

## ABSTRACT

BROWN, ALECIA RAEKIAH. Physiological and Immunological Responsiveness of Cattle Consuming Endophyte-Infected Tall Fescue. (Under the direction of Dr. Daniel H. Poole).

Tall fescue is the most commonly utilized forage in the Southeastern portion of the United States due to its favorable agronomic qualities such as high yield, drought tolerance, pest resistance, and general survivability. Varieties of tall fescue such as 'Kentucky-31' are infected with an endophyte that produces toxins such as ergovaline and causes a common condition known as fescue toxicosis in livestock. These animals face adverse health symptoms and performance losses such as hypoprolactemia, heat stress, reduced average daily gain, vasoconstriction, and compromised immunity. This thesis research was conducted to examine the performance and immune response of animals exposed to endophyte-infected tall fescue and the use of pulmonary arterial pressure measurements as a means of animal selection. In chapter 2, the effects of protein supplementation on the cytokine response after exposing steers to 185  $\mu\text{g}/\text{kg}$  ergovaline, the primary toxin produced by endophyte-infected tall fescue, was evaluated. Thirty-two Angus steers were randomly assigned to receive one of four treatments with diets consisting of endophyte-free (EF) or endophyte-infected (EI) fescue seed and 14% or 18% crude protein. EI steers were observed to have greater serum concentrations of pro-inflammatory cytokines (TNF- $\alpha$ , IFN- $\alpha$ , IFN- $\gamma$ , IL-1 $\alpha$ ), chemokines (CCL2, CCL4, MIG), anti-inflammatory cytokines (IL-2, IL-13, IL-15, IL-21, IL-36), and multiple growth factors (FGF-1, IGF-1, VEGF-A) compared to EF steers ( $P < 0.05$ ). These results demonstrate that exposure to ergovaline resulted in an increase in cytokines and chemokines to cause a hyperactive immune response. Chapter 3 examines cytokine profiles in heifers exposed to ergovaline that possess the heat tolerant slick hair genotype compared to wildtype controls. Twenty Angus X Senepol heifers were blocked by hair coat genotype, Slick

(S) or Normal (N), and randomly fed a novel endophyte fescue (EN) or endophyte-infected fescue (EI) diet. Heifers with the slick genotype had greater concentrations of pro-inflammatory cytokines (IFN- $\alpha$ , IL-1-F1), anti-inflammatory cytokines (IL-2, IL-21), chemokines (CCL4), and growth factors (bFGF, GPRASP-1) when compared to heifers with the wildtype hair genotype ( $P < 0.05$ ). These results suggest an interesting interaction between the slick hair genotype, ergot alkaloids, and immune response. In chapter 4, pulmonary arterial pressure testing was explored as a potential tool to identify susceptible animals to pulmonary hypertension and its possible relationship with fescue toxicosis tolerance. Twenty postpartum beef cows were previously identified as tolerant ( $n=10$ ) or susceptible ( $n=10$ ) to fescue toxicosis and had been exposed to ergot alkaloids previously. Cows from the fescue toxicosis tolerant group had an increased jugular mean arterial pressure, an increased right ventricular mean arterial pressure, and a greater pulmonary artery mean arterial pressure compared to cows from the susceptible group ( $P < 0.05$ ). Chapter 5 summarizes chapters 2, 3, and 4 in an effort to draw conclusions about the preceding results and offer potential applications of this research to producers. Overall, these studies provide interesting results investigating the physiological and immunological effects of endophyte-infected tall fescue exposure in cattle.

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Physiological and Immunological Responsiveness of Cattle Consuming Endophyte-Infected Tall  
Fescue

by  
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**DEDICATION**

This is dedicated to my family, friends, and past educators. I am grateful for all the love, help, and support.

## **BIOGRAPHY**

Alecia Raekiah Brown was born and raised in Portsmouth, Virginia to Steven Brown and Angela Bee alongside her two older sisters, Asjah Fezio and Randy Hopkins. Her enthusiasm for science began at a young age with a fascination for living organisms. Her interest led her to receiving a Bachelor of Science degree in Biology with a minor in Chemistry from Virginia Commonwealth University in May of 2017. During her time at VCU, she discovered an interest in reproductive physiology.

Throughout the four years of Alecia's undergraduate career, she worked as an Office Assistant at VCU's Rehabilitation Research & Training Center where she assisted on many projects aimed to assist individuals with disabilities through research, training, and resources. Upon completion of her bachelor's degree, she was hired full time as a Research Associate and continued her work there until the summer of 2018.

After persuasion from her sister Asjah, who studies veterinary medicine, Alecia explored her options for a graduate education in Animal Science. In the Fall of 2018, Alecia began a Master of Science degree program in Animal Science with a concentration in reproductive physiology at North Carolina State University under the direction of Dr. Daniel Poole. After completing her master's degree, Alecia plans to continue to work within the science field doing what she loves.

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## **Chapter 1: Review of Literature**

## Introduction

Beef cattle operations are one of the many sources of agricultural revenue in the Southeastern region of the United States. The overall health of the animals at these establishments is essential from a management and welfare perspective. It is essential that cattle remain healthy in order to maintain optimal growth, reproductive success, and overall profitability. Environmental stressors may lead to a variety of symptoms, performance loss, as well as an overall compromised immune system. Suppressed immunity can then lead to decreased growth performance and potentially more health issues.

Pastures within the Southeastern United States utilized for grazing purposes are often covered by a cool season perennial grass known as tall fescue. It is an immensely popular forage to utilize due to its extensive survivability and its capacity to thrive in the southeast climate. Kentucky-31, a variety of tall fescue, was popularized in the 1940s and quickly spread across pastures, parks, and other landscapes (Hoveland, 2009). This is due to the desirable qualities it possesses such as ease of establishment, persistence through drought, pest resistance, and ability to withstand a variety of soil conditions (Blodgett, 2001; Christensen & Voisey, 2009). Very little management is necessary for the plant to thrive. These favorable qualities are caused by the symbiotic relationship it has with an endophytic fungus known as *Epichloë coenophiala*. This endophyte produces ergot alkaloids which causes adverse effects to livestock that graze the forage. These detrimental effects lead to a condition known as fescue toxicosis. Some symptoms attributed to fescue toxicosis include vasoconstriction, increased heat stress, reduced reproductive performance, decreased weight gain, and a compromised immune system (Strickland et al., 2009).

Another environmental stressor imposed on cattle is high mountain disease, also known as brisket disease, which occurs in domestic cattle that reside in high-altitude areas. The most obvious

symptom is the accumulation of edematous fluid that occurs in the tissue surrounding the parasternal muscles or the brisket region of the animal. This is caused by the hypoxic conditions of being at high elevation and leads to pulmonary artery hypertrophy. Heart failure occurs to the right ventricle and hypertension increases the hydrostatic pressure resulting in edema. Animals often are lethargic, have reduced feed intake, and display poor performance (Holt & Callen, 2007).

Pulmonary arterial pressure testing is one method that can be utilized to determine existence of pulmonary hypertension. This may be especially useful for cattle that are susceptible to developing high mountain disease and provide valuable insight into the health of cattle being raised at higher elevations. Cattle that show indication of high mountain disease can then be transported to lower elevation and removed from the herd. Interestingly, this may also be useful for developing breeding strategies to select for cattle that are less susceptible of developing the condition (Holt & Callen, 2007). Fescue toxicosis and high mountain disease both negatively affect the cardiovascular system of cattle and cause a reduction in productivity and profitability for producers. The relationship between pulmonary arterial pressure testing and fescue toxicosis susceptibility may be of interest for potential future selection practices.

Developing a better understanding of how the immune and cardiovascular systems respond to exposure of ergot alkaloids commonly found in endophyte infected tall fescue may lead to management improvement strategies and interventions for cattle grazing endophyte-infected pastures. Exploration of the tolerance of animals following exposure to environmental stressors could prove useful to mitigate the effects of infected tall fescue consumption. Detecting degrees of tolerance for ailments such as fescue toxicosis may prove useful in efforts to select for animals experiencing other diseases as well. The relationship between fescue toxicosis, high mountain

disease, and genetic selection for tolerance may prove interesting in future efforts to improve the sustainability of the beef industry in the Southeastern United States.

### **Endophyte-Infected Tall Fescue**

Tall fescue [*Lolium arundinaceum* (Schreb.) Darbysh.] is a popular forage that can be found throughout the Southeastern United States. It is estimated that at least 14 million hectares of tall fescue cover the United States (Gunter and Beck, 2004). It is a cool season, perennial plant that was introduced to the United States in the late 19<sup>th</sup> century. Perennial grasses are considered a major asset to productive animal-forage systems. It is the dominant vegetation in a perennial pasture system and are sought for their growing capabilities. With management and optimal conditions, these grasses have the ability to grow indefinitely and that survivability is appealing to producers for their pasture systems (Kahn and Cottle, 2014).

Today, the most commonly utilized variety of tall fescue is called 'Kentucky-31' (Blodgett, 2001). Dr. E.N. Ferguson, a professor at the University of Kentucky, is credited for the immense popularization of tall fescue. Dr. Ferguson observed the plant's tolerance to withstand harsh conditions and collected seed samples for trial. By 1943, Kentucky 31(KY-31) was available to the public and quickly spread throughout the Southeast (Hoveland, 2009). Kentucky-31 gained popularity due to its favorable qualities which attracted various individuals to utilize the plant within their farms, parks, roadside turf, and communities (Hoveland, 2009; Saikkonen et al., 2015). This plant has the ability to withstand environmental stressors such as drought and flooding as well as severe temperature changes. It flourishes in many soil conditions despite soil composition or pH and is resistant to pests (Blodgett, 2001). Kentucky-31 is also easy to establish, has a long grazing season, and can be stockpiled for later use. Since tall fescue has an optimal growing season in the spring and autumn months, stockpiling during the fall is common so that one may reduce

the costs of a winter-feeding system (Poore & Drewnoski, 2010). Kentucky-31 also has a high nutritive value specifically in crude protein, digestible dry matter, and mineral content, which appeals to producers to incorporate it in pasture systems (Blodgett, 2001). All these advantages make it economically advantageous to producers to incorporate into pastures for beef cattle.

### ***Epichloë coenophiala***

*Epichloë* is a genus of species from the Clavicipitaceae family of fungi which form endophytic symbiosis with a variety of grasses (Clay, 1988). Fungal endophytes live internally and display no outward symptoms of inhabitation (Saikkonen et al., 2015). This fungal organism resides within Kentucky-31 tall fescue and survives using its plant host. Tall fescue and this endophytic fungus have what is referred to as a mutualistic symbiosis which has direct effects on the plant's survivability and persistence (Christensen & Voisey, 2009). The endophyte benefits from this relationship by receiving nutrients, protection, reproductive means, and dissemination (Hoveland, 2009).

These endophytes consist of elongated hyphal filaments which absorb nutrients directly from the plant's hyphal wall (Christensen & Voisey, 2009). The fungal hyphae grow throughout the plant's tissues and remains in the intercellular spaces for a lifetime. *Epichloë* are capable of asexual vertical transmission through the adult plants into its seeds. Varieties of the species with sexual life cycles can also infect grasses through horizontal distribution. True sexual capable varieties may also spread the fungus by ascospore transmission (Saikkonen et al., 2015). Once the ovaries of the plant are infected, the endophyte penetrates the embryonic axis and shoot apical meristem zone. Within the shoot apical meristem, hyphae extend into the tissues which new leaves originate. New tillers grow from the auxiliary buds from these areas and are colonized by the endophyte. The inflorescence at the apex of the shoot apical meristem is also colonized which

enables the vertical transmission through the produced seeds of the maternal plant (Christensen & Voisey, 2009; Clay, 1988). Once hyphae are established within the leaves of the plant, it invades all parts including the tip, blade, and sheath. The prevalence of hyphae within leaves is dependent on temperature as well as the species of host. Hyphae presence is at its lowest during the cold winter months and increases as temperature warms; however, they continue to live and remain metabolically active for the plant's lifetime (Christensen & Voisey, 2009). The host plant (i.e. tall fescue) benefits from this relationship by receiving traits that enhance its competitiveness with other plants in its environment. The ability for widespread growth and resistance to environmental stressors give it more survivability than other species lacking this endophytic relationship (Saikkonen et al., 2015). One attribute given to the plant is the deeper root system enabling tolerance of drought conditions. This root system is also what prevents detrimental stress from continuous grazing (Hoveland, 2009).

### **Ergot Alkaloids**

Alkaloids are compounds commonly isolated from plant material which consist of one or more nitrogen atoms in a heterocyclic ring (Kukula-Koch & Widelski, 2017). There are three classifications of alkaloids; pyrrolizidines (lolines), peramines (pyrrolopyzarine), and ergot alkaloids. The endophyte found in varieties of tall fescue produces ergot alkaloid compounds. Ergot alkaloid production is dependent on a variety of factors including season, drought conditions, soil nitrogen, and plant nutrition (Bacon et al., 1986). Ergot alkaloids may be classified into three groups including clavines, ergopeptines, and amides of lysergic acid (Money, 2016).

The endophyte found in tall fescue produces several toxins such as peramines, lolines, and ergopeptine alkaloids. Peramine causes the insect deterrence trait found in tall fescue but it is otherwise harmless to animals after consumption. Loline enhances tolerance to drought and also

contributes to insect resistance but is linked to some toxicity in animals. Ergopeptine can be categorized into further subtypes including ergotamine, ergosine, ergocristine, ergovaline, ergocryptine, ergonine, and ergocornine. Approximately 84-97% of ergopeptines found in tall fescue is classified as ergovaline. For cattle, ergovaline concentrations that are greater than 200ppb in tall fescue is considered toxic (Blodgett, 2001).

Ergot alkaloids have the ability to disrupt biological processes within the body due to its tetracyclic structure and ability to interact with receptors. Ergot alkaloids are ligands for the monoamine neurotransmitters, and thus activate serotonin (5-hydroxytryptamine) receptors, dopamine receptors, and adrenoreceptors. The tetracyclic structure of ergot alkaloids allows them to act as agonists, partial agonists, or antagonists at these receptors and ergot alkaloids are also capable of interacting with more than one receptor site. These complex and variable interactions are what lead to the adverse outcomes that have been observed after consumption (Klotz, 2015; Pertz & Eich, 1999).

### **Fescue Toxicosis**

During the middle ages, ergot contaminated rye consumption led to death and sickness. Then referred to as “St. Anthony’s Fire,” ergotism results in a variety of symptoms including hallucinations, convulsions, and gangrene (Fiserova & Pospisil, 1999; Strickland et al., 2011). Today, the human population has minimal risk of developing ergotism due to modern advances in grain production. However, livestock animals face the challenges associated with consumption of endophyte infected tall fescue varieties. In animals, ergotism is referred to as “fescue toxicosis”. Soon after the widespread utilization of Kentucky 31, narratives of poor animal performance surfaced. Producers observed poor performance in their animals as early as the 1950s. After years of research and speculation, endophyte produced ergot alkaloids were determined to be the source

of fescue toxicosis (as reviewed by Aiken & Strickland, 2013). The effects of exposure, however, are highly variable due to the interactions between animal, endophyte, environment, plant, and toxins. These factors affect the severity and manifestation of the syndrome (Klotz, 2015). The complex effects of a multitude of symptoms as well as the varied levels of severity contribute to the tremendous economic losses faced by the beef industry.

Once an animal has been exposed to endophyte infected tall fescue, ingested the toxin, and the toxin has reached the molecular target site, a range of physiological responses ensue. The ergot alkaloids are first absorbed either passively or actively through the gastrointestinal epithelia or the rumen epithelia in the case of cattle. The ergot alkaloids can then be transferred to the lymphatic system eventually leading to the systemic circulation. Currently, very little is known concerning the detection and analysis of specific ergot alkaloids such as ergovaline from bodily fluids or within tissues of animals grazing endophyte-infected tall fescue (Strickland et al., 2011).

Animals suffering from fescue toxicosis experience an array of symptoms including reduced average daily gain, heat stress, hypoprolactemia, reduced reproductive success, and vasoconstriction. Animals exposed to endophyte infected tall fescue may also develop what is referred to as “fescue foot”. This condition consists of swelling, tenderness, and discomfort of the fetlock and hoof region. Animals often become lame and may suffer tissue necrosis of the ears or tail and cattle may experience a loss of the tail switch. These symptoms manifest from the impacts of ergot alkaloids on the vascular system resulting in reduced or lack of blood flow to organs and tissues. Summer slump is another term used to describe animals experiencing fescue toxicosis and refers to poor weight gain, rough hair coat, heat stress, and the subsequent behaviors displayed by the animals such as seeking shade, laying in mud, and reduced grazing (Strickland et al., 2009).

Many of the symptoms attributed to fescue toxicosis such as retained winter hair coat and reduced milk production post calving are caused by a decrease in serum prolactin concentrations. This has been a key indicator in animals suffering from fescue toxicosis and is a persistent finding within the literature (Aiken et al., 2011). Prolactin is a polypeptide hormone that is released from the specialized cells known as lactotrophs of the anterior pituitary gland. It is also synthesized by the central nervous system, reproductive tissues, the uterus, and mammary gland. Prolactin is found throughout the body within the nervous system, immune system, uterus, mammary gland, tissues of conception, and various other tissues. Prolactin is responsible for many biological mechanisms such as lactation, homeostasis maintenance, reproductive processes, hair follicle cyclicity, and hair shedding (Freeman et al., 2000; Littlejohn et al., 2014). Most prolactin secretion is regulated by hypothalamic prolactin-inhibiting factors. Dopamine is understood to be a key inhibitor of prolactin and most dopamine receptors on pituitary lactotrophs are dopamine  $D^2$  receptors (Freeman et al., 2000; Blodgett, 2001). As mentioned previously, ergovaline possesses an ergoline ring structure that acts as an agonist to many receptors such as dopamine  $D^2$ . When the dopamine  $D^2$  receptor is activated by ergovaline, the inhibitory G protein cascade is stimulated, adenylate cyclase activity is blocked, and intracellular cAMP concentrations are decreased. The similar ring structure allows ergovaline to bind to the dopamine  $D^2$  receptor domain, inhibit activity, and therefore reduce prolactin secretions (Strickland et al., 2011). Prolactin has been associated with mammary gland development and lactation initiation. Activation of the dopamine  $D^2$  receptor by ergovaline may explain why reduced milk production is commonly observed in cows consuming ergot alkaloids. It was observed in lactating cows that animals consuming endophyte infected tall fescue had a 45-60% decrease in milk production and similar results have been concluded in other studies (Schmidt & Osborn, 1993). Another study by Bernard et al. (1993)

found that cows exposed to endophyte infected tall fescue had reduced prolactin concentrations by 30% preceding parturition. The quantity of milk was not affected; however, concentrations of milk constituents such as fat decreased. Although decreased prolactin has not been shown to directly affect milk quantity, it is still of interest for its involvement in milk production as well as the role of other fescue toxicosis effects such as reduced vascularity, heat stress, and a decrease in feed intake on milk supply.

Prolactin also has a role in the immune response and is considered to be a mediator of the immune-neuroendocrine network. There has been increased interest in exploring the role of prolactin as a cytokine which mediates immune response. Interestingly, prolactin has been found to be synthesized and secreted by lymphocytes in mammals such as mice. It has also been found that the prolactin receptor is found to be ever-present on hematopoietic cells, stem cells that develop into other blood cells, which suggests involvement of prolactin in the regulation of proliferation, differentiation, and/or apoptosis of vital immune cells and ultimately function (Yu-Lee, 1997). Previous studies have reported that dopamine receptor agonists suppress natural killer cell activity in a way that demonstrates prolactin dependence after ergot alkaloids were consumed (Cross, 2003). Prolactin is thought to be stimulatory of the immune system. A study by Hiestand et. al (1986) found that administration of bromocriptine, an ergoline derivative and dopamine agonist, decreased lymphocyte responsiveness in vivo as well as in vitro after antigenic stimulation in rodents. Stimulation of prolactin also seemed to reverse the immunosuppressive effects of bromocriptine administration. Similarly, it has also been demonstrated that hypophysectomized rats had a compromised humoral and cell-mediated immunity but was restored with prolactin reintroduction. Prolactin irregularities have also been reported for auto-immune disorders and may play a role in the autoimmune system (Reber, 1993).

Prolactin and its receptor are also known to participate in the regulation of hair growth. Hair growth is regulated by an array of hormones including estrogen, testosterone, prolactin, and growth hormone. It has been shown that prolactin is involved in pelage replacement in a variety of mammals during seasonal changes. Cattle produce hair seasonally and a decreasing daylength stimulates growth of the winter hair coat and increasing daylength produces the short summer hair coat. These seasonal photoperiod changes affect the circulating hormones such as melatonin and prolactin inversely. When prolactin is high, the growth of the summer coat is initiated, while low concentrations of circulating prolactin are associated with growth of a dense winter coat (Dicks et al., 1994). Duncan and Goldman (1984) demonstrated that administration of bromoergocryptine to suppress prolactin substantially inhibited the spring molt of Djungarian hamsters. In a study by Dicks et al. (1994), it was found that prolactin secretion is involved in the molting of cashmere goats. An increase in prolactin, which usually occurs in the spring played a role in the reactivation of primary and secondary hair follicles. These findings further suggest the importance of circulating prolactin for maintenance of appropriate hair growth and shedding in cattle.

Fescue toxicosis also affects the vascular system, resulting in vasoconstriction and reduced blood flow to the extremities. Vasoconstriction is the narrowing of blood vessels as a result of constricting muscles of the vessel wall which limits the flow of blood. This process helps to regulate blood pressure, body temperature, and other bodily functions.

Ergot alkaloid interaction and persistent stimulation of adrenergic receptors result in continual constriction of the vascular system seen in fescue toxicosis (Klotz, 2015). Once ergot alkaloids such as ergovaline bind to the biogenic amine receptors on smooth muscle of the vascular system, it will act as an agonist and stimulate a vasoconstrictive response. These circumstances of vasoconstriction and limited blood flow are what lead to issues such as fescue foot. Fescue foot is

the occurrence of lameness, loss of ear tips, tail switch, and necrosis of feet and hooves due to endophyte-infected tall fescue consumption. It occurs primarily during the winter months when temperatures have decreased (Schmidt & Osborn, 1993). The reduced peripheral blood flow associated with fescue toxicosis results in an inability to properly regulate body heat. Core body temperatures remain elevated as efficiency of surface heat dissipation is reduced (Strickland et al., 2009). Vasoconstriction at the extremities has been attributed to the occurrence of fescue foot and thrombosis. Previous studies using post-mortem exams (Jensen et al., 1956) determined that reduced blood flow and vasoconstriction were responsible for the condition. Vasoconstriction, thickening of the medial layer of blood vessels, ischemia, endothelial damage, thrombosis, and gangrene are all symptoms attributed to the vasoconstrictive aspects of fescue toxicosis. Hyperplasia of the smooth muscle is the source of the observed thickened medial blood vessel layer. Klotz and McDowell (2017) describe hemodynamic responses using color Doppler ultrasonography in heifers treated with endophyte infected tall fescue seed compared to those on a non-toxic variety. The endophyte treated group showed vasoconstriction on the tail artery within 4 hours of initial consumption. Klotz and McDowell (2017) detected a 20% decrease in heart rate in the endophyte infected heifers when compared to the control group and stroke volume of the caudal artery was reduced from the baseline measurements. The combined effects of caudal artery vasoconstriction and reduced heart rate contributed to the 55% reduction seen in blood flow rate. Vasoconstriction has also been reported to affect the uterine and ovarian blood vessels in cattle consuming ergot alkaloids (Poole et. al., 2018). The diameter of ovarian veins and arteries showed a significant decrease for days 10 and 17 of the estrus cycle in ergot alkaloid exposed animals. This finding suggests that constriction of the ovarian vasculature could have an impact on ovarian and uterine hormone communication thereby affecting reproductive regulation. Interestingly, this

long-term stimulation of receptors on the vascular tissues can also reduce receptor sensitivity and restricts the target receptor from its designated response (Klotz, 2015).

The vasoconstrictive responses due to ergot alkaloid toxicity also contributes to thermal stress. Vasoconstriction decreases blood flow to the peripheral tissues and leads to reduced ability to dissipate heat at the body's surface (Strickland et al., 2009). The transport of blood to the peripheral tissues is a necessary process for efficient thermoregulation through evaporative cooling. This inability to dissipate body heat perpetuates the heat stress seen in animals suffering from fescue toxicosis. This symptom in conjunction with hypoprolactemia and retention of a rough, winter hair coat can disrupt normal thermoregulation and lead to symptoms such as reduced average daily gain, elevated rectal temperatures, salivation, and elevated respiration (Klotz, 2015). Symptoms related to heat stress and fescue toxicosis are often interconnected in a way that is difficult to distinguish. This leads to often confounding results because of the close interactions observed. Browning, Jr. & Leite-Browning (1997) induced fescue toxicosis by injecting ergot alkaloids into beef heifers to study performance. The heifers received all treatments, one for every week of the trial, and were administered ergotamine tartrate, ergonovine maleate, or saline as a vehicle control. Heifers were observed to have decreased skin temperatures, heart rate, and circulating prolactin concentrations when receiving the ergot alkaloid treatments compared to controls. Heifers also exhibited increased respiration rates as well as decreased blood pressure compared to controls.

As previously discussed, hypoprolactemia results in the retention of a rough, winter hair coat instead of the animal efficiently sloughing off excess hair for warmer summer months. Most of the rough hair coat is retained from the winter coat however some of it is newly emerged during the summer and grows even more in length. Extraneous hair then acts to insulate the elevated

internal body temperature of these animals already experiencing the effects of vasoconstriction. This disruption of the hair follicle due to low prolactin plays a crucial role in the exacerbation of heat stress for livestock grazing endophyte-infected fescue during the summer months when ambient temperature and humidity rise (Aiken et al. 2011).

Warmer weather often intensifies signs of fescue toxicosis and the combined stressors inducing hyperthermia in these animals cause observable behavioral changes as well. Cattle that experience heat stress are often found seeking shade in an effort to cool. Cattle may spend more time wading into water or mud if available and have a poor appetite. Schmidt and Osborn (1993) found that steers spent less time grazing and were more likely to graze at night when consuming a high-endophyte diet compared to steers consuming a low-endophyte diet. The complex relationship seen between fescue toxicosis and heat stress are what lead to behavioral symptoms and results in an overall reduced average daily gain. The reduction in average daily gain observed in cattle is a result from an overall decrease in feed intake (Schmidt & Osborn, 1993), and potentially an increase in water intake. In a study conducted by Hoveland et al. (1983), cattle grazing 94% endophyte infected pastures had an average daily gain (ADG) of 0.50 kg compared to an ADG of 0.83 kg in cattle grazing 5% endophyte-infected pastures. Those cattle grazing the 94% infected pasture also had greater rectal temperatures (+0.8 C) and visibly rougher hair coats than steers on low-endophyte pasture.

Cross (2003) summarizes multiple reports of cattle average daily gains when consuming ergot alkaloids and found a 44% mean reduction of ADG for steers consuming endophyte-infected tall fescue compared to those grazing non-infected pastures. Since the majority of tall fescue pastures are grazed by postpartum cows, Cross also measured the mean ADG for beef cows. Cows grazing endophyte free pastures had a mean ADG of 0.25 kg/cow/day while endophyte-infected

cows had a mean ADG of just 0.02 kg/cow/day. This demonstrates how additional stressors such as ergot alkaloids on the taxing period of lactation can negatively impact gains which subsequently can affect the calves of those dams.

Another major concern for animals suffering from fescue toxicosis is reproductive success. Reproduction is a vital aspect of any cow-calf operation in order to maintain profitability and production. Reports of reduced reproductive performance due to endophyte-infected tall fescue has been noted; however, there is a complex network of hormones, organs, and other variables such as age, genetics, environment, and nutrition that play a role. Other factors include management procedure, length of exposure to the ergot alkaloids, and the amount of ergot alkaloid being consumed. Reproductive tissues such as the ovary, corpus luteum, embryo, or fat stores may be vitally impacted. Ergot alkaloids may influence the hypothalamus, pituitary, and pineal glands, impacting the functionality of reproductive processes. Reproductive success can be compromised at the cellular or molecular level as well therefore all these factors produce a difficult subject of study (Strickland et. al., 2011).

Pregnancy rates can be compromised in cattle by heat stress alone so the complex effects of ergot alkaloids can have direct and indirect effects on reproduction. Factors such as hair coat retention and vasoconstriction lead to additional heat stress and reduced feed intake may lead to nutritional stress; therefore, these can then contribute to pregnancy loss and decreased productivity. Reduced pregnancy rates with fescue toxicosis has been an area of interest; however, published studies have shown varying results. Many studies have reported no differences in pregnancy rates between cattle exposed to ergot alkaloids compared to non-toxic diets (Drewnowski et al., 2009; Burke et al., 2001.) There is still concern surrounding the occurrence of pregnancy loss, conception rates, and even time of puberty for cattle exposed to ergot alkaloids.

Interestingly, it has been speculated that heifers are more susceptible to ergot alkaloids due to the overlapping growth demands (Burke and Rorie, 2001.)

Studies have found that many important hormones associated with reproductive success are reduced in animals suffering from fescue toxicosis. Concentrations of circulating prolactin, progesterone, estrogen, follicle stimulating hormone, and luteinizing hormone have been shown to be altered due to fescue toxicosis (Strickland et al., 2011). It is interesting to note that ergot alkaloid exposed cattle have shown to have reduced concentrations of serum cholesterol, a precursor to steroid hormones such as progesterone. The specific mechanisms for reduced reproductive hormones is still not fully understood (Strickland et al., 2011).

Decreased prolactin concentrations in cattle with fescue toxicosis also impacts reproductive processes. As previously mentioned, prolactin has been shown to affect lactation and overall gain performance which are involved in reproductive success; however, the extent of prolactin involvement remains unknown. While prolactin plays a role in gonadotropin release in sheep and mares, direct evidence of this mechanism has not been found in cattle. However, it has been found that cattle possess prolactin receptors on the corpus luteum and granulosa cells which indicates some possible role in folliculogenesis and reproduction (Strickland et al. 2011).

Progesterone, which is a hormone responsible for the establishment and maintenance of pregnancy, is also reported to be decreased in cattle exposed to endophyte-infected tall fescue. Plasma progesterone concentrations have been found to be greater during the mid-to-late luteal phase before ovulation in heifers on an endophyte free diet compared to those on an endophyte infected diet. Reduced concentrations of progesterone may participate in failure to maintain pregnancy after day 12 of pregnancy recognition (Jones et al., 2003). The secretion of progesterone by corpora lutea in vitro regardless of endophyte treatment suggests that perhaps in vivo decreases

of progesterone concentrations are caused by vasoconstriction of the surrounding reproductive tract and subsequently limiting circulation in the peripheral blood (Jones et al., 2003; Poole et al., 2018). Ergot alkaloid exposed heifers had reduced ovarian and uterine artery area compared to those that were not exposed however blood pressure was not affected. This reduction in blood flow without an increase in blood pressure may indicate a decrease in necessary movement of nutrients or steroid precursors leading to reproductive insufficiency (Poole et al., 2018). These observations support the theory of decreased hormone circulation due to ovarian vasoconstriction.

Environmental conditions have been shown to have an effect on reproductive measures in cattle exposed to ergot alkaloids. Burke et al. (2001) conducted an experiment in which heifers received a diet consisting of endophyte-infected fescue seed or endophyte-free fescue seed in combination with heat stress or thermoneutral conditions. Heat stressed heifers on endophyte infected tall fescue seed had a decreased in average corpus luteum diameter and serum progesterone levels compared to those in thermoneutral conditions. This reduction of serum progesterone was seen in heifers on non-toxic diets under heat stress but was more prominent in the heifers also receiving the infected seed diet. Heat stressed heifers on the endophyte-infected diet also had reduced concentrations of prolactin; however, thermoneutral heifers on the endophyte diet did not. Both the heat-stressed and endophyte-infected treatment groups of heifers displayed a decrease in total cholesterol as well. There was also an association with reduced preovulatory follicle diameter when heat stress and infected seed diet were treated simultaneously. Both heat stress treatments and endophyte-infected diets resulted in decreased serum estradiol and consumption of endophyte-infected seed caused a reduction in large follicles during the estrous cycle of those heifers. These findings further suggest the role that environmental conditions and additional stressors have on reproductive processes in animals consuming ergot alkaloids. Further

research is necessary to grasp the full extent of the involvement of ergot alkaloids and reproductive processes, whether directly or indirectly and the complex interactions from other factors associated with compromised reproduction.

In cattle, a dominant genetic syndrome was observed that resulted in excessive hair, issues with thermoregulation, and lactation dysfunction. After further investigation by Littlejohn et al. (2014), it was determined that a mutation was the cause and that the excessively hairy, dysfunctional condition was caused by a mutation which disrupts the integrity of the structure of the prolactin hormone. Further genetic mapping identified a similar mutation which results in the slick coat of the thermotolerant Senepol cattle breed. Littlejohn et al. (2014) determined that the slick mutation was caused by a frameshift mutation due to a single base deletion that creates a stop codon prematurely of the prolactin receptor and is known as the PRLR p.Leu462 mutation.

While the Senepol was the first breed identified to possess this slick phenotype, the slick hair trait has been traced to the Carora breed. The gene has been found on chromosome 20 and results in the inheritance of a short, sleek, hair coat if the dominant allele is passed down. These genetics have been introduced to Holstein cows because it improves the thermoregulatory ability of cows that possess this trait. Slick cattle are able to regulate body heat better than cattle with a wild-type hair coat because animals with this coat are able to sweat more thereby improving conductive and convective cooling. Dikmen et al. (2008) showed that the slick hair phenotype improves thermoregulatory abilities in a group of Holstein cows. Vaginal temperatures of the wild-type control group experiencing indoor heat stress were above 39°C which is considered greater than the thermoneutral zone for lactating cows. The outdoor heat stress group was also seen to have increased vaginal and surface temperature, respiration rate, sweating rate compared to controls. The slick cows in either environment could regulate their body temperature better than

the wild-type group (Dikmen et al. 2008). Similarly, Dikmen et al. (2014) conducted an additional study in which cows possessing the slick genotype had decreased vaginal temperatures than wild-type cows and had less of an increase in rectal temperatures or respiration rate. As seen previously, slick cattle also had a greater sweating rate than the wildtype counterparts.

### **The Immune System**

The immune system is a complex network of cells and molecules which defend the body from identified foreign material such as viral infection. This network is regulated by two response systems, the innate (natural) immune response and the acquired (adaptive) immune response. The innate response enforces the same defense mechanisms with the same methods every time an infectious agent is detected. The acquired immune response however is capable of improving its response to any given identified infection upon repeated exposure (Mackey et al., 2000). The immune system consists of lymphocytes which are the white blood cells that are produced from the bone marrow and can be found within the blood and lymphatic tissues. Lymphocytes are the body's main immune cells and work cohesively to defend the body from foreign substances. Lymphocytes can be classified into types based on site of maturation such B lymphocytes and T lymphocytes which can be further be classified into many varieties of immune cells which may have regulatory, cytotoxic, or roles for recognition. Cells and molecules of the immune system enter the bloodstream and can enter tissues by penetrating the capillary walls (Mackey et al., 2000; Jerne, 1973).

The innate immune system works quickly to identify and eliminate any threats to the body throughout a normal day such as injury. If a pathogen does cross the first barrier of defense, phagocytic lymphatic cells such as macrophages or neutrophils act to recognize, ingest, and destroy pathogens they encounter. Macrophages are large mononuclear phagocytic cells which are

found in most tissues of the body are play a key role in host defense. When macrophages encounter a pathogen, its surface receptors detect and bind to them. This activates the macrophage to engulf the material and secrete signaling molecules known as cytokines. Cytokines are small proteins and respond once binding to specific receptors to affect the behavior of other cells. Macrophages also release chemokines which are a type of cytokine with chemoattractant properties which elicit movement of specific cells towards the site of chemokine production. Cells with chemokine receptors that are attracted including neutrophils, monocytes, neutrophils and effector cells to enter sites of infection from the bloodstream (Janeway et al., 2001).

Cytokines are a diverse group of molecules with differing origins, functions, and structures with many newly discovered cytokines emerging often. Cytokines may be classified functionally as anti-inflammatory or pro-inflammatory, but these proteins elicit a range of effects on various types of cells. Cytokines may act in an autocrine behavior in which they affect the cells that released them or in a paracrine behavior in which they affect other adjacent cells. Additionally, cytokines may even act in an endocrine manner which affects more distant cells but that is dependent on many factors such as blood circulation. Cytokines induce inflammation in which heat, pain, swelling, or redness may occur. This is accomplished by local dilation of blood capillaries which allows for the flow of more blood plasma into the connective tissue through the permeable endothelium meanwhile clotting downstream to ensure pathogens are not allowed to travel from the site (Parham, 2015). Therefore, cytokines are classed according to their structure into three major families. The hematopoietin family is involved in both the innate and adaptive immune system and includes many interleukins. The TNF family is also involved in both innate and adaptive immunity and includes some membrane bound components. Lastly is the chemokine family which as mentioned recruits and activates various effector cells (Janeway et al., 2001).

Pro-inflammatory cytokines elicit fever and inflammation in an effort to combat infection. Some symptoms of fescue toxicosis as well as the overall state of inflammation observed suggests that pro-inflammatory cytokines play a role and increase production. Some examples of pro-inflammatory cytokines include but are not limited to tumor necrosis factor alpha (TNF- $\alpha$ ), interferon alpha (IFN- $\alpha$ ), interferon gamma (IFN- $\gamma$ ), and interleukin 1 alpha (IL-1-F1) (Janeway et al., 2001). Neural cell adhesion molecule (NCAM) or cluster of differentiation (CD56) is a prototypic marker for Natural killer (NK) cells and thought to be involved in pathogenic recognition for the innate immune system. Pro-inflammatory cytokines IL-1 and TNF- $\alpha$  have been associated with the symptom of decreased serum prolactin. (Ziegler et al., 2017).

Chemokines are pro-inflammatory mediators that promote the movement of effector cells towards the sites of infection. Some chemokines include chemokine ligand 9 (CXCL- 9) also known as monokine induced by interferon  $\gamma$  (MIG), chemokine ligand 2 (CCL2), chemokine ligand 4 (CCL4), and chemokine ligand 10 or CXCL-10 (IP-10). Many of these are also secreted by natural killer cells and modulate the behavior of leukocyte movement (Ziegler et al., 2017). Chemokine ligand 2 is responsible for attracting monocytes and memory T cells (Daly & Rollins, 2003). Chemokine ligand 4 also participates in recruiting monocytes and immune cells however CCL4 promotes the release and production of pro-inflammatory cytokines including IL-1, IL-6, and tumor necrosis factors (Bishara, 2012).

Growth factors are proteins that mediate cell interactions and regulate survival, migration, proliferation, and differentiation. Cytokines and growth factors have similar functions and thus the terms are often used interchangeably. Some growth factors include fibroblast growth factor (FGF-1), basic fibroblast growth factor (bFGF), Insulin-like growth factor (IGF-1), and vascular endothelial growth factor (VEGF)-A which is responsible for contributing to the permeability of

the vasculature and is regarded as a vasodilator (Janeway et al., 2001). G Protein-Coupled Receptor Associated Sorting Protein (GPRASP)-1 is thought to be a ubiquitous tumor marker and regulates G-protein coupled receptors in cells (Zheng et al., 2012).

Anti-inflammatory cytokines function to inhibit major proinflammatory cytokines and prevent further inflammation. Anti-inflammatory and Pro-inflammatory cytokines act to balance inflammation induced by the immune system. Anti-inflammatory modulators assist in preventing excessive inflammatory response which would subsequently result in injury while pro-inflammatory factors ensure risk from infection and insufficient response is avoided (Opal & DePalo., 2000). Some interleukins considered to be anti-inflammatory include IL-2, IL-4, IL-13, IL-15, IL-21, and IL-1-F5. IL-4 affects a variety of cells and has been shown to inhibit LPS-induced formation of proinflammatory cytokines. It is important to note however, evidence has shown that some cytokines are multifunctional and are not limited to just one classification. For example, IFN alpha is considered to act both as pro-inflammatory as well as anti-inflammatory.

The adaptive immune system is activated once infection breaks past the innate system line of defense. Often, the adaptive immune response is delayed 4-7 days while the innate system carries out its role of protection. The adaptive immune response establishes a long-lasting effective defense mediated by memory to combat infection and establish immunity. It responds to the antigen with antigen specific lymphocytes and is mediated by clonal selection of those lymphocytes. Recruitment of antigen specific effector cells and memory cells specifically attack the infection and prevent re-infection from occurring (Janeway et al., 2001).

### **The Immune System and Fescue Toxicosis**

It has been hypothesized that ergot alkaloids have negative effects on the immune system resulting in an overall suppression of immune response and decreased responsiveness to

vaccinations. Thus, fescue toxicosis would increase risk to infection or disease and thus be very detrimental to a cattle operation. The implication of prolactin's role in immune regulation also raises questions about the extent of ergot alkaloid's role however limited evidence has been provided to support this theory. Dawe et al. (1997) found that consumption of endophyte infected fescue seed resulted in decreased serum antibody titers in rats compared to rats exposed to an endophyte-free diet. Another study conducted by Filipov et al. (1999) saw an increase in proinflammatory cytokine production in male mice treated with ergotamine compared to controls. Other factors such as the presence of alpha adrenoceptors on B cells are suggested to interact with ergot alkaloids. Decreased concentrations of prolactin have been shown to possibly be immunosuppressive and may be involved in autoimmunity (Dawe et al., 1997; Reber, 1993). Additionally, Schultze et al. (1999) demonstrated that consumption of ergot alkaloids in cattle over the course of two years resulted in a decrease in immunoglobulins alpha and gamma globulin fractions.

### **Mitigation Strategies**

Since fescue toxicosis leads to decreased feed intake and a reduced ADG (Cross, 2003), nutritional supplementation may improve growth performance and may mitigate some of the negative effects of ergot alkaloids. Other options to mitigate fescue toxicosis includes the introduction or dilution of other cultivars such as clover. This may help prevent the consumption of such heavy loads of toxin and thus remedy some of the negative effects observed (Roberts & Andrae, 2004). Dietary supplementation with high protein diets has been shown to alleviate some effects of fescue toxicosis. Forcherio et al. (1995) supplemented cows consuming endophyte infected tall fescue with energy and protein and found that cows with greater ruminal degraded protein produced more milk and their calves subsequently gained more weight than cows that had

more ruminal undegraded protein. Another study observed that steers that were grazing endophyte infected tall fescue had an increased ADG when supplemented with energy and protein compared to those in the control group. This however had no effect on performance feedlot ADG in these steers afterward (Elizalde et al., 1998).

Early efforts to alleviate fescue toxicosis involved removing the endophyte, but this proved to be insufficient because the plant lacked its desired persistence (Aiken & Strickland, 2013). The association between the toxicity of the plant and its agronomic traits was evident (Aiken & Strickland, 2013). Retaining the favorable characteristics of the plant while alleviating the symptoms associated with fescue toxicosis would be ideal. Once it was established that one of the primary alkaloids produced by the endophyte caused fescue toxicosis, modifications involving the removal of only the toxin producing alkaloid were of interest. This new strain would produce alkaloids to benefit the plant survivability but lack the ergot alkaloid metabolite that causes toxicity. This strain is now known as novel endophytes. Currently, a variety of novel tall fescue has been marketed and is commercially available as Jesup-Max-Q however pasture renovation has proved immensely difficult. Toxic endophyte infected pastures retain their tremendous persistence so transitioning to non-infected pastures is labor heavy and economically taxing on producers (Phillips & Aiken, 2009). Phillips & Aiken (2009) found that use of novel endophyte-infected tall fescue would result in improved growth in stocker cattle when compared to cattle grazing toxic fescue. Cattle consuming novel endophyte-infected tall fescue displayed a 47% greater average daily gain compared to cattle consuming toxic endophyte-infected tall fescue. However, costs for novel endophyte-infected tall fescue renovation on pastures would not see economic return for 3-7 years and persistence in the southern United States is still in question (Gunter & Beck, 2004). Efforts to assuage the complex issues found with endophyte-infected tall fescue pastures continue

to be researched from the agronomic perspective however an array of alternative management efforts and genetic selection is of interest as well (Phillips & Aiken, 2009).

### **High Mountain Disease**

High mountain disease, otherwise known as “brisket disease,” is a condition that commonly occurs in domestic cattle populations raised in high-altitude regions (>1524 m). High mountain disease leads to adverse changes to the vascular system much like fescue toxicosis. The tissue covering the parasternal muscles, which is the brisket, accumulates edematous fluid. This is caused by pulmonary arterial hypertrophy due to the hypoxic conditions of high elevation. Edema is caused by an increase of hydrostatic pressure caused by heart failure of the right ventricle and hypertension of the veins. Cattle may also present symptoms such as distention of the jugular vein and edema in the limbs, mandible, or ventral abdomen. The animals commonly show lethargy and reduced feed intake. The latter stages of high mountain disease result in malnourished, weak animals that have little movement (Holt & Callen, 2007).

High mountain disease was investigated in the early 1900s by George Glover and Isaac Newsome. Within a few years, Glover and Newsome came to the conclusion that altitude was the primary cause through “exhaustion of the heart”, and that lowland cattle moved to higher altitudes were more susceptible. Cattle were suggested to be acclimated slowly and bred with “native” highland bulls (reviewed by Rhodes, 2005). The specific area of cardiac failure was discovered later, and hypoxia was shown to constrict the arteries, cause medial hypertrophy, thicken the vascular adventitia, reduce the diameter of the pulmonary artery lumen, and shrink small pulmonary arteries and arterioles. These alterations of the vascular system then lead to the hypertension, hypertrophy of the right ventricle, congestive heart failure, and fatality of the animal. Animals suffering from high mountain disease are likely to have increased heart and respiratory

rates and a murmur may be detected if enlargement of the right ventricle has resulted in valve insufficiency. The heart becomes enlarged due to the hypertrophy and increased dilation of the right ventricle. Other clinical indications include a rise in hepatic enzymes such as aspartate transaminase or L-iditol dehydrenase. Animals usually have a normal blood count but any excessive fluid in the pleura cavity, pericardium, or peritoneum may have low protein or low cellularity (Holt & Callen, 2007). Hypoxia, inadequate oxygen at the tissue level, is a strong stimulus for pulmonary hypertension (Newman et. al., 2011). Pulmonary hypertension occurs as a result of the narrowing of the pulmonary arterioles due to hyperplasia. This narrowing causes hypertension and subsequently, right ventricular hypertrophy is of consequence. This may further develop into right ventricular congestive or dilatory cardiac failure. (Holt & Callen, 2007).

### **Pulmonary Arterial Pressure**

Pulmonary arterial pressure (PAP) measurement is one option that can be applied to pulmonary hypertension detection and diagnosis. Developing tools to explore susceptibility to high mountain disease would be valuable for the treatment and health of cattle being raised at high elevations. Pulmonary arterial pressure testing may also be a suitable approach for developing breeding strategies to select for cattle that have a greater tolerance to high altitude regions. Pulmonary arterial pressure testing is performed by inserting a catheter into the jugular vein and gently fed downwards. The end of the catheter is connected to an external pressure transducer and the catheter is advanced to the distal jugular vein and jugular pressure may be recorded. The catheter passed into the right atrium and pressure is measured again. Once through the right atrioventricular valve, ventricular pressure may be recorded. From the right ventricle, the catheter is moved through the pulmonary valve and into the pulmonary artery where the mean PAP measurement may be obtained once the pressure is stabilized. The mean PAP should be around

34-44 mmHg for animals kept at altitudes between 1524 to 1981 meters, 5000 to 6500 feet respectively. Pulmonary artery pressure measurements in the 48-213 mmHg range are indicative of cattle experiencing pulmonary artery hypertension (Holt & Callen, 2007).

Some factors that may influence PAP measurements and result in variation are age, breed, body condition, environment, genetics, and concurrent illness. Age is a component because animals less than 12 months of age are less predictable and display greater variation in PAP score. Test results are only reliable in young animals when the PAP measurement is high but does not test well for low measurements. Pulmonary artery pressure testing in older animals have more consistent and accurate results. Breed also plays a role in PAP testing variability. Some breeds shown increased resistance to the effects of high mountain disease although no breed is completely tolerant. This finding may be the effects of specific genetic aspects of the different breeds (Holt & Callen, 2007). Environmental factors such as temperature also affect PAP testing. Will et al. (1978) found that cold winter conditions resulted in pulmonary hypertension at a mere altitude of 1,524 m. Similarly, placement of calves into temperature-controlled hypobaric chambers at 3,048 m or 1,524 m concluded that temperature and altitude interact to potentiate vasoconstriction of the pulmonary artery and veins (Busch et al., 1985). Concurrent illness is also of concern for variation in PAP measurement. Since PAP testing only measures pulmonary blood resistance at the time of the procedure, any cause including temporary illness may alter results. Many conditions such as asthma, intestinal parasites, lung worm, or respiratory diseases such as bovine viral diarrhea (BVDV) or infectious bovine rhinotracheitis (IBR) may predispose animals to developing pulmonary hypertension. Suspected or known illness should be accommodated and animals should be retested once symptoms have resided. It is important to note that these animals may still be of greater risk for high mountain disease even after recovery. High elevations can exacerbate

pulmonary disease and reintroduction to high altitude conditions put cattle at high risk to develop High Mountain Disease (Holt & Callen, 2007; Angel & Tyler, 1992).

Recent studies have shown evidence that cattle susceptible to pulmonary hypertension due to hypoxic conditions is heritable. A study from Newman et al. (2011) analyzed DNA and RNA from cattle suffering severe pulmonary hypertension and those with normal measurements. After a 10K bovine SNP array, four possible genes of interest were identified which were related to human pulmonary arterial hypertension and located proximal to SNPs of animals with severe cases of pulmonary hypertension and pulmonary arterial hypertension resistant cattle from the herd. NADH dehydrogenase (ubiquinone) flavoprotein 2 (NDUFV), myosin heavy chain 15 (MYTH15) and myocardial signaling protein (FKBP1A) were genes identified.

Another area of interest applicable to a PAP based breeding program raises the question of possible effects to growth performance traits. Selection for high altitude tolerance needs to comply with current growth standards for a productive cattle operation. Shirley et al. (2008) concluded that PAP measurements were moderately heritable, but the genetic correlation found directly affected growth unfavorably. It was concluded that a selection for growth at low altitude would result in elevated susceptibility to high altitude disease. However, Crawford et al. (2016) saw that PAP measurement had weak genetic correlations with growth performance of yearling Angus bulls. PAPs were weakly correlated with direct and maternal birth weight, yearling weight, and postweaning gain, which suggests that selection for PAP measurements should have limited influence on the growth performance of young cattle.

Interestingly, recent studies have explored that measuring tolerance for high altitude disease through PAP testing may be more beneficial at lower altitudes (<1,600m). Pauling et al. (2018) found that PAP testing at high elevation is not absolutely genetically correlated. Instead,

measurements collected at an altitude of 1,219 to 1,600 m could be useful in finding a correlated trait from a multi-trait evaluation. This contradicts the previous conclusions by Holt and Callen (2007) which stated selection for PAP as a predictor for susceptibility for pulmonary hypertension should be conducted at elevations  $>1,600$  m. Pauling et al. (2018) found that PAP measurements from high elevation and moderate elevation are both moderately heritable however using a bivariate model to predict sire at high elevation PAP supported the use of lower elevations for observations. Based on these findings, PAP observations performed at  $<1,600$ m should be considered a useful tool for indication of risk for animals developing pulmonary hypertension and ultimately high mountain disease.

It can be concluded that cattle raised at high elevations with low PAP measurements may prove acceptable for breeding a more tolerant herd at high elevation. Any cattle that has been tested and report a PAP measurement of 49 mmHg or greater should be monitored and are likely to experience high mountain disease. These animals should not be considered for breeding programs or be maintained in the herd. Currently, there is much variation in PAP testing due to the complex environmental, physiological, and genetic discrepancies that result in the individuality of response in cattle. Research identifying genetic markers and heritability will be useful for managing the prevalence of HMD in cattle at high elevations (Holt & Callen, 2007). Due to the changes in the vascular system as a result of ergot alkaloid exposure, developing a better understanding of how PAP changes in response to ergot alkaloid exposure may provide insight into a feasible method to identify and select cattle tolerant to fescue toxicosis.

## **Conclusions**

The effects of ergot alkaloids can be summarized as a complex, multifaceted issue that interacts with a multitude of physiological variables within the body. This complexity has resulted

in an incomplete understanding of the severity and specific pathway in which ergot alkaloids negatively affect livestock animals that are exposed. Fescue toxicosis negatively affects the beef cattle production systems by compromising immunity, reproduction, and overall performance. The role that ergot alkaloids play on immunity and response from various cytokines and chemokines is still not widely understood and thus further exploration may shed insight on the full extent that fescue toxicosis may have on that system. The occurrence of high mountain disease also warrants further investigation in an effort to alleviate issues with cattle raised at high elevations. The identification of susceptible cattle to pulmonary hypertension would prove useful to implement breeding protocols and avert the impacts that high mountain disease can cause to an operation's productivity and profitability. Thus, the focus of this thesis research was to explore the effects of endophyte infected tall fescue consumption on the performance and immune response in beef cattle. Additionally, this research examines the relationship between pulmonary arterial pressure and cattle susceptibility or resistance to fescue toxicosis as a means of consideration for selection in management protocols.

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## **Chapter 2: Changes of Cytokine Profiles in Response to Ergovaline Exposure and Protein Supplementation in Steers**

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## Abstract

The consumption of endophyte-infected tall fescue is known to adversely affect the health and performance of cattle. Previous studies have shown that protein supplementation can reduce some of the negative effects of fescue toxicosis in weaned steers. The aim of this study was to evaluate the effect of protein supplementation on cytokine response in steers exposed to 185  $\mu$ g/kg ergovaline, the primary toxin produced by endophyte-infected tall fescue. Thirty-two Angus steers were fed a total mixed ration (TMR) and randomly assigned to receive one of four treatments for 56 days in and assigned in a 2 X 2 factorial. Treatment groups were endophyte-free seed and 14% CP (**EF-14**; n=8), endophyte-free seed and 18% CP (**EF-18**; n=8), endophyte-infected seed and 14% CP (**EI-14**; n=8), and endophyte-infected seed and 18% CP (**EI-18**; n=8) respectively. Steers were vaccinated against infectious bovine rhinotracheitis (IBR) and bovine viral diarrhea virus (BVDV) types 1 and 2 on day 28 of the trial and then received a booster on day 42. Blood samples were taken for cytokine analysis on days 0, 28, 31 (72 hr post-vaccine), and 42. Serum cytokine concentrations were evaluated using Quantibody® Bovine Cytokine Arrays. Data were analyzed using PROC MIXED of SAS with repeated measures. Statistical significance was determined at  $P < 0.05$  and a tendency at  $0.05 < P < 0.10$ . Interestingly, concentrations of pro-inflammatory cytokines (TNF- $\alpha$ , IFN- $\alpha$ , IFN- $\gamma$ , IL-1 $\alpha$ ), chemokines (CCL2, CCL4, MIG), anti-inflammatory cytokines (IL-2, IL-13, IL-15, IL-21, IL-36), and multiple growth factors (FGF-1, IGF-1, VEGF-A) were greater in EI steers compared to EF steers ( $P < 0.05$ ). Furthermore, steers from the EI-14 group had greater concentrations of VEGF-A and IGF-1 on day 28 of the trial when compared to all other steer groups ( $P < 0.05$ ). Overall, these data demonstrate that ergovaline exposure increased cytokines and chemokines within these animals to induce a chronic immune response.

## **Introduction**

Tall Fescue (*Lolium arundinaceum* [Schreb.] Darbysh.) is a popular forage utilized in the Southeastern portion of the United States. Its prevalence in pasture systems can be attributed to the favorable qualities which make it appealing for grazing animals such as cattle. Its persistence, high yield, pest resistance, and survivability is attributed to the symbiotic relationship it has with an endophytic fungus (Blodgett, 2001). This endophyte produces ergot alkaloids which allow for the agronomic characteristics but also produces toxins which has adverse effects on livestock that graze it. Animals that consume ergot alkaloids develop a syndrome known as fescue toxicosis. A multitude of symptoms manifest from this condition including hypoprolactemia, retention of the winter hair coat, reduced average daily gain, compromised reproduction, and vasoconstriction (Strickland et al., 2009). These symptoms provide for complex physiological interactions with the toxin and attribute to poor animal performance and economic losses. Fescue toxicosis may have a major influence on the immune system by increasing cytokine secretion and compromising immune response. Previous studies have shown that protein supplementation may alleviate some of the performance loss due to reduced feed intake seen in cattle exposed to ergot alkaloids. Furthermore, it has been previously shown that additional protein incorporation into the diet can decrease the response of pro-inflammatory cytokine secretion in beef heifers exposed to endotoxins (Kahl et al., 1997). Thus, the objective of this study is to examine the effects of ergovaline exposure and supplemental protein on changes to cytokine response.

## **Materials and Methods**

This study was conducted in Bahama, North Carolina at the Butner Beef Cattle Field Laboratory (BBFCL). All procedures involving animal use was approved by the North Carolina State University Institutional Animal Care and Use Committee (17-043-A).

## Animals and Experimental Design

Data collected on feed and steer performance was conducted from early May to the beginning of July 2017 (Figure 2.1). Angus steers (n=36) were weaned on April 3, 2017 (day -35) at about 6 months of age. The animals were allowed ad libitum access to non-toxic fescue hay on a dry lot until the trial commenced. Steers were then grouped by weight ( $196.1 \pm 3.6$  kg) and assigned to one of twelve pens randomly. Each pen was assigned 3 steers respectively and randomly assigned to receive a diet consisting of endophyte-infected fescue seed (EI; treatment, 185  $\mu$ g ergovaline/kg of BW) or non-infected “endophyte-free” fescue seed (EF; control, 0  $\mu$ g ergovaline/kg of BW). The pens were then assigned randomly to receive protein supplementation (18% CP, soybean meal) or no protein supplementation (14% CP, 100% NRC requirements) in their total mixed ration. This resulted in the following four treatment assignments for individuals: endophyte-free 14% CP (**EF-14**; n = 9), endophyte-free 18% CP (**EF-18**; n = 9), endophyte-infected 14% CP (**EI-14**; n = 9), and endophyte-infected 18% CP (**EI-18**; n = 9).

Measurements taken weekly included body weight (BW), body condition score (BCS; scale of 1 to 9; adapted from Whitman, 1975), heart rate, caudal blood pressure, respiration rate, caudal artery and vein diameter, hematocrit, rectal temperatures, and surface temperature (Previously described in Poole et al., 2019). Hair coat shedding scores (HSS; adapted from Olsen et al., 2003) and hair coats scores (HCS; adapted from Olsen et al. 2003) were conducted by two personnel each week.

A thermal imaging camera (Fluke Ti45FT IR Flexcam, Fluke Corporation, Everett, WA) was used to collect surface temperature data weekly by taking images within a 18x20 cm square clipped of hair on the left shoulder. The highest, lowest, and average temperature measured were documented (SmartView 3.5 Thermal Imager Software; Eisemann et al., 2014; Poole et al., 2018).

Each week, heart rate and caudal blood pressure was measured by a 16-24cm blood pressure cuff (LifeSource A&D Engineering Inc., San Jose, CA) three times. Doppler ultrasonography (M-Turbo, SonoSite Inc, Bothell, WA) was used to measure caudal artery and vein diameter.

On day 14 of the feeding period, each steer was hair-clipped, anesthetized locally with 10 mL of 2% lidocaine and calibrated iButton temperature data loggers were inserted into the neck of each steer surgically. The data loggers were inserted under the skin and then sutured using a medical stapler. The temperature data loggers were surgically removed at the end of the trial on day 56.

Samples of blood were collected using a 10mL sterile vacutainer serum blood collection tube without additive (Vacutainer; Becton, Dickinson and Company, Franklin Lakes, NJ) from the jugular vein of the animal. The samples were placed on ice immediately and centrifuged later at  $1,500 \times g$  for 20 minutes at 4°C. The serum was then transferred into 5 ml polystyrene vials (BD Falcon, Franklin Lakes, NJ) and stored in a -80°C freezer for analysis.

All steers were vaccinated against bovine rhinotracheitis (IBR) and bovine viral diarrhea viruses (BVDV) types 1 and 2 on day 28. Steers also received 5 mL subcutaneous injection of Dectomax injectible (Zoetis Inc., Kalamazoo, MI) for internal and external parasite treatment, 2 mL subcutaneous injection of Bovi Shield One Shot (Zoetis Inc.) for vaccination against *Mannheimia haemolytica*, and a 2mL subcutaneous injection of Ultrabac 7 (Zoetis, Inc) for vaccination against *clostridium*. On day 42, two weeks later, each steer received a booster of 2mL subcutaneous injection of Bovi Shield Gold 5 (Zoetis Inc.) and Ultrabac 7 (Zoetis Inc.).

Each week, ambient temperature and humidity were documented throughout data collection and weather records were collected from the National Weather Service Henderson Oxford Airport station nearby BBCFL. Temperature-humidity index (THI, Buffington et al., 1977) was calculated

using the following formula:  $THI = T_{db} - [0.55 - (0.55 \times RH / 100)] \times (T_{db} - 58)$  In which  $T_{db}$  represents dry bulb temperature ( $^{\circ}F$ ) and RH represents relative humidity.

### **Animal Diet**

Animals from the 18% CP, protein supplementation group, received a total mixed ration (TMR) of 61.45% corn silage, 20.5% soybean meal, 13.75% fescue seed (EF or EI), 3.8% commodity pellets, 0.3% trace mineral salt, and 0.2% limestone on a dry matter basis. Animals from the 14%, not supplemented additional protein group, received a diet of 61.45% corn silage, 11.75% soybean meal, 13.75% fescue seed (EF or EI), 8.75% ground corn, 3.8% commodity pellets, 0.3% trace mineral salt, and 0.2% limestone on a dry matter basis. The EI diet consisted of Piedmont Tall Fescue seed (Southern States Cooperative, Richmond, VA) targeted to reach a dietary level of 500  $\mu g/kg$  of total ergot alkaloid content. The EF diet consisted of Cajun II Tall Fescue seed (King's Agri Seed Inc., Ronks, PA) which contained a level of 0  $\mu g/kg$  total ergot alkaloid (Agrinostics, Ltd., Watkinsville, GA). Post hoc analysis revealed that EI diet contained 185  $\mu g/kg$  ergovaline and the EF diet contained 0  $\mu g/kg$  ergovaline (MU Veterinary Medical Diagnostic Lab; Rottinghaus, 1993). These diets were formulated to adhere to the National Research Council (1996) requirements for 1 kg/d ADG at a feed intake of 2.35% of body weight to prevent refusals. Feed was adjusted each week according to average pen body weight and silage dry matter. Fresh water was provided to pens daily ad libitum. Every 28 days, samples of feed from each treatment were analyzed for nutrient composition (Cumberland Valley Analytical Services, Waynesboro, PA) and any refusals were weighed weekly to calculate an accurate dry matter intake.

### **Data Analysis: Animal Selection**

Before performance of serum cytokine concentration arrays, one steer from each treatment group was removed due to data logger incision infection resulting in a total of 32 animals remaining (n=32).

### **Cytokine Analysis**

Concentrations of serum cytokines were evaluated on days 0, 28 (vaccine), 31 (72 hr post-vaccine), and 42 (booster). Concentrations were analyzed through use of the Multiplex ELISA Bovine Cytokine Arrays Q1 and Q2 (Quantibody® Cytokine Arrays; RayBiotech, Inc.; Norcross, GA). Bovine Cytokine Array Q1 detects the following cytokines and chemokines: interferon alpha (IFN- $\alpha$ ), interferon gamma (IFN- $\gamma$ ), interleukin (IL)- 13, IL-1 $\alpha$ , IL-1-F5, IL-21, and tumor necrosis factor alpha (TNF-  $\alpha$ ), chemokine ligand 9 (CXCL)- 9 (MIG), CXCL-10 (IP-10), and chemokine ligand 4 (CCL4). Bovine Cytokine Array Q2 detects the following cytokines and chemokines: fibroblast growth factor (FGF)-1, basic FGF (bFGF), G Protein-Coupled Receptor Associated Sorting Protein (GPRASP)-1, Insulin-like growth factor (IGF)-1, IL-2, IL-4, IL-15, CCL2, cluster of differentiation (CD) 56, and vascular endothelial growth factor (VEGF)-A. Arrays were executed using the RayBiotech company's provided procedure. Serum samples were diluted 1:2 as suggested from manufacturer procedure. Sample slides were refrigerated at 4° C and blocked from light for storage. Samples were then shipped overnight on ice for laser scanning and extraction and absorbance values were recorded as 'F532 Medium – B532' by the manufacturer. Serum concentrations in pg/mL were determined in-house using Q-Analyzer Software for QAB-CYT (RayBiotech, Inc.; Norcross, GA).

## Statistical Analysis

Data were analyzed using the MIXED procedure of SAS 9.3 (SAS Inst. Inc., 1996) with repeated measures. Pen was the experimental unit and the model for steer performance included fescue treatment (EF vs. EI), protein (14% vs. 18%), time of sample collection, and any interactions. Results were recorded as least squares means  $\pm$  SEM with statistical significance reported at a  $P \leq 0.05$ . A tendency was reported at a  $P > 0.05$  and  $\leq 0.10$ . Terms with a significance value of  $P > 0.20$  were removed from the complete model in a stepwise manner to acquire the final reduced model for each of the variables.

## Results

Steers from the EI group had increased concentrations of pro-inflammatory cytokines such as TNF- $\alpha$  (1.756 vs.  $0.689 \pm 0.272$  ng/ml), IFN- $\alpha$  (1.027 vs.  $0.212 \pm 0.262$  ng/ml), IFN- $\gamma$  (0.347 vs.  $0.152 \pm 0.061$  ng/ml), and IL-1-F1 (0.319 vs.  $0.140 \pm 0.060$  ng/ml) when compared to steers from the EF treatment group ( $P < 0.05$ ). For the pro-inflammatory cytokine CD56, no differences were observed between treatment groups ( $P > 0.05$ ). It was also observed that steers from the EI group had increased concentrations of chemokines such as CCL2 (140.37 vs.  $42.10 \pm 23.25$  ng/ml), CCL4 (1.027 vs.  $0.212 \pm 0.262$  ng/ml), and MIG (1.413 vs.  $0.589 \pm 0.217$  ng/ml) when compared to steers from the EF group ( $P < 0.05$ ). There were no differences observed for the chemokine IP-10 ( $P > 0.05$ ). Steers from the EI group also had increased concentrations of anti-inflammatory cytokines such as IL-2 (14.79 vs.  $2.89 \pm 3.60$  ng/ml), IL-13 (0.917 vs.  $0.380 \pm 0.138$  ng/ml), IL-15 (3.56 vs.  $1.04 \pm 0.59$  ng/ml), IL-21 (1.74 vs.  $0.67 \pm 0.27$  ng/ml), and IL-1-F5 (0.464 vs.  $0.172 \pm 0.077$  ng/ml) when compared steers in the EF group ( $P < 0.05$ ). No differences were seen for anti-inflammatory cytokine IL-4 ( $P > 0.05$ ). EI steers had increased concentration of growth factor including FGF-1 (27.31 vs.  $13.53 \pm 4.52$  ng/ml), IGF-1 (4.62 vs.  $2.81 \pm 0.49$  ng/ml), and VEGF-

A ( $90.36$  vs.  $29.48 \pm 12.50$  pg/ml) when compared to EF steers ( $P < 0.05$ ). There was also a significant difference found for EI-14 steer which had increased concentrations of VEGF-A ( $231.9$  vs.  $52.5, 26.9, 99.0 \pm 30.1$  pg/ml) for EF-14, EF-18, and EI-18 and IGF-1 ( $10.3$  vs.  $5.8, 5.2, 6.5 \pm 1.2$  ng/ml for EF-14, EF-18, and EI-18) on day 28 of the trial when compared to other steer groups ( $P < 0.05$ ; Figure 2.2). There were no differences found on day 0 or 42 of the trial ( $P > 0.05$ ). A tendency was observed for EI steers to have greater concentrations of GPRASP-1 when compared to EF steers ( $2.87$  vs.  $2.64 \pm 0.09$  ng/ml). Furthermore, there was a fescue by protein interaction with EI-18 steers having greater concentrations of the growth factor FGF-2 when compared to EF-18 and EI-14 steer groups ( $P < 0.05$ ). No differences were observed when compared to EF-14 steers ( $P > 0.05$ ). Lastly, a decrease in CD56 concentrations was observed 72 hr post-vaccination in EF-14, EF-18, and EI-14 steers ( $P < 0.05$ ), however no differences were seen in EI-18 steers ( $P > 0.05$ ). A decrease in IGF-1 concentrations was also observed 72 hr post-vaccination in EI-14 steers ( $P < 0.05$ ), however no differences were seen in the other steer groups ( $P > 0.05$ ). No other differences were observed for any cytokines 72 hr post-vaccination ( $P > 0.05$ ). All steer cytokine data is summarized in Table 2.1.

## Discussion

In this study, the data demonstrates an elevated immune response in which pro-inflammatory and anti-inflammatory cytokines concentrations were increased in steers consuming endophyte-infected tall fescue. The pro-inflammatory cytokines TNF- $\alpha$ , IFN- $\alpha$ , IFN- $\gamma$ , and IL-1-F1 had increased concentrations in animals exposed to ergot-alkaloids compared to controls. These pro-inflammatory cytokines illicit fever and inflammation in efforts to stimulate the innate immune response and combat infection. In this study, the concentrations of pro-inflammatory chemokines were increased in steers consuming ergot alkaloids including CCL2, CCL4, and MIG. Chemokines

are pro-inflammatory mediators, often recruited and secreted by macrophages or even receiving the signal to be released by other pro-inflammatory cytokines. They recruit various effector cells to the site of infection (Janeway et al., 2001). Some of the inflammation, heat stress, or poor thermoregulation in cattle suffering from fescue toxicosis may be attributed to the pro-inflammatory immune response resulting in fever and inflammation. An increase in anti-inflammatory cytokines were also observed in cattle exposed to ergot alkaloids compared to controls. This is not unexpected because anti-inflammatory cytokines act to mediate pro-inflammatory immune response. Anti-inflammatory cytokines IL-2, IL-13, IL-15, IL-21, and IL-1-F5 were increased in steers consuming ergot alkaloids. Anti-inflammatory cytokines act to inhibit pro-inflammatory cytokines and keeping immune response in balance by modulating and preventing excessive inflammation (Opal & DePalo, 2000).

Steers receiving a diet of ergot alkaloids had an increased amount of GPRASP-1 as well. This is a protein that modulates lysosomal sorting and functional down regulation of G-protein coupled receptors. It is known to interact with many receptors including the dopamine D2 receptor. Studies have shown that these proteins interact with D2 receptors and limit the resensitization in order to down-regulate (Bartlett et al., 2005). This relationship with the D2 receptor may have an influence on circulating prolactin by limiting the activation of the receptor by ergot alkaloid interaction and subsequently preventing a decrease in circulating prolactin.

This study also found that steers consuming ergot alkaloids increased levels of growth factors in steers consuming ergot alkaloids including growth factors FGF-1, IGF-1, and VEGF-A. Growth factors have been associated with vasodilation and angiogenesis. It has been found that FGF-1 decreases blood pressure when administered intravenously and inducing dilation of pial arterioles by increasing nitric oxide (Rosenblatt et al., 1993). FGF has also been documented to

decrease blood pressure in rabbits and cause hypotension (Cuevas et al., 1991). On days 0 and 28 of the study, EI-14 steers had a increased concentration of VEGF-A and IGF-1 which align with previous reports involving elevated skin and body temperatures on this steer trial (Eisemann et al., 2014; Poole et al., 2018).

Overall, exposure to ergot alkaloids seems to have stimulated a prolonged innate immune response through recruitment of anti-inflammatory and pro-inflammatory cytokines, chemokines, growth factors, and associated modulators. It seems that steers fed to requirement and then supplemented with additional protein did seem to alleviate some aspects fescue toxicosis however the effects were minimal but may be useful in preventing weight or performance loss. Thus, increased concentrations of pro-inflammatory and anti-inflammatory cytokines seen in steers consuming ergot alkaloids appear to illicit a hyperactive immune response which can compromise overall immunity in beef steers

## TABLES

**Table 2.1:** Serum concentrations of cytokines (ng/ml unless otherwise specified) in beef steers consuming endophyte-infected (EI) or endophyte-free (EF) tall fescue with 14% crude protein (14) or 18% crude protein (18).

Item	Treatment <sup>1,2</sup>				SEM	Fescue	<i>P-value</i>	
	EF-14	EF-18	EI-14	EI-18			Protein	Interaction
<b>Pro-inflammatory</b>								
TNF- $\alpha$	0.793 <sub>a</sub>	0.583 <sub>a</sub>	1.802 <sub>b</sub>	1.710 <sub>b</sub>	0.379	0.007*	0.696	0.879
IFN- $\alpha$	0.155 <sub>a</sub>	0.269 <sub>a</sub>	0.872 <sub>b</sub>	1.182 <sub>b</sub>	0.364	0.030*	0.568	0.791
IFN- $\gamma$	0.183 <sub>ab</sub>	0.121 <sub>a</sub>	0.298 <sub>ab</sub>	0.396 <sub>b</sub>	0.084	0.025*	0.834	0.351
IL-1-F1 <sub>3</sub>	0.182 <sub>ab</sub>	0.097 <sub>a</sub>	0.360 <sub>b</sub>	0.278 <sub>ab</sub>	0.083	0.037*	0.326	0.983
CD56	25.21	28.36	23.33	22.04	2.63	0.130	0.728	0.411
<b>Chemokines</b>								
CCL2	48.14 <sub>a</sub>	36.07 <sub>a</sub>	186.78 <sub>b</sub>	93.96 <sub>a</sub>	32.31	0.004*	0.115	0.223
CCL4	0.106 <sub>a</sub>	0.146 <sub>ab</sub>	0.418 <sub>ab</sub>	0.477 <sub>b</sub>	0.151	0.040*	0.750	0.952
MIG	0.553 <sub>a</sub>	0.626 <sub>a</sub>	1.725 <sub>b</sub>	1.100 <sub>ab</sub>	0.302	0.009*	0.372	0.259
IP-10	0.666	0.470	0.691	0.690	0.095	0.206	0.309	0.315
<b>Anti-inflammatory</b>								
IL-2	2.80 <sub>a</sub>	3.00 <sub>a</sub>	8.80 <sub>a</sub>	20.77 <sub>b</sub>	5.00	0.022*	0.236	0.251
IL-4	4.02	0.32	0.90	0.33	2.03	0.454	0.306	0.452
IL-13	0.448 <sub>a</sub>	0.313 <sub>a</sub>	0.770 <sub>ab</sub>	1.064 <sub>b</sub>	0.192	0.008*	0.687	0.277
IL-15	0.844 <sub>a</sub>	1.229 <sub>a</sub>	4.792 <sub>b</sub>	2.334 <sub>a</sub>	0.819	0.003*	0.217	0.092 <sub>†</sub>

**Table 2.1** (continued)

IL-21	0.782 <sup>a</sup>	0.558 <sup>a</sup>	2.022 <sup>b</sup>	1.452 <sup>ab</sup>	0.369	0.006*	0.293	0.646
IL-1-F5 <sup>4</sup>	0.212 <sup>ab</sup>	0.132 <sup>a</sup>	0.460 <sup>b</sup>	0.469 <sup>b</sup>	0.108	0.009*	0.746	0.687
<b>Growth factors</b>								
FGF-1 <sup>5</sup>	11.25 <sup>a</sup>	15.82 <sup>ab</sup>	31.70 <sup>b</sup>	22.92 <sup>ab</sup>	6.29	0.034*	0.743	0.300
FGF-2 <sup>6,7</sup>	6.41 <sup>ab</sup>	3.10 <sup>a</sup>	3.38 <sup>a</sup>	8.44 <sup>b</sup>	1.56	0.480	0.590	0.012*
IGF-1	2.82 <sup>a</sup>	2.79 <sup>a</sup>	4.72 <sup>b</sup>	4.53 <sup>ab</sup>	0.69	0.011*	0.878	0.915
VEGF-A <sup>7</sup>	29.27 <sup>a</sup>	29.69 <sup>a</sup>	114.84 <sup>c</sup>	65.88 <sup>b</sup>	17.37	0.001*	0.174	0.166
GPRASP-1	2.67 <sup>ab</sup>	2.61 <sup>a</sup>	2.96 <sup>b</sup>	2.77 <sup>ab</sup>	0.15	0.093 <sup>†</sup>	0.358	0.629

<sup>a,b,c</sup> Within row, means without a common superscript significantly differ ( $P \leq 0.05$ )

<sup>1</sup> Values are reported as least square means for the experiment

<sup>2</sup> EF-14: endophyte-free 14% crude protein; EF-18: endophyte-free 18% crude protein; EI-14: endophyte-infected 14% crude protein; EI-18: endophyte-infected 18% crude protein

<sup>3</sup> Also known as interleukin (IL)-1 $\alpha$

<sup>4</sup> Also known as IL-36

<sup>5</sup> Also known as acidic fibroblast growth factor (a-FGF)

<sup>6</sup> Also known as basic fibroblast growth factor (b-FGF)

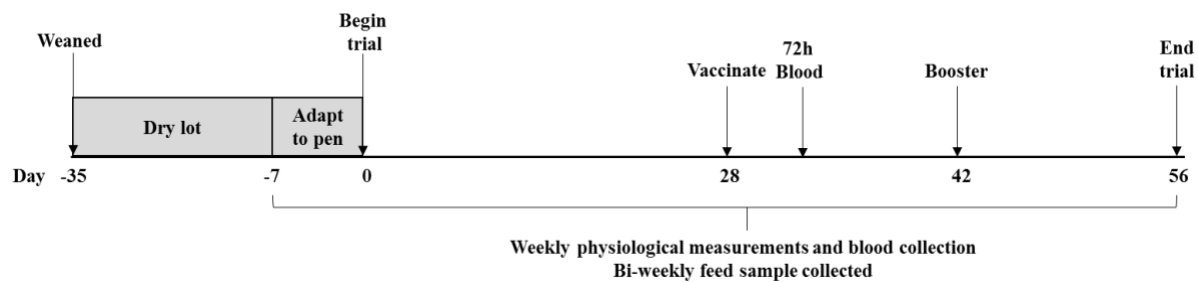
<sup>7</sup> Reported in pg/ml

\* $P$ -values  $\leq 0.05$  determined significant

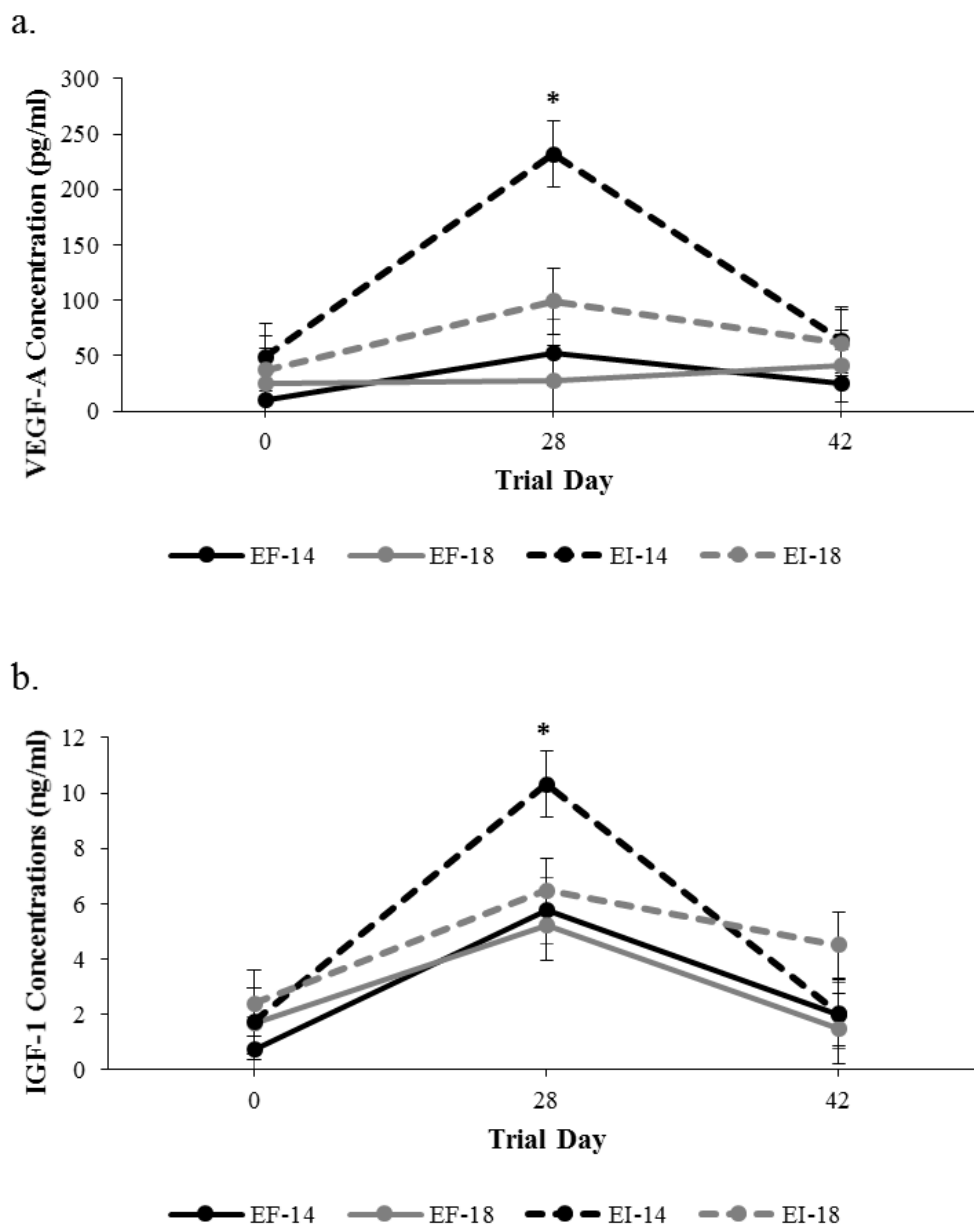
<sup>†</sup> $P$ -values  $0.05 > P \leq 0.10$  determined a statistical tendency

## FIGURES

**Figure 2.1:** Experimental timeline for steers consuming endophyte-infected (EI) or endophyte-free (EF) tall fescue with 14% crude protein (14) or 18% crude protein (18).



**Figure 2.2:** Vascular endothelial growth factor-A (VEGF-A) concentrations (a) and insulin-like growth factor (IGF)-1 concentrations (b) in steers consuming endophyte-free fescue seed with 14% CP (EF-14) or with 18% CP (EF-18) and endophyte-infected fescue seed with 14% CP (EI-14) or with 18% CP (EI-18). EI-14 steers had increased concentrations of VEGF-A (231.9 vs. 52.5, 26.9, 99.0  $\pm$  30.1 pg/ml) for EF-14, EF-18, and EI-18 and IGF-1 (10.3 vs. 5.8, 5.2, 6.5  $\pm$  1.2 ng/ml for EF-14, EF-18, and EI-18) on day 28 of the trial when compared to other steer groups ( $P < 0.05$ ). There were no differences found on day 0 or 42 of the trial ( $P > 0.05$ ).



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**Chapter 3: Changes in Cytokine Profiles in Response to Ergovaline Exposure  
in Slick vs. Normal Hair Coat in Beef Heifers**

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**Abstract**

Fescue toxicosis is a syndrome that commonly occurs in cattle grazing toxic endophyte infected tall fescue. Our lab has previously demonstrated that chronic exposure of ergot alkaloids adversely effects the innate immune response. Thus, the objective of this study was to examine changes in cytokine profiles in heat tolerant heifers exposed to ergot alkaloids from endophyte-infected tall fescue compared to wildtype controls. Angus X Senepol heifers (n=20) were blocked by weight and hair coat genotype, Slick (S) or Normal (N) then randomly fed novel endophyte fescue (EN) or endophyte-infected fescue (EI) haylage in a total mixed ration for 63d with n=5 animals in each treatment and hair coat genotype respectively. Weekly measurements were collected to monitor physiological responses during exposure to ergot alkaloids. Blood samples were collected for cytokine analysis on d 0, 14, 28 and 56. Cytokine concentrations were quantified using Quantibody® Bovine Cytokine Arrays. Data were analyzed using PROC MIXED of SAS with repeated measures. Statistical significance was determined at  $P < 0.05$  and a tendency at  $0.05 < P < 0.10$ . Heifers with the slick genotype had greater concentrations of pro-inflammatory cytokines (IFN-  $\alpha$ , IL-1-F1), anti-inflammatory cytokines (IL-2, IL-21), chemokines (CCL4), and growth factors (bFGF, GPRASP-1) when compared to heifers with the wildtype hair genotype ( $P < 0.05$ ). Interestingly, these findings contrast from previous data demonstrated by our lab. Exposure to ergovaline via endophyte-infected fescue did not induce an innate immune response, rather heifers with the slick hair genotype had elevated cytokine concentrations. Overall, developing a better understanding of the interaction between ergot alkaloids and the slick hair genotype will give insight to improve cattle health management.

## Introduction

The presence of Kentucky-31, an endophyte-infected variety of tall fescue, on much of the pasture of the southeast region of the United States is an ongoing issue for many cattle producers. Endophyte-infected tall fescue produces ergot alkaloids which are the causative agent of a multifaceted condition known as fescue toxicosis. The symptoms of fescue toxicosis lead to economic losses and poor animal performance. Some symptoms include but are not limited to increased heat stress, hypoprolactemia, reduced average daily gain, decreased reproductive success, and a compromised immune system (Strickland et al., 2009). Heat stress is a major component of the detrimental effects that fescue toxicosis has on cattle health. Vasoconstriction limits the ability to dissipate heat and reduced circulating prolactin leads to retention of the rough winter hair coat during the summer months (Strickland et al., 2009; Dicks et al., 1994). These symptoms work together to exacerbate heat stress in cattle experiencing fescue toxicosis.

Interestingly, a mutation on the prolactin receptor has been found to cause the slick haired trait seen in the Senepol breed of cattle (Littlejohn et al., 2014). Cattle that possess the slick hair trait are better able to regulate body heat than wildtype counterparts due to increased convective and conductive cooling as well as an increased sweat rate (Dikmen et al., 2008). Compromised immunity is another concern for cattle consuming endophyte-infected tall fescue. Previous research has suggested that fescue toxicosis compromises immunity and decreased prolactin has been shown to be immunosuppressive (Dawe et al., 1997; Schultze et al., 1999). A possible mitigation strategy to alleviate some of the detrimental stress on animals experiencing fescue toxicosis is the incorporation of the slick hair gene. The reduction of hair perpetuating heat stress as well as the possible effects of the prolactin mutation gene may prove beneficial to cattle experiencing the effects of ergot alkaloids. Thus, the aim of this study is to examine the effects of

ergot alkaloid exposure and its interaction with the slick hair genotype and resulting changes in cytokine response.

## **Materials and Methods**

This study was conducted in Bahama, North Carolina at the Butner Beef Cattle Field Laboratory (BBFCL). All procedures involving animal use was approved by the North Carolina State University Institutional Animal Care and Use Committee (13-093-A).

### **Animals and Experimental Design**

Thirty-one ( $n=31$ ) Angus X Senepol heifers were used for this study at BBCFL after being delivered from the Cherry Research Farm at the Center for Environmental Farming System in Goldsboro, North Carolina. These heifers were not previously exposed to endophyte infected tall fescue and were ranked by haircoat phenotype and weight and received a treatment group of either endophyte-infected fescue (EI;  $421\mu\text{g}$  ergovaline/kg of BW) or novel endophyte fescue (EN; control,  $36\mu\text{g}$  ergovaline/kg of BW) at random during a previous study (Eisemann et al., 2014; Poole et al., 2019). The heifers were previously assigned a haircoat phenotype (slick or wildtype) at birth. Animals were later genotyped as Slick (S, possesses mutation in PRLR gene) or wildtype (W) (Recombinetics, Inc. St. Paul, MN). Treatment groups were as follows: novel endophyte wildtype (**EN-W**;  $n = 7$ ), novel endophyte slick (**EN-S**;  $n = 8$ ), endophyte-infected wildtype (**EI-W**;  $n = 6$ ), and endophyte-infected slick (**EI-S**;  $n = 9$ ). A heifer from the EI-S group was later removed from the trial due to cystic ovaries.

Body weight (BW), body condition score (BCS; scale of 1 to 9 adapted from Whitman, 1975) were measured weekly. Surface temperature, rectal temperature, heart rate, caudal blood pressure, caudal artery and vein diameter, respiration rate, and hematocrit were also collected each week to assess physiological response. Two personnel recorded hair coat scores (HCS; adapted

from Olsen et al., 2003) as well as hair shedding score (HSS; on a scale of 1 [slick] to 5 [rough winter coat]; adapted from Gray et al., 2011) for a weekly average. A thermal imaging camera (Fluke Ti45FT IR Flexcam, Fluke Corporation, Everett, WA) was used to measure surface temperature. Images were taken from a 18x20 cm clipped square behind the left shoulder to record the highest, lowest, and average surface temperature (SmartView 3.5 Thermal Imager Software; Eisemann et al., 2014). Each week, caudal blood pressure and heart rate were measured using a 16-24 cm blood pressure cuff (Life Source A&D Engineering Inc., San Jose, CA) three times for each animal. Doppler ultrasonography (M-Turbo, SonoSite Inc, Bothell, WA) was used to measure caudal artery and vein diameter. Twenty heifers ( $n = 20$ ; EN-S:  $n=5$ , EN-W:  $n=5$ , EI-S:  $n=4$ , and EI-W:  $n=6$ ) also had internal temperatures recorded by attaching a calibrated iButton temperature data logger to a progesterone-free controlled internal drug release device (CIDR; Zoetis, Parsippany, NJ) and inserted vaginally. Internal temperature was recorded every 10 minutes for two 7-day intervals of the experimental trial and was blocked every two hours for data analysis. Blood samples were collected from the jugular vein into a 10mL sterile vacutainer serum blood collection tube without additive (Vacutainer; Becton, Dickinson and Company, Franklin Lakes, NJ.) The samples were placed on ice immediately and centrifuged later at  $1,500 \times g$  for 20 minutes at 4C. The serum was then transferred into 5 ml polystyrene vials (BD Falcon, Franklin Lakes, NJ) and stored in a -80C freezer for analysis.

Each week, ambient temperature and humidity were documented throughout data collection and weather records were collected from the National Weather Service Henderson Oxford Airport station nearby BBCFL. Temperature-humidity index (THI, Buffington et al., 1977) was calculated using the following formula:  $THI = T_{db} - [0.55 - (0.55 \times RH / 100) \times (T_{db} - 58)]$ , in which  $T_{db}$  represents dry bulb temperature ( $^{\circ}F$ ) and RH represents relative humidity.

## Animal Diet

Animals were fed a Total mixed ration (TMR) based on either Kentucky-31 Tall Fescue (EI, 851  $\mu\text{g}/\text{kg}$  total ergot alkaloid and 175  $\mu\text{g}/\text{kg}$  ergovaline) or non-toxic infected tall fescue (MaxQII Texoma, EN, 66  $\mu\text{g}/\text{kg}$  total ergot alkaloid and 60  $\mu\text{g}/\text{kg}$  ergovaline) haylage. Haylage was collected in Fall of 2014 and preserved in AG bags after processing. The TMR diet was formulated according to the National Research Council (1996) requirements for 0.9 kg/day ADG when limited to a dry matter intake of 2% of body weight (71% TDN and 14% CP). Dry matter intake was readjusted as heifers gained every two weeks. The TMR for the EI group consisted of 75% fescue haylage, 11.9% ground infected fescue seed (Piedmont Tall Fescue, Southern States Cooperative, Richmond, VA), 8% corn, 1.3% soybean meal, and 3.1% commodity pellet. On day 31, about halfway through the trial, a new stock of endophyte-infected fescue seed was used which had a decreased ergot alkaloid concentration. The EI diet was then reformulated to consist of 75% endophyte-infected haylage, 19% ground infected fescue seed, 4% corn, and 1% soybean meal. The endophyte novel group TMR diet consisted of 63% novel fescue haylage, 19% commodity pellet, 12% corn, and 5% soybean meal. Each diet also contained a vitamin mineral prefix, salt, and limestone to meet the requirements for heifers. Ad libitum access to fresh water was provided for the duration of the trial. In order to observe individual feed intake, animals used individual feeding gates (American Calan, Northwood, New Hampshire). To reach a dietary level of 1,000  $\mu\text{g}/\text{kg}$  of total ergot alkaloid, infected tall fescue seed was incorporated into the EI TMR. The EN TMR consisted of 40  $\mu\text{g}/\text{kg}$  total ergot alkaloid (Agrinostics, Ltd., Watkinsville, GA). Further analysis of the ergovaline content of fescue haylage and seed revealed that EI TMR contained 421  $\mu\text{g}/\text{kg}$  ergovaline, the EN TMR contained 36  $\mu\text{g}/\text{kg}$  ergovaline. Ergovaline was the only ergot alkaloid found in the analysis (MU Veterinary Medical Diagnostic Lab; Rottinghaus, 1993).

### **Data Analysis: Animal Selection**

Twenty heifers (n=20) were selected for cytokine concentration analysis. A cohort of animals (n=5) were selected from each treatment group to analyze cytokine profiles throughout the trial on days 0, 14, 28, and 56 of the study. The cohort of animals were chosen based on treatment group and were selected to accommodate cytokine array availability. Days 0, 14, 28, and 56 were selected for cytokine analysis so that a comprehensive cytokine profile could be examined. Day 0 was before exposure to ergot alkaloid treatments and thus serves as a preliminary measurement. Fescue toxicosis has been found to take around 21 days to develop clinical symptoms so day 28 displays a measurement after fescue toxicosis should have developed in the endophyte-infected treatment group. In order to examine any physiological compensation to alleviate immune response, day 56 was also studied. Treatment groups were as follows: novel endophyte wildtype (**EN-W**;  $n = 5$ ), novel endophyte slick (**EN-S**;  $n = 5$ ), endophyte-infected wildtype (**EI-W**;  $n = 5$ ), and endophyte-infected slick (**EI-S**;  $n = 5$ ).

### **Cytokine Analysis**

Concentrations of serum cytokines were evaluated on days 0, 14, 28, and 56. Concentrations were analyzed through use of the Multiplex ELISA Bovine Cytokine Arrays Q1 and Q2 (Quantibody® Cytokine Arrays; RayBiotech, Inc.; Norcross, GA). The Bovine Cytokine Array Q1 detects the following cytokines and chemokines: interferon alpha (IFN- $\alpha$ ), interferon gamma (IFN- $\gamma$ ), interleukin (IL)- 13, IL-1 $\alpha$ , IL-1-F5, IL-21, and tumor necrosis factor alpha (TNF- $\alpha$ ), chemokine ligand 9 (CXCL)- 9 (MIG), CXCL-10 (IP-10), and chemokine ligand 4 (CCL4). Bovine Cytokine Array Q2 detects the following cytokines and chemokines: fibroblast growth factor (FGF)-1, basic FGF (bFGF), G Protein-Coupled Receptor Associated Sorting Protein (GPRASP)-1, Insulin-like growth factor (IGF)-1, IL-2, IL-4, IL-15, CCL2, cluster of

differentiation (CD) 56, and vascular endothelial growth factor (VEGF)-A. Arrays were executed using the RayBiotech company's provided procedure. Serum samples were diluted 1:2 as suggested from manufacturer protocol. Sample slides were refrigerated at 4° C and blocked from light for storage. Samples were then shipped overnight on ice for laser scanning and extraction and absorbance values were recorded as 'F532 Medium – B532' by the manufacturer. Serum concentrations in pg/mL were determined in-house using Q-Analyzer Software for QAB-CYT (RayBiotech, Inc.; Norcross, GA).

### **Statistical Analysis**

Data were analyzed using the MIXED procedure of SAS 9.3 (SAS Inst. Inc., 1996) with repeated measures and the data were log transformed for analysis. Individual animal was the experimental unit and the model for performance data included fescue treatment (EN vs. EI), haircoat (Slick vs. Wildtype), time of sample collection, and any interactions. Results were recorded as least squares means  $\pm$  SEM with statistical significance reported at a  $P \leq 0.05$ . A tendency was reported at a  $P > 0.05$  and  $\leq 0.10$ . Terms with a significance value of  $P > 0.20$  were removed from the complete model in a stepwise manner to acquire the final reduced model for each of the variables.

### **Results**

Heifers with the slick genotype had greater concentrations of pro-inflammatory cytokines including IFN-  $\alpha$  ( $211.7 \pm 7.19$  vs.  $11.6 \pm 7.19$  ng/ml, SLICK vs. WT, respectively) and IL-1-F1 ( $50.6 \pm 4.15$  vs.  $8.4 \pm 4.15$  ng/ml, SLICK vs. WT, respectively) when compared to heifers with the wildtype hair genotype ( $P < 0.05$ ). Pro-inflammatory cytokine TNF- $\alpha$  was greater in EN-slick ( $659.1 \pm 28.0$  ng/ml) heifers compared to EN-wildtype, EI-slick, and EI-wildtype heifers ( $88.8 \pm 28.0$ ;  $189.2 \pm 28.0$ ; and  $273.9 \pm 28.0$  ng/ml, respectively;  $P < 0.05$ ). Concentrations of IFN-  $\alpha$  were

greater in EN-slick ( $281.0 \pm 117.1$  ng/ml) heifers compared to EN-wildtype or EI-wildtype heifers ( $3.8 \pm 117.1$ ;  $158.1 \pm 117.1$ ;  $31.5 \pm 117.1$  E ng/ml, respectively;  $P < 0.05$ ). Heifers with the slick hair genotype tended to have greater concentrations of pro-inflammatory cytokines such as TNF- $\alpha$  ( $360.1 \pm 55.5$  vs.  $158.5 \pm 55.5$  ng/ml, SLICK vs. WT, respectively) and IFN- $\gamma$  ( $56.7 \pm 8.96$  vs.  $20.551 \pm 8.96$  ng/ml; SLICK vs. WT, respectively;  $0.05 < P < 0.10$ ). Pro-inflammatory cytokine IFN- $\gamma$  tended to be greater in EF-slick heifers ( $112.9 \pm 78.6$  ng/ml) than heifers in all other treatment groups ( $30.8 \pm 78.6$ ;  $27.0 \pm 78.6$ ;  $13.821 \pm 78.6$  ng/ml, respectively;  $0.05 < P < 0.10$ ). It was also observed that EF-slick heifers ( $90.1 \pm 64.3$  ng/ml) tended to have greater concentration of IL-1-F1 when compared to EF-wildtype heifers ( $27.4 \pm 64.3$ ;  $4.6 \pm 64.3$ ;  $14.9 \pm 64.3$  ng/ml, respectively;  $0.05 < P < 0.10$ ). Heifers with the wildtype genotype had greater concentrations of pro-inflammatory cytokine CD56 ( $2554.5 \pm 136.1$  vs.  $1742.9 \pm 136.1$  ng/ml; WT vs. SLICK, respectively) when compared to heifers with the slick genotype ( $P < 0.05$ ). Wildtype heifers also tended to have greater concentrations of CD56 on days 0 ( $2454.0 \pm 262.9$  vs.  $1736.7 \pm 262.9$  ng/ml; WT vs. SLICK, respectively), 28 ( $2459.7 \pm 262.9$  vs.  $1313.1 \pm 262.9$  ng/ml, WT vs. SLICK, respectively), and 56 ( $2794.8 \pm 262.9$  vs.  $1716.2 \pm 262.9$  ng/ml, WT vs. SLICK, respectively) of the trial ( $0.05 < P < 0.10$ ). No difference was found for CD56 on day 14 ( $2522.5 \pm 262.9$  vs.  $2338.2 \pm 262.9$  ng/ml, WT vs. SLICK, respectively) of the trial.

Chemokine CCL4 concentrations were observed to be greater in heifers with the slick genotype ( $55.1 \pm 2.43$  vs.  $3.5 \pm 2.43$  ng/ml, SLICK vs. WT, respectively) when compared to wildtype heifers ( $P < 0.05$ ). It was observed that EN-slick heifers ( $202.1 \pm 100.6$  ng/ml) had greater concentrations of chemokine IP-10 than EN-wildtype heifers ( $23.3 \pm 100.6$ ;  $80.0 \pm 100.6$ ;  $94.1 \pm 100.6$  ng/ml, respectively;  $P < 0.05$ ). Additionally, slick heifers ( $128.5 \pm 18.4$  vs.  $48 \pm 18.4$  ng/ml,

SLICK vs. WT, respectively) tended to have greater concentrations of IP-10 than wildtype heifers ( $0.05 < P < 0.10$ ). No differences were seen for chemokine CCL2 or MIG ( $P > 0.05$ ).

Anti-inflammatory cytokines IL-2 ( $193.2 \pm 23.3$  vs.  $65.2 \pm 23.3$  ng/ml, SLICK vs. WT, respectively) and IL-21 ( $513 \pm 65.2$  vs.  $179.2 \pm 65.2$  ng/ml, SLICK vs. WT, respectively) concentrations were greater in heifers with the slick genotype compared to wildtype heifers ( $P < 0.05$ ). Concentrations of IL-21 in EN-Slick heifers ( $839.4 \pm 369.9$  ng/ml) were also observed to be greater than EN-Wildtype heifers ( $109.6 \pm 369.9$ ;  $305.6 \pm 369.9$ ;  $286.3 \pm 369.9$  ng/ml, respectively;  $P < 0.05$ ). It was observed that concentrations of anti-inflammatory cytokines IL-2 tended to be greater in EN-slick heifers ( $378.4 \pm 178.4$  ng/ml) than all other treatment groups ( $93.9 \pm 178.4$ ;  $52.2 \pm 178.4$ ;  $81.1 \pm 178.4$  ng/ml; respectively) and IL-1-F5 tended to be greater in EN-slick heifers ( $94.7 \pm 52.5$  ng/ml) than heifers from the EN-wildtype treatment ( $12.5 \pm 52.5$ ;  $50.9 \pm 52.5$ ;  $57.6 \pm 52.5$  ng/ml, respectively;  $0.05 < P < 0.10$ ). No differences were observed in anti-inflammatory cytokines IL-4, IL-13, or IL-15 ( $P > 0.05$ ).

Growth factors bFGF ( $0.760 \pm 0.027$  vs.  $0.014 \pm 0.027$  pg/ml, SLICK vs. WT, respectively), and GPRASP-1 ( $243.2 \pm 8.6$  vs.  $194.6 \pm 8.6$  ng/ml, SLICK vs. WT, respectively) were observed to have significantly greater concentrations in slick heifers when compared to heifers with the wildtype hair genotype ( $P < 0.05$ ). It was also observed that FGF-1 concentrations were greater in EN-slick ( $10254.8 \pm 2451.0$  ng/ml) heifers when compared to EN-wildtype heifers ( $4468.7 \pm 2451.0$ ;  $6253.1 \pm 2451.0$ ;  $9048.3 \pm 2451.0$ , respectively;  $P < 0.05$ ). Interestingly, growth factor IGF-1 had greater concentrations in wildtype heifers compared to slick heifers on days 28 ( $284.5 \pm 176.3$  vs.  $70.1 \pm 176.3$  ng/ml, WT vs. SLICK, respectively) and 56 ( $450.8 \pm 176.3$  vs.  $78.1 \pm 176.3$  ng/ml, WT vs. SLICK, respectively) of the trial ( $P < 0.05$ ; Figure 3.1). No differences were found for IGF-1 on days 0 or 14 ( $P > 0.05$ ). A tendency was also observed in which

concentrations of IGF-1 tended to be greater in EI-wildtype heifers ( $429.6 \pm 77.2$  ng/ml) compared to EI-slick heifers ( $264.6 \pm 77.2$ ;  $216.8 \pm 77.2$ ;  $146.3 \pm 77.2$  ng/ml, respectively;  $0.05 < P < 0.10$ ). Heifers from the EN-slick treatment group ( $8.8 \pm 5.6$  ng/ml) also tended to have greater amounts of growth factor VEGF-A when compared to EI-wildtype heifers ( $0.853 \pm 5.6$ ;  $4.4 \pm 5.6$ ;  $6.1 \pm 5.6$  ng/ml, respectively;  $0.05 < P < 0.10$ ). It was also observed that slick heifers had greater concentrations of GPRASP-1 than wildtype heifers on days 0 ( $173.8 \pm 15.6$  vs.  $283.8 \pm 15.6$  ng/ml, WT vs. SLICK, respectively), and day 14 ( $207.0 \pm 15.6$  vs.  $292.9 \pm 15.6$  ng/ml, WT vs. SLICK, respectively;  $P < 0.05$ ; Figure 3.2). No differences were observed on days 28 or 56 for GPRASP-1 ( $P > 0.05$ ). All heifer cytokine data is summarized in Table 3.1.

## Discussion

Contrary to previous work demonstrated by our lab, the results from this study did not indicate the activation of a hyperactive immune response in heifers consuming ergovaline. Heifers possessing the slick hair genotype instead had elevated cytokine concentrations compared to wildtype controls. Heifers with the slick genotype had greater concentrations of pro-inflammatory cytokines IFN- $\alpha$  and IL-1-F1 compared to wildtype controls. Pro-inflammatory cytokines TNF- $\alpha$  and IFN- $\gamma$  tended to be greater in slick heifers compared to wildtype as well. As previously mentioned, pro-inflammatory cytokines stimulate fever and inflammation in response to infection. The pro-inflammatory chemokine CCL4 was also elevated in slick heifers compared to wildtype animals. This protein is a chemoattractant for monocytes, natural killer cells, and other immune-involved substances (Janeway et al., 2001). Slick heifers also tended to have greater IP-10 than wildtype controls. An increase of anti-inflammatory cytokines IL-2 and IL-21 was also observed in slick cattle compared to wildtype controls. This is likely due to the mediatory role of anti-inflammatory cytokines on the inflammatory response of the immune system. Growth factors such

as bFGF and GPRASP-1 were greater in slick heifers compared to wildtype. As mentioned in chapter 2, GPRASP-1 down-regulates the G-protein coupled receptors including the dopamine D2 receptor (Bartlett et al., 2005). There was also a time by haircoat interaction in which wildtype heifers demonstrated increased concentrations of IGF-1 than slick heifers on days 28 and 56 of the study (Figure 3.1). These findings may be the result of a heat stress response as wildtype heifers began to experience the heat related symptoms associated with ergot-alkaloid exposure. As mentioned previously, it takes about 21 days for the symptoms of fescue toxicosis to take effect and these dates comply with this circumstance.

Interestingly, slick heifers from the EN diet group also indicated increased concentrations of various pro-inflammatory and anti-inflammatory cytokines when compared to other treatment groups which suggests further that the slick hair genotype is responsible for the cytokine results demonstrated. The slick haircoat is caused by a frameshift mutation of the PRLR gene which alters the protein by truncating a portion of the cytoplasmic domain (Littlejohn et al., 2014). While the slick haircoat has been identified as the PRLR p.Leu462 mutation, this receptor has a variety of mutations associated with its structure. Some cattle possess slick hair traits despite not possessing the PRLR p.Leu462 mutation and other genetic variants are thought to be involved in this trait. In fact, almost 90% of slick hair coats found in cattle were not associated with the allele found in Senepol cattle (Porto-Neto et al., 2018). This discrepancy of the true molecular compositions of the slick trait found in cattle raises further question on the physiological effects it may have. As previously hypothesized, the slick haircoat may be effective in alleviating the heat stress experienced by animals suffering from fescue toxicosis due to the thermoregulatory improvement (Dikmen et al., 2008). However, further research is necessary to understand how the slick genotype is interacting with the immune system.

One consideration when comparing the cytokine response between chapters 2 and 3 in the presence of sex steroids that have been shown in previous studies to alter immune responsiveness (Grossman, 1984). Chapter 2 utilized gonadectomized animals and therefore would have minimal circulating concentrations of estrogen, testosterone or progesterone, whereas chapter 3 utilized intact heifers that displayed normal estrous cycles, thus having greater circulating concentrations of estrogen, testosterone or progesterone. Interestingly the steers in chapter 2 displayed a hyperactive innate immune response when chronically exposed to ergot alkaloids, which was modified in the heifers in chapter 3. Sex steroid hormones have been established to alter immunological responses including cytokine production, antigen expression, and apoptotic factors. These effects may be carried out by directly interacting with immune cells. There has also been documentation of sex steroid involvement on the thymus of rodents and birds which interact with specific binding sites for estrogen and androgens. Estradiol has been found to have immunoenhancing effects on humoral immunity while testosterone has been shown to be immunosuppressive (Bilbo & Nelson, 2001). Progesterone is known to have immunosuppressive effects however the molecular mechanism is not truly defined. Studies have reported that this immunosuppressive effect is not mediated by the classic progesterone receptor (Schust et al., 1996). These findings further imply complex molecular mechanisms may be occurring between reproductive hormones and the immune system.

The regulation of immune response due to hormones such as estrogen and progesterone as well the combination of these hormones warrants further investigation. Overall, sex steroid hormones have a diverse effect on the immune system functionally and differs between species. Additional research is needed to understand how those hormones may affect immunity within

female cattle. Further investigation is also necessary to fully understand the slick hair genotype and its possibly complex interactions with the immune system

## TABLES

**Table 3.1:** Serum concentrations of cytokines (ng/ml unless otherwise specified) in beef heifers consuming endophyte infected (EI) or endophyte novel (EN) tall fescue identified with slick (S) or wildtype (W) haircoats.

Item	Treatment <sup>1,2</sup>				SEM	Fescue	<i>P</i> -value	
	EN-S	EN-W	EI-S	EI-W			Hair coat	Interaction
<b>Pro-inflammatory</b>								
TNF	659.1 <sub>a</sub>	88.8 <sub>b</sub>	189.2 <sub>b</sub>	273.4 <sub>b</sub>	28.0	0.816	0.085	0.0140
IFNA	281.0 <sub>c</sub>	3.8 <sub>a</sub>	158.1 <sub>bc</sub>	31.5 <sub>ab</sub>	117.1	0.4263	0.0002	0.0904
IFNG	112.9 <sub>a</sub>	13.8 <sub>b</sub>	27 <sub>b</sub>	30.8 <sub>b</sub>	78.6	0.5087	0.0815	0.0551
IL-1-F <sub>13</sub>	90.1 <sub>a</sub>	4.6 <sub>b</sub>	27.4 <sub>ab</sub>	14.9 <sub>ab</sub>	64.3	0.8569	0.0051	0.0594
CD56	1676	2491	1812	2619	131.8	0.407	<0.0001	0.8679
<b>Chemokines</b>								
CCL2	13592.8	21496.8	20071.2	18819.1	5166.9	0.7208	0.5792	0.4605
CCL4	66.2	1.0	45.8	10.3	48.3	0.3434	0.0011	0.1438
MIG	306.8	91.1	249.8	205.5	205.5	0.6563	0.281	0.4391
IP-10	202.1 <sub>a</sub>	23.3 <sub>b</sub>	80 <sub>ab</sub>	94.1 <sub>ab</sub>	100.6	0.7317	0.0599	0.029
<b>Anti-inflammatory</b>								
IL-2	378.4 <sub>a</sub>	52.2 <sub>b</sub>	93.9 <sub>b</sub>	81.1 <sub>b</sub>	178.4	0.3011	0.0354	0.0662
IL-4	63.7	19.6	42.3	22.2	41.7	0.8031	0.145	0.6599
IL-13	155.0	240.3	152.9	50.0	74.4	0.1065	0.5178	0.1127

**Table 3.1** (continued)

IL-15	1193.1	577.7	799.5	1287.6	500.9	0.6425	0.7801	0.1604
IL-21	839.4 <sub>a</sub>	109.6 <sub>b</sub>	305.6 <sub>ab</sub>	286.3 <sub>ab</sub>	369.9	0.873	0.0322	0.0441
IL-1-F5 <sub>4</sub>	94.7 <sub>a</sub>	12.5 <sub>b</sub>	50.9 <sub>ab</sub>	57.6 <sub>ab</sub>	52.5	0.4894	0.1146	0.0741
<b>Growth factors</b>								
FGF-1 <sub>5</sub>	10254.8 <sub>a</sub>	4468.7 <sub>b</sub>	6253.1 <sub>ab</sub>	9048.3 <sub>a</sub>	2451	0.69	0.359	0.018
FGF-2 <sub>6,7</sub>	2.3	0.005	0.221	0.032	3.4	0.6587	0.0129	0.1763
IGF1	264.6	216.8	146.3*	429.6*	77.2	0.8641	0.2135	0.0746
VEGFA <sub>7</sub>	8.8 <sub>a</sub>	0.8530 <sub>b</sub>	4.4 <sub>ab</sub>	6.1 <sub>a</sub>	5.6	0.3882	0.1504	0.0567
GPRASP-1	245.2	202.8	241.3	186.7	15	0.4319	0.0006	0.5971

<sup>a,b,c</sup> Within row, means without a common superscript significantly differ ( $P \leq 0.05$ )

<sup>1</sup> Values are reported as least square means for the experiment

<sup>2</sup> EN-S: endophyte-novel slick; EN-W: endophyte-novel wildtype; EI-S: endophyte-infected slick; EI-W: endophyte-infected wildtype

<sup>3</sup> Also known as interleukin (IL)-1 $\alpha$

<sup>4</sup> Also known as IL-36

<sup>5</sup> Also known as acidic fibroblast growth factor (a-FGF)

<sup>6</sup> Also known as basic fibroblast growth factor (b-FGF)

<sup>7</sup> Reported in pg/ml

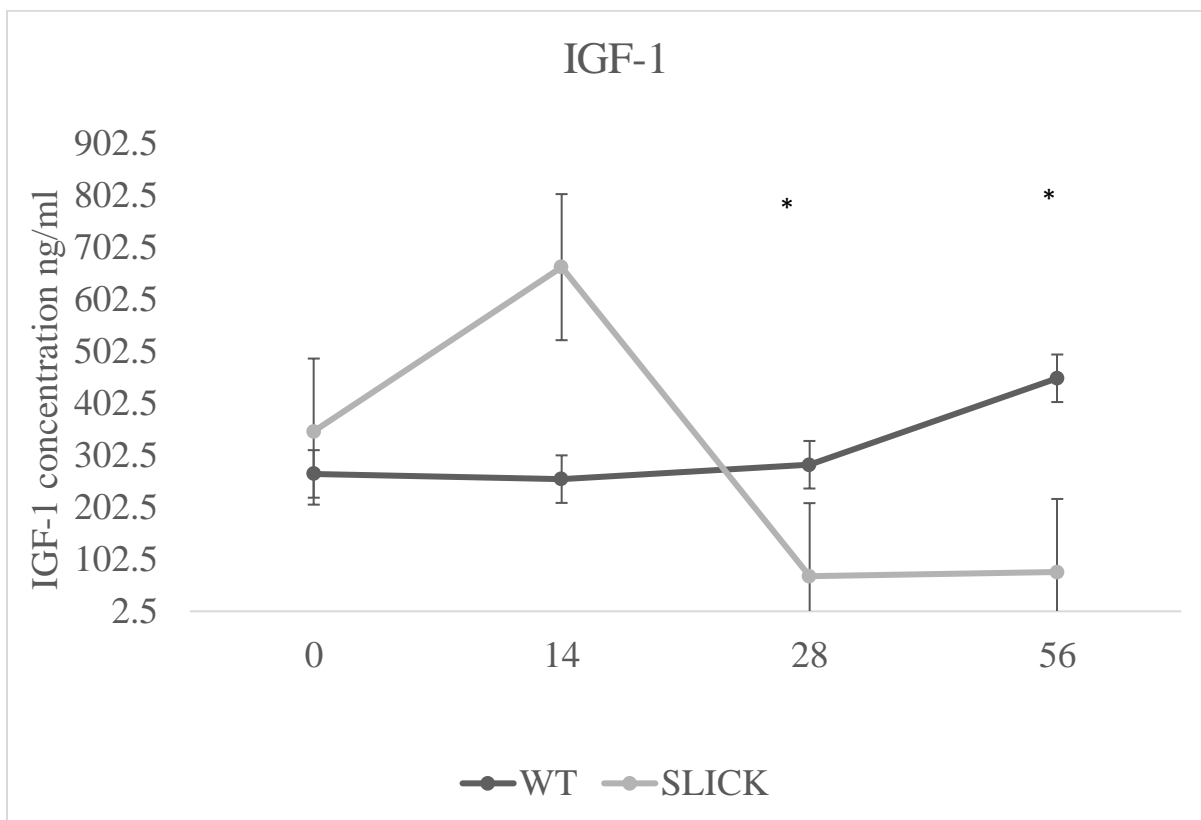
\* $P$ -values  $\leq 0.05$  determined significant

† $P$ -values  $0.05 > P \leq 0.10$  determined a statistical tendency

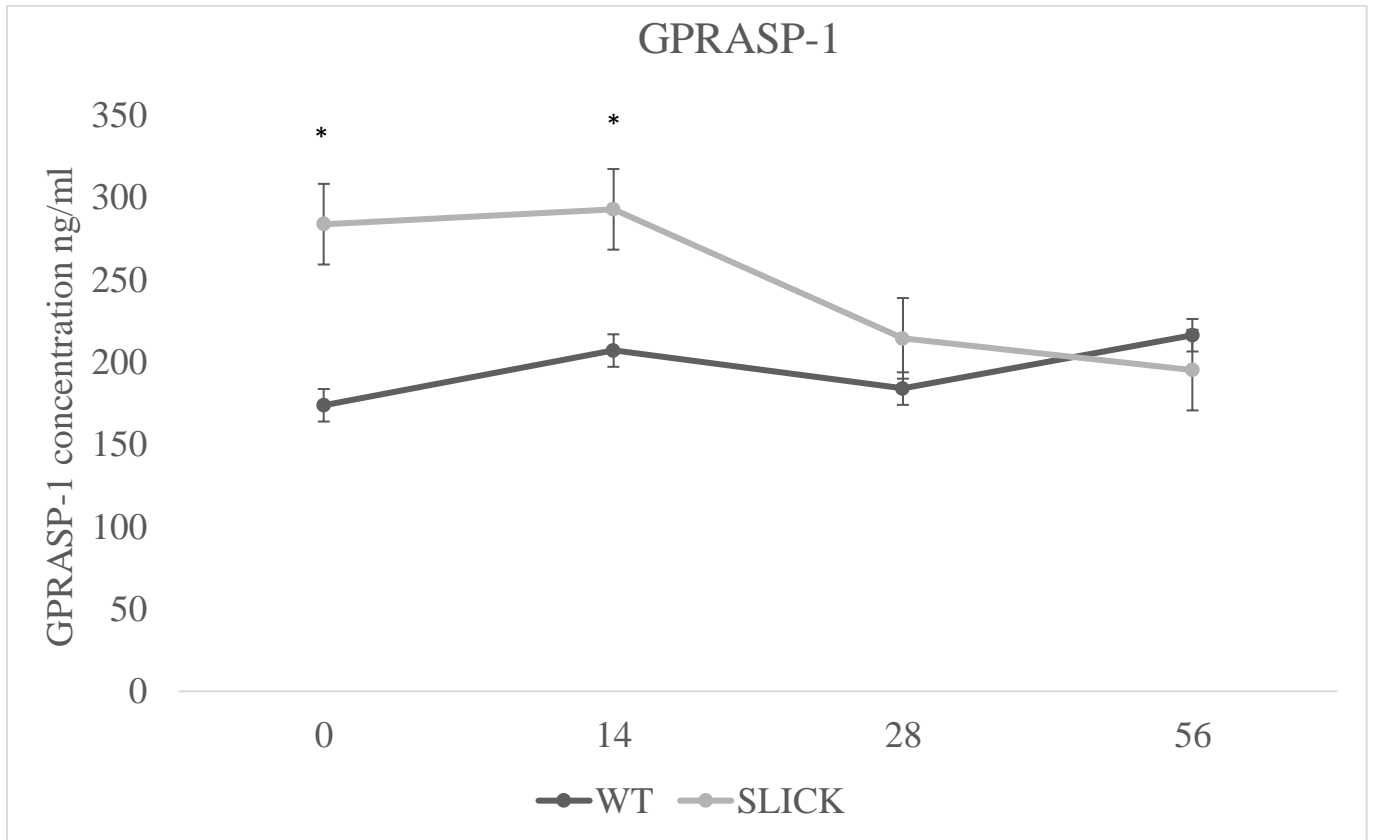
## FIGURES

**Figure 3.1:** IGF-1 concentrations in beef heifers with the slick or wildtype haircoat.

Growth factor IGF-1 had greater concentrations in wildtype heifers compared to slick heifers on days 28 ( $284.5 \pm 176.3$  vs.  $70.1 \pm 176.3$  ng/ml, WT vs. SLICK, respectively) and 56 ( $450.8 \pm 176.3$  vs.  $78.1 \pm 176.3$  ng/ml, WT vs. SLICK, respectively) of the trial ( $P < 0.05$ ). No differences were found for IGF-1 on days 0 or 14 ( $P > 0.05$ ).



**Figure 3.2:** GPRASP-1 concentrations in beef heifers with the slick or wildtype haircoat. Slick heifers had greater concentrations of GPRASP-1 than wildtype heifers on days 0 ( $173.8 \pm 15.6$  vs.  $283.8 \pm 15.6$  ng/ml, WT vs. SLICK, respectively), and day 14 ( $207.0 \pm 15.6$  vs.  $292.9 \pm 15.6$  ng/ml, WT vs. SLICK, respectively;  $P < 0.05$ ). No differences were observed on days 28 or 56 for GPRASP-1 ( $P > 0.05$ ).



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**Chapter 4: Investigating Genetic Resistance to Fescue Toxicosis in Beef Cattle  
by Utilizing Pulmonary Arterial Pressures**

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**Abstract**

A common condition occurring in domestic cattle raised at high altitudes (>1524 m) is known as high mountain disease. Reduced oxygen availability leads to the development of hypoxia induced pulmonary hypertension and subsequent right heart failure. Pulmonary arterial pressure (PAP) testing is a tool utilized to detect and diagnose pulmonary hypertension. PAP testing may be useful for the identification of susceptible or tolerant animals to high mountain disease. Thus, the purpose of this study is to examine the complex interactions of ergot alkaloids and the vascular system and its relationship to pulmonary arterial pressure scores for the identification of susceptible animals and the development of useful selection practices. Twenty postpartum beef cows (n=20) were previously identified as tolerant (n=10) or susceptible (n=10) to fescue toxicosis and had been previously exposed to ergot alkaloids. Pulmonary arterial pressure was measured and recorded for each animal three times from late April to late August of 2019 while grazing endophyte-infected tall fescue pastures throughout the study. Data were analyzed using PROC MIXED of SAS with repeated measures. Statistical significance was determined at  $P < 0.05$  and a tendency at  $0.05 < P < 0.10$ . Cows from the tolerant group had an increased jugular mean arterial pressure ( $14.4 \pm 0.55$  vs.  $12.5 \pm 0.54$  mmHg,  $P < 0.05$ ), a significantly greater right ventricular mean arterial pressure ( $31.3 \pm 1.2$  vs.  $27.8 \pm 1.1$  mmHg,  $P < 0.05$ ), and a greater pulmonary artery mean arterial pressure ( $36.0 \pm 0.7$  vs.  $33.4 \pm 0.7$  mmHg,  $P < 0.05$ ),) than cows from the susceptible group ( $P < 0.05$ ). The complex interactions between ergot alkaloids and the vascular system and its relationship to pulmonary arterial pressure scores may be of interest for the development of animal selection practices.

## **Introduction**

High mountain disease, otherwise known as “brisket disease,” occurs in domestic cattle raised in high altitude conditions (>1524 m). This condition is caused by hypoxia induced pulmonary arterial hypertension. Hypoxia causes a reduction of the pulmonary arteries and arterioles resulting in hypertension, hypertrophy of the right ventricle, congestive heart failure, and can lead to animal fatality. A common symptom of high mountain disease is the accumulation of edematous fluid in the tissues covering the parasternal muscle, resulting in the distention of the brisket region. This fluid buildup is caused from right ventricular heart failure causing an increase of hydrostatic pressure and movement of fluid into the tissues (Holt & Callen, 2007).

Pulmonary arterial pressure (PAP) testing is a tool that may be useful in determining the susceptibility or resistance of high mountain disease in cattle herds raised at high altitude. Selection for resistant animals would improve the performance of cattle raised in these conditions and be a valuable option for producers. A PAP measurement of 34-44 mmHg is considered to be normal for adult cattle at altitudes of 1524 to 1981 meters, 5000 to 6500 feet respectively. Animals that fall into the 48-213 mmHg PAP score range are considered to be experiencing pulmonary artery hypertension (Holt & Callen, 2007). Studies have suggested that hypoxia induced pulmonary hypertension is heritable (Newman et al., 2011) and that selection for PAP score performance does not negatively correlate with weight gain (Crawford et al., 2016). The identification of susceptible cattle to pulmonary arterial hypertension can be utilized as a useful management tool to implement improved breeding protocols for the selection of improved animal performance at high altitudes. The interaction of ergot alkaloids with the vascular system and the changes in PAP measurements as a result of ergot alkaloid exposure may provide insight on a method of selection for cattle tolerant of fescue toxicosis.

## **Material and Methods**

This study was conducted in Reidsville, North Carolina at the Upper Piedmont Research Station (UPRS). All procedures involving animal use was approved by the North Carolina State University Institutional Animal Care and Use Committee (17-043-A).

### **Animals and Experimental Design**

Postpartum cows (n=20) that were previously identified as either tolerant or susceptible to ergot alkaloid commonly found in endophyte-infected tall fescue (Mayberry, 2018) were utilized for this study at the Upper Piedmont Research Station in Reidsville, North Carolina. The city of Reidsville is located 804 ft. above sea level. Pulmonary artery pressure was measured and recorded for each animal for a total of three times from late April to late August of 2019. These animals grazed endophyte-infected tall fescue previous to the study and throughout its duration. Cows were given ad libitum access to fresh water throughout the trial. Body weight was measured for each of the three PAP measurement days using a digital livestock scale as animals were moved through a squeeze chute. Body condition scores (BCS; scale of 1 to 9; adapted from Whitman, 1975) were evaluated by two trained personnel for an average score on each date. Blood samples were collected from the jugular vein using a sterile 10.0 mL vacutainer tube with no additive (Vacutainer, Becton, Dickerson and Company, Franklin Lakes, NJ). Blood was collected during the PAP procedures after jugular puncture and blood flowed freely before catheters were inserted as described in the following PAP procedure section. Blood samples were placed on ice immediately and centrifuged once returning to the lab for 20 minutes at  $1,500 \times g$  for 20 minutes at 4C. Serum samples was then transferred into 5 ml polystyrene vials (BD Falcon, Franklin Lakes, NJ) and stored in a -80C freezer for future analysis.

## **Animal Selection**

In a previous study from our lab (Mayberry et al., 2018), animals on this study were ranked based on tolerance or susceptibility to fescue toxicosis. Animals were linearly ranked using growth performance for “selection based on residuals.” Average weekly gain (AWG) was utilized with estimates grouped by three window periods: week 1 through 13 (ES), weeks 1 through 7 (P1), and weeks 7 through 13 (P2). This was used to estimate the animals’ response to exposure to ergot alkaloids. A model consisting of parity, location, and the partial regression coefficient for the covariate of initial BW was used to determine the most extreme tolerant or susceptible animals at Upper Piedmont Research Station (n=148 for combined locations). Each of the three time periods described produced differing results of the most extreme tolerant or susceptible forty animals. Further analysis determined the best time period to represent the effects of fescue toxicosis on animal performance. Animals were selected from the P1 period due to the presence of the greatest additive genetic variance, a greater amount of extreme AWG residual values, and the greatest percentage of fescue being in this period. Forty animals from P1 was thus used to evaluate 88 performance and resistance to fescue toxicosis. This method of selection is can be described as the Fescue Toxicosis Selection Method (FTSM). At the Upper Piedmont Research Station, ten animals were described to be extremely tolerant and ten animals were identified as extremely susceptible. This method of selection is can be described as the Fescue Toxicosis Selection Method (FTSM). Due to animals being sold or moved to other locations, ten cows (n=10) from the top thirty susceptible cattle on the property and ten cows (n=10) from the top tolerant cattle were selected for this study for a total of twenty postpartum cows (n=20).

### **Diet and Forage Measurements**

Cattle grazed pastures known to be endophyte infected, toxic tall fescue for the entirety of the study. Cattle were rotationally grazed every two weeks at each location among select pastures to continue adequate forage management as well as ensure sufficient forage was continually available. Prior to cattle being moved, pastures were sampled in an established manner and composite samples taken from each pasture to evaluate nutrient quality and percentage of available forage that was fescue. Nutrient quality samples will be analyzed for nutrient content (North Carolina Department of Agriculture Forage Laboratory, Raleigh, NC). Cattle were provided ad libitum access to water throughout the experimental period.

### **Pulmonary Artery Catherization**

Each animal was secured and haltered to expose the lateral jugular furrow. An injection of 2% lidocaine was injected subcutaneously at the site of catherization. The site was then scrubbed with soapy water to remove any debris and hair was clipped. The skin was then cleansed using Betadine Surgical Scrub (7.5% Povidone-iodine) and allowed to dry. Pressure was applied to the jugular vein at the level of the thoracic inlet or just proximal to this area to allow blood flow resistance causing jugular vein distension. Then the proximal jugular vein was punctured with a 12 gauge, 9cm needle until blood freely flowed from the needle. The needle was then gently threaded down the jugular leaving 1 cm of needle out of the skin to allow needle control. An intramedic polyethylene catheter (internal diameter 1.19mm, outside diameter 1.7mm) was then passed through the needle and into the jugular vein. The catheter tubing was flushed with 0.9 % NaCl (approximately 1 ml) prior to putting in the needle and was flushed while the catheter was being gently advanced down the jugular vein. The constant flushing of the 0.9 % NaCl aided in moving the catheter down the vein. The external end of the catheter was then connected to the

transducer via 3-way stopcocks after approximately 1-1.5 feet of catheter had been passed into the jugular vein. The transducer was placed at the level equal to the base of the heart, which is approximately just caudal to the point of the elbow. The transducer and catheter were flushed again to ensure no air had entered the system. The catheter was then advanced to the distal jugular vein and the first pressure taken to help evaluate catheter location. The catheter was passed into the right Atrium through the right A-V valve and into the right ventricle of the heart where the second measurement was taken. The catheter was then advanced from the right ventricle, through the pulmonary valve, and into the main pulmonary artery. The catheter remained in this location until the pressure held constant, approximately 5 seconds. The location of the catheter within the animal's vasculature as well as the pressure reading was done by monitoring the pressure changes and characteristics of the pressure wave lengths on a data scope. This allowed accurate knowledge of the location of the end of the catheter at all times. Once the catheter reached the pulmonary artery and pressure stabilizes then the mean, systolic, and diastolic pressure were recorded. After recording the pulmonary artery pressure, the catheter was slowly pulled back and removed from the animal followed by removal of the needle from the jugular vein. These animals were monitored twice daily for any signs of pain or distress for 72 hours following this procedure.

### **Statistical Analysis**

Data were analyzed using the Type 3 test of fixed effects of SAS 9.3 (SAS Inst. Inc., 1996) with repeated measures. Individual animal was the experimental unit and the model for performance data included Tolerant vs. Susceptible (Tol vs. Sus), day of pulmonary arterial pressure measurement, and any interactions. Results were recorded as least squares means  $\pm$  SEM with statistical significance reported at a  $P \leq 0.05$ . A tendency was reported at a  $P > 0.05$  and  $\leq 0.10$ .

Terms with a significance value of  $P>0.20$  were removed from the complete model in a stepwise manner to acquire the final reduced model for each of the variables.

## Results

Cows from the tolerant group had an increased jugular mean arterial pressure compared to cows in the susceptible group ( $14.4 \pm 0.6$  vs.  $12.5 \pm 0.5$  mmHg, respectively;  $P<0.05$ ; Figure 4.1). Cows from the tolerant group also had a significantly greater right ventricular mean arterial pressure compared to cows in the susceptible group ( $31.3 \pm 1.2$  vs.  $27.8 \pm 1.1$  mmHg, respectively;  $P<0.05$ ; Figure 4.2). For the pulmonary artery mean arterial pressure, tolerant cows displayed increased PAP compared to cows than cows from the susceptible group ( $36.0 \pm 0.7$  vs.  $33.4 \pm 0.7$  mmHg, respectively;  $P<0.05$ ; Figure 4.3). There were no significant differences or tendencies found between treatment groups for average daily gain, body weight, body condition score, or hair coat scores ( $P>0.10$ ; Summarized in Table 4.1).

## Discussion

An increased pulmonary arterial pressure occurs when pulmonary arteries become narrowed, reducing the ease of blood flow, and thus raising the pressure within that artery. This abnormal pressure then applies additional pressure onto the right ventricle of the heart which can lead to the right heart failure seen in animals with high mountain disease. High mountain disease and fescue toxicosis both significantly impact the vascular system through vasoconstrictive response. In cattle, pulmonary vascular shunting can be much more severe than what is seen in other animals. The large body and small lung ratio contribute to this condition and is considered highly heritable. It is expected that concurrent illness such as fescue toxicosis would elevate PAP scores (Holt & Callen, 2007).

Interestingly, cattle from the tolerant group had a greater jugular mean arterial pressure, right ventricular mean arterial pressure, and pulmonary mean arterial pressure. These results were unexpected but raise compelling questions about how fescue toxicosis susceptibility interacts with pulmonary arterial pressure performance. The animals from this study were ranked based on average weekly gain to determine if they were susceptible or tolerant to fescue toxicosis. This selection has demonstrated that animals susceptible to fescue toxicosis seem to perform better on PAP assessment than animals deemed tolerant.

It is important to note that pulmonary arterial pressure score is influenced by a variety of factors as mentioned previously. This group of cows (n=20) is a smaller sample size with only ten animals per treatment group. Multiple factors such as breed, genetics, and concurrent illness are known to cause PAP measurement variability. This study was also conducted at a low elevation (804 ft. above sea level) as suggested by Pauling et al., (2018) can be a beneficial tool for detecting tolerance to high mountain disease. Holt & Callen, (2007) however, claim that testing at a high elevation after substantial exposure may increase the accuracy of PAP testing for determining susceptibility. While low elevation PAP testing may prove useful for detecting cattle sensitive to hypoxic conditions or hypertensive, increasing elevation may give better insight to true susceptibility to high mountain disease.

Concurrent illness is a variable which heightens pulmonary arterial pressure scores especially those which affect the respiratory or vascular system negatively. As previously discussed in chapter 1, fescue toxicosis causes a range of symptoms such as vasoconstriction of the peripheral vasculature and heat stress. Fescue toxicosis has been attributed to cause vasoconstriction, medial blood vessel thickening, ischemia, endothelial damage, thrombosis, and gangrene (Klotz & McDowell, 2017). The vasoconstriction contributes to the heat stress

experienced as does hair coat and warmer environmental conditions. This heat stress then leads to increased breathing rates or panting and animals may consume more water than usual to cool. Panting is described as an increase in the frequency of respiration and subsequent decrease in tidal volume of the lungs. This mechanism is utilized by animals as an attempt to elevate respiratory evaporation and dissipate heat (Robertshaw, 2006). During panting, cardiac output, heart rate, and mean arterial blood pressure increase (Jennings et al., 1973). Heat stress without panting would result in activation of the parasympathetic nervous system which would reduce overall blood pressure by decreasing the heart rate and dilating blood vessels. It has been demonstrated that cattle experiencing chronic, moderate heat stress display a decreased heart rate while increased heart rates occur during short-term heat exposure (as reviewed by Neuwirth et al., 1979).

Cattle susceptible to fescue toxicosis would likely experience this symptom while grazing ergot alkaloids on pasture during the summer months as demonstrated in this study however this was not observed. It could be anticipated that vasoconstriction caused by endophyte infected tall fescue exposure would perpetuate an increased PAP score result for fescue toxicosis susceptible cattle however that was not observed either.

The PAP results for this study oppose these hypotheses but we may be observing the outcome of a hyperactive immune response in fescue toxicosis susceptible cattle. As previously discussed, cytokines are involved with inflammation and many growth factors contribute to vasodilation. For example, FGF-1 decreases blood pressure and induces dilation of pial arterioles after intravenous administration (Rosenblatt et al., 1993). Growth factors are also associated with angiogenesis which is the process from which new blood vessels form from pre-existing ones. Fescue toxicosis susceptible animals may be experiencing a hyperactive immune response much like what was observed in ergot alkaloid exposed cattle in chapters 2 and 3. Prolonged, moderate

heat stress and the activation of growth factors may be responsible for vasodilation and angiogenesis in susceptible cattle causing a compensatory circumstance to lead to reduced pulmonary arterial pressure scores. Cattle deemed tolerant would then not experience this hyperactive immune response and thus result in greater PAP scores when compared to the susceptible animals. Since no significant differences were found between treatment groups for average daily gain (ADG), body weight, or body condition score, these results correlate with previous studies which conclude that selection for PAP measurement should have minimal influence on cattle gain performance.

In conclusion, there appears to be underlying physiological mechanisms resulting in fescue toxicosis susceptible cows to have reduced PAP scores compared to the tolerant cattle group. Additional analysis of ergot alkaloid content in grazed pastures and blood analysis may provide some insight to these mechanisms. Overall, further investigation is warranted to explore the complex relationship between fescue toxicosis, genetic tolerance, and pulmonary arterial pressure.

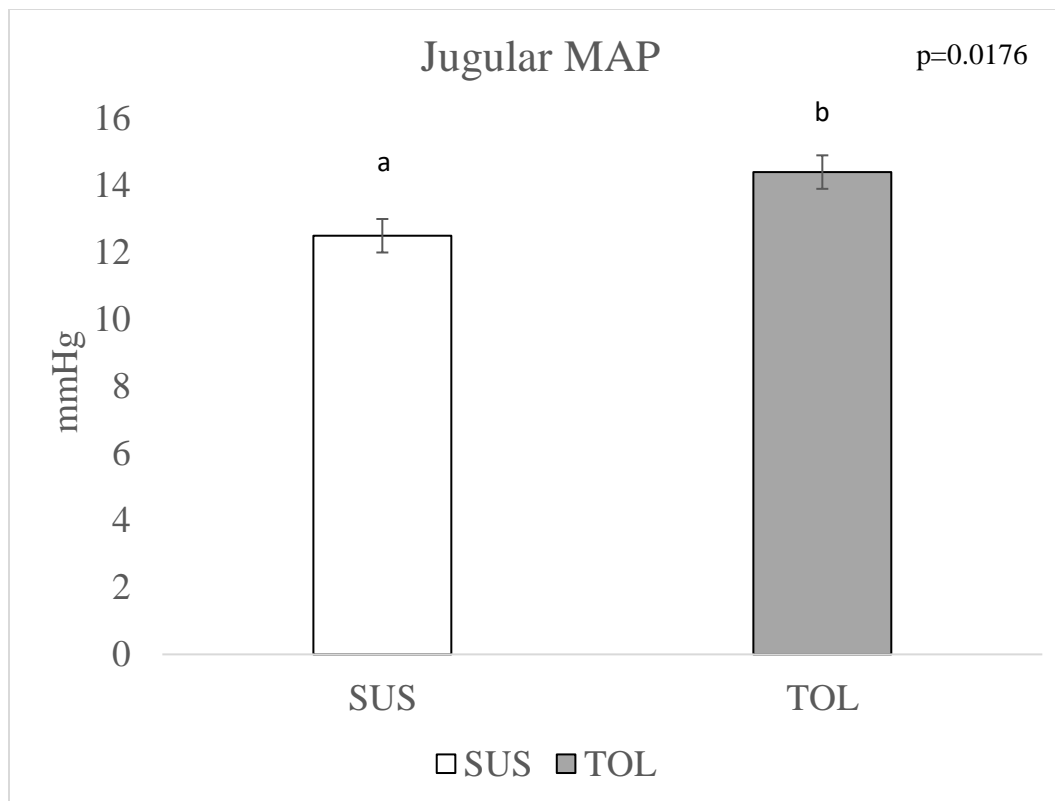
## TABLES

**Table 4.1:** Average daily gain, body weight, body condition score, and hair coat score of postpartum beef cows identified as tolerant or susceptible to fescue toxicosis from late-April to late-August 2019.

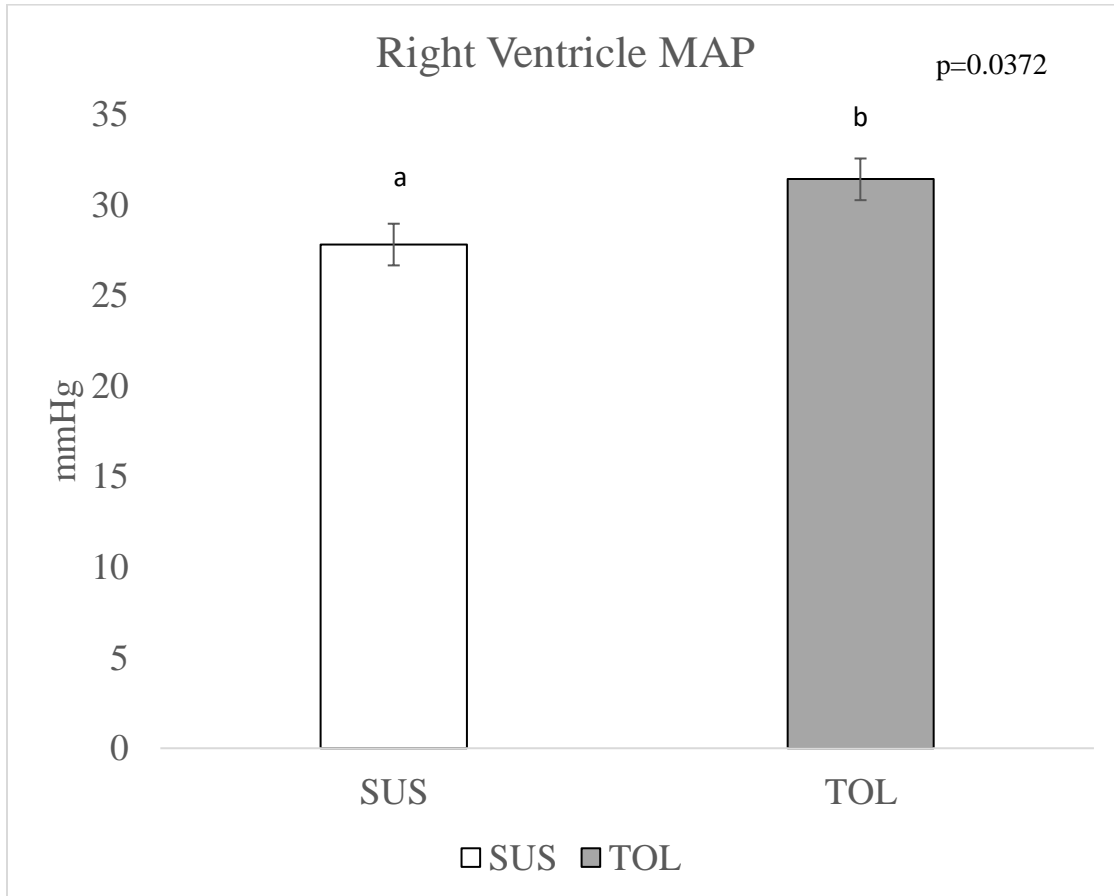
	P values					
	Tolerant	Susceptible	SEM	Treatment (T)	Date (D)	TxD Interaction
<b>ADG</b>	56	43.9	6.8	0.2273	-	-
<b>Body Weight (kg)</b>	549.8	546.8	10.2	0.8345	0.0002	0.9442
<b>BCS</b>	5.45	5.58	0.07	0.2618	<0.0001	0.5896
<b>HCS</b>	2	2.2	0.18	0.4944	0.0006	0.7864

## FIGURES

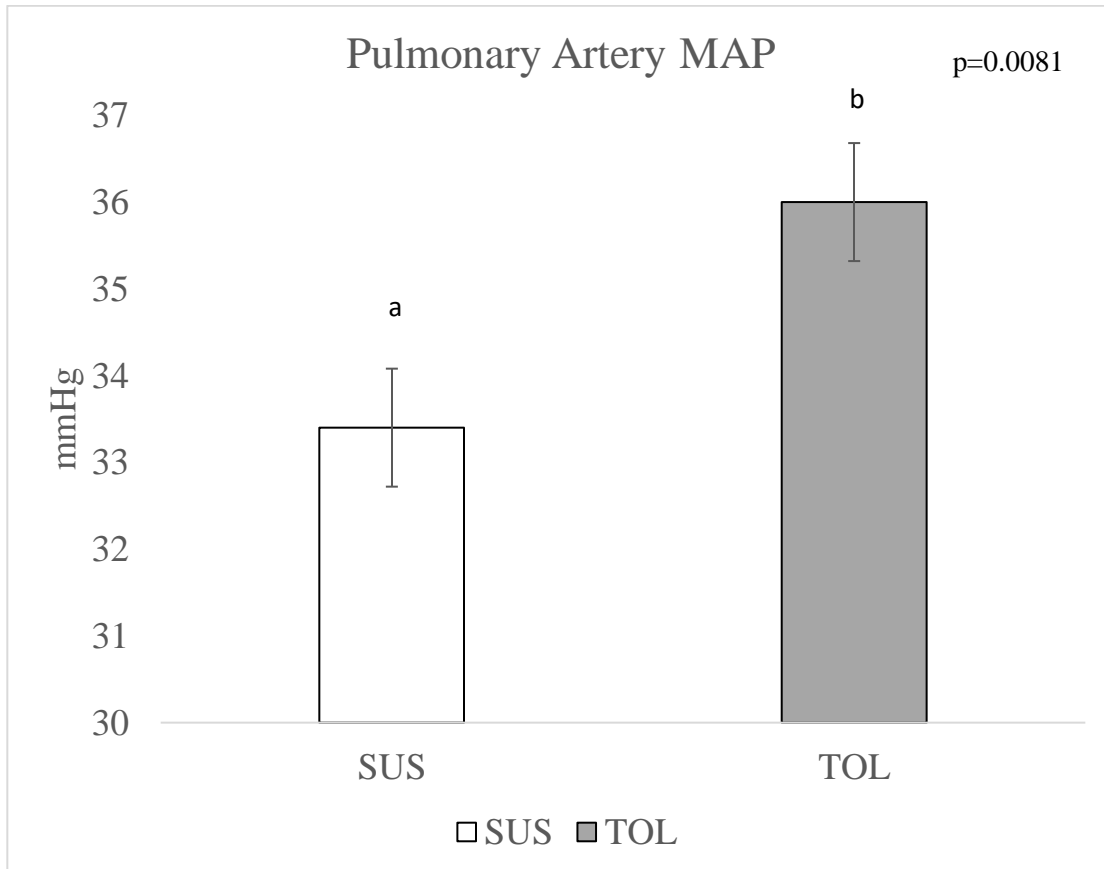
**Figure 4.1:** Jugular mean arterial pressures by treatment (mmHg). Tolerant animals had a greater jugular MAP than susceptible animals ( $14.4 \pm 0.6$  vs.  $12.5 \pm 0.5$  mmHg, respectively;  $P < 0.05$ ).



**Figure 4.2:** Right ventricular mean arterial pressures by treatment (mmHg). Tolerant animals had greater right ventricular MAP than susceptible animals ( $31.3 \pm 1.2$  vs.  $27.8 \pm 1.1$  mmHg, respectively;  $P < 0.05$ ).



**Figure 4.3:** Pulmonary artery mean arterial pressures by treatment (mmHg). Tolerant animals had greater pulmonary artery MAP than susceptible animals ( $36.0 \pm 0.7$  vs.  $33.4 \pm 0.7$  mmHg, respectively;  $P < 0.05$ ).



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## **Chapter 5: Conclusions and Implications**

The extensive physiological and immunological effects of fescue toxicosis is yet to be entirely defined. This reality in conjunction with the difficulty of endophyte-infected pasture renovation make selection practices and methods to alleviate the adverse effects of fescue toxicosis of interest. In chapter 2, a hyperactive innate immune response was observed in steers exposed to ergovaline. Steers consuming endophyte-infected tall fescue displayed elevated concentrations of pro-inflammatory cytokines, anti-inflammatory cytokines, chemokines, and growth factors. Protein supplementation had minimal effects however could be utilized by producers as a method inhibit some of the performance lost.

In chapter 3 however, heifers did not display a hyperactive immune response from exposure to ergot alkaloids. Animals which possessed the slick hair trait instead demonstrated elevated cytokine response compared to other treatment groups. Cattle with a slicker haircoat have been observed to have improved thermoregulation so incorporation of this gene may still be of interest. Producers residing in warmer climates or that have noticed heavy retention of winter coats in cattle exposed to ergot alkaloids may benefit from the incorporation of the slick gene. Heat stress alleviation may appeal to producers due to the negative effects thermal stress has on weight gain and a reduction of hair may improve the visual appearance as well. The time by haircoat interaction for IGF-1 reported in chapter 2 further suggests that the slick haircoat may be beneficial in the alleviation of heat related symptoms associated with fescue toxicosis. Both studies involving investigation of the immune response in chapters 2 and 3 further suggest the intricate physiological response that ergot alkaloids and individual animal variation can have. While producers may not observe some of the more visual complications associated with ergot alkaloids in their herd, these studies show that there may be underlying issues that one may not be aware of. While animals may

not be affected by physically displayed symptoms, cattle could be more susceptible to infection, respiratory disease, or parasites.

Cattle experiencing fescue toxicosis display similar vascular conditions that are observed with high mountain disease, which is a condition that affects the productivity and profitability of cattle systems at high altitudes. Pulmonary arterial pressures are currently regarded as a method to identify animals susceptible to pulmonary hypertension. Pulmonary hypertension may exacerbate the occurrence of fescue toxicosis or high mountain disease. Thus, in chapter 4, the relationship between fescue toxicosis and pulmonary hypertension was examined. Fescue toxicosis tolerant animals had increased PAP score than those identified as susceptible to the detrimental effects of ergot alkaloids. As previously mentioned, PAP scores have been suggested to be heritable and thus selection for tolerant animals may improve overall herd health. This selection tool may be effective for preventing high mountain disease but also improve recovery from various disease. Cattle which are less likely to develop pulmonary hypertension may have a better chance of recovery from diseases which target the cardiovascular system. Selection for animals which perform well on PAP evaluations could improve the herd genetics and prevent losses. The relationship between ergot alkaloids, pulmonary hypertension, and genetic selection for tolerance may prove useful in future efforts to improve the sustainability of the beef industry in the Southeastern United States.