes are affected by fescue toxicosis. Early work in sheep suggested ewes on endophyte-infected fescue exhibited delayed conception after introduction of the ram but neither body weight gains, gestation length, average number or lambs born, lamb birth weight, lamb survival, nor gain to weaning was affected by the endophyte (Bond et al., 1988). Bond et al. (1998) also showed

delayed conception due to embryonic mortality for ewes grazing endophyte infected (E+).

In cattle, it has been suggested the alkaloids effect on the corpus luteum (CL) includes larger luteal cells with a greater number of mitochondria, lipid droplets, and secretory granules (Ahmed et al., 1990). This combined with reduced circulating prolactin only decreases the chance of cows to maintain a pregnancy.

Prolactin is a leutotropic hormone secreted from the anterior pituitary and known for controlling lactation and mammary development. Consistently referenced in literature, serum prolactin is reduced in animals with fescue toxicosis. A study completed by Aiken et al. (2001) concluded serum prolactin concentrations gradually increased once steers were removed from tall fescue, and then concentrations stabilized after 3 days. It is estimated that prolactin is involved in over 300 different functions (Ben-Jonathan et al., 1996). Additionally, regulation of prolactin is also influenced by day length. One would expect prolactin to increase during late gestation, but studies in a variety of species, have shown the consumption on ergot alkaloids drastically reduces prolactin during late gestation (Duckett et al., 2014).

Reproduction in female animals is not only affected by fescue toxicosis, but it has been shown that a bulls' reproductive efficiency can also be altered. It is suggested that scrotal circumference is negatively impacted in bulls grazing toxic tall fescue due most likely to vasoconstriction of the testicular vein (Stowe et al., 2014). Semen in bulls grazing E+ also has been shown to have decreased motility than the E- counterparts.

Therefore, sperm physiology may be altered due to consumption of toxic tall fescue (Stowe et al., 2014).

Concentration of ergovaline is most prominent in forages during spring, which is subsequently a critical time for conception in spring calving herds. When animals fail to conceive, producers lose money and when multiplied by the amount of beef producers in the U.S. the problem is magnified.

### **ALKALOID DIGESTION AND ABSORPTION**

Investigations into the alkaloid metabolism once ingested by the animal are lacking conclusive evidence on how each alkaloid may be modified and absorbed. Although lacking a lot of evidence, alkaloids are thought to be modified in the rumen for the digestive tract and absorbed in various tissues, or excreted. Ergovaline, considered the most potent vasoconstrictor, is metabolized into lysergic acid in the rumen (Eckert et al., 1978) However, due to the lack of methods, it is unclear to what degree ergot alkaloids are metabolized and deposited into other tissues. Hill et al. (2001) showed absorption through ruminal, reticular, and omasal tissues in parabiotic chambers in sheep. Due to the complex nature of the reticulo-rumen, absorption and degradation of the alkaloids are challenging to determine. Laboratory experiments by Harlow et al. (2017) demonstrated that rumen hyper-ammonia-producing bacteria (HAB) degrade ergovaline and that these bacteria can adapt to increase ergovaline degradation when repeatedly exposed. Hill et al. (2001) determined alkaloid transport is greater in ruminal tissues compared to reticular tissues and that lysergic acid and lysergol had a greater transport potential within the

rumen. Furthermore, the omasal tissues seemed to pass ergopeptide alkaloids in greater quantities than lysergic acid and lysergol (Hill et al., 2001).

Harlow et al. (2017) also documented the ability of some toxic fescue naïve steers to adapt by increasing rumen degradation of ergovaline. Similar results by Studemann et al. (1998) saw a disappearance of ergot alkaloids within 12 h from switching to a E+ diet. Another method used to determine the bioavailability of ergot alkaloids is to examine urinary and biliary excretory patterns of the alkaloids (Eckert et al., 1978). Excretion has been documented at various levels in feces, urine and bile (Westendorf et al., 1993; Studemann et al., 1998; Schultz et al., 2006; DeLorme et al, 2007; Merrill et al., 2007; Schuman et al. 2009) in various species (cattle, horse and sheep). However, most studies have had a difficult time accounting for all the consumed alkaloids in excretion. In all studies, more ergovaline was consumed than lysergic acid, but less ergovaline was excreted than lysergic acid. This leads us to believe the animal is either storing and/or converting ergovaline in the digestion and absorption processes. Hill et al. (2001) suggest that circulating alkaloids in ruminants grazing tall fescue are either 1) converted from the ergopeptide form before excretion, 2) absorbed as the ergoline alkaloids, or 3) absorbed as both the metabolized to the ergoline prior to excretion.

Little information has been discovered about the distribution of the ergot alkaloids in the tissues of grazing livestock. Ergot alkaloids have been found in adipose tissue by Realini et al. (2005). Other studies (Schumann et al., 2007; 2009) have been inconclusive. Inconclusive results may be due to the sensitivity of detection methods. As methods become more sensitive and selective for target metabolites in place of parent compounds,



reports of alkaloid residues in the tissues of grazing animals might increase (Strickland et al., 2011).

Small concentrations of ergot alkaloids may bioaccumulate in animal tissues and if mobilized may show signs of fescue toxicosis. Ingestion of alkaloids tends to affect animals rather quickly, and younger animals more drastically. Aiken et al. (2009) completed a study that showed endophyte-naïve cattle exhibited a vasoconstrictive response to ergot alkaloids within 51 h after initial daily feeding of diets containing 0.4 g ergovaline plus its epimer, ergovalanine.

Not only can alkaloids be converted in the rumen and deposited into muscle (Realini et al., 2005) and adipose tissue, there are many negative effects on milk production. A mass-balance study revealed that the toxin concentration in ruminal fluid apparently increases over time (De Lorme et al., 2007). These unexpected findings resulted in the hypothesis that the rumen fermentation processes can liberate non-extractable toxins (escaping the initial feed analysis) and metabolize ergovaline into lysergic acid that might be even be absorbed through the ruminal wall (Hill et al., 2001).

Various mycotoxins have the ability to modify the rumen flora due to their antimicrobial activity. This may decrease the degrading capacity of the rumen resulting in an unexpected passage rate of intact toxins from other sources. A comparable effect can be also be expected in cases in which the rumen flora is affected in the course of metabolic diseases, as, for example, rumen acidosis. In conclusion, dairy cows are protected against exposure to mycotoxins by their rumen flora (Fink-Gremmels, 2008). Various myotoxins, however, pass this barrier or are converted into metabolites that

retain biological activity. The assessment of undesirable effects exerted in ruminants should include the antimicrobial activity of various mycotoxins that results in an impairment of the function of the rumen flora, followed by a poor feed utilization and reduced weight gain and productivity (Fink-Gremmels, 2008). It appears that that ruminants have a greater tolerance to ergot alkaloid exposure due to microbial or hepatic metabolism or both (Strickland et al., 2011). Additionally, researchers (Tor-Agbidye et al., 2001 ; Zbib et al., 2014) confirmed a greater resistance of sheep to fescue toxicosis than cattle.

As for the alkaloids effect on milk there has been data that shows Although no data is available on the effects of EV on milk production and quality in sheep, hay containing 782 µg EV/kg was reported to decrease fat and protein contents in dairy cows (Kim et al., 2007). Zbib et al. (2014) found no ergovaline residue in milk of ewes consuming toxic treatments after 28 days of exposure. This again may be due to digestive fermentation that is modifying the ergot alkaloids prior to reaching the mammary tissue. Additional research into determining methods to analyze different tissues and excretion fluids are needed in order to quantify and identify ergot alkaloids in different locations.

### **IDENTIFIED MANAGEMENT PLANS**

Mitigation efforts have been prescribed by Extension personnel to livestock producers for over thirty years. Early plans included destroying endophyte infected (E+) stands and replacing pastures with endophyte free (E-) tall fescue. However, E- tall fescue could not compete when it came to disease and drought pressures. Prescriptions

also included extensive management plans that include timely fertilization, rotation of grazing animals, dilution with legumes, weeds or non-toxic plants.

Most strategies are aimed at reducing the amount of ergots consumed by grazing animals. The concentration of ergot alkaloids within tall fescue varies throughout the year. Highest concentrations have been observed in the early autumn as regrowth occurs, spring due to seed head concentrations with the lowest during mid summer when the grass is dormant. Concentrations can also greatly differ in the different parts of the plant. An early study by Rottinghaus et al. (1991) determined high concentrations were three times higher in the seed head than in the leaf blade. Later, it was determined there is greater ergot alkaloid concentrations in leaf sheaths than in blades, due to greater mycelia mass (vegetative part of the fungus) in the sheaths. When choosing the mitigation plan that is right for a particular grower it is important to weigh out how each plan or combination of plans that will work with a particular farm set up, climate, number of animals, and finances.

#### *Renovating Pastures with Endophyte Free (E-) Varieties*

When tall fescue was determined to be toxic to grazing animals, much research during the 1980's was directed at developing non-toxic varieties. Most of these new varieties could not withstand harsh environmental stresses due to not having the endophyte that advantages the plant.

Endophytes are most concentrated in the seed head and are transmitted maternally through seed. Endophyte free cultivars can be developed by only allowing endophyte free plants to produce seeds for following generations (Hopkins et al., 2009). However, when

producers planted these new endophyte free varieties, they only lasted for about 3 to 4 years due to intolerances of drought and overgrazing (Gunter and Beck, 2004). Due to stand failure many livestock producers see no use in planting endophyte free varieties. This option that was thought of as initial fix to fescue toxicosis turned out to not be a viable option at all.

### *Renovating Pastures with Novel Fescue Varieties*

A non-toxic or novel tall fescue variety contains endophyte that does not produce the toxic alkaloids that are most detrimental for production in grazing animals. The identification of ergovaline as the most prominent alkaloid causing the negative effects (Klotz, 2015) on grazing livestock has allowed researchers to remove the toxic endophyte strains and replace them with endophytes that do not produce alkaloids such as ergovaline that are detrimental to the animal.

Researchers at the University of Georgia developed the first two varieties for U.S production using Pennington Seed's® Max Q endophyte: Jesup, and Georgia-5, in 1995 and 1993, respectively. Jesup tall fescue was released in 1995 by UGA's Agricultural Experiment Station as a clone of Kentucky 31 collected from a pasture in Jesup, Georgia in 1981. Jesup comes in two versions: endophyte infected (Max-Q technology) or endophyte free (GA-Jesup Improved-EF). The Jesup and AR542 (later known as Max-Q) novel endophyte strain combination provided the greatest survival under mob grazing and was eventually commercially released as Jesup-MaxQ (Aiken and Strickland, 2013).

Experiments by Bouton et al. (2002) investigated re-infecting Jesup and Georgia-5 tall fescue varieties with non-alkaloid producing endophyte strains and compared to

those treatments to endophyte free. When wether lambs were grazed on novel or E+ varieties the lambs on novel treatment had lower rectal temperature than those grazing E+ cultivars (Parish et al., 2003). Other studies showed Jesup possessed greater yield and strand survival when compared to E- cultivars. Lambs consuming Jesup with AR542 gained on average 124 g/d which was equal to the E- treatment, but was 57 percent greater than E+ treatment. Additionally, Parish et al., (2003) found beef cattle grazing AR542, and endophyte-free tall fescue pastures did not exhibit decreased prolactin concentrations or increased rectal temperatures indicative of fescue toxicosis as did in cattle on endophyte infected pastures. Based on these studies, using novel varieties is a viable option for producers looking to completely renovate pastures, as the endophytes strains are present in the grass but are not those harmful to livestock. Studies suggest gains of animals on novel endophyte varieties are similar to endophyte free, but without the toxic effects. Both Jesup varieties can only be sold as a class of Certified Seed and by individuals licensed by Georgia's Integrated Cultivar Release System (GICRS) and the University of Georgia's Research Foundation (UGARF) (Cultivar Release, 1993).

Max Q technology has been inoculated into many high producing tall fescue varieties adapted to different climate zones. This technology was developed in New Zealand by AgResearch Limited as the first non-ergot producing strain of fungus named AR542. Pennington Seed® also offers Texoma Max Q II, which was released more recently in 2001. Texoma Max-Q II was released for climates in the southern-central U.S., such as Texas, Oklahoma, Arkansas, Louisiana, and Mississippi.

An initial study by Bouton et al. (2002) concluded responses from ewes grazing E+ forage compared to E- or AR542 (now Max-Q technology) effected weight gain, serum prolactin, and rectal temperature within 2 weeks of exposure (Bouton et al., 2002). Parish et al. (2003) reported 43% greater intake by steers grazing endophyte-free and Jesup-MaxQ tall fescues than those grazing toxic endophyte infected Jesup.

Developing novel and endophyte free varieties is a complicated and lengthy task. In general, it takes around eight years for a new variety of seed to be developed. As with much of the seed in the United States, tall fescue varieties must go through the processes of obtaining patents, and Plant Variety Protections (PVP's) to ensure the seeds progeny will be contaminant free and true to type. Seed must also be grown, harvested, and packaged through a state approved Seed Certification Program. These programs hold growers to a strict set of standards for each variety of grass by inspections of the crop in the field, harvesting equipment, and seed cleaning and bagging equipment. Seed is not tagged and sold until it also passes germination and purity tests by a state approved and certified seed lab.

Planting novel varieties has been deemed a reliable option, however, it does have its complications. When grazing novel varieties, grazing management plans need to be altered in periods of low growth. Novel varieties cannot withstand harsh grazing pressures that toxic endophyte infected can. Further grazing recommendations can be found by accessing the Fescue Toxicosis and Management Handbook (Roberts & Andrae, 2010) and are outline below.

For producers to establish novel varieties in formally planted E+ pastures, the process is a costly and lengthy operation. There are four steps in a renovation program: 1) prevent seed head production in the existing E+ infected stand, 2) destroy the existing stand, 3) seed the new variety, and 4) manage the new planting (Handcock & Andrae, 2009). To start this process, a producer should plan for a mid-September to October planting date. Due to the endophyte concentration being highest in the seed head, proper and timely mowing needs to be done in order to prevent seed head formation. Two methods of spraying have been identified “spray-smother-spray” and/or “Spray-spray-plant” methods is widely accepted by using a herbicide such as Glyphosate based product.

However, this is another costly part of this type of management plan. After the second herbicide application, novel seed should be planted. These new seedlings need special attention to obtain a good stand being sure not to overgraze or mow will help the stand survival. Novel stands cannot withstand the grazing pressures like endophyte infected varieties, so a management plan to reduce the pressures especially during the hottest months of the year is necessary.

Although the results for novel have been successful, adoption of planting novel varieties is slow to be accepted. Currently, the cost to renovate exceeds \$600/ha, most economic analyses show it to be a good long-term investment, especially on fields with high forage production potential (Kallenbach, 2015). Often times, producers have land leases rather than ownership, so their return on investment may not be worth-while. There is still a need to educate producers about the new technologies of the novel

varieties on the market, and the long-term return on investment it could have in the right situation.

### *Dilution*

Dilution has been the most highly accepted mitigation strategy. Legumes such as red and white clover, alfalfa and lespedeza are often prescribed to be inter-seeded with tall fescue to reduce the effects of fescue toxicosis. Interseeding tall fescue with legumes colonized by N-fixing *Rhizobia* bacteria is an alternative N source, but most legumes do not persist under continuous, intensive frequent grazing (Aiken & Strickland, 2013). Thompson et al. (1993) combined grazing studies to show that ADG was not affected despite adding clover in stands of fescue with high infection levels compared to stands of fescue with low infection levels and no clover. Suggesting, the most improvement can be seen in stands with lower infection levels. Contrary to this study, it has been shown that dilution does improve the quality of forage in the pasture, and could explain increased animal gains that have been observed

Managing pastures to favor growth of other grasses can dilute toxic tall fescue. If tall fescue is grazed closely in the spring it will prevent other species from getting shaded out, as tall fescue is a clump-type growing grass. Also, applying fertilizer in the summer will also support growth of other species of forages growing alongside tall fescue. The dilution option has proved to be a cost-efficient way for many producers in trying to manage the adverse effects of this crop. However, highly beneficial for cow/calf producers, it is not a long-term solution to the fescue toxicosis problem. Legume stands have poor growth in the summer in the deep south, and will eventually disappear in time.



### *Supplementation.*

Supplementation of concentrates has been another widely used method to combat fescue toxicosis. A study by Aiken et al. (2001) determined it takes as little as 3 days to decrease rectal temperature to a normal range in steers exhibiting fescue toxicosis. Stress on cattle before transport is harder on cattle exhibiting signs of fescue toxicosis. Results from Aiken et al. (2001) concluded placing cattle on a endophyte free diet 3 to 4 days prior to transport will help reduce mortality and enhance feedlot adjustment and performance.

Supplementation has been shown to increase average daily gain of cattle grazing endophyte infected pastures. Research led by Aiken has investigated supplementing with a broiler litter ground corn mixture (1998) and soybean hulls (2008) concluded ADG increased for cows supplemented with these mixtures compared to grazing E+ without supplementation (Aiken et al. 1998; Aiken et al. 2008). Forcherio et al (1993) concluded providing ruminally degradable protein to lactating young cows grazing E+ did not enhance calf growth, but did promote additional cow weight gain.

Supplementation may have a positive impact due to decreasing total forage intake. In an early study by Hannah et al. (1989) supplementation of corn or corn gluten to steers grazing E+ pastures decreased grazing time from 7.8 h to 4.6 hours. Supplementation basically acts like a dilution factor when consuming tall fescue. If cattle are eating other sources of nutrients, they will be consuming less toxins, when forced to graze E+.

### *Close Grazing.*

A study by Belesky and Hill (1997) concluded that frequent, intensive grazing can reduce ergot alkaloid concentrations in vegetative tillers. The leaves and pseudostems of uncut plants of two tall fescue genotypes yielded twice the ergot alkaloid concentrations of those harvested from plants cut 5 to 10 cm heights at 7-d intervals for 6 wk period (Aiken & Strickland, 2015). Low ergot alkaloid concentrations in leaf blade tissues relative to other plant parts (Rottinghaus et al., 1991) indicate that intake of ergot alkaloids could be reduced by adopting grazing management practices that maximize consumption of leaf blades (Aiken & Strickland, 2013).

#### *Fertilization.*

Since the late 1980's researchers have been experimenting with the effect of nitrogen fertilization on ergot alkaloid concentrations first with Lyons et al. (1990) who conducted experiments in the greenhouse, and later Belesky and Hill (1997) followed up by conducting field experiments. When using the close grazing technique to control endophyte consumption, pastures will require additional nitrogen fertilization to assist the plant in continued growth. However, if producers are trying to reduce toxin loads in the field, they should not fertilize E+ tall fescue pastures with high rates of nitrogen.

Alkaloids contain nitrogen, and ergot alkaloid concentrations increase rapidly following nitrogen fertilization application (Rottinghaus, 1991). Studies have shown an increase in fat necrosis and fescue foot in cattle grazing recently fertilized E+ pastures. In today's market, one ton of nitrogen fertilizer would cost about \$1,110.00 in South Carolina. The costs for fertilization once, and possibly more per year, can quickly accumulate.

#### *Ammonization of hay.*

Hay can be treated with anhydrous ammonia can partially detoxify the hay. When low-quality grass hay is treated, the ammonia breaks down cell wall linkages to render the forage more digestible (Roberts & Andrae, 2010). Low quality hay bales are stacked and covered in plastic with 3% anhydrous ammonia applied. The reaction takes 2 to 4 weeks to occur. With ammonization, hay is still toxic, but has been shown to be at half of the alkaloid concentration (Roberts et al., 2009).

### *General Management*

Many producers have used management plans including the above-mentioned applications, as well as simply utilizing rotational and strip grazing. Rotational grazing for animals that have to be on tall fescue should aim at getting the animals off the fescue at times of highest ergot alkaloid concentrations. So, during the spring when seed heads are present, animals should be removed from tall fescue pasture. Late spring and summer are also the time when livestock animals combat heat stress and generally have a lag in performance. Roberts and Andrae (2010) suggest moving cattle from toxic fescue in times of highest ergot alkaloid concentration to warm season grasses such as Bermuda. Rotating cattle to warm-season perennial grass pastures is likely realized with warmer, drier summers when incidence of ergot alkaloid-induced heat stress is greater and there is minimal growth of tall fescue (Aiken & Strickland, 2013).

Another general management strategy is to stock pile fescue for winter grazing. This allows an extension of the grazing period through the winter months. To take advantage of the stockpiling method a producer would want to begin the process in late summer to early fall. This process involves removing cattle from the pasture, applying

40-80 lbs of Nitrogen per acre and allowing the grass to accumulate growth until November or December (Ball et al., 2015). In a Missouri study, stockpiling reduced the period of hay feeding by half (120 to 6 d). The alkaloid levels in stockpiled forage decline over time (Kallenbach et al., 2003). The combination of lowered ergovaline concentrations in stockpiled toxic fescue and the decreased ambient temperatures may reduce animal production losses due to fescue toxicosis.

### **CATTLE GENOME**

Currently, symptoms for fescue toxicosis is determined by phenotypic traits such as; rough hair coat, heat stress, reduced gains, reduced milk production, and peripheral vasoconstriction. Due to advances in technology it is important for us to explore a genetic approach to fescue toxicosis. The human genome project was a federally funded project that started in the 1990's and is always undergoing modifications. This project later sparked the interest into developing a complete cattle genome.

Genome wide association studies (GWAS) are a new method of genetic testing that use single nucleotide polymorphisms (SNP). Within these studies phenotypic performance data are compared to an array of SNP's and an analysis is performed. During the 2007 Welcome Trust Case Consortium, it was declared that increasing evidence demonstrates that GWAS is extremely powerful method to identify genes that are involved in diseases (Campbell et al., 2014). This method of technology is becoming very useful in identifying potential genes that play a role in various diseases and complex traits.

The sequencing of the bovine genome allowed researchers to collectively put together 50,000 SNPs that are located throughout the entire genome. The bovine genome is similar in size to the human genome with an estimated 3 billion base pairs, 83% are identical to humans.

Single Nucleotide Polymorphism's that were polymorphic in many populations were primarily derived by comparing whole-genome sequence reads representing five taurine and one indicus breed to the reference genome assembly obtained from a Hereford cow (Elsik et al., 2016).

High-density SNP genotyping or genome sequencing is now available as a diagnostic tool for predicting individual predisposition to heritable genetic diseases, ushering in the era of "personal genomics". The objective in using the genome wide study of cattle is to use genomic data to supplement extensive sets of performance data to predict genetic merit values that are used for selection decisions by producers.

### **DOPAMINE NEUROTRANSMITTER (DRD2)**

The dopamine receptor has five different subclasses (D1-D5) identified by particular properties. D1 and D2 have been most widely studied. Toxic effects by ingesting ergot alkaloids have been linked to D2 within the striatum which inhibits acetylcholine and dopamine release, carotid body depressing chemosensory activity, the sympathetic nerve terminals inhibiting norepinephrine release and the anterior pituitary stimulating prolactin and  $\alpha$ -melanocyte-stimulating hormones (Cooper et al., 1991).

The Dopamine 2 (DRD2) gene is located on the bovine chromosome 15 and has been shown to play a role in prolactin secretions (Civelli et al., 2013). Dopamine is

transported to the anterior pituitary via the hypothalamic/ hypophysial portal system (Guyton, 1986; Cross & Strickland 1995). Dopamine exerts its inhibitory action on prolactin secretion through interaction with the D2 dopamine receptor located on the lactotrophic cell. Compounds interacting with this receptor, as agonists, will cause suppression in prolactin secretion. Prolactin is essential to initiate lactogenesis.

Therefore, with decreased serum prolactin concentrations, lactation in cattle is decreased, and almost eliminated entirely in the horse (Klotz, 2015). Furthermore, it has been shown that the dopamine 2 receptors may be involved in the effects on insulin regulation (Garcia-Tornadu et al., 2010). An early study completed by Strickland et al. (1992), isolated pituitary cell preparations and provided evidence that the alkaloids of tall fescue are acting as dopamine agonists to reduce prolactin secretion. Much research has focused on preventing DRD2 stimulation by ergot alkaloids, and has been found successful in horses. Administration of Domperidone®, a dopamine receptor antagonist has been shown to prevent signs of fescue toxicosis in late gestating mares grazing toxic tall fescue (Cross et al., 1995).

Due to the nature of fescue toxicosis and its affects on different mammalian animals, research has now taken a turn toward a genetic approach to fescue toxicosis. There is not one single trait that can be linked to fescue toxicosis, but rather many genes that play a role within the disease. Hohenboken and Blodgett (1997) completed the first published research on a genetic approach. They selected eight generations of mice for resistance or susceptibility, then fed E+ seed. They evaluated growth in these generations. The results suggested growth in the resistant line was not retarded and the enzymes

involved in detoxification reaction were higher than in the susceptible line (Hohenboken & Blodgett, 1997). More recently, (2012) researchers at the University of Tennessee and the University of Missouri teamed up to apply the D2 gene with fescue toxicosis in Angus-based cattle. DRD2 was a good candidate gene to investigate due to its role in regulation of prolactin, and could contain a polymorphism that would serve as a marker for resistance to fescue toxicosis (Campbell et al., 2014). They found an intronic single-nucleotide polymorphism (SNP) at position 534 within the DRD2 gene. The 'A' allele was shown to be associated with increased serum prolactin concentrations and decreased hair coat scores. Through their series of experiments, they genotyped multiple cattle herds at research stations at each University using tail hair follicles, extracted and amplified by PCR. Results indicated steers (n=42) grazing Novel or E+ tall fescue prolactin concentrations were lower in G|G steers grazing E+ in May, while A|A steers had higher prolactin levels grazing E+. These results showed that the A|A genotype may have a resistance to fescue toxicosis compared to GG steers when grazing toxic grass.

Additional research is warranted to investigate and define the genes that play a role with fescue toxicosis in ruminant species. Definitively discovering the genes that are involved, could allow a new management system to be implemented in those herds who have no other choice but to graze toxic fescue. Due to the lack of research investigating a genetic association with fescue toxicosis, the research contained in this dissertation aimed to determine some impacts a particular gene may be having in association with fescue toxicosis in beef cattle as well as sheep.

## **FESCUE TOXICOSIS IN OTHER SPECIES**

### *Equine.*

The effects of fescue toxicosis in horses are quite different than other species, as the majority of its symptoms and negative effects are reproduction related. Due to the nature of the equine digestive tract, alkaloids are not broken down by microbial fermentation as in ruminants, therefore ingested alkaloids in equine have greater effects. One difference in horses when suffering from fescue toxicosis is horses do not have increased body temperatures. It is thought this is due to horses sweating more freely than ruminant animals that allow them to cool easier (Cross, 2009).

Fescue foot exhibited in cattle caused by vasoconstriction has not been observed in horses. However, pregnant females are severely affected in late gestation. When mares are experiencing fescue toxicosis, gestation can be in excess of 13 months, when the normal gestation length is 11-12 months (Cross, 2009). This phenomenon is thought to be attributed to the inhibition of cortisol-releasing hormone (CRH). Dystocia is widely observed in horses and cattle alike. Increases in foal skeletal size has been widely observed in mares on E+ diets during late gestation, due to increased time in utero. These large framed foals are hard for the mares to foal out resulting in countless mortality of mare and/or foal through the years. Milk production is effected in all species studied, with horses exhibiting complete agalactia due to reduced prolactin secretion from the pituitary lactotrophic cells (Cross, 1995). When compared to ruminants who show a reduction in milk production (Klotz, 2015). A study completed by Monroe et al. (1998) found that 88% of mares on E+ diets suffered from complete agalactia after foaling.



Toxic effects of horses grazing tall fescue are most detrimental in the last stage of gestation with pregnant mares. Alleviating fescue toxicosis in mares can be controlled well by removing the mares from E+ pastures during the last stage of gestation. A study completed by Taylor (1993) examined reactions from mares removed from E+ treatment at 10, 20, or 30 d before estimated foaling date. Mares removed 30 d before foaling where similar to endophyte free treatment in lactation, foaling and hormonal patterns (Taylor, 1993).

In an effort to help mares, researchers sought to find a dopamine receptor antagonist. Redmond et al. (1992) administered domperidone orally (1.10 mg/kg BW) to gravid mares grazing E+ tall fescue and saw an increased serum prolactin and progesterone concentrations. Mares appeared to have a complete recovery from tall fescue toxicosis without any side effects from the drug. Treated mares had milk, live foals, and correct gestation length. Veterinarians and/or horse owners reported the drug to be 94.5% effective in prevention of the signs of fescue toxicosis (Cross et al., 1995). Domperidone is now a commercially available drug to combat fescue toxicosis in gravid mares.

### *Sheep.*

The effects of fescue toxicosis are less widespread than in cattle. Perhaps it is due to the small number of sheep operations in the south where toxic fescue is prevalent. Fewer studies have been completed on the impacts of fescue toxicosis on sheep than in cattle. However, the responses of these studies utilizing sheep as a model indicate similar responses as found in cattle.

Duckett et al. (2014) showed exposure to ergot alkaloids during gestation reduces fetal growth in sheep. Ewes were fed E+ fescue seed or E- seed mixed in with a total mixed ration and tracked through parturition. Male lambs were euthanized and measurements were taken. E+ diets appeared to reduce gestation length by about 4 days. Birth weight of lambs from ewes consuming E+ diets was reduced by 37% compared to E-. Organ and muscle weights were also reduced. This could be attributed to intrauterine growth restriction, as the E+ ewes were not producing as much blood flow to the fetus and ewes on E- seed. Additionally, intrauterine growth restriction of the fetus during the second trimester of gestation reduces the formation of secondary muscle fibers. Exposure *in utero* to ergot alkaloids altered skeletal muscle formation by reducing the ratio of secondary to primary myofibers, myofiber hypertrophy *in utero*, and protein content of muscles. In conclusion, Britt et al. (2014) pinpointed that late gestation was the most detrimental stage of gestation to the fetus from ewes experiencing fescue toxicosis.

N. Zbib et al. (2015) found low concentrations of ergovaline in tissues and high concentrations of ergovaline in the liver and high concentrations of lolitrem in fat tissue in ewes fed endophyte infected ryegrass hay. These new studies suggest the alkaloids are being deposited in other tissues throughout the body. Alkaloids in milk was also measured and conclusions were made that excretion in milk were very low which aligns with another study in dairy cattle that found only .23% of ingested alkaloids to be found in the milk (Finch et al., 2013).

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## CHAPTER TWO

### **DOES GENOTYPE PLAY A ROLE IN THE REISTANCE TO FESCUE TOXICOSIS IN OVINE?**

#### **ABSTRACT**

The objectives of this study were to: 1) identify single nucleotide polymorphism (SNP) in the dopamine receptor 2 (DRD2) gene in the ovine, and 2) determine how DRD2 genotype altered response of ewes fed endophyte-infected (E+) or endophyte-free (E-) tall fescue seed during two stages of gestation (MID, d 35 to 85 or LATE, d 86 to 133). Pregnant ewes (n=32) were divided into four treatments receiving either endophyte-free tall fescue seed (E-; 0 µg ergovaline + ergovalinine /hd/d) or endophyte infected tall fescue seed (E+; 1772 µg ergovaline + ergovalaline/ hd/d) during MID (d 35- d85) and/or LATE (d 86 – d 133) gestation in a 2 X 2 factorial arrangement. Ewes were individually fed the same amount of feed plus seed (E+ or E-) from d 35 to 133 of gestation, when terminal surgeries were performed. Genomic DNA was isolated from liver samples collected after terminal surgeries on ewes at d 133 of gestation and used to screen the ovine DRD2 gene for SNP. One SNP, g270a, in exon 1 was discovered and investigated further. Allele frequencies were favorable so a genotyping assay was designed, tested and found to be 100 percent concordant with sequencing results. Data were analyzed with genotype (A|A, A|G, G|G), fescue treatment (E+ or E-) at two stages of gestation (MID or LATE), day of collection (serum assays only), and all interactions in the model. Prolactin concentrations tended to differ ( $P = 0.089$ ) by genotype and day of collection. Ewes with G|G and A|G genotypes had lower prolactin concentrations than

AlA ewes on d 50 and 105 but genotypes were similar at other time periods (d 30, 85, 130). Consumption of E+ fescue seed reduced ( $P < 0.001$ ) prolactin levels at MID and LATE gestation but interactions with genotype were non-significant ( $P > 0.44$ ). Cortisol, triiodothyronine (T3), and thyroxine (T4) concentrations were elevated ( $P < 0.05$ ) in AlA genotype compared to GlG or AlG but were not altered ( $P > 0.20$ ) by fescue treatment, stage of gestation, or interactions with genotype. Total lamb fetal weight at d 133 of gestation was lower ( $P < 0.05$ ) for ewes fed E+ seed during LATE gestation and greater ( $P < 0.05$ ) in ewes with GlG genotype compared to AlG or AlA; however, the interactions between fescue treatment and ewe genotype was non-significant ( $P > 0.50$ ). Genotype at the ovine DRD2 genotype appears to be associated with circulating hormone concentrations in the ewe and fetal birth weight, regardless of fescue treatment.

Keywords: SNP, Tall Fescue Toxicosis, Sheep, Dopamine Receptor

## INTRODUCTION

Effects of fescue toxicosis are estimated to cause a one billion dollar loss to the livestock industry each year (Roberts & Andrae, 2004). *Lolium Arundinaceum*, otherwise known as tall fescue, is the primary grazing forage in the Southeastern United States (Roberts, 2000). It is planted due to its tolerance in harsh environmental conditions such as heat stress, pest and disease pressures. Tall fescue is infected with an endophytic fungus, *Epichloë coenophiala* formerly known as *Neotyphodium coenophialum*, that has a symbiotic relationship with the plant (Porter et al., 1979; Young et al., 2014). The fungus produces ergot alkaloids that help with the hardiness of the grass to stressful conditions but negatively affects grazing animals. The alkaloids can also bind to adrenergic, serotonergic, and dopaminergic receptors which in turn causes vasoconstriction, reduced fetal weight, retained placentas, rough hair coat and fescue foot collectively referred to as fescue toxicosis (Strickland et al., 2011). Of all the ergopeptide alkaloids produced, ergovaline is present in the greatest quantity (Lyons et al., 1986) and is believed to be the primary causative agent of fescue toxicosis (Joost, 1995).

Current recommendations for producers to combat grazing on toxic fescue pastures include: re-planting pastures with endophyte-free or novel-endophyte cultivars, or extensive management practices such as rotational grazing, dilution with clovers, or feeding supplements. However, many of these options are costly, labor intensive and slow to become adopted, leading researchers to explore a genetic based approach to livestock management. Campbell et al. (2014) examined a DRD2 SNP in Angus based cattle herds and concluded that a DRD2 SNP could be informative to identify cattle as

being tolerant or susceptible to fescue toxicosis. The objectives of this study were to: 1) identify single nucleotide polymorphisms (SNP) in the dopamine receptor 2 (DRD2) gene in the ovine, and 2) determine how DRD2 genotype altered response of ewes fed endophyte-infected (E+) or endophyte-free (E-) tall fescue seed during two stages of gestation (MID, d 35 to 85 or LATE, d 86 to 133).

## MATERIALS AND METHODS

*Experimental Design.* Suffolk ewes, naïve to toxic tall fescue, were purchased in Northeast Iowa and shipped to Clemson University 90 d prior to the start of this study. Suffolk ewes ( $n = 57$ ;  $BW = 78.2 \pm 9.5$  kg) were weighed and BCS scored (1-5; 5 = fattest, 1 = thinnest) and divided into 10 groups ( $n = 5-7$ /group) for estrus synchronization to facilitate a surgery schedule. Each week, one group of ewes was synchronized using an intravaginal controlled internal drug release (CIDR) insert (Eazi-Breed CIDR, Zoetis Animal Health) for 7 d. Upon CIDR removal, ewes were given prostaglandin F<sub>2</sub> $\alpha$  (12.5 mg i.m.; Lutalyse, Pfizer, New York) and turned in with a purebred Suffolk ram. The ram was fitted with a marking harness and crayon that was changed weekly. Ewes were checked twice daily for marks to estimate breeding dates. Ewes were confirmed pregnant at d 30 of gestation using real-time ultrasound and a transrectal probe.

Thirty-two pregnant Suffolk ewes ( $78.24$  kg  $\pm$  9.5) were assigned to one of two dietary treatments (endophyte-infected tall fescue seed, **E+**; fed at 1772  $\mu$ g ergovaline + ergovalinine/hd/d or endophyte-free tall fescue seed, **E-**; fed at 0.0  $\mu$ g ergovaline + ergovalinine/hd/d) fed during two stages of gestation (d 35-85, MID or d 85-133, LATE)

in a 2 x 2 factorial arrangement. E+ or E- seed was mixed into total mixed ration (TMR) and individually fed at equal amounts to each ewe. Ewes were weighed and blood samples obtained at d 30, 50, 85, 105, and 133 of gestation. Terminal surgeries were performed on d 133 of gestation to assess maternal and fetal characteristics at the same gestation age. Additional information on the study can be found in Britt et al. (2018).

***DNA Extraction.*** Liver samples were collected from each ewe after euthanasia on the day of surgery. Liver samples were immediately frozen in liquid nitrogen and stored at -80°C. Genomic DNA was isolated from frozen liver tissues (n = 32) using a DNeasy Blood and Tissue Kit from Qiagen (Germantown, MD).

***Identification of the DRD2 SNP.*** Primers were designed to amplify overlapping sequences within the ovine DRD2 coding sequence. A SNP (g270a) was located within exon 1 (Table 3.1), and was most promising. A genotyping assay was designed (Table 3.2), tested (Figure 3.1) and found to be 100% concordant with sequencing results.

***Genotyping.*** TaqMan SNP genotyping Assay (Applied Biosystems) was used for genotyping the DRD2 SNP in the 32 ewes. This procedure was completed by the Department of Animal Science Lab at University of Tennessee, Knoxville, TN.

***Blood Collection.*** Blood samples were obtained from the jugular vein into 10 ml serum and plasma vacutainers. Serum tubes were allowed to clot at room temperature for 30 min and then at 4°C overnight. Then they were centrifuged at 2000 x g for 20 min at 4°C to obtain serum. Plasma tubes were stored on ice and then centrifuged at 2000 x g for 20 min at 4°C to obtain plasma.

**Hormone Assays.** Prolactin assays were conducted on serum samples from all ewes and days of collection in this study. Prolactin concentrations were measured using RIA procedures of Bernard et al. (1993) at the University of Tennessee. The intra-assay variance for prolactin was 5.99% and the inter-assay variance was 4.47%. For other hormone assays, a subsample of ewes (n = 16; n = 4/fescue treatment/stage of gestation) was selected based on average response to the whole treatment group. The distribution of genotype for the subsample was 4 A1A, 5 A1G, and 7 G1G. Serum was analyzed for cortisol, T4 (Triiodothyronine), levels were measured using an ovine-specific ELISA assay (Abnova, Taoyua City, Tiawan). The cortisol ELISA had an intra-assay variance of 6.09 and a limit of detection at 0.1 ng/mL. The T4 ELISA had an intra-assay variance of 9.9% and a limit of detection at 2.0 ng/mL. Serum was analyzed for T3 using a bovine ELISA (Abnova) with an intra-assay variance of 8.8% and limit of detection of 0.2 ng/mL. Insulin serum concentrations were analyzed using an ovine-specific insulin ELISA (Mercodia, US, NC) with an intra-assay variance of 5.22% and limit of detection at 0.025 µg/L. Insulin-like growth factor (IGF-1) was also measured using an human-based ELISA kit (Enzo Life Sciences, Farmingdale, NY) with an intra-assay variance of 5.06% and a limit of detection of 50 pg/mL. Glucose concentrations were analyzed using a colorimetric hexokinase assay (Pointe Scientific Canton, MI) with an intra-assay variance of 8.12% and a limit of detection of 0.6 mg/dl.

**Statistical Analysis.** Data were analyzed using the mixed procedure of SAS as a 3 x 2 x 2 factorial arrangement of treatments with three genotypes (A1A, A1G, G1G), two fescue seed treatments (E+ or E-) fed at two stages of gestation (MID or LATE), and all

interactions tested. For hormone assays, day of collection was also included in the model and all interactions tested. Serum prolactin concentration data were log transformed to normalize and analyzed as described above. Significance was determined at  $P < 0.05$ .

## RESULTS

### *DRD2 SNP identification*

Seven DRD2 SNPs were identified for ovine. The SNP at Exon 1, g270a appeared the most important with allele frequencies of 0.64 for G and 0.36 for A. For the 32 ewes genotyped, genotypic frequencies were 47% GlG, 34% AlG, and 19% AlA. This gave us the following distribution of genotypes: 8 AlA, 10 AlG, and 14 GlG (Figure 2.1).

### *Serum Hormones*

The interaction between ewe genotype and day of collection tended to differ ( $P = 0.089$ ) for prolactin (Fig. 2.2). On d 50 and 105 of gestation, serum prolactin levels were lower for GlG and AlG compared to AlA genotypes but levels did not differ on d 30, 85 or 130. The interaction between fescue seed treatment and stage of gestation was significant ( $P = 0.046$ ) for prolactin. Serum prolactin levels were lower ( $P < 0.001$ ) in ewes fed E+/E+ and E-/E+ compared to those fed E-/E- or E+/E- treatments across gestation. All interactions between fescue treatment, stage of gestation, day of collection, and genotype were non-significant ( $P > 0.05$ ). Serum cortisol, T3 and T4 concentrations were greater ( $P = 0.047$ ,  $P = 0.016$  and  $P = 0.021$ , respectively) in ewes with AlA genotypes compared to GlG and AlG (Fig. 2.3). Serum T4 concentrations also differed ( $P < 0.05$ ) by day of collection with concentrations increasing during gestation. Serum IGF-1 concentrations tended to differ ( $P = 0.068$ ) by genotype. Serum IGF-1 levels were

lower for AIA genotype compared to GI|G or AI|G. Insulin and glucose concentrations did not differ ( $P > 0.05$ ) by genotype or any interaction with fescue treatment or stage of gestation.

### ***Maternal Genotype on Lamb Fetal Weight***

Total fetal weight per ewe at d 133 of gestation differed ( $P < 0.05$ ) by genotype and fescue treatment fed during LATE gestation (Figure 3.4); however, all interactions were non-significant ( $P > 0.05$ ). Ewes with the GI|G genotype had total fetal weights that were heavier ( $P = 0.048$ ) by 6% than ewes with AI|G and AIA genotype. Ewes consuming E+ fescue seed during LATE gestation had lower ( $P = 0.0095$ ) total fetal weights by 15% compared to ewes fed E- fescue seed.

## **DISCUSSION**

Fescue toxicosis negatively impacts animal performance in addition to affecting reproductive performance and the performance of offspring (Paterson et al., 1995). Campbell et al. (2014) conducted a series of experiments in Angus-based cattle identifying an AI|G SNP within the DRD2 gene that altered the animal's response to toxic tall fescue. They found that steers with GI|G genotype had lower serum prolactin concentrations and higher hair coat scores compared to AIA when grazing E+ fescue. These results suggest that DRD2 genotype may play a role in how the animal responds to toxic tall fescue and we decided to examine this in the sheep.

We identified seven SNPs in the DRD2 gene in Suffolk ewes, naïve to toxic tall fescue that were exposed to E+ or E- fescue seed at two stages of gestation. One SNP had allelic frequencies that were favorable and was examined further. Results show that



DRD2 genotype was associated with altered serum prolactin and cortisol levels regardless of fescue seed treatment. The A1A ewes had higher prolactin concentrations on d 50 and 105 compared to A1G and G1G ewes. Ergovaline is a dopamine agonist and interacts with DRD2 to reduce prolactin production (Sibley and Creese, 1983; Klotz, 2015). Prolactin concentrations were lower with E+ fescue, especially in LATE gestation. It has been well documented in multiple species (ovine, equine and bovine) that as parturition approaches maternal prolactin concentrations increase (Edgerton et al., 1973; Worthy et al., 1986; Duckett et al., 2014). A reduction in serum prolactin concentrations is a classic response to E+ tall fescue consumption and has been reported in cattle (Emile et al., 2000; Koontz et al., 2012), horses (McCann et al., 1992), and sheep (Emile et al., 2000; Parish et al., 2003).

Concentrations of cortisol, T3 and T4 were also higher for A1A ewes compared to A1G and G1G ewes. Qiu et al. (2016) also identified SNPs within the DRD2 gene (SNP483 in exon 3, SNP939 in exon 6) that were associated with personality and behavioral traits in Merino sheep selected over 20 generations for calm or nervous temperament. Sheep with the SNP939-calm genotype had lower agitation scores during the isolation box test. In ACTH challenge, sheep with SNP939 genotype did not have altered cortisol levels but sheep with CYP17 SNP (gene involved in cortisol production) did have an greater cortisol response under challenge. Blood cortisol concentrations increase in response to stress in sheep and cattle. Under periods of heat stress, animals have reduced ability to dissipate body heat, and blood flow to peripheral (rib skin), core-body (duodenum colon), and brain (cerebellum) tissues is decreased (Paterson et al.,

1995). One might expect animals to have increased concentrations of cortisol during periods of stress induced by consuming ergot alkaloids. Others (Hurley et al. 1981; Aldrich et al., 1993) showed cortisol levels were not affected by fescue treatment (E+ or E-) in cattle. In our study, fescue treatment (E+ vs. E-) did not alter cortisol concentrations.

The thyroid produces T3 and T4, but T4 in much greater amounts. When T4 reaches organs and body tissues it is converted to T3. T3 is the active hormone known for regulating metabolism. Thyroid system development is most similar between human and ovine fetuses (Polk, 1995) and its role is well described in neonatal brain development. Research shows that their presence is essential for the changes in the hypothalamus responsible for the onset of anestrus (Koibuchi and Chin, 2000). A study in humans outlining normal thyroid concentrations post partum demonstrated trends of T3 and T4 rising from the first trimester to the second and then declining in the third (Glinour, 1997). Contrary to humans, Polk (1995) determined T3 and T4 concentrations increase progressively in the sheep during the last half of gestation. In this study, T3 and T4 concentrations were 50% higher in AIA ewes.

Total fetal weight per ewe was lower in AIA and AIG ewes when compared to GIIG ewes. In humans, Jackson and Beaver (2015) found an association between low birth weight and hyperactivity that was influenced by dopaminergic polymorphisms. These results suggest that DRD2 genotype may alter lamb birth weights and increase productivity of ewes, regardless of fescue treatment. Ewes with GIIG genotype produced heavier fetal lamb weights compared to other DRD2 genotypes, regardless of fescue

treatment (E- or E+). Total fetal weight per ewe was also lower in ewes fed E+ fescue seed during LATE gestation. Duckett et al. (2014) also reported reduction in lamb birth weight in ewes fed E+ fescue seed.

The interest in this study was to determine if DRD2 genotype influenced the response to E+ fescue. We did not find any interactions between fescue treatment and DRD2 genotype; however, it is important to note that genotyping was conducted after the completion of the study. Additional research to explore the role of the DRD2 genotype with circulating hormone levels in the ewe and fetal lamb weights is warranted and may be beneficial in sheep production systems.

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Table 2.1 SNP g270a's location in the DRD2 Gene.

tcgtcagcctcgctgtggctgacctct{g/a}gtggccacactggtcatgccctgggtgtcta



Table 2.2 Primers for the DRD2 gene that were used to identify the SNP.

<b>Oligo</b>	<b>Sequence</b>
Forward primer	CCACCACCAACTACCTGATCGT
Reverse primer	TAGACCACCCAGGGCATGAC
VIC probe	ACCTCCTAGTGGCC
FAM probe	TGGCTGACCTCCTGGT

Figure 2.1 Allelic frequencies between A/A, A/G and G/G genotypes for the Suffolk ewe flock.

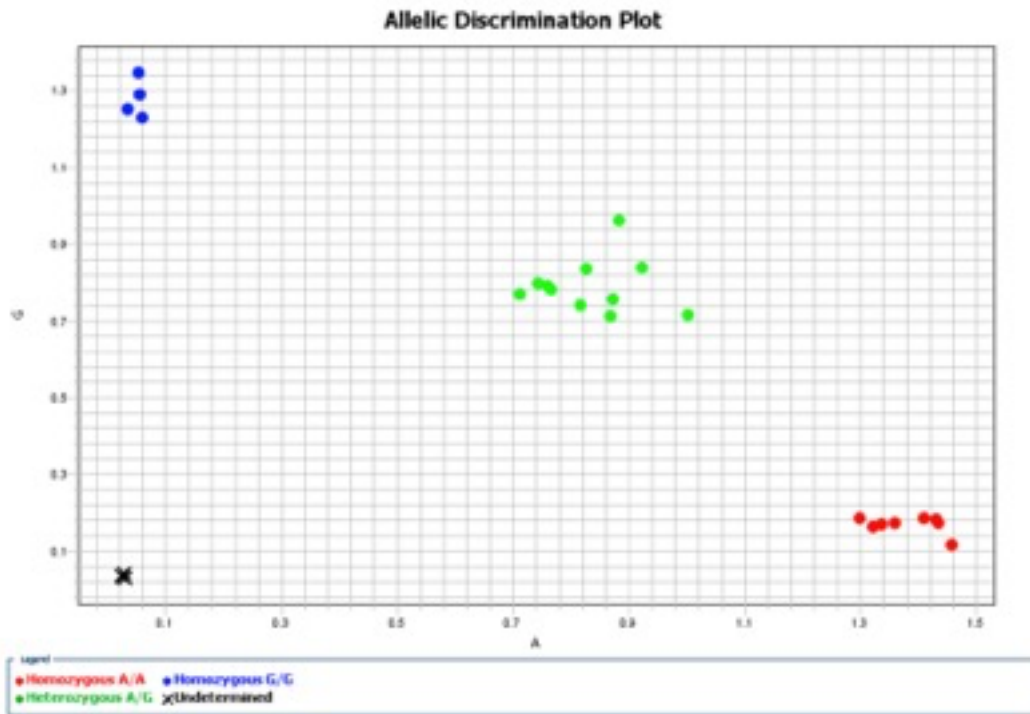


Figure 2.2 Prolactin concentrations in ewes with different DRD2 genotype (A/A, A/G, G/G) fed endophyte-infected (E+) or endophyte-free (E-) tall fescue seed during d 35 to 85 (MID) gestation and/or d 86 to 133 (LATE) gestation. \*Denotes significance ( $P < 0.05$ ) by genotype compared to A/A at that stage of gestation and between dietary fescue treatments compared to E-/E-.

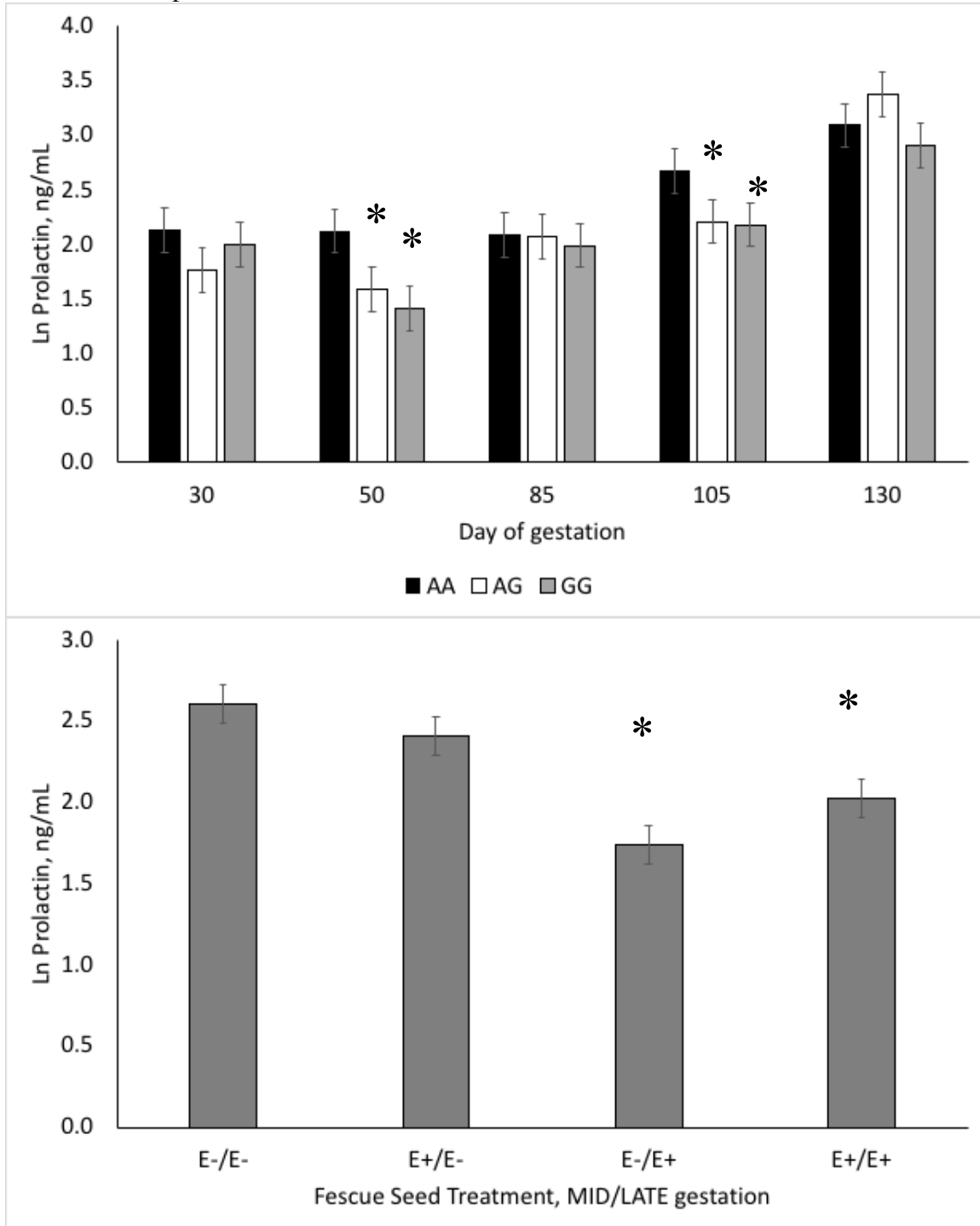
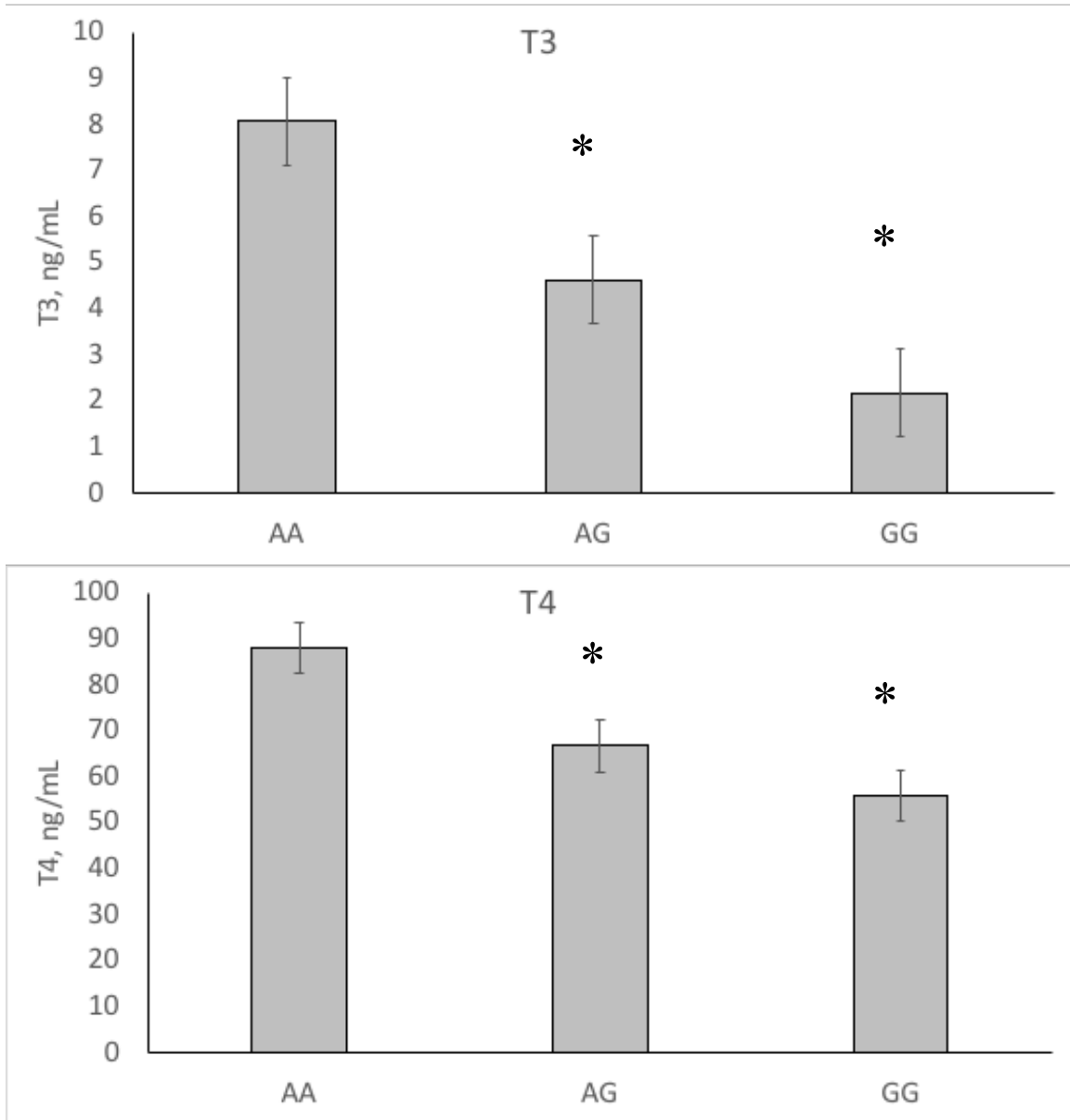


Figure 2.3 . Cortisol, triiodothyronine (T3), thyroxine (T4), and IGF-I concentrations by ewe DRD2 genotype. \*Denotes significance ( $P < 0.05$ ) between genotypes compared to A/A.



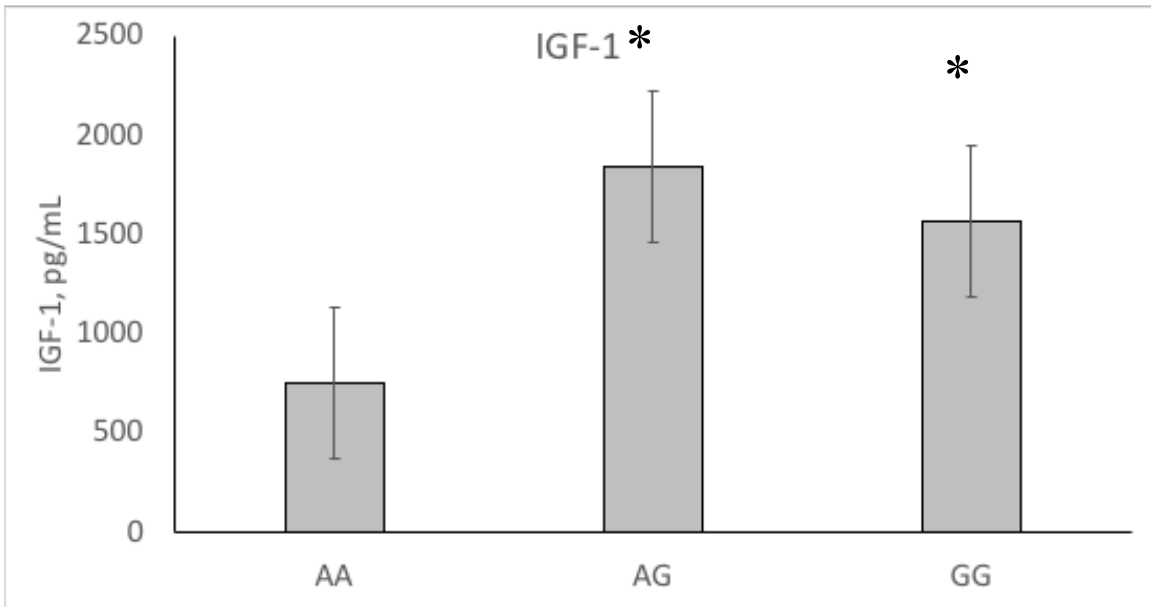
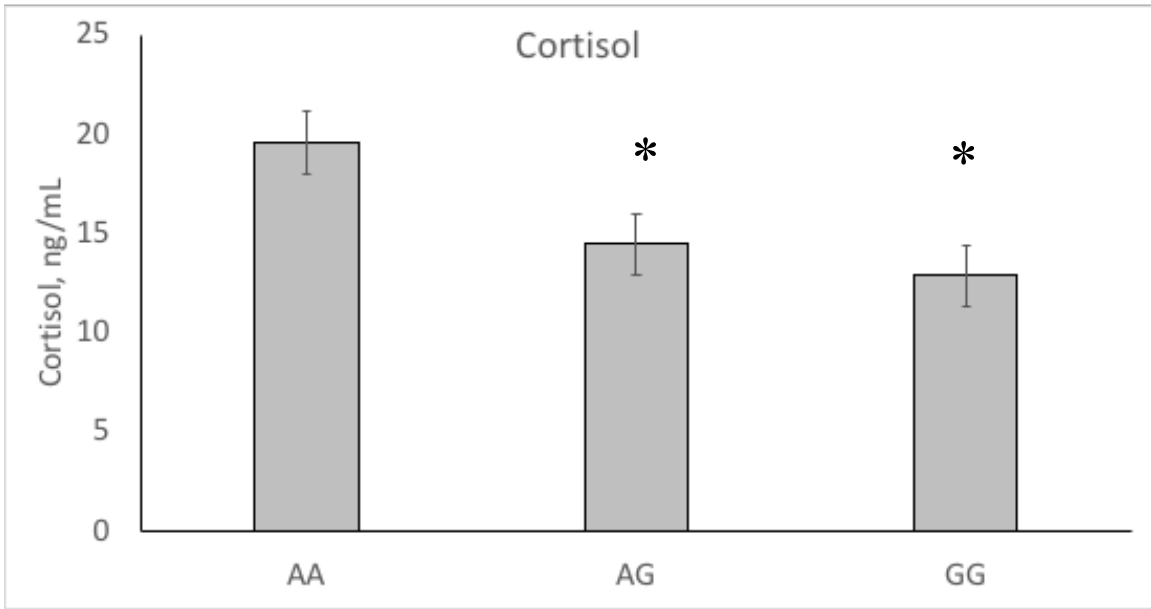
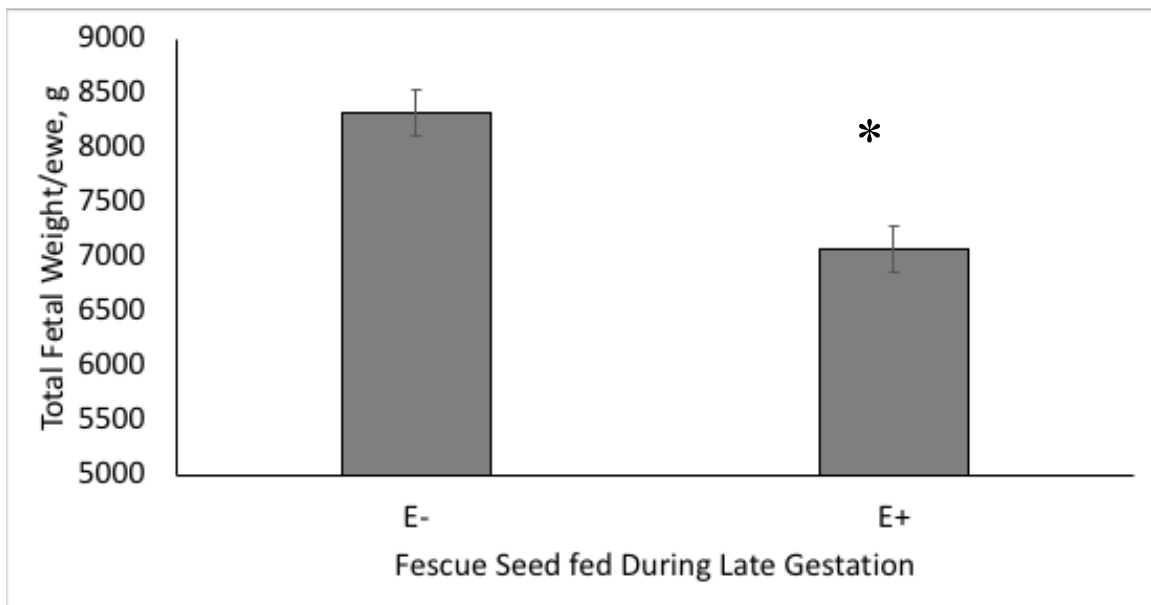
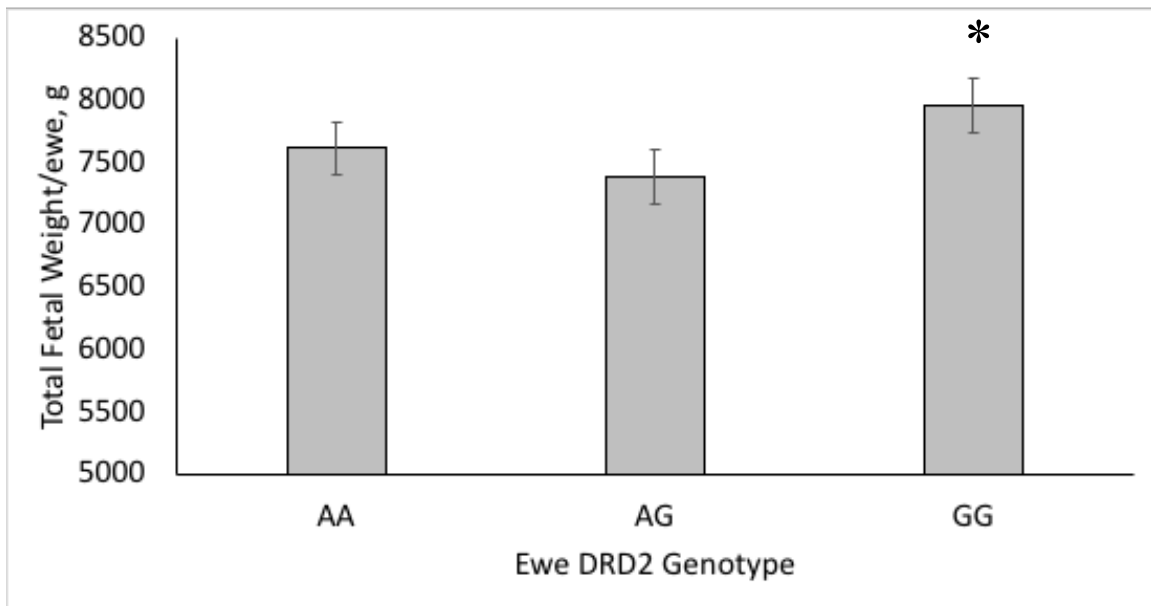


Figure 2.4. Fetal lamb weight at d 133 of gestation in ewes with different DRD2 genotype (A/A, A/G, G/G) fed endophyte-infected (E+) or endophyte-free (E-) tall fescue seed during d 35 to 85 (MID) gestation and/or d 86 to 133 (LATE) gestation. \*Denotes significance ( $P < 0.05$ ) between genotypes compared to A/A and between dietary fescue treatments compared to E-.



## CHAPTER THREE

### **THE EFFECTS OF MATERNAL DRD2 SNP G270A ON SUBSEQUENT LAMB WEIGHT AND GROWTH CONSUMING TOXIC FESCUE.**

#### **ABSTRACT**

Two experiments were conducted to investigate the role of a single nucleotide polymorphism (SNP) in the ovine dopamine receptor 2 (DRD2) gene and fescue toxicosis during gestation on fetal lamb weight and growth. In experiment 1, pregnant ewes (n=61) were genotyped (A|A, A|G, or G|G; DRD2, SNP g270a) then divided into four treatments receiving either endophyte-free tall fescue seed (E-) or endophyte infected tall fescue seed (E+; 1772 µg ergovaline + ergovalaline/ hd/d) during MID (d 35- d85) and LATE (d 86 – d 133) gestation in a 3 X 2 X 2 factorial. Results of this study indicate ewes with genotype G|G had heavier fetal weights as well as higher average daily gain (ADG) ( $P<.05$ ) and heavier lambs at weaning ( $P<.05$ ). Toxic fescue (E+) treatment during LATE gestation reduced ( $P<0.05$ ) fetal lamb weight but there was no interaction between genotype and fescue treatment. Results indicated production benefits for ewes carrying one or two copies of the G allele. Therefore, experiment 2 (n=22) was designed to determine production advantages of lambs with homozygous maternal genotypes A|A and G|G. Results indicate maternal genotype G|G produced heavier lamb birth weight ( $P<.05$ ). Maternal genotype G|G lambs continued to have an advantage at the time of lamb weaning ( $P>.05$ ). ADG and weight per day of age (WDA) tended to be greater for lambs from maternal genotype G|G from birth to weaning ( $P<.10$ ). Conclusions can be made that DRD2 SNP g70a may provide producers with an additional tool for selective

breeding. Choosing ewes with one or two copies of the G allele at this SNP site may provide a production benefit.

Keywords: SNP, DRD2, ovine, production, sheep management



## INTRODUCTION

Previous studies have suggested genetic variations in multiple species (Campbell et al., 2014, Bovine; Wilbanks et al., 2018, Ovine) using a Single Nucleotide Polymorphism (SNP) in the dopamine 2 gene (site g270a). Dopamine has been shown to be associated with reduced maternal function in response to consuming toxic tall fescue, as ergot alkaloids produced by the endophytic fungus infecting toxic fescue binds dopamine 2 receptors resulting in decreased serum prolactin concentrations and vasoconstriction (Strickland et al., 2011).

Currently, symptoms for fescue toxicosis are determined by phenotypic traits such as; retained hair coat, heat stress, reduced gains, reduced milk production, and peripheral vasoconstriction. Recent advances in genetic technology have enabled us to explore a genetic approach to fescue toxicosis. SNP g270a, located in exon 1 of the dopamine 2 gene, was identified within the ovine genome (Wilbanks et al., 2018). Preliminary data suggested the possibility of ewe's genotyped as G|G being more productive by producing heavier lambs at d 133 of gestation than the other genotypes when consuming toxic tall fescue (Chapter 3; Wilbanks et al., 2018). The objectives of these studies were to: 1) assess responses in ewes stratified by genotype to E+ or E- tall fescue during MID (d 35-85) or LATE (d 86- parturition) gestation on ewe/lamb production and 2) determine response in ewes of A|A and G|G genotype in normal production systems on ewe/lamb production.

## MATERIALS AND METHODS

### *Experiment 1.*

**Genotypes.** All ewes (n = 61) were used to screen for polymorphisms in the DRD2 gene. Genotypes were determined using genomic DNA isolated from blood samples by a DNeasy Blood and Tissue Kit from Qiagen (Germantown, MD). Extracted DNA was then shipped to the Department of Animal Sciences at the University of Tennessee. There, a TaqMan SNP genotyping Assay (Applied Biosystems) was used for genotyping the DRD2 SNP in the 61 ewes. Information on the identification of the DRD2 SNP g270a is available in Wilbanks et al. (2018). Maternal genotypes at the DRD2 SNP were A|A, A|G, or G|G with a genotypic frequency of 21.4% A|A, 50% A|G, and 28.6% G|G.

**Design.** Ewes were weighed, assigned BCS scores (1-5; 5 fattest, 1 = thinnest) and divided into 5 groups (n= 10-16/group) for estrus synchronization using intravaginal controlled internal drug release (CIDR) insert (Eazi-Breed CIDR, Zoetis Animal Health) for 7 d. Upon CIDR removal, ewes were given prostaglandin F2 $\alpha$  (12.5 mg i.m.; Lutalyse, Pfizer, New York) and turned in with a purebred Suffolk ram (A|G DRD2 genotype). The ram was fitted with a marking harness and the crayon was changed weekly. Ewes were checked twice daily for marks to estimate breeding dates. Ewes were confirmed pregnant at d 30 of gestation using real-time ultrasound and transrectal probe. Pregnant ewes carrying twin fetuses were assigned by DRD2 genotype to one of two dietary treatments; endophyte-infected tall fescue seed (E+; Black Magic Turf-type tall fescue seed or endophyte free tall fescue seed, E-; Bull turf-type tall fescue seed) fed

during two stages of gestation (d 35-85, MID or d 85-133 LATE) in a 3 X 2 X 2 factorial arrangement.

Prior to treatment, endophyte levels were analyzed based on the methods of Aiken et al. (2009). Toxic fescue (E+) seed contained 4.14 µg/g DM of ergovaline and ergovalaline supplying a dose level of 1772 µg ergovaline + ergovalaline/hd/d. Endophyte-free (E-) treatment was fed at the same level but supplied 0.0 µg ergovaline + ergovalaline/hd/d. E+ or E- seed was mixed into total mixed ration (TMR) and individually fed at equal amounts to each ewe. Ewes were weighed and blood samples obtained at d 30, 50, 85, 105, 133 and at parturition. Additional information on feeding procedures can be found in Britt et al. (2018).

Ewe weights and whole blood samples were collected at d 30, 50, 85, 105, 133 and prior to parturition. Blood for serum samples was drawn from the jugular vein into 10 ml serum and plasma vacutainers. Serum tubes were allowed to clot at room temperature for 30 min. and then at 4°C overnight. Then they were centrifuged at 2000 x g for 20 min at 4°C to obtain serum. Prolactin assays were conducted on serum samples from all ewes and days (d0, 35, 55, 85, 105, 133 and parturition) of collection in this study. Prolactin concentrations were measured using RIA procedures of Bernard et al. (1993) at the University of Tennessee (intra-assay variance % CV= 6.53, inter-assay variance % CV =6.19).

At parturition, ewes were allowed to lamb naturally, then lambs were processed. Data collected on lambs at birth included: a 3-5 cc sample of whole blood collected via jugular vein (processed as noted above), birth weight, crown-rump length measurement,

sex, as well as dystocia and vigor scores. Additionally, all lambs were ear tagged, and navels were dipped in iodine and an umbilical clamp was secured. Within 7 d post birth, males were surgically castrated, tails were banded, and vaccinations were administered (tetanus, antitoxin). Lamb weights and FAMACHA© scores were collected every 2 weeks post birth until weaning. At parturition, all ewes were removed from treatment and maintained on TMR for 21 d during the duration of the milk production test to minimize potential dietary impacts on lactation.

A two-day milk production test was conducted following parturition, based on the methods of Benson et al. (1999). Ewes were milked out by hand and a dividing panel was positioned within the lambing jug to allow nose-to-nose contact, but prevent nursing. After three hours, dams were milked and the weight of the milk was recorded. Lambs were bottle-fed the collected milk and the dividing panel was removed. After the two-day milk production test, dams and their lambs were removed from the jug and managed collectively. This procedure was also repeated at d 21 following the above noted procedure. Milk production test results are presented as production capacity within 24 hours. Following this, ewes were moved to non-fescue pasture and supplemented during the remaining lactation period prior to weaning. All lambs were weaned at 75 d, at which time ewe and lamb were separated and managed independently.

**Statistics.** Data were analyzed using the mixed procedure of SAS as a 3 x 2 x 2 factorial arrangement of treatments with three genotypes (A|A, A|G, G|G), two fescue seed treatments (E+ or E-) fed at two stages of gestation (MID or LATE), and all interactions tested. Prolactin concentrations were log transformed to preserve normality.

Data were analyzed by repeated measures procedure with dietary treatment (E- or E+), time of gestation (MID or LATE) and day in the model. Fetal number (single, twin, triplet) and sex were included as a covariate when significant ( $P < 0.05$ ). Significance was determined at  $P \leq 0.05$ , and trends declared at  $P > .05$  to  $P \leq 0.10$ .

## **Experiment 2**

**Design.** Suffolk ewes ( $n = 22$ ) with A|A or G|G genotypes from experiment 1 were used in experiment 2, with the addition of 2 virgin ewe lambs. Genotyping on additional ewes was conducted with blood samples, as outlined in section 3.1. Only A|A and G|G genotypes were used in this study. The distribution of genotypes were 45.45% A|A and 54.54% G|G (Figure 4.7).

Twenty-two Suffolk ewes (BW=  $83.5 \pm 10.3$  kg) were weighed and BCS scored (1-5; 5 = fattest, 1 = thinnest). Prior to breeding, ewes were flushed with whole corn at .45 kg/ h/d for 14 d prior to breeding and until completion of breeding season. Ewes were turned in with a purebred Suffolk ram for 2 breeding cycles. Rams were fitted with a marking harness and crayon that was changed weekly. Ewes were checked twice daily for marks to estimate breeding dates. A Southdown ram was used to cover any ewes not marked after two full cycles (33 days), and stayed with the ewes for a total of 27 days. Ewes were confirmed pregnant at d 30 of gestation using a BCF abdominal ultrasound machine.

Ewes were allowed to graze non-toxic forages including rye, E- fescue, and non-fescue grass hay, ad libitum throughout gestation until parturition. Ewes were weighed prior to study, nearing parturition and at weaning of lambs. Ewes were regularly

FAMACHA© and BCS scored and de-wormed with as needed. Ewes were allowed to lamb naturally. Lambs were processed within 12 h of birth. Lambs were ear tagged, navels were dipped in iodine and a naval clamp was applied. Measurements including birth weight, crown-rump length, sex, and birth date were collected. Dystocia and vigor scores were collected where possible. Male lambs were banded for castrations, and all lambs had a tail band applied for tail docking within 7 d post birth. Lambs were weighed again at weaning. After parturition, ewes continued to graze the same forages until lambs were weaned.

**Statistics.** Data were analyzed with genotypes (A|A or G|G) as fixed effects. Significance was determined at  $P < 0.05$ , and trends declared at  $P > 0.05$  to  $P < 0.10$ .

## RESULTS

**Ewe/ Lamb weight and growth.** The distribution of genotypes in experiment 1 consisted of 14 A|A, 29 A|G and 18 G|G ewes (Figure 3.1). Experiment 1, lamb birth weight differed ( $P=0.007$ ) by ewe genotype with G|G genotypes producing lambs about 11% heavier (Figure 3.3) than those with one or two A alleles. Additionally, ewes consuming E- dietary treatments produced heavier ( $P=0.010$ ) lambs at birth than ewes consuming E+ seed. Birth weights were on average about 8% heavier for lambs born to E- ewe dietary treatments (Figure 3.4).

At weaning, ewes with G|G genotypes produced lambs who weighed more ( $P=0.046$ ) than lambs with maternal genotype A|G or A|A (Figure 3.2 ). Lamb weaning weight tended to be reduced for lambs born to ewes on E+ during MID and LATE gestation ( $P=0.075$ ).

Lamb average daily gain (ADG) until weaning tended to be greater for lambs born to maternal genotype A|G, with maternal genotype A|A gaining the least amount and G|G genotypes being intermediate ( $P < 0.045$ ) (Figure 3.5). An interaction for MID by LATE gestation was observed. At weaning, lambs born to ewes consuming E+ diet throughout gestation had lighter weaning weights. Of the four treatments, lambs born to ewes who consumed E- diets in MID gestation and were switched to E+ diets through LATE gestation, tended to have the greatest ADG from birth to weaning ( $P = 0.096$ ) (Figure 3.6).

In the production study, experiment 2, ewe weight collected at breeding was greater for ewes genotyped as G|G ( $P = 0.01$ ). Birth weight for lambs did not differ by dam genotype or dam dietary treatment ( $P = 0.180$ ) (Figure 3.7). At the time of lamb weaning ewe weight was greater ( $P = 0.01$ ) by about 17.5 kg. for ewes who were genotyped as G|G. Lamb weaning weight was heavier ( $P = 0.059$ ) for lambs by maternal genotype G|G when compared to lambs from A|A genotyped ewes (Figure 3.8). Weight per day of age (WDA) for lambs tended to be greater for lambs from G|G ewes ( $P = 0.096$ ). Additionally, maternal genotype G|G tended to produce lambs who had greater ADG ( $P = 0.079$ ) than lambs from A|A genotyped ewes (Figure 3.9). Maternal genotype did not have an effect on crown-rump measurement or vigor scores at birth.

***Serum Prolactin.*** The consumption of ergot alkaloids are known to reduce prolactin concentrations and induce vasoconstriction. For ewes consuming E- dietary treatments in experiment 1, there were no differences in prolactin concentrations ( $P > 0.05$ ), but for those assigned to E+ seed, prolactin concentrations were reduced in MID ( $P$

= 0.001) and LATE ( $P = 0.001$ ) gestation (Figure 3.10). Furthermore, ewes genotyped as A|A and consuming E+ diets had a more significant reduction in prolactin concentrations in MID and LATE gestation than ewes genotyped as G|G ( $P = 0.01$ ).

## DISCUSSION

Previous to these two experiments, a SNP within the DRD2 receptor was identified by Campbell et al. (2014) in Angus-based beef steers and by Wilbanks et al. (2018) in sheep. Based on these studies it was shown that DRD2 genotypes in steers decreased prolactin concentrations and increased hair coat score in steers with G|G genotypes. When they looked into the association of the genotypes and fescue toxicosis on first year heifers they realized the majority of their herd was A|A genotype suggesting that selection pressures had already been applied to these herds prior to the knowledge of any genotype associations. The increase of HCS and the reduction in serum prolactin concentrations suggested the DRD2 SNP may have a use in selecting animals against fescue toxicosis. In 2018, Wilbanks et al. wanted to determine if a locating a SNP in the DRD2 receptor was a possibility in the Ovine genome. A DRD2 SNP was found by extracting DNA from Clemson University's Suffolk ewe flock ( $n=32$ ) and screening for possible SNP sites in the Dopamine gene. One SNP (g270a) located in exon was found to be promising so a genotyping assay was designed. Each ewe was then assigned a genotype at this SNP site. Fescue toxicosis has been shown to be the most detrimental during late gestation for maternal and fetal function (Britt et al., 2018). When ewes were genotyped, and fed either E+ or E- seed, the results suggested the possibility of production benefits of one DRD2 genotype (G|G) shown to produce heavier fetal lamb



weights as well as influencing cortisol and thyroid hormones (Triiodothyronine, thyroxine). However, the results of the study did not have equal genotype stratification as genotyping occurred after the completion of the study outlined in Britt et al. (2018). The intent of these two experiments was to expand upon previous work by Wilbanks et al. (2018) to determine the postnatal effects on lambs by ewe genotype in regards to lamb weight and growth. Experiment 1 of this study was able to represent all genotypes, and measure any E+ associations in ewes on subsequent lambs as Duckett et al. (2014) showed the reduction in fetal growth when ewes were fed E+ tall fescue seed during gestation. The intent of experiment 2 was to investigate the production advantages DRD2 SNP g70a may have when E+ was taken out of the equation.

The DRD2 receptor was chosen first by Campbell et al. (2014) in beef steers, and then by Wilbanks et al. (2018) in sheep due to its regulation in prolactin secretion. However, prolactin has more physiological functions than the other pituitary hormones all combined (Freeman et al, 2010), with the current estimation standing at approximately 300 different biological actions in vertebrates (Bole-Feysot et al., 1998). Prolactin measured in experiment 1, showed a suppression of prolactin for ewes consuming E+ treatment when compared to E- treatments, as one would expect. Interesting enough though, ewes with G|G genotypes consuming E+ treatments-had a greater reduction in serum prolactin concentrations than ewes with A|A or A|G genotypes. Thus suggesting ewe genotypes play a role in prolactin regulation during gestation.

Additionally, early milk production was measured in experiment 1 at d 1 and d 2 and again at d 21 and 22. There were no differences in the amount of milk produced for

ewes consuming E+ or E- treatment, nor by genotype. Leading one to believe that growth advantages of lambs from ewes with specific genotypes are due to the individual lamb and not related to a difference in ewe milk production.

When no associations of genotype to fescue treatment were present, the experimental design for experiment 2 was implemented with the objective of determining what role ewe DRD2 genotype played in a normal production setting without E+ fescue on lamb weight and growth. Data suggest the G|G genotype produces heavier lambs at birth, and weaning. As well as greater ADG, and WDA. Based on these results we can conclude ewes of G|G genotypes outperform other genotypes in terms of postnatal growth until weaning.

Currently, selective breeding is being extensively used to increase performance, meat and wool quality, however selections are largely based upon phenotypic traits. These studies are helping determine how to combat abiotic and biotic stressors that contribute to production losses by using a genetic approach. Using this new information, producers may have the potential to strengthen their flock by practicing keep/cull on ewes for each year breeding term, with the aim of keeping ewes with 2 copies of the G allele at SNP g270a and culling those with one or two A alleles. However, larger and replicated studies that include other breeds of sheep would be needed in order for a test to be made commercially available for producers.

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34.

Figure 3.1. Genotypic distribution of A|A, A|G, and G|G genotypes for the Suffolk Ewe flock in experiment 1

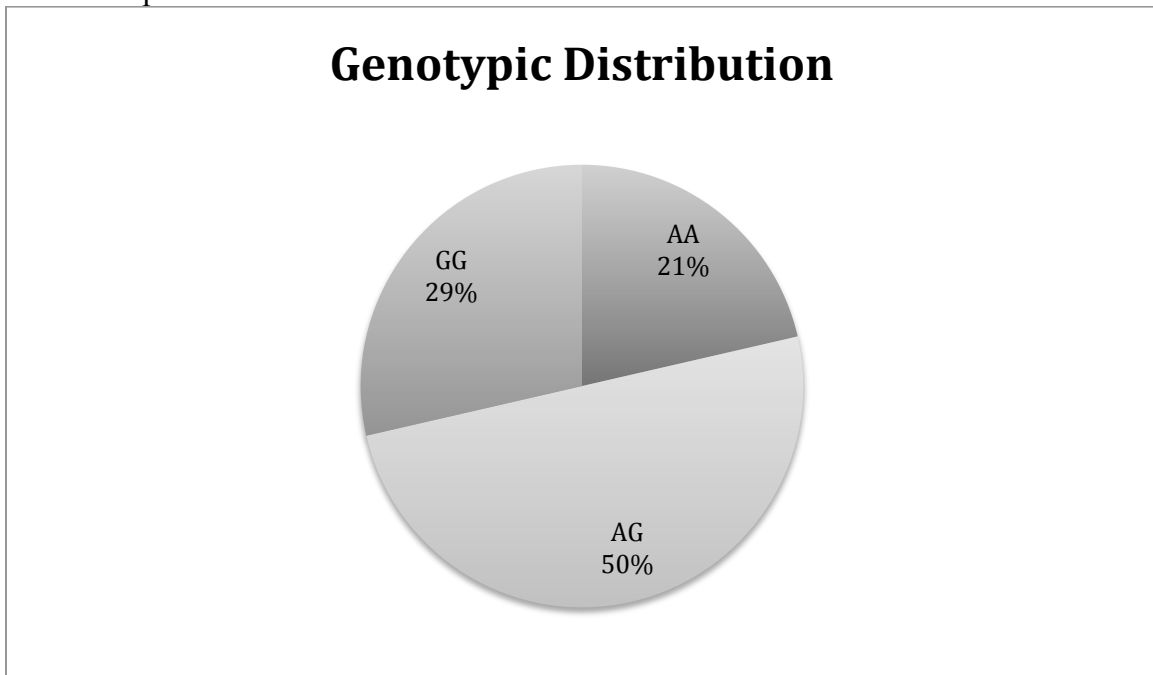


Figure 3.2. Genotypic distribution of A|A, A|G, and G|G genotypes for the Suffolk Ewe flock in experiment 2.

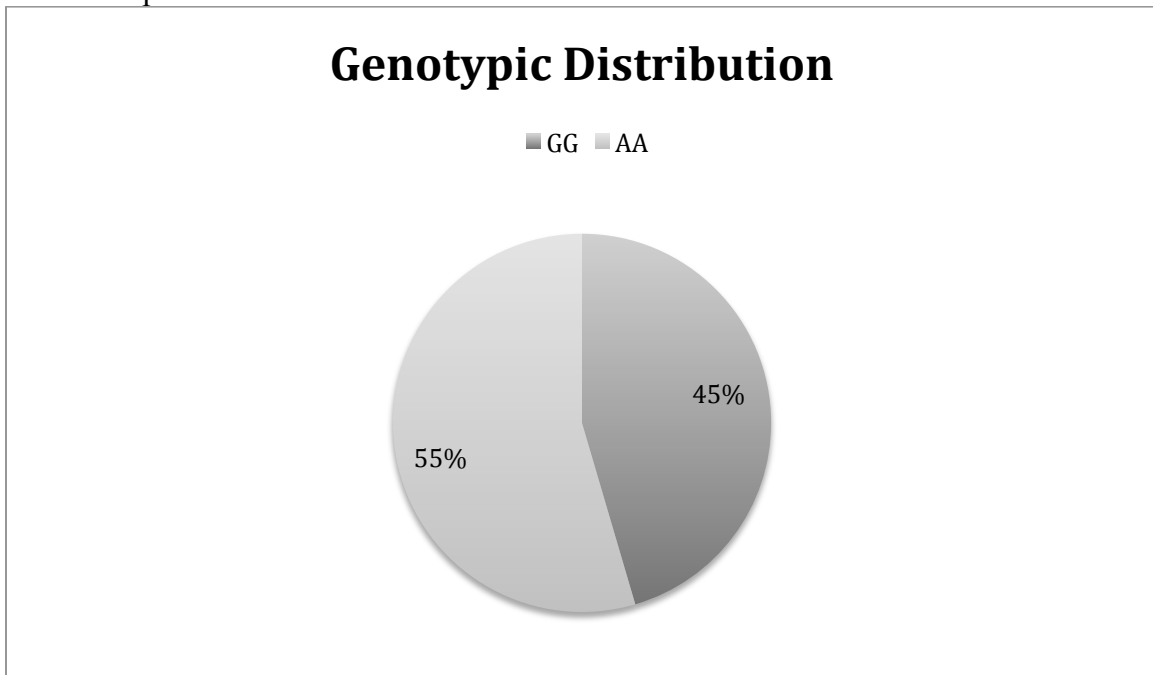


Figure 3.3. Lamb birth and weaning weight by maternal genotype (A|A, A|G or G|G) in experiment 1.

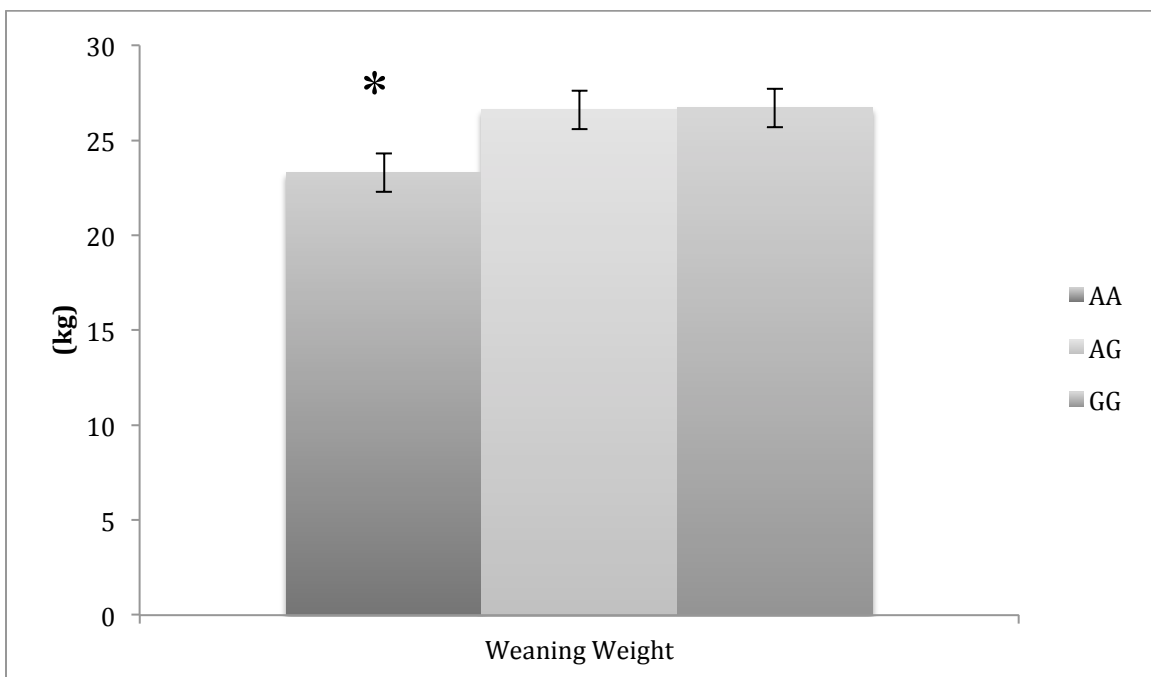
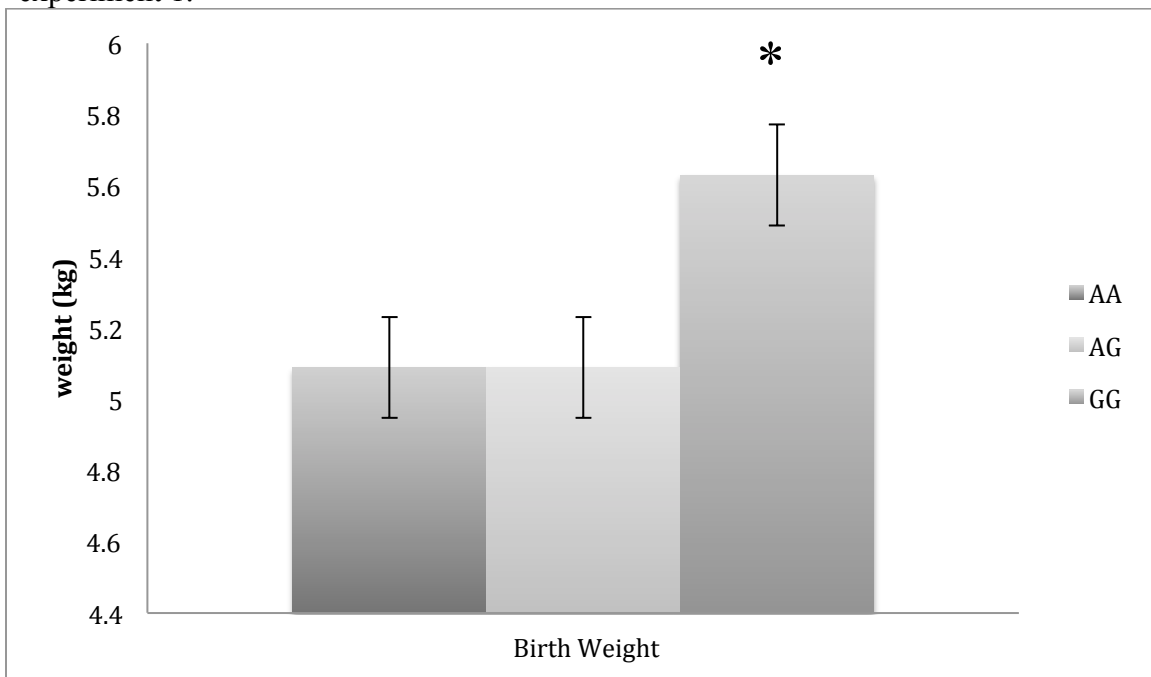




Figure 3.4. Birth weight of lambs by dietary treatment (E+ or E-) of ewes fed during d 35-85 (MID) or d 86- 133 (LATE) gestation in experiment 1.

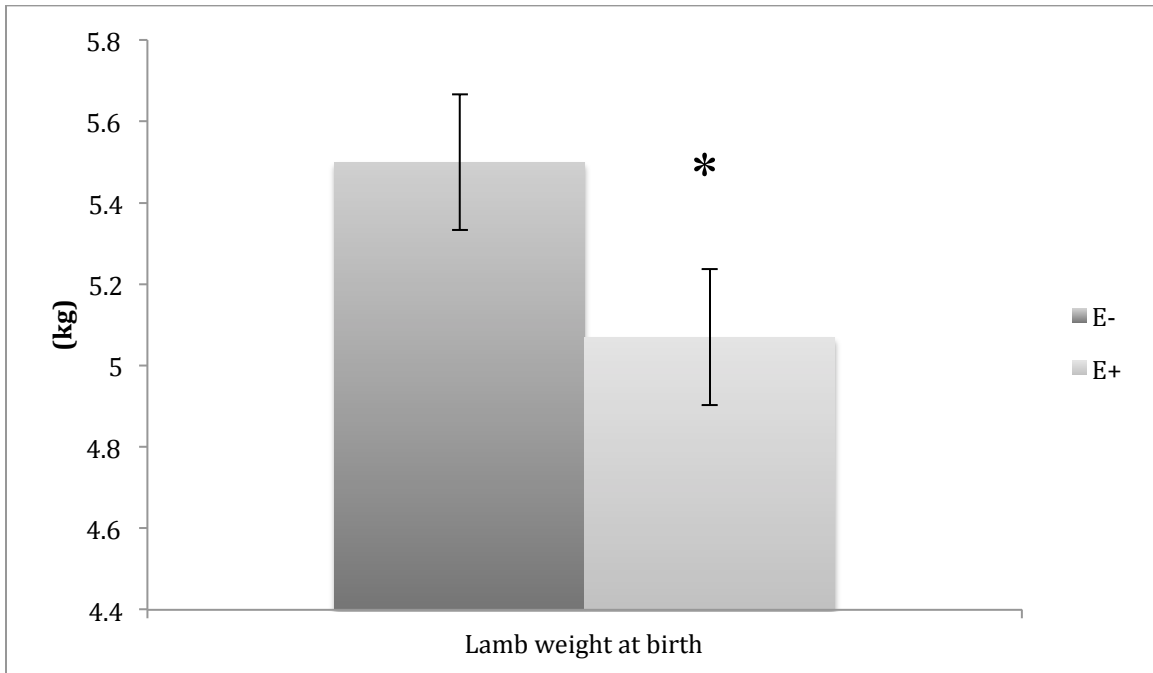


Figure 3.5. Lamb ADG from birth to weaning by maternal genotype (A|A, A|G or G|G) in experiment 1.

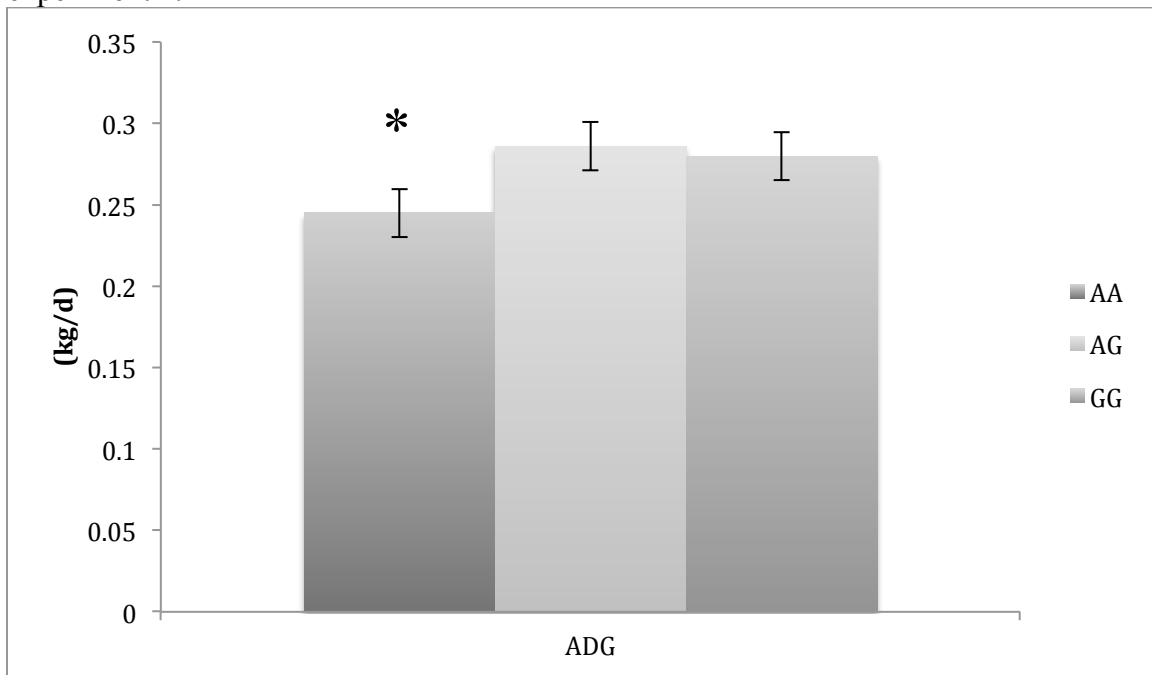


Figure 3.6. Average Daily Gain (ADG) of lambs at the time of weaning from ewes consuming endophyte infected (E+) or endophyte free (E-) seed during d 35-85 (MID) or d 86-133 (LATE) gestation in experiment 1.

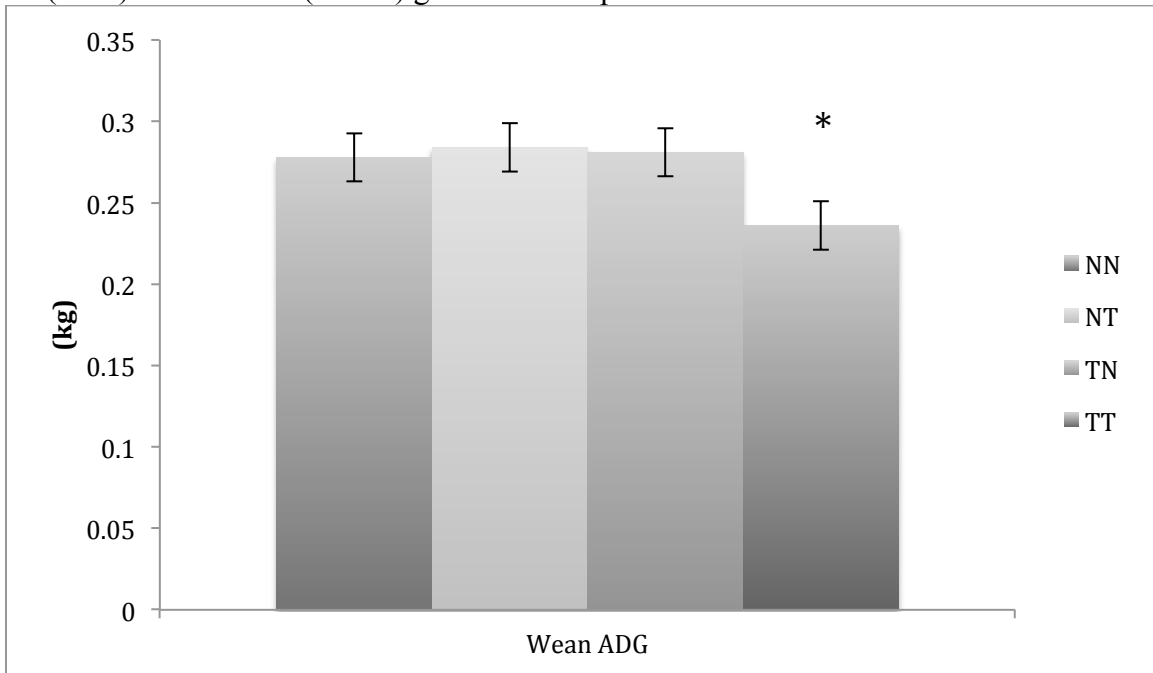


Figure 3.7. Lamb birth weights from maternal genotype (A|A or G|G) in Experiment 2.

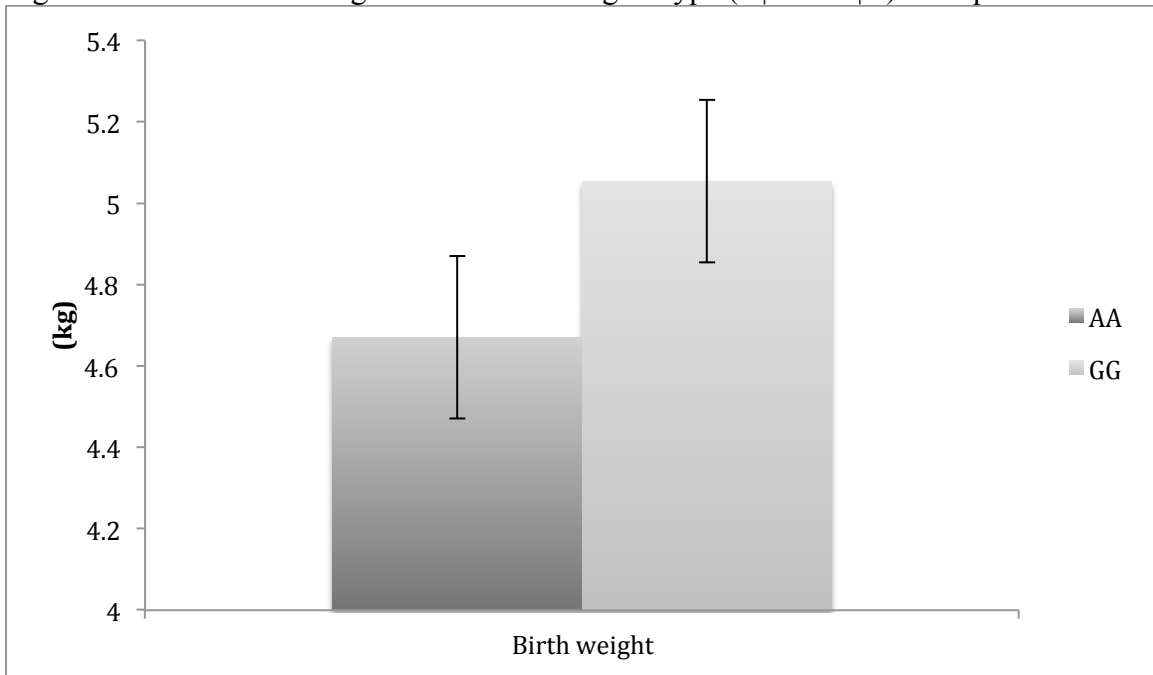


Figure 3.8. Lamb weaning weights by maternal genotype (A|A or G|G) in Experiment 2.

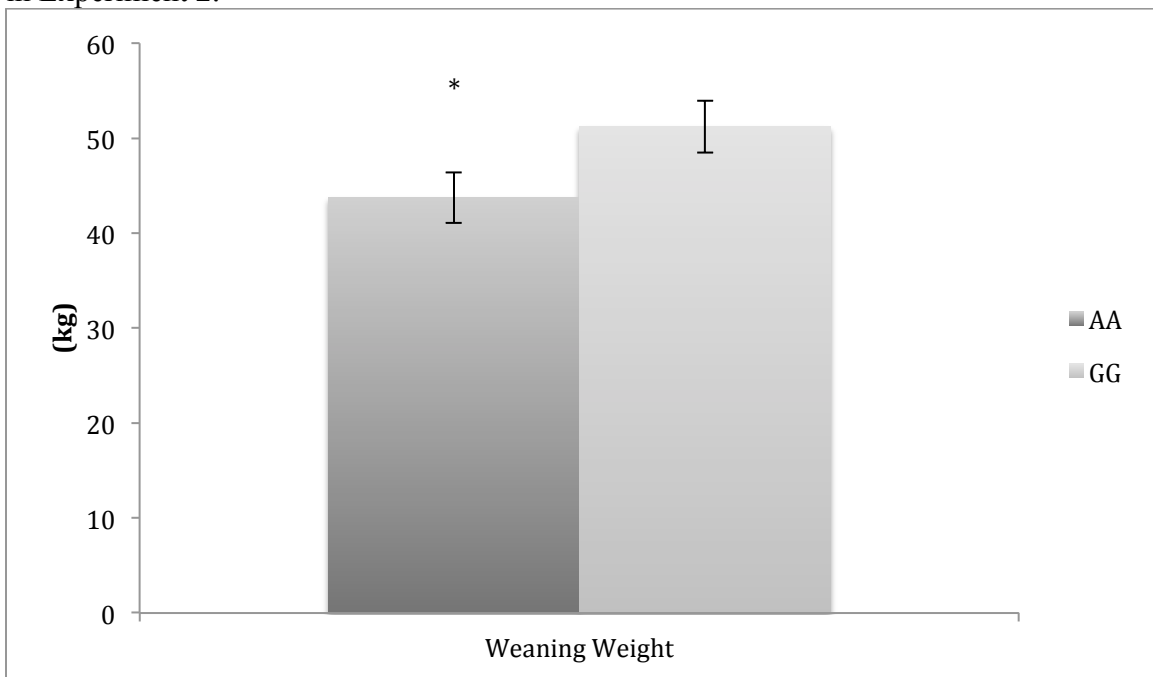


Figure 3.9. Lamb ADG by maternal genotype (A|A o G|G) from birth to weaning in experiment 2.

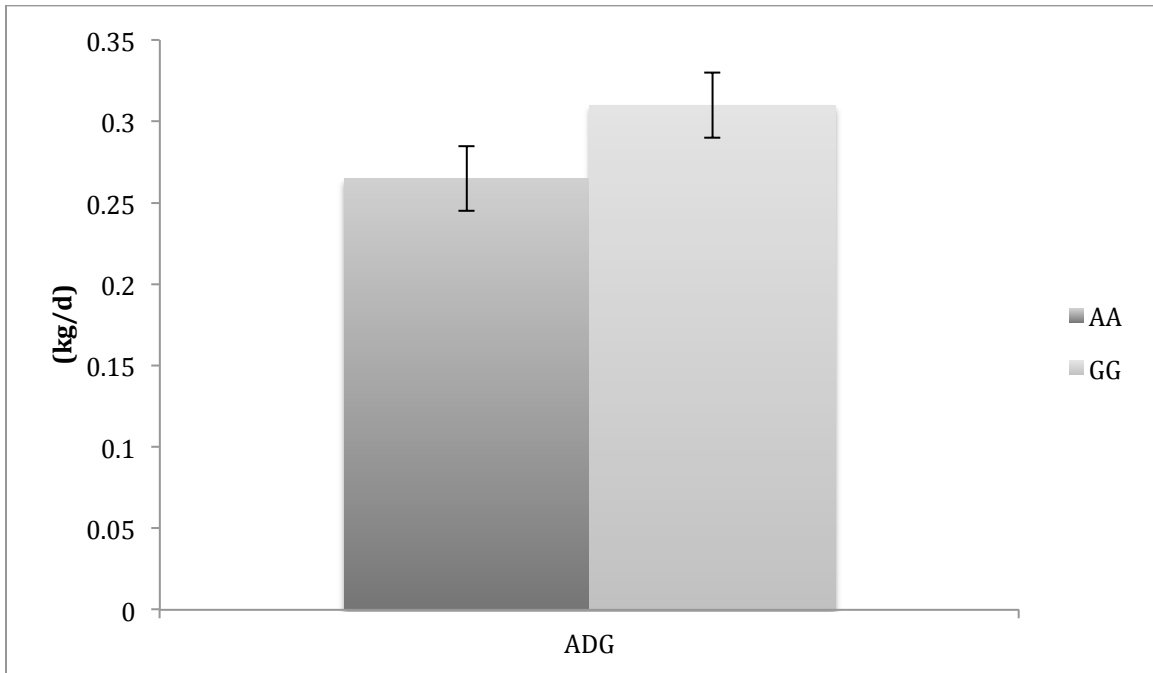
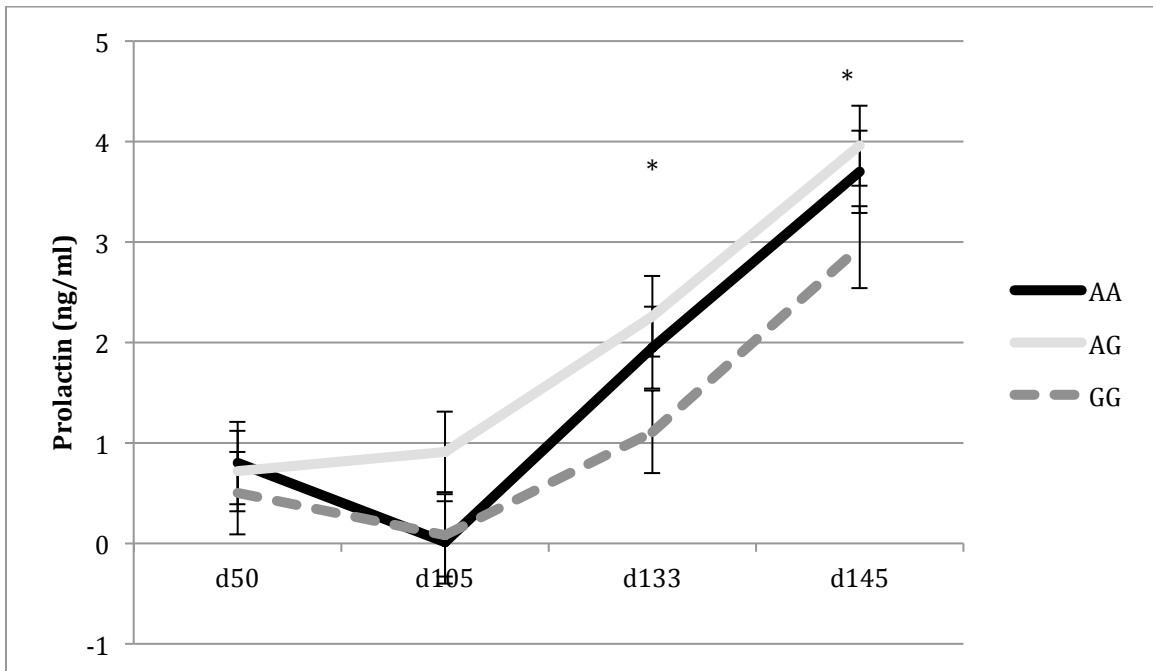
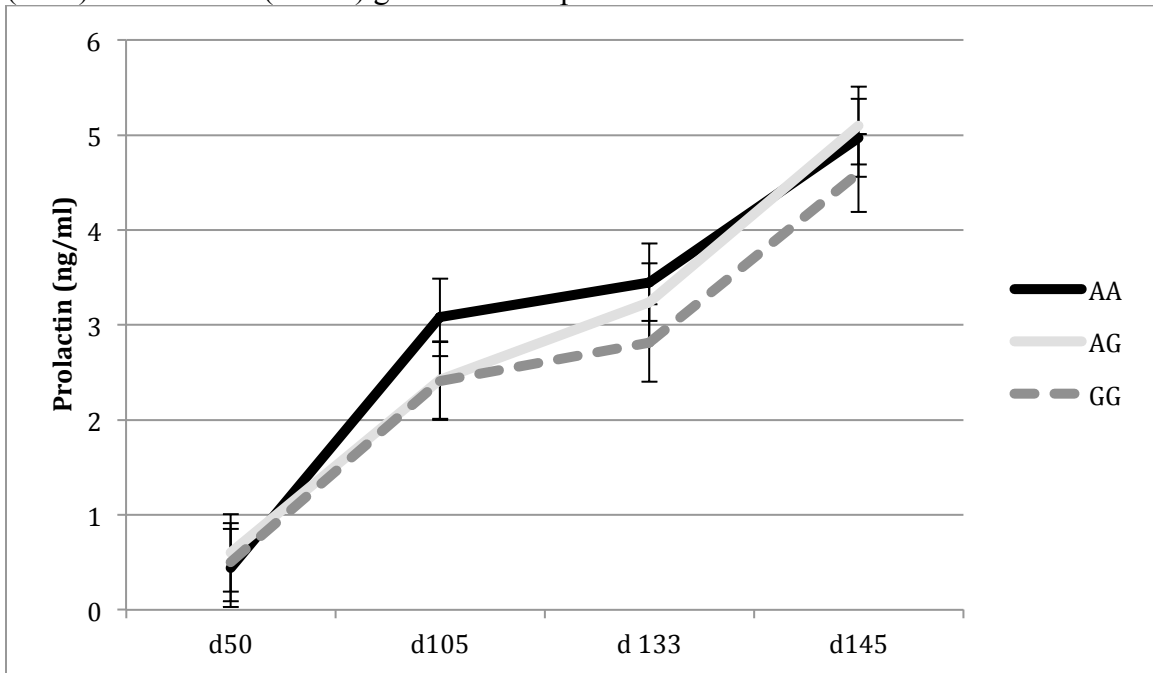


Figure 3.10. Ewe prolactin concentrations in ewes consuming endophyte free (E-) or endophyte infected (E+) tall fescue seed by genotype (A|A, A|G, or G|G) during d 35-85 (MID) or d 86- 133 (LATE) gestation in experiment 1.



## CHAPTER FOUR

### **EFFECTS OF MATERNAL GENOTYPE AND FESCUE PASTURE TYPE GRAZED DURING THE LAST TRIMESTER ON COW/CALF PRODUCTION**

#### **ABSTRACT**

The objective of this study was to investigate effects of dopamine 2 receptor (DRD2) single nucleotide polymorphism (SNP; rs41749780) and fescue pasture type grazed during the last trimester on cow and calf performance. Pregnant Angus cows (n = 225) were genotyped for the DRD2 SNP (A|A, A|G, G|G). For this study, we used cows with an A|A (n = 39) or G|G genotype (n = 35) and assigned them randomly within genotype to fescue pasture type (E+, Kentucky 31 or NOV, Texoma MaxQ2) with two replicates per pasture type. During times when pasture was limiting, E+ or E- hay was provided in each pasture replicate. At 30 d increments, hair coat score (HCS), body condition score (BCS), cow body weight (BW), and real-time ultrasound measures of rump fat and rump muscle depth were collected on each cow. At birth, calf birth weight was obtained and at 30 d post calving weigh-suckle-weigh was performed with a 3 h cow-calf separation. Data were analyzed in a 2 x 2 factorial with two dietary fescue types (E+ or NOV), two DRD2 genotypes (A|A or G|G), and the interaction in the model. Cows grazing E+ treatment had lower ( $P < 0.05$ ) BW gain during the initial 30 d of grazing but did not differ ( $P > 0.05$ ) in BW at d 60 or 90. Cows grazing E+ fescue pastures had greater ( $P < 0.05$ ) hair coat scores at d 90 compared to NOV. Cows with G|G genotype had greater ( $P < 0.05$ ) amount of rump fat deposition compared to GG cows. Grazing E+ fescue pastures decreased ( $P < 0.05$ ) serum prolactin levels at d 30, 60



and 90 of grazing. Cows with DRD2 genotype of G|G tended to have lower ( $P = 0.07$ ) prolactin levels at d 0 and 90 of grazing. Adjusted calf birth and weaning weights did not differ ( $P > 0.05$ ) by fescue pasture type or cow genotype. Milk production was estimated using weigh-suckle-weigh at d 30 post-calving. Fescue pasture type and cow genotype did not alter ( $P > 0.05$ ) milk production. In evaluation of calf sire, there tended to be an interaction ( $P = 0.07$ ) between fescue pasture type and cow genotype in calves that were sired by natural service. Adjusted birth weight was lower in calves from A|A cows that grazed E+ fescue pastures compared to NOV but did not differ by fescue pasture type for calves born to G|G cows. Adjusted weaning weights were higher for calves born to A|A cows grazing E+ fescue and lower for calves born to G|G cows grazing E+ fescue. Grazing toxic tall fescue during late gestation reduced cow BW, rump fat deposition, and serum prolactin levels but did not alter adjusted birth or weaning weights of all calves. However when evaluated by calf sire, adjusted birth and weaning weight for natural service sired calves showed that response to grazing E+ fescue during late gestation depended on cow DRD2 genotype.

Keywords: Fescue Toxicosis, Single Nucleotide Polymorphism, milk production

## INTRODUCTION

Fescue toxicosis has plagued beef producers for decades and is estimated to cause a loss of 1 billion dollars to the livestock industry each year (Roberts et al., 2010; Strickland et al., 2011). Cows grazing toxic tall fescue can have lower weight gains (Peters et al., 1992; Paterson et al., 1995), conception rates (Porter and Thompson, 1992), calving rates (Caldwell et al., 2013), birth weights (Watson et al., 2004), milk production (Peters et al., 1992), and weaning weights (Peters et al., 1992; Caldwell et al., 2013). Currently there are viable mitigation plans for producers to incorporate into their grazing programs, but due to cost, land ownership, and return on investment concerns producers are not quick to incorporate the plans.

Strickland et al. (2011) suggested a need for a genetic approach to this problem. Campbell et al. (2014) identified the DRD2 SNP site in Angus-based cattle and suggested polymorphisms in the Dopamine Receptor 2 (DRD2) genes may modulate responses to fescue toxicosis. Investigating the dopamine receptor 2 gene, in particular SNP rs41749780, will provide more information about the role genotype at this location plays in cow-calf operations. The DRD2 gene found on bovine chromosome 15 was chosen to be investigated to do its role in regulating prolactin secretion (Civelli et al., 1993) making it a good candidate gene for containing a polymorphism to serve as a marker for resistance to fescue toxicosis (Campbell et al. 2014). Building on the findings of Campbell et al. (2014) the objectives of this study were to investigate effects of DRD2 SNP, rs41749780, grazing toxic or non-toxic tall fescue during the last trimester on cow and calf performance.

## MATERIALS AND METHODS

**Genotyping.** Mature Angus cows (n = 225) were genotyped for DRD2 SNP rs41749780 (Campbell et al., 2014) using a TaqMan Genotyping Assay (Applied Biosystems, Foster City, CA). Hair follicles (5-10) were collected from tail switches of each dam and DNA was extracted using Quick Extract™ DNA Extraction Solution (Epicentre, Madison, WI). Extracted DNA was sent to The University of Tennessee, Department of Animal Sciences for genomic amplification by isolating the DNA samples using the GenomiPhi DNA amplification kit (GE Healthcare, Piscataway, NJ) based on the methods outlined in Bryant et al. (2004). Following PCR, 5 µl of amplified product was subjected to a 2h digestion reaction at 65°C with 2.5 units of Tfi (USB Biolabs, Boston, MA) in a total reaction volume of 20µl. Half of the reaction volume was used in agarose gel electrophoresis against DNA size ladder (Promega, Madison, WI) and genotypes were determined based on fragment size (Campbell et al., 2014). Each dam is one of three possible genotypes: A|A, A|G or G|G. Dams with homozygous alleles were assigned to the project and heterozygotes (A|G) were not used. Genotype stratification included 39 A|A dams and 35 G|G dams (Figure 5.1). A timeline of events for this study can be found in Figure 5.2.

**Design.** Pregnant cows (n = 74, 3 to 7 yr of age, 540 ± 68 kg BW) were assigned to grazing treatment (Kentucky 31, E+ or Texoma MaxQ II, NOV, Pennington Seed, Madison, GA) during the last trimester of pregnancy. Dams were randomly assigned within DRD2 genotype to fescue pasture type replicate. There were two pasture replicates (10 ha pastures/rep) per fescue pasture type. Cows started on grazing treatments were

started 90 d prior to estimated calving date. Cows were maintained on non-fescue pastures during the first and second trimester of gestation. Cows were routinely treated for parasites and fly control based on the Clemson University Farm SOP.

In April, all cows were synchronized using the fixed-time AI (TAI) 7-day Co-Synch + CIDR protocol for timed AI to a single sire (Connealy Mentor 7374, Select Sires, registration # 15832714). At seven days post TAI, two clean up bulls were added and cows were exposed to the clean-up bulls for 70 d. Cows were pregnancy checked by rectal palpation at d 65 d and 95 after TAI by an experienced technician. Estimated breeding dates were calculated based on palpation results.

Pastures were fertilized 15d prior to cows being allocated to treatment with 27.2 kg of nitrogen per acre. A strip grazing management practice was implemented through the duration of the project, and forage grab samples were collected each time cattle were moved to a new strip of each pasture.

***Forage Analysis.*** Forage samples were analyzed for total ergot alkaloid levels (Agrinostics Limited Co., Watkinsville, GA). All forage samples were dried at 55°C for a minimum of 3 d, and ground using a 1-mm screen Wiley cutting mill (Arthur H. Thomas, Philadelphia, PA).

***Data Collection.*** Blood was collected on dams at d 0, 30, 60, and 90 of late gestation in 10 mL vacutainer serum tubes (Covidien Ltd., Monoject; Dublin, Ireland) via the coccygeal (tail) vein. Calf serum samples were obtained from the jugular vein within 12 h of birth using a 10 mL vacutainer serum tube (Covidien Ltd., monoject; Dublin, Ireland). All serum samples were allowed to clot at room temperature and then

stored at 4°C overnight. Then samples were centrifuged at 2,000 x g for 20 min at 4°C to obtain serum. Serum was stored at -20°C for subsequent prolactin analysis. Prolactin assays were conducted on serum samples from all dams every 30 d during the last trimester of pregnancy. Prolactin concentrations were measured using RIA procedures of Bernard et al. (1993) at the University of Tennessee. The intra- and inter- assay coefficients of variation were 5.59 and 5.75 % respectively.

Body condition scores (BCS) and hair coat scores (HCS) and rump fat measurements were collected on d 0, 30, 60, and 90 of LATE gestation. The BCS scoring system ranged from 1 = emaciated to 9 = obese based on the methods of Wagner et al. (1998). HCS were collected based on the methods of Saker et al. (2001) and dams were scored from 1= slick to 5= unshed. Both, BCS and HCS were recorded by the same trained technician. Real-time ultrasound measurement were collected using a Aloka 500-V ultrasound unit (Corometrics Medical Systems, Wellingford, CT) equipped with a 17-cm, 3.5-MHz linear probe to estimate subcutaneous fat thickness and depth. All images were interpreted using a Biosoft toolbox (Biotronics, Inc., Ames, IA) by the same ultrasound technician.

At parturition, calves were processed within 12 h of birth at 0800 and 1600. Data collected included birth weight, sex, and date of birth. Calves were ear tagged, tattooed, and males were castrated. Calves were injected intramuscularly with 500,000 units of Vitamin A, D and E. Calves were given Clostridial, TSV-2, and Leptospirosis 5-way in May. Calves were weaned on September 11 and weaning weight was collected. Birth and

weaning weights were adjusted according to the Beef Improvement Federation Guidelines (BIF, 2018) based on age of the dam and sex of the calf.

**Statistics.** Data were analyzed as a 2 x 2 factorial arrangement of treatments with genotype (A|A or G|G), fescue treatment (E+ or NOV), and two-way interaction as fixed effects. Pasture replicate was the experimental unit (n=8). For calf data, sex was included in the model as a covariate when significant ( $P < 0.05$ ). Significance was determined at  $P < 0.05$ , and trends at  $P < 0.15$ .

## RESULTS AND DISCUSSION

Genotype testing of our mature Angus cows (n = 225) at the Simpson Research and Education Center for DRD2 showed a distribution of 56% A|G, 20% G|G and 20% A|A (Fig. 4.1). For this study we used the A|A and G|G genotype only and exposed them to endophyte-infected toxic tall fescue (E+) or novel endophyte-infected tall fescue (NOV) during the last trimester of gestation (180 to 270 d of gestation; 90 d). The last trimester was chosen due to results from previous research in sheep that found exposure to ergot alkaloids during late gestation had the greatest impact of fetal growth and development (Britt et al., 2018). In Campbell et al. (2014), DRD2 SNP rs41749780 located in the Dopamine 2 gene was identified and then DRD2 genotypes were assigned to herds of Angus-based steers and heifers and associations were made in regards to rectal temperature, HCS, and prolactin concentrations on genotype. However, the influence of dam genotype on calf growth were not evaluated. This study allowed us to investigate if any maternal benefits exist by selecting for a genotype that is more resistant to fescue toxicosis.

Pasture grab samples were collected monthly throughout the study and analyzed for total ergot alkaloid levels (Table 4.1). For this study, cows grazed stockpiled fescue pastures with either endophyte-infected, toxic (E+) or endophyte-infected, novel (NOV) tall fescue. Pastures with E+ fescue had higher total ergot alkaloid levels compared to NOV (1902 to 2631 ng/g for E+ vs. 86 to 462 ng/g for NOV). Kallenback et al. (2003) suggested livestock producers could reduce toxicosis problems by stockpiling forages for winter grazing. When pasture availability was limited, hay of similar fescue types were provided. Total ergot alkaloid levels in the E+ hay were lower than pasture samples (718 ng/g). Roberts et al. (2009) has shown the concentration of ergovaline in hay can drastically reduce within the first 3 d after mowing and continue to decrease as stored. In our study, ergot alkaloid levels were reduced considerably ranging in average from 2283 ng/g on stockpiles fescue pastures to 718 ng/g in hay, a 68% reduction in ergot alkaloid level. These changes in ergot alkaloid levels with hay may have reduced the impact of ergot alkaloids on fetal growth.

During the first 30 d on treatment, dams grazing E+ fescue had lighter ( $P = 0.05$ ) BW by about 25 kg; however at d 60 to d 90 of grazing, there were no differences ( $P > 0.30$ ) in body weight of dams grazing E+ fescue compared to NOV (Table 5.2). Overall, the change in BW tended to be lower ( $P = 0.088$ ) for cows grazing E+ fescue compared to NOV over the 90 d treatment period. Cows with G|G genotypes tended ( $P = 0.08$ ) to have heavier BW at calving (d 90 on treatment). There were no interactions ( $P > 0.05$ ) between fescue type and DRD2 genotype in BW. Body condition score did not differ ( $P > 0.05$ ) among cows grazing different forage types or by genotype at any time

during the treatment period. Hair coat score tended to be higher ( $P = 0.11$ ) for E+ cows after grazing for 90 d. Hair coat scores did not differ ( $P > 0.05$ ) by maternal genotype or the interaction with fescue type. It has been well documented the consumption of E+ forage result in production loss (Patterson et al., 1995). Cattle have gained from 30% to 100% less on ergot alkaloid-containing endophyte-infected tall fescue compared to cattle consuming an endophyte-free tall fescue diet (Patterson et al., 1995; Hoveland et al., 1983; Boling, 1985; Schmidt et al., 1986). As expected, in our study, dams consuming E+ fescue demonstrated a loss of BW gain during late gestation compared to dams consuming the NOV treatment. Reduction in BW gain for cattle consuming toxic fescue has been attributed to a number of things, but most often attributed to the reduction in intake (Bond et al., 1984). Watson et al (2004) concluded that grazing tall fescue pastures infected with AR542, a novel fescue variety, may give significant advantages in cow-calf growth rates and BCS over grazing E+ pastures.

Rump fat measures tended to be greater ( $P < 0.15$ ) for dams on NOV treatments at d 0 and 90 but the change in rump fat deposited tended to be higher ( $P = 0.13$ ) for E+ due to the lower rump fat starting levels compared to NOV. Cows with G|G genotypes had greater ( $P = 0.012$ ) rump fat deposition from d 0 to 90 than dams with A|A genotypes. Rump depth tended to be lower ( $P = 0.08$ ) on d 90 of grazing E+ fescue than NOV. Cow genotype did not alter ( $P > 0.70$ ) rump depth. This translated to a trend for a greater ( $P = 0.072$ ) reduction in rump depth during the 90 d treatment period for cows grazing E+ fescue. Klotz (2015) suggested a potential interaction between an individual's genetic predisposition, prior exposure to ergot alkaloids, or prior health issues such as



damage to hepatic or respiratory tissues that may reduce alkaloid tolerance in an individual. Perhaps, having a G|G genotype in conjunction with the consumption of ergot alkaloids are altering signals in dams to partition energy into the storage of fat rather than for the fetus.

At the start of this experiment (d 0), serum prolactin concentrations were similar between E+ and NOV fescue cows. At d 30, 60 and 90 on fescue, cows grazing E+ pastures had reduced ( $P < 0.001$ ) serum prolactin levels compared to NOV. Cows with an A|A genotype tended to have higher ( $P = 0.074$ ) serum prolactin concentrations when compared to dams of G|G genotype at the start of the study (d 0) and at the end of the study (d 90); however during the middle treatment period (d 30 and 60), prolactin concentrations did not differ ( $P > 0.05$ ) by cow genotype. There were no interactions ( $P > 0.05$ ) between fescue pasture type and cow genotype. Prolactin depression has been well documented in animals experiencing fescue toxicosis (Aiken et al, 2001; Duckett et al., 2014) and results for these dams were not different. Dams consuming E+ diets during the last 30 d of gestation had reduced serum prolactin concentrations ( $P < 0.05$ ) when compared to those consuming NOV treatment. As far as genotype, at d 0 of LATE gestation, prolactin concentrations for all dams tended to differ ( $P = 0.07$ ) with dams of A|A genotypes having higher serum prolactin concentrations when compared to dams of G|G genotypes. As gestation progressed, (d 30 to d 9 of LATE gestation) prolactin concentrations were not different by genotype, but were depressed for dams on E+ treatment ( $P < 0.01$ ) Prior to parturition (d 90 to LATE gestation), prolactin concentrations were influenced by genotype, again with dams of A|A genotypes having higher

concentrations ( $P=0.05$ ).

At parturition, dystocia scores were collected from all dams. Fescue pasture type or cow genotype did not alter dystocia scores. At 30 d post calving, a weigh-suckle-weigh was conducted on a subsample of cows/calves. Calves were separated from the cow for 3 h, allowed to nurse, and then weighed to estimate milk production according to the method of Radunz et al. (2010). Milk production did not differ ( $P > 0.40$ ) by fescue pasture type or cow genotype. Similar to the results in our study, a study by Shoup et al. (2015) designed to determine the effects of the interaction of level of supplementation and age at weaning on cow pre- and post partum and calf performance showed no difference in milk production nor in calf BW. However, it is possible that an error in weigh-suckle-weigh pertaining to scale sensitivity contributed to a discrepancy in these results. Bluntzner and Sims (1976) caution that urination and defecation of the calf between weight and reweigh can contribute error to the milk production estimate as well as the possibility that the calf may not consume all of the milk that the cow produces.

Rebreeding rate was lower ( $P = 0.034$ ) for cows that grazed E+ fescue pastures during the last trimester of gestation and the first 30 d post-calving compared to NOV. Cow genotype did not alter ( $P = 0.54$ ) rebreeding rate. There are conflicting reports on the effect of grazing E+ tall fescue on reproductive performance of the beef cow. Some studies (Gay et al., 1988; Brown et al., 1992; Porter and Thompson, 1992); reported decreased reproductive performance while others (Mahmood et al., 1994; Rorie et al., 1998; Burke et al., 2001; Watson et al. 2004) have failed to observe any reduction did not see reproductive differences for dams grazed on NOV fescue versus E+ throughout

gestation. It seems that reproductive dysfunction may only be evident when a certain set of conditions prevail, which may include factors such as climate, cow genetics, alkaloid concentrations, and nutritional management (Watson et al., 2004). In addition, it is not clear as to what mechanisms of the reproductive system the endophyte may impact such as oocyte quality, fertilization processes, or early embryonic development. It has been shown that vasoconstriction was observed in the caudal artery but not in the caudal vein of fescue naïve heifers fed E+ seed (Poole et al., 2018). Poole et al. (2018) also collected reproductive measurements such as ovarian structures, uterine and ovarian vessel diameter, and hormone concentrations; concluding no difference in antral follicle counts, corpus luteum area or circulating progesterone levels in the E+ fed heifers. However, they did find a significant diameter decrease in the arteries and veins servicing the ovary and uterus, which may contribute to reduced ovarian function and pregnancy rates.

Furthermore, early development and survival has been shown to be compromised in embryos from heifers fed ergotamine tartrate (Schuenemann et al., 2005), but effects were ameliorated by administration of domperidone, a selective D2 receptor antagonist (Jones et al., 2003). Campbell et al. (2014) suggested this is due to modulated dopamine signaling serving to regulate prolactin secretion which has been implicated in the onset of puberty and appeared to reduce the effects of fescue toxicosis on A1A heifers allowing them to grow and obtain puberty faster. In general, dams of spring-born calves would have to deal with greater endophyte load while lactating and breeding in April-June, than dams of fall-born calves. Campbell et al. (2014) hypothesized the allelic and genotypic frequencies would favor the A allele in spring

calving but not fall calving herds. In this study, following calving in January through March, dams were re-bred in April. Eleven of the 74 cows were open after TAI and 75 d with a clean-up bull. Eight of those 11 dams were on toxic fescue prior during their last pregnancy. Conception rates have been previously shown to decrease (up to 41% lower) for cows exposed to ergot alkaloids via tall fescue grazing than those grazing an ergot alkaloid-free pasture (Schmidt et al., 1986).

In our study, adjusted calf birth and weaning weights did not differ ( $P > 0.23$ ) by fescue pasture type or cow genotype (Table 4.3). Because the cows were bred TAI or by natural service from a clean-up bull, we did not examine sire differences on calf weights. There was a trend for an interaction ( $P = 0.07$ ) between cow genotype and fescue pasture type for adjusted calf birth and weaning weights (Fig. 4.3). For cow with A|A genotype, adjusted calf birth weight was lower when cows grazed E+ fescue compared to NOV; however for G|G cows, there was not difference in adjusted calf birth weights by fescue pasture type. At weaning, cows with A|A genotype had heavier adjusted weaning weights when they grazed E+ fescue during late gestation and early lactation compared to NOV. In contrast, cows with G|G genotype that grazed E+ fescue had lower adjusted weaning weights compared to NOV. These results closely align with Campbell et al (2014) demonstrating the possibility for dams carrying two copies of the A allele to be more tolerant of fescue toxicosis. Sire DRD2 genotype is unknown, however other heritable traits may affect growth parameters such as birth and weaning weights. When analyzed by breeding procedure (AI or Natural), Calves sired by TAI did not differ ( $P > 0.05$ ) by fescue pasture type or cow genotype for adjusted birth and weaning weight. However, for

calves sired by natural service bulls, adjusted birth weight and weaning weight tended to have a treatment by genotype interaction ( $P=0.07$ ). It has been shown in humans that fetal environment influences subsequent adult health (Barker, 1990) and can impact muscle fiber size and postnatal muscle fiber growth in beef cattle (Du et al., 2013) and sheep (Duckett et al. 2014). Fetal growth restrictions are highly correlated with uteroplacental growth and development and are exasperated in cases of over and under nutrition and other environmental conditions that reduces blood flow and nutrient uptake essential for fetal growth (Reynolds and Redmer, 1995, 2001). In 2014, Duckett et al. concluded that lambs exposed to ergot alkaloids in utero had a lower secondary to primary muscle fiber ratio in the semitendinosus muscle compared to E-. Muscle fiber number is set before birth, but muscle fiber growth is due to hyperplasia, which has been shown to be complete by about 180 d in the cow (Albrecht et al., 2013) and 105 d in the sheep (Du et al., 2010). However, postnatal growth is predominately through hypertrophy of existing muscle fibers, a reduction of secondary fiber number also impacts postnatal growth.

In conclusion, these data align with other research showing the production problems from cows consuming E+ forages, this study's cows having decreased serum prolactin concentrations, dam body weight gain, and increased HCS. The purpose of this study was to determine any genotypic associations in the DRD2 gene (rs41749780) grazing toxic or non-toxic tall fescue during the last trimester on cow and calf performance. Based on the results from this study, it appears there may be a genotypic advantage to calf growth from dams of A|A genotypes. However, there are many other

influences that may influence these results, therefore a second-year study is currently being implemented using the same dams in order to generate more conclusive results.

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Figure 4.1. Genotypic distribution of mature Angus cows (n = 225) at Simpson Farm.

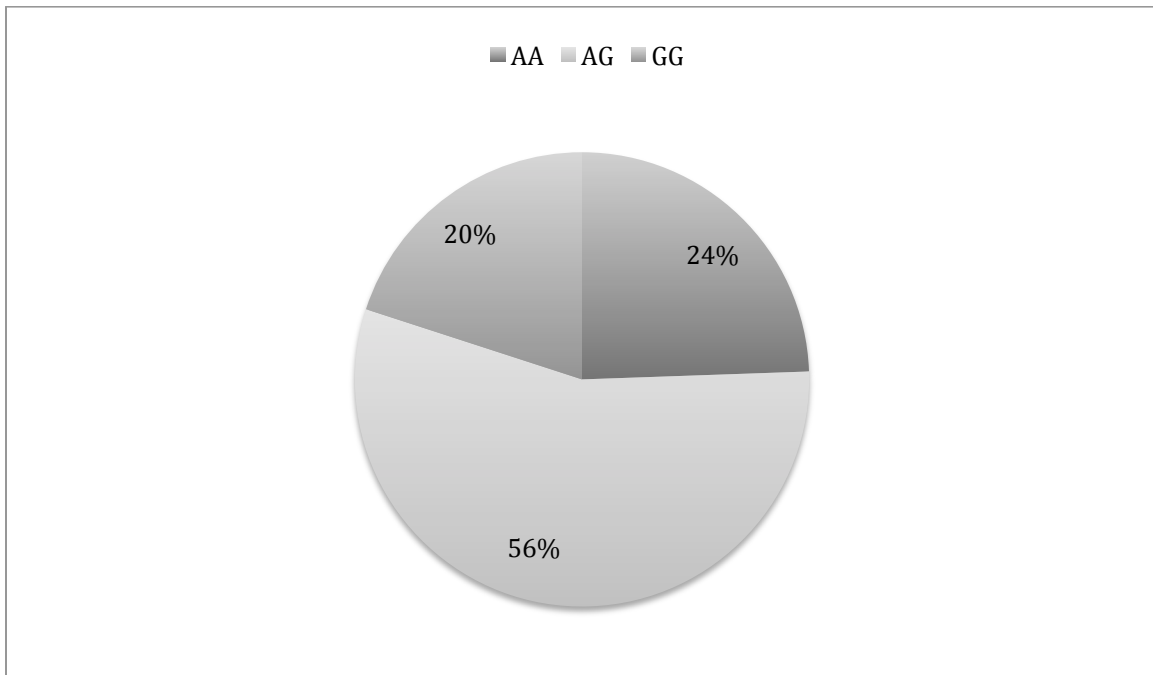


Figure 4.2. A timeline of events including genotyping the Simpson herd to the weaning of subsequent calves.

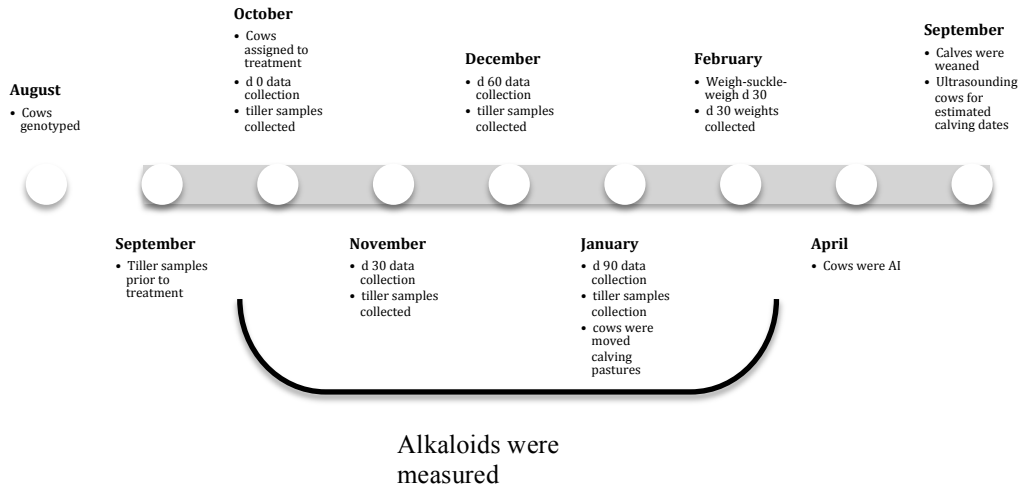


Table 4.1. Total ergot alkaloid levels for each pasture replicate and hay in this study.

Ergots, ng/g	d 0	d 30	d 60	d 90
<b>Pastures</b>				
NOV, Rep 1	511	462	400	226
NOV, Rep 2	127	187	185	243
E+, Rep 1	1906	2213	2286	1902
E+, Rep 2	2325	2631	1829	1829
<b>Hay</b>				
NOV, Rep 1				167
NOV, Rep 2				170
E+, Rep 1				716
E+, Rep 2				720

Table 4.2. Effects of dam DRD2 genotype and fescue pasture type on BW, prolactin, BCS and HCS during LATE gestation.

	Fescue Type		DRD2 Genotype		SEM	Fescue	P-Value	
	E+	NOV	AA	GG			Genotype	Interaction
n (pasture rep)	2	2	2	2				
Body wt (kg)								
d 0	597.1	592.1	591.0	598.2	7.42	0.37	0.72	0.77
d 30	610.5	635.1	620.3	625.3	8.34	0.054	0.40	0.77
d 60	630.6	629.3	627.4	632.4	6.84	0.84	0.94	0.42
d 90	644.9	656.6	643.3	657.6	16.29	0.32	0.077	0.96
Change	46.34	64.45	52.73	58.03	3.22	0.088	0.44	0.74
BCS <sup>1</sup>								
d 0	4.98	5.14	5.08	5.04	0.084	0.41	0.78	0.75
d 30	5.25	5.29	5.31	5.22	0.017	0.70	0.17	0.17
d 60	5.28	5.27	5.39	5.17	0.17	0.97	0.33	0.53
d 90	5.36	5.44	5.37	5.44	0.041	0.86	0.45	0.17
Change	0.40	0.31	0.30	0.40	0.13	0.11	0.69	0.60
HCS <sup>2</sup>								
d 0	1.27	1.23	1.24	1.26	0.25	0.45	0.88	0.67
d 30	2.28	2.03	2.18	2.14	0.10	0.52	0.83	0.70
d 60	2.31	2.07	2.21	2.17	0.12	0.38	0.91	0.80
d 90	2.77	2.28	2.53	2.52	0.064	0.11	0.92	0.32
Change	1.49	1.05	1.28	1.26	0.0025	0.19	0.19	0.74
Rump Fat, cm								
d 0	1.08	1.25	1.27	1.06	0.014	0.07	0.54	0.49
d 90	1.46	1.59	1.55	1.51	0.18	0.080	0.88	0.42
Change	0.40	0.34	0.29	0.44	0.0018	0.13	0.012	0.39
Rump Depth, cm								
d 0	9.19	8.99	9.07	9.12	0.10	0.33	0.78	0.86
d 90	8.30	8.81	8.66	8.46	0.11	0.080	0.88	0.42
Change	-0.84	-0.21	-0.42	-0.64	0.33	0.072	0.72	0.69
Prolactin (ng/ul)								
d 0	3.77	4.97	5.99	2.75	1.23	0.50	0.074	0.73
d 30	0.43	5.55	3.44	2.54	0.99	0.004	0.52	0.62
d 60	1.10	8.14	4.39	4.85	0.98	0.001	0.74	0.60
d 90	11.32	23.66	20.93	14.04	2.34	0.0006	0.049	0.25
Dystocia <sup>3</sup>	1.15	1.02	1.07	1.11	0.055	0.17	0.65	0.32
Milk d 30, kg	9.56	11.21	10.63	11.49	0.79	0.79	0.45	0.92
Rebreeding,%	79.6	91.3	93.2	77.8	0.12	0.034	0.54	0.91

<sup>1</sup>Body condition score (BCS): 1 = thin to 9 = obese

<sup>2</sup>Hair coat score (HCS): Scoring system

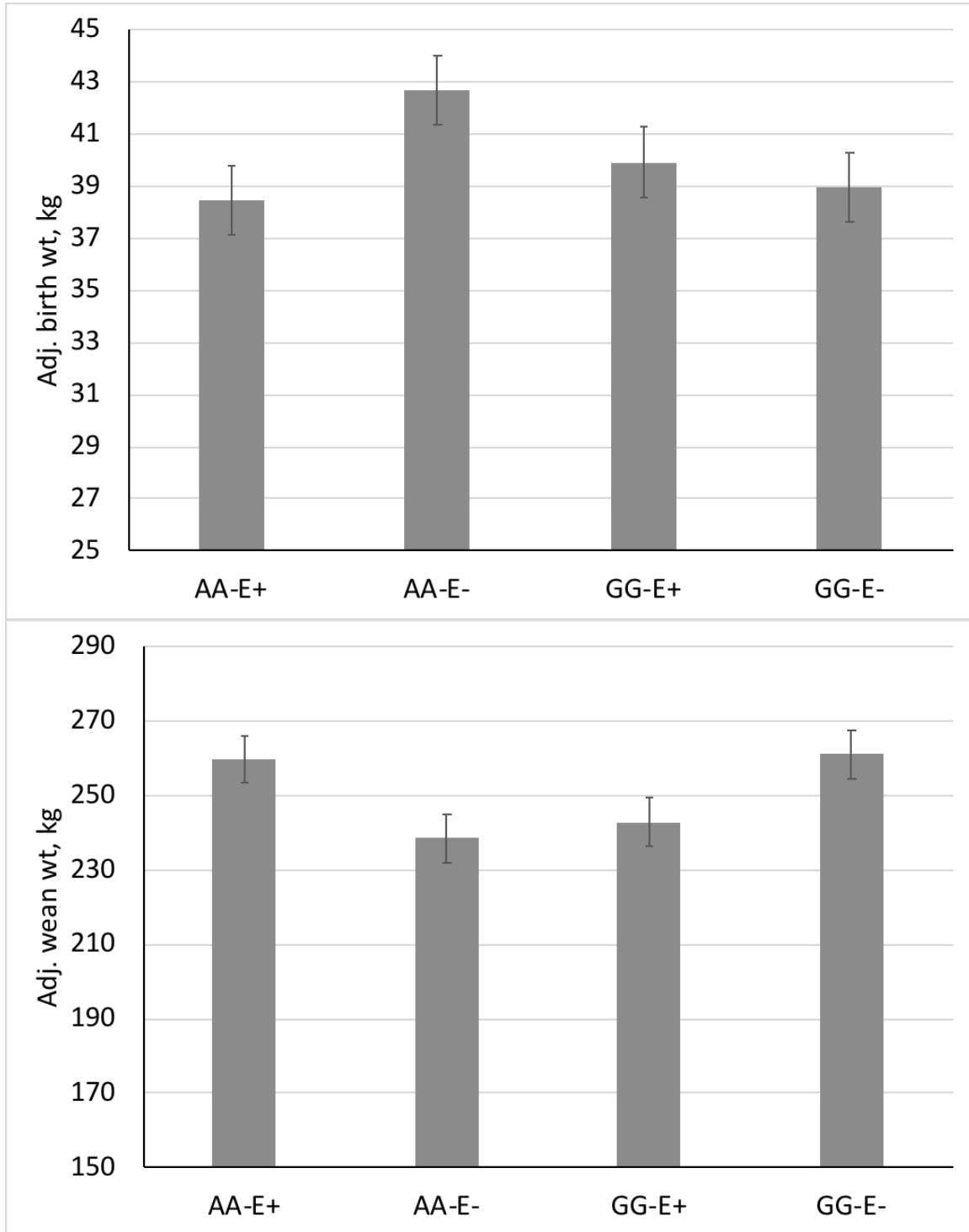
<sup>3</sup>Dystocia: scoring system

Table 4.3. Effects of dietary fescue treatment and DRD2 genotype on adjusted calf birth weight and weaning weight of calves

	<b>Fescue Type</b>		<b>DRD2 Genotype</b>		<b>SEM</b>	<b>P-Value</b>		
	<b>E+</b>	<b>NOV</b>	<b>AA</b>	<b>GG</b>		<b>Fescue</b>	<b>Genotype</b>	<b>Interaction</b>
n (pasture rep)	2	2	2	2				
Adj. birth wt, kg	40.75	40.20	41.21	39.74	0.38	0.58	0.23	0.19
Adj. weaning wt, kg	275.0	275.6	277.0	273.2	1.06	0.75	0.68	0.34



Figure 4.3. Fescue type by maternal DRD2 genotype on birth and weaning weights for natural service calves only. The interaction between fescue type and maternal genotype tended to be significant ( $P = 0.07$ ).



## CHAPTER 6

### CONCLUSIONS

Fescue toxicosis has been an ongoing problem for cattle producers for nearly fifty years. Research has provided an understanding of how to properly manage cattle when the only option is to graze toxic tall fescue. Additionally, with the advent of novel endophyte varieties another option for the Southeast was present and proven to work. However due to cost, and other limiting factors, producers have been slow to adopt these practices. With the identification and the sequencing of the cattle genome along with emerging technologies it was only fitting for a genetic based approach to fescue toxicosis to be the next objective for researchers.

The works comprising this dissertation include the identification of a DRD2 SNP in ovine and the application of the SNP in flocks of Suffolk ewes in order to determine if there were any associations with consuming toxic fescue. When there were none, a production-based study was conducted.

In ovine, the SNP located in exon 1 of the Dopamine 2 gene, appears to have no relationship to having a 'susceptibility' or 'tolerance' to fescue toxicosis. However, it does appear genotype G|G produces lambs who grow out to heavier weights by weaning. The studies completed to determine this lead us to believe that selecting for a particular genotype (G|G) could be advantageous to sheep producers regardless of animals consuming toxic or non-toxic fescue. Producers could use this knowledge, to genotype their flocks and work towards a plan of keeping ewes with two copies of the G allele at this SNP site. The theory would be, those ewes would produce heavier lambs with

heavier weaning weights, in turn allowing the producers to make more profit. However, in order for this to be an option for producers, a test available to the public would have to be commercialized.

Furthermore, in cattle, a SNP within the DRD2 gene has been identified by other researchers and applied to herds of steers and first year heifers with the conclusion that selecting for a particular genotype (A|A) would allow producers to identify cows who were more tolerant to the implications of fescue toxicosis.

The work in this dissertation on cattle sought to determine if any genotypic associations existed in a cow/calf production setting. This was chosen, as most of the cow/calf operations are located in the South, and that is the same location as the majority of toxic fescue. This study also only tracked the last trimester of gestation as the studies that were completed in sheep at Clemson pinpointed the toxic effects of ergot alkaloids during late gestation as being the primary time effecting fetal growth. This study showed all the typical signs (prolactin depression, lower birth weights, conception rates, etc.) for the cows consuming toxic fescue. However, when looking at genotype a trend for pasture by genotype interaction existed for adjusted calf birth and weaning weights. With the A|A genotype had heavier adjusted birth and weaning weights. Dam did not estimate milk production, nor prolactin concentrations different by genotype.

With a study designed such as this many uncontrollable factors are present. Environmental factors existed with a considerable amount of summer rainfall that lead to a stockpile foraging situation over the winter months. Additionally, the decrease of the ergovaline load in hay fed to the cows nearing parturition was also a factor. These factors

may have not allowed a genotypic advantage to show. A different finding might occur under different environmental effects. Therefore, a second year study is currently being implemented to determine if genotype plays a role in the cow/calf setting in late gestation in cows grazing toxic fescue.

## Appendix A

### Timeline for Fescue Toxicosis Research

The following timeline is a “snapshot” of the research that relates directly or indirectly to the identification and management of fescue toxicosis in ruminants. The purpose of this timeline is to assist in telling the story of how the research relating to fescue toxicosis has evolved and lead us to the need for a genetic approach to combat this problem.

**50 C.E.** The Holy Bible mentioned the “Sowing of Tares” (Bacon, 1995)

**600 B.C.** A “noxious pustule in the ear of grain” was referred to on an Assyrian cuneiform tablet (Strickland et al., 2011)

**98-55 BC** The Roman historian Lucretius called erysipelas “Ignis sacer”, i.e. Holy Fire, which was the name given in the Middle Ages to ergotism (Van Dongen & de Groot, 1995). People of the Middle Ages would suffer gangrene, problems of the central nervous system, and death from ingesting *C. purpurea* through eating rye bread.

**994 A.D.** Epidemic in Aquitaine, France where 40,0000 perished. Survivors were those who fled and received non-contaminated (Barger G., 1931). Later ergotism, was known as “St. Anthony’s Fire” as it was depicted as a burning sensation of the limbs.

- 1630** The first animal experiment concerning ergots was performed, by administering “cornicula nigra” to chickens, geese and pigs which all died (Van Dongen & de Groot, 1995).
- 1787 to 1822** The practice of administering ergots to women in childbirth for oxytocic effects. This practice ended in the 1800’s due to the number of stillbirths, uterine ruptures, and maternal deaths (Van Dongen & de Groot, 1995).
- 1880** Tall Fescue originated from Europe, and was then established in eastern Kentucky by shipments of infected meadow fescue seed before 1880 (Vinall, 1909).
- 1890** Kentucky 31 was found growing in the mountainous regions of eastern Kentucky. By the late 19<sup>th</sup> century Tall Fescue was described as being “an exceedingly valuable grass for mowing or pasture” (Lamson-Scriber, 1896).
- 1906** The first Ergot alkaloid ergotamine was isolated by Barger and Dale.
- 1918** Ergotamine was isolated and identified by Arthur Stoll (De Costa, 2002)
- Plant selections for a tall fescue variety to later be known as *Alta* was started. This variety was later released by the Oregon Experimental Station/USDA, and was widely planted in Pacific Northwest and western US.
- 1931** E.N. Fergus a professor at the University of Kentucky discovered Kentucky-31 in Kentucky and obtained seed for trials. He was impressed with how the grass stayed green all winter. Grass trials in Kentucky and Virginia denoted superior growth, height, competitive ability, and drought tolerance compared to meadow fescue (Garman, 1900) (Kennedy, 1900).
- 1940** Tall Fescue dissemination as a pasture grass.

**1940-1950** Tall fescue (KY-31) transformed the landscape, which previously was mostly barren and brown during the winter season.

**1943** Kentucky 31 (KY-31) was released as a cultivar (Fergus, 1952). This variety's advantages were: adaptability to a wide range of soils, dependability and provided grazing over much of the year.

**1970-1980** The cause of what we now know to be fescue toxicosis was discovered in the late 1970's to be from alkaloids produced by endophytic fungi (Glenn et al., 1996).

**1977** Researchers at USDA Russell Research Center in Athens, GA reported a fungus inside the grass that was suspected to be associated with fescue toxicosis in grazing animals (Bacon et al., 1977).

**1979** Tall Fescue occupied close to 15 million ha in the United States and was deemed the most important pasture grass in the U.S. (Buckner et al., 1979).

**1983** A grazing experiment conducted by Hoveland et al. with steers grazing fescue with low and high endophyte infections confirmed that ergot alkaloids produced by an endophyte were the cause of fescue toxicosis.

**1984** Bond et al. (1984) reported that steers which grazed an experimental line of tall fescue (G1-307) containing high levels of the endophyte showed signs of fescue toxicosis and grazed 20% less than steers grazing low endophyte lines or cultivars.

- 1985** Yates et al. (1985) used Mass Spectrometry to identify significant levels of ergopeptine alkaloids in crude extracts of tall fescue from pastures producing clinical signs of fescue foot in cattle.
- 1986** Lyons, Plattner, and Bacon (1986) determined ergovaline is the most active alkaloid produced by the endophyte, accounting for 84 to 97 percent of the total alkaloid present, during experiments at the University of Georgia along with USDA. Furthermore, they conducted greenhouse experiments to determine if varying rates of nitrogen and different Nitrogen sources would effect ergot alkaloid concentrations in the plant. It was concluded that plants grown at high rates of nitrogen had higher total alkaloid concentrations
- After determining the cause of fescue toxicosis, much research focused on planting endophyte free cultivars. Many where then releases in the late 1980s, however they couldn't hold up to grazing, insect and heat pressures of endophyte infected, Kentucky 31. The stands quickly deteriorated.
- 1987** Endophyte detection using microscope was developed (Shelby and Dalrymple, 1987)
- 1988** Yates et al. (1988) developed a high-pressure liquid-chromatography (HPLC) method for alkaloid detection. However, it was not useful in fresh plant tissue. Belesky et al. (1988) conducted grazing studies in Georgia showing ergopeptine alkaloid concentrations increased in late spring to coincide with anthesis, decreased in the summer as plants enter dormancy, and increased again to



maximum concentration in early autumn at the initiation of fall regrowth. Later done in Missouri (1991) with similar results.

Bond et al. (1988) showed consumption of fungus-infected fescue decreased plasma prolactin and delayed the average time from ram introduction to conception.

**1989** Crawford et al. (1989) concluded endophyte levels higher than 20-30% can effect livestock production.

**1990** Cornell et al., (1990) determined the minimum concentration of 50 ug/kg to produce clinical signs of fescue toxicity in animals subject to heat stress.

Lugsby et al. (1990) found that cattle previously grazed on endophyte-infected fescue pastures can make significant compensatory gains during the first 50 days in the feedlot.

**1991** Rottinhaus et al. (1991) developed a HPLC method for alkaloid (ergovaline) detection in live plant tissues. Also reported increasing ergovaline concentrations in leaf blade, sheaths and stems with fertilization.

**1993** Studemann et al. (1993) completed an experiment looking at steer performance and behavior. Steers spent more time grazing low or high alkaloid levels during the hottest part of the day. Additionally, suggestions of a carry over effects of steers when moved from low to high endophyte levels. Average daily gains were higher for steers grazing low endophyte levels.

A research team at Clemson University determined taking pregnant mares away from E+ fescue 30 d prior to estimated foaling dates, almost eliminated effects of

- fescue toxicosis. Later, James Strickland and Dee Cross at Clemson University patented domperidone as a method of treating fescue toxicosis in animals. (Cross, D.L.,2009)
- 1994** Hill & Agee (1994) detected ergoline alkaloid in endophyte-infected fescue by immunoassay.
- 1995** Dr. Joe Bouton at The University of Georgia developed the tall fescue variety Jesup, an endophyte free variety or available with Max-Q technology.
- 2001** Hill et al. (2001) found that ergopeptine alkaloid transport across gastric tissues was lower than transport of lysergic acid and lysergol, with the implication that the latter alkaloids were the cause of observed physiological responses in livestock.
- 2002** Bouton et al. completed an experiment re-infection Jesup and Georgia 5 tall fescue varieties with non-ergot alkaloid producing endophyte strains. Different cultivar-strain combinations were tested against the E+ and E- versions of the same cultivars for stand survival and dry matter yield, then assessed for toxicity in lambs. It was concluded that Jesup (AR542) possessed highest yield and stand survival better than E- checks, and similar to E+ checks. Lambs gained more on cultivars containing AR542, which was equivalent to E-, forage but with 57 percent greater than gains on E+ forage.
- 2005** Realini et al (2005) reported the presence of alkaloids in beef tissues.
- 2006** A group of researchers sought to determine if a newly marketed product from ADM Alliance Nutrition called Endo-Fighter would “reduce the severity of fescue toxicosis in cattle grazing tall fescue”. Through two experiments; one using

heifers and the other steers the conclusion included no significant differences between treatments for animal performance, hair coat score, body condition score, or serum prolactin concentration. “Feeding Endo-Fighter under the conditions of these studies did not appear to reduce the signs or severity of fescue toxicosis”.

(Norman, R. et al., 2010)

**2007** Duckett et al. (2007) evaluated animal performance, carcass quality and economics of cattle finished after grazing endophyte-infected, endophyte-free or non-ergot alkaloid producing endophyte-infected tall fescue. Conclusions included evidence that producers have an economic incentive of about \$160/ha or about 2:1 to replace E+ pastures with Novel AR542.

Ruminal metabolism of ergovaline to lysergic acid was studied in ewes ingesting E+ straw. A model of ergopeptide degradation was established. (Duringer, J.M., 2007)

**2010** Fescue Toxicosis and Management Manual authored by Craig Roberts and John Andrae focusing on strategies for incremental alleviation and alkaloid management was published for producers. (Roberts & Andrae, 2010)

**2011** Strickland et al. (2011) published a review paper on St. Anthony’s Fire in livestock, covering the history, causes, mechanisms, and current solutions to the disease.

**2013** Foote et al. (2013) showed a reduction in blood flow to the stomachs of steers.

Koontz et al (2013) found that the consumption of ergot alkaloids caused decreased metabolism.

**2014** Exposure to ergot alkaloids in late gestation in sheep shown to reduce fetal growth.

(Duckett et al, 2014).

Stowe et al. reported on tall fescue effects on bull growth, semen characteristics, and breeding soundness exams (BSE). They found bulls consuming tall fescue seed did not differ in body weight, or body condition score, nor percentage passing BSE exams. However, E+ bulls did have lower serum prolactin concentrations and smaller scrotum circumference. (Stowe, et al., 2014)

XKR4 gene, was reported by Bastin et al. (2014) to be significantly correlated to circulating prolactin concentrations in 592 mixed-breed beef cattle grazing toxic tall fescue.

Ely et al. (2014) reported cows testing homozygous AA for the DRD2 SNP and not homozygous GG for the XKR4 SNP had greater serum prolactin concentrations than did cows without this genetic profile.

Campbell et al (2014) suggested the DRD2 SNP (rs41749780) may have a use in selecting animals resistant to fescue toxicosis.

Koontz et al (2015) determined the consumption of ergot alkaloids did not negatively impact the overall energy balance of digestion of feed, and also showed a decrease in passage rate of gut contents.

**2018** Trotta et al. (2018) showed results that indicated ergopeptide alkaloid exposure influences contractility of bovine ruminal and mesenteric blood vessels through serotonin receptor subtype 5HT2A by acting as both an agonist and antagonist.

Poole et al (2018) paper concluded reduced ovarian function and pregnancy rates in heifers could be due to the significant decrease in the diameter of arteries and veins servicing the ovary and uterus in fescue naïve heifers.

## Appendix B

### Literature Cited

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