## ABSTRACT

BOSTIAN, AMANDA WARD. Chronic Exposure to Ergot Alkaloids Impacts Growth and Performance in Steers. (Under the direction of Daniel H. Poole).

Tall fescue [*Lolium arundinaceum* (Schreb.) Darbysh] is the predominant grass cattle graze throughout the southeastern United States. Due to association with the fungus, *Epichloë coenophiala,* the grass becomes endophyte-infected and produces ergot alkaloids. Ergot alkaloid exposure causes a whole host of physiological problems in cattle including increased body temperature and heart rate, as well as decreased growth and blood flow to the extremities. Together, these symptoms are called fescue toxicosis and the exact pathway by which it occurs is not well understood. Crossbred Angus steers (n=8) were placed in Calan gates and were randomly assigned to receive either endophyte-infected fescue seed (EI) or non-infected fescue seed (EF; control) for 63 days. Fescue seed was fed at 18% of the diet on a dry matter (DM) basis, with the remainder of the diet being composed of corn silage (57% DM) and concentrate (25% DM). The EI diet had an ergovaline infection rate of 509.4 ppb in the fescue seed with no other ergot alkaloids present. The EF diet had no ergot alkaloids present. In the first study, weekly measurements and blood samples were taken to monitor the steer's physiological responses during exposure to ergot alkaloids. The animals were humanely euthanized at the completion of the study and tissue samples were frozen for use in later studies. It was hypothesized that growth would be reduced in the EI group with reduced fescue toxcicosis symptoms present due to the presence of strictly ergovaline versus a combination of ergot alkaloids in the seed that the animals consumed. In the second of the experiment, serum collected from the steers was analyzed for concentrations of Luteinizing Hormone (LH), Growth Hormone (GH), Insulin-Like Growth Factor 1 (IGF-1) and Cortisol. It was hypothesized that LH would not be different between the treatment groups, while both GH and

IGF-1 would be reduced with Cortisol being elevated. In the first experiment, respiration rate, rectal temperature, surface temperature assessed by thermal camera, temperament, hair coat and shedding scores (1-5 scale) did not differ between treatment groups (P>0.05). Systolic and diastolic blood pressure as well as caudal vein diameter were not different (P>0.05). However, there was decreased heart rate (67.43 vs. 73.72 beats/min) and increased caudal artery diameter (35.58 vs. 34.38 $mm^2$) in EI animals compared to EF (P<0.05). Body weight and average daily gain (BW 425.8 vs 409.5 kg; ADG 0.99 vs 1.14 kg/d) tended to increase in the EF group compared to EI (P=0.09). Body condition score tended to be greater in the EF group compared to EI (5.46 vs, 5.39; P=0.07). Hematocrit was not different between treatment groups (P>0.05). Heart, liver, spleen, kidney and pancreas weights did not differ between treatment groups (P>0.05). In the second experiment, circulating LH concentrations were no different between treatment groups (P>0.05) during chronic exposure to ergot alkaloids whereas, circulating growth hormone (GH) concentrations were reduced in the EI (1.3 ng/ml) group when compared to the EF controls (7.2 ng/ml; P<0.05). Circulating IGF-1 concentrations also tended to be reduced in the EI (28 ng/mL) when compared to the EI group (95.7 ng/mL; P=0.10). There was a tendency for a date by treatment interaction to reduce Cortisol concentrations in the EI group (489.2 ng/mL) when compared to the EF group (511.4 ng/mL; P=0.11). Reductions in circulating growth hormone and I insulin-like growth factor-1 could play an important role in the reduced weight gain that is seen in animals that are chronically exposed to ergot alkaloids. Determining exactly how ergot alkaloids are functioning along the hypothalamic-pituitary axis to alter both GH and IGF-1 production could help to further elucidate the mechanisms that leads to losses associated with fescue toxicosis.

# Chronic Exposure to Ergot Alkaloids Impacts Growth and Performance in Steers

by
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## DEDICATION

This dissertation is dedicated to my husband Kyle. It has been a long road to get to this point and I appreciate all the support that you have provided me with. Thank you for loving me unconditionally and realizing that I could accomplish my dreams, even when I wasn't sure that I could. I love you!

## BIOGRAPHY

Amanda Ward Bostian was originally born in Aliquippa, Pennsylvania but spent most of her life growing up in Mooresville, North Carolina. She graduated from South Iredell High School in 2002 and attended North Carolina State University where she attained a B.S. degree in Animal Science with an industry concentration, graduating with honors in 2006. Amanda actively sought out the program of Dr. J. Lannett Edwards at the University of Tennessee-Knoxville to pursue her M.S. degree in Animal Science with a concentration in reproductive physiology. Her research focused on the effects of heat stress and the use of protein-free culture medium on the production of in vitro produced bovine embryos. Upon graduation in August of 2008, Amanda began to pursue her career as a clinical human embryologist in Austin, Texas. During the next several years, she grew and trained in her career and became certified as a Technical Supervisor in embryology by the American Board of Bioanalysts. Life brought Amanda back to Raleigh for work and in January of 2012, she started to pursue a Ph.D. under the direction of Dr. Daniel Poole with a focus on the physiology of fescue toxicosis. Amanda has continued to further her career during her program is currently employed as an embryology laboratory consultant while completing her degree.

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# CHAPTER 1

Literature Review: Fescue Toxicosis, Steers, and their Growth and Development

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## INTRODUCTION

Tall fescue [*Lolium arundinaceum* (Schreb.) Darbysh] is the predominant grass growing throughout the Southeastern United States and is preferred by producers. This is because it is drought-resistant, tolerant to over grazing and does not require a high degree of management. It is also very pest-resistant and does not require intensive pasture management on the part of the farmer. It is easy to establish and adapts well to different environments. It stands up well to being over-grazed and offers an extended grazing season when compared to other varieties However, most the grass is associated with the fungal endophyte *Epichloë coenophiala* and this infects the grass causing it to produce ergot alkaloids which cause a whole host of physiological problems in cattle that ingest it. Symptoms include increased body temperature, reduced growth, reduced food intake and decreased blood flow to extremities. These symptoms grouped together are known as fescue toxicosis and the underlying mechanics that causes it are not well understood. Unfortunately, none of the benefits to the plant are offered with endophyte-free fescue, even though it would alleviate the detrimental impact on the animal. It does not grow as prolifically, does not stand up to heavy grazing, and is easily overtaken by endophyte-infected seed. In fact, when both endophyte-infected and endophyte-free fescue were planted together on a native grassland, the endophyte-infected fescue showed significantly growth and reproductive abilities. Additionally, it is expensive and that is a difficult sell to producers.

## ERGOT ALKALOIDS

An alkaloid is a heterocyclic, amino acid derived compound that is of low molecular weight (Schläger and Dräger, 2016). Alkaloids can be either plant or animal derived and there are currently approximately 10,000 of them identified (Koskinen, 2012). Initially

identified in 1819, alkaloids are thought to infer a benefit upon the organism that is producing in them. That benefit can be in the form of anti-fungal, anti-bacterial or anti-viral properties for the host organism (Fattorusso, 2008). The alkaloids can be divided into 5 subgroups which are heterocyclic alkaloids (most known for medicinal uses), alkaloids with an exocyclic nitrogen (hallucinogenics), polyamine alkaloids, terpene alkaloids and the peptide alkaloids, which ergot alkaloids belong to (Koskinen, 2012).

Ergot alkaloids belong to the peptide alkaloid classification because its structure is comprised of two or more amino acids that are linked by peptide bonds (Koskinen, 2012). Ergovaline is one of the most well characterized ergot alkaloids. Its structure is a D-lysergic acid attached to an amide composed of L-valine-L-alaine-L-proline. This only differs by one amino acid from lysergic acid where the L-valine is switched out for an L-phenylalanine (An, 2004; Jakubczyk et al., 2014). Initially called ergotoxine in 1906, a combination of ergot alkaloids were isolated and the effects on the nervous system of cats began to be studied (Barger and Dale, 1907). Further study continued and it was found that there were three different metabolites of ergot alkaloids: clavines, amides of lysergic acid and ergopeptines (Panaccione et al., 2014). Ergot alkaloids were first identified in the sclerotia of ryegrass and were being produced by *Claviceps*. It was soon found out that many different classes of fungi were capable of producing ergot alkaloids, including *Neotyphodium*, which is commonly associated with the root of tall fescue (*Lolium arundinaceum* Schreb. Darbysh.) (Jakubczyk et al., 2014).

## TALL FESCUE

Tall fescue [*Lolium arundinaceum* (Schreb.) Darbysh] is the predominant cool-season grass grown in the transition zone, with the most popular cultivar being Kentucky-31. The

transition zone stretches from Indianapolis, Indiana to Macon, Georgia (Siegel et al., 1984). Kentucky-31 started to be grown and quickly gained popularity in the United States in 1942. It is now estimated to be grown on 35 million acres of land (Bacon and Siegel, 1988; Ball et al., 2015). Producers have shown a preference for this cultivar over other tall fescue cultivars as it is extremely hardy, stands up well to over-grazing and can withstand drought. Kentucky-31 has extensive root development, the ability to adjust for water conservation, increase its sugar accumulation and can roll its leaves which furthers its abilities to conserve water (Ball et al., 2015; Strickland et al., 2011).

## BENEFITS AND PITFALLS ASSOCIATED WITH TALL FESCUE

While Kentucky-31 grew extremely well, what producers did not see was *Neotyphodium*, the fungus that was growing in the intercellular spaces, on the roots of the grass that was utilizing it as a host in a form of "defensive mutualism". Defensive mutualism is a type of relationship whereby the host plant (tall fescue) provides shelter and a food source to the fungus while the endophyte provides protection from predators to the host plant (Clay, 1988). Association with the endophyte is what gives tall fescue its vigor, however there is no visual way for a producer to identify an endophyte-infected field from an endophyte-free field (Panaccione et al., 2014). Presence of the endophyte does not alter the appearance of the tall fescue. The alkaloids that are being produced by these endophytes, however, are thought to be what confers all of the positive effects seen on the growth and hardiness of endophyte-infected tall fescue (Clay, 1988; Panaccione et al., 2014).

By 1948, Kentucky-31 was thriving throughout the transition zone and was the predominant forage source for many beef producers. Producers noticed that the animals grazing Kentucky-31 did not gain weight as well and were in general not as healthy as the

animals that were not on the same pasture. On paper, this made no sense as tall fescue appeared to be an excellent forage source with high digestible dry matter, crude protein, amino acid and mineral content (Hoveland, 1993). Poor animal performance was not immediately associated with an endophyte, however, because this grass did not produce the sclerotia that is seen with the fungus, *Claviceps purpurea*, which can also be found in tall fescue (Bacon and Siegel, 1988; Hoveland, 1993; Strickland et al., 2011).

## STEER PERFORMANCE ON TALL FESCUE INFECTED WITH ERGOT ALKALOIDS

Reduced serum prolactin is one of the hallmark symptoms of cattle suffering from fescue toxicosis, as it is thought that ergot alkaloids are acting as a dopamine agonists which in turn reduces the level of prolactin in circulation (Aiken et al., 2011; Bacon and Siegel, 1988; Browning et al., 1998, 1997; Lipham et al., 1989; Porter and Thompson, 1992). It is well documented that animals grazing endophyte-infected fescue have depressed prolactin concentrations (Browning et al., 1997; Lipham et al., 1989; Porter and Thompson, 1992; Strickland et al., 2011; Thompson et al., 1987). There are occasional studies where reduced serum prolactin is not observed, but this could be partially explained due to the time of year when the study was performed as prolactin concentrations do change with photoperiod (Browning et al., 1998).

The observed decline in prolactin occurs due to the structural similarity of the various ergot alkaloids to the biogenic amines (such as dopamine, norepinephrine and epinephrine). The ergot alkaloids can bind to the biogenic amine receptors and cause a response. One pathway that they are able to act via is to bind the D2 dopamine receptor, acting as an agonist, which eventually reduces the release of prolactin into circulation (Lipham et al.,

1989; Strickland et al., 2011). When Metoclopramine, a dopamine antagonist, was administered to steers for 10 weeks grazing endophyte-infected pasture, it did improve circulating prolactin concentrations. It was, however, more effective on animals grazing pastures with a low infection rate versus a pasture with high infection rate which indicates that this might not be the only pathway involved in reducing prolactin concentrations (Lipham et al., 1989).

Prolactin concentrations can be linked to hair growth in the animals. Since steers have reduced serum prolactin, the concentrations may be too low to keep the animal from growing hair at inappropriate times of the year (Aiken et al., 2011). This could contribute to the rough haircoat that is associated with animals suffering from fescue toxicosis, along with the fact that winter haircoats appear to not shed out in a timely fashion (Bond et al., 1984; Porter and Thompson, 1992; Schmidt and Osborn, 1993). After clipping all old hair in the summer, one study found that 80% of rough-coated animals grew new long day hair after clipping. This would indicate inappropriate timing of hair growth and that animals are not in fact retaining old hair but growing new hair throughout the year when grazing endophyte-infected tall fescue (Aiken et al., 2011).

The retained haircoat may potentially act as an insulating layer that does not allow the steer to dissipate heat. Animals exhibiting the characteristic rough haircoat while grazing endophyte-infected fescue were noted to exhibit an increased rectal temperature when grazing at an ambient temperature (Aiken et al., 2011). Increased rectal temperature is another characteristic of animals suffering from fescue toxicosis. The animals more easily become hyperthermic, are less tolerant of heat and any negative effects of fescue toxicosis are exacerbated by elevated temperature due to this (Ball et al., 2015; Klotz, 2015; Porter and

Thompson, 1992; Schmidt and Osborn, 1993). There are studies with data indicating no differences in rectal temperature for cattle that have been exposes to various ergot alkaloids, but many times these animals have had intravenous injections of the ergot alkaloid and very short term exposure to it ( Browning and Leite-Browning, 1997; Browning, 2000; McClanahan et al., 2008). Additionally, clipping the rough haircoats of animals grazing endophyte-infected pasture did not cause a decrease in rectal temperature when compared to unclipped cattle grazing the same pasture (McClanahan et al., 2008).

Sweating is also reduced in animals that are suffering from fescue toxicosis. Over the course of a study where steers grazed endophyte-infected pasture, sweat production decreased from 51.8 $g/m^2$/hour on day 28 to 29 $g/m^2$/hour at day 104 of the study (McClanahan et al., 2008). Skin temperature is decreased at the extremities as well (1.4% at the tailhead and 1.9% at the tip of the tail, when compared to core body temperature), which indicates that the animal may not be able to dissipate body heat (Browning, 2000). Excessive salivation is also observed, which is likely caused by the animal panting in an attempt to rid themselves of the extra core body heat that has been created (Bond et al., 1984; Schmidt and Osborn, 1993).

Vasoconstriction is one other commonly observed symptom in cattle grazing endophyte-infected tall fescue. Vasoconstriction, caused by activity of the ergot alkaloids at the adrenergic receptors, can contribute to decreased skin temperature observed at the periphery (Browning, 2000; Klotz, 2015), but also leads to increased blood pressure. Ergot alkaloids are noted to have a vasoconstrictive effect (Porter and Thompson, 1992) and this results in decreased blood flow. Decreased blood flow caused by ergot alkaloids is due to damage caused to the endothelial lining of blood vessels which leads to edema and

thrombosis (Klotz, 2015). Both systolic and diastolic blood pressure are increased by exposure to ergot alkaloids when compared to control animals and this would indicate that there is vascular resistance and/or vasoconstriction present (Browning and Leite-Browning, 1997; Browning, 2000). Heart rate is typically reduced in animals suffering from fescue toxicosis, however this effect has been difficult to repeat in studies. Oftentimes there is no difference in heart rate (Browning and Leite-Browning, 1997; Browning, 2000), or even an increase in heart rate (Bond et al., 1984). This could have to do the fact that many studies that chose to evaluate heart rate chose to use intravenous ergot alkaloids injections and only observed the animals for 1-2 hours.

In contrast to the variability observed in heart rate, increased respiration rate is a reliable indicator when observing an animal (Bond et al., 1984; Klotz, 2015; Porter and Thompson, 1992) as it is one of the variables that is consistently observed in animals that are suffering from fescue toxicosis. This was also seen after intravenous administration of ergot alkaloids when the animals were observed for 2 hours (Browning et al., 1998), but was not always the case. When the same lab performed the same study on a group of heifers with intravenous injection of ergonovine or ergotamine, respiration rate remained unchanged (Browning and Leite-Browning, 1997).

As these cattle have proven themselves to be heat-intolerant with trouble thermoregulating (Porter and Thompson, 1992), it should come as no surprise that grazing behavior is also altered. The animals will seek shade, spend less time grazing and tend to stand in water in an effort to reduce body temperature (Schmidt and Osborn, 1993). When steers were grazed on either an endophyte-infected or endophyte-free pasture for 3 years, those on the endophyte-infected pasture spent substantially less time grazing and more time

lying in the shade. It should be noted that when the ambient temperature was high, all animals, regardless of treatment, spent more time lying in the shade than grazing (Bond et al., 1984). Another important consideration is that when pastures are at not suitably stocked and an endophyte-infected pasture grows to seedhead, the cattle will selectively graze them. This will cause the animals to suffer even more severe effects of fescue toxicosis (Schmidt and Osborn, 1993).

While all of the above mentioned symptoms are detrimental to animal performance, the most troubling side effect of fescue toxicosis to the producer is the reduced weight gain that is observed (Porter and Thompson, 1992). When rats were fed a diet of infected fescue seed, as the percentage of diet comprised of seed increased, average daily gain decreased (Neal and Schmidt, 1985). Both animal weight gains and feed intake typically decrease when animals are grazing endophyte-infected fescue. Poor weight gain is typically associated with the fact that the animal is not eating enough to meet its nutritional needs (Bond et al., 1984; Klotz, 2015; Schmidt and Osborn, 1993). When examined, there is more dry matter remaining in the rumen which could indicate decreased blood flow to the rumen. In fact, when ergotamine and ergovaline were given intravenously, rumen and reticulum contractions were immediately stopped (Klotz, 2015).

With dry matter not moving well through the digestive tract and the animals not eating as much as would be expected, there is no surprise to see decreased average daily gains (ADG). When evaluated, ADG decreased from 0.76 kg/day at day 28 to 0.51 kg/day at day 104 of a study evaluating the effects of grazing a pasture with >61% endophyte infection (McClanahan et al., 2008). It is estimated that there is a 10% loss in ADG for every 10% increase in ergovaline present in tall fescue (Porter and Thompson, 1992). When steers were

treated with a dopamine antagonist while grazing a highly-infected tall fescue pasture, ADG improved to 0.314 kg/day compared to 0.150 kg/day for animals that were not treated (Lipham et al., 1989).

While this is all very dire, the negative effects on ADG can be reversed and generally see anywhere from a 30-100% improvement when animals are moved from endophyte-infected to endophyte-free pasture. Compensatory gains are thought to be best in the first 7 weeks after removal (Hoveland, 1993; Paterson et al., 1995). Additionally, when endophyte infection rate is low (<20%), animals can still have normal average daily gain and may still consume normal amounts (Schmidt and Osborn, 1993).

## DISEASES ASSOCIATED WITH GRAZING TALL FESCUE AND OTHER COMMON FORAGES

What the producers were seeing is a disease now known as fescue toxicosis which is estimated to cost the beef industry $1 billion annually in reproductive and performance losses (Strickland et al., 2011). It is far from the only disease that affects cattle grazing pasture. Symptoms of fescue toxicosis include decreased weight gain, less time spent grazing, increased time spent in the shade, increased blood pressure & respiration rate, decreased heart rate, elevated rectal temperature, excessive salivation, lameness, rough haircoat, and reduced serum prolactin (Aiken et al., 2011; Bacon and Siegel, 1988; Bond et al., 1984; Hoveland, 1993; Neal and Schmidt, 1985; Porter and Thompson, 1992; Strickland et al., 2011). Animals are exposed to ergot alkaloids upon consumption of the seed or leaf sheath of a plant that is associated with the endophyte (2.20 mg/g and 4.60 mg/g plant dry weight, respectively). There is very little toxin found in the remaining vegetative part of the tall fescue plant (Neal and Schmidt, 1985; Siegel et al., 1984).

Pastures with an infection rate of as little as 22% can elicit signs of fescue toxicosis (Aiken et al., 2011). Exposure of the animal to as little as 200 ppb of ergovaline can be toxic to cattle (Canty et al., 2014). Ergot alkaloids are ingested and absorbed through the mucosal membranes in pH neutral environments. In ruminants, this occurs via the rumen or small intestine. Most absorption is thought to occur in the rumen because the pH is neutral and the microbial activity breaks ergot alkaloids down to simpler molecules that can then be absorbed. Once across the epithelium, ergot alkaloids enter the lymphatic system and eventually, general circulation (Strickland et al., 2011; Stuedemann et al., 1998). Once in circulation, ergot alkaloids are able to act as an agonist to D2 receptor of Dopamine and elicit effects throughout the body (Lipham et al., 1989; Strickland et al., 2011; Stuedemann et al., 1998). This is due to the presence of a tetracyclic ergoline ring that is very similar in structure to the biogenic amines (Klotz, 2015). For elimination of the ergot alkaloids, a majority occurs through the urine. When animals were grazed on infected pasture for 169 days, 20 times more ergot alkaloids were excreted through the urine than what was found in the bile (Stuedemann et al., 1998).

Symptoms of fescue toxicosis will present within 10-21 days of animals being turned out onto endophyte-infected pastures (Schmidt and Osborn 1993). To get rid of symptoms, an animal will need to be removed immediately from all forage that contains the endophyte. This would include any tall fescue could have been baled when it was endophyte-infected. When hay was baled in Arkansas, it exhibited an ergovaline infection rate of 352 μg/kg. After 9 months of storage, ergovaline was still present at 238 μg/kg, which is still sufficient to elicit fescue toxicosis (Norman et al., 2007).

Ergot poisoning and fescue toxicosis share a lot of similarities, however, ergot poisoning is caused by the fungus *Claviceps purpurea* and can infect a wide variety of plants. These plants can include rye (which is the most susceptible) and other forages like bluegrass, fescue, brome and even ryegrass. Ryegrass will be discussed later as it is also host to *Neotyphodium lolii* which is a much larger problem for it. Ergotamine and ergonovine are the most prevalent ergot alkaloids produced by this relationship and anywhere from 200-600 ppb can have a negative effect on the animals. The fungus produces a hard, black sclerotia, as is seen with Dallisgrass, that contain varying amount of ergot alkaloid (Osweiler, 2016).

Symptoms caused by ergot poisoning result from grazing seedheads or consuming hay which was baled with seedheads. If a producer was to go into the field or examine hay to look for the presence of an infestation, appearance of dark brown, purple or black bodies in seed heads would be looked for (Randle and Griffin, 2013). Symptoms of ergot poisoning are like those of fescue toxicosis. The first visual sign to the producer will be lameness, typically in a hindlimb starting 2 to 6 weeks after grazing an infected pasture or eating infected hay. Vasoconstriction is a serious symptom because the ergot alkaloids act directly on the muscles lining arterioles by causing thrombosis which eventually causes damage to the muscles if the animal is not removed from the pasture and is continually exposed to ergot alkaloids. This will slow blood flow to the extremities, which can lead to necrosis. Much like is seen with fescue toxicosis, animals suffering from ergot poisoning will be heat intolerant and have decreased serum Prolactin concentrations. They will also have increased body temperature and respiration rates (Osweiler, 2016). Unlike fescue toxicosis, however, the animals do not suffer from reduced weight gain or a poor hair coat (Hoveland 1993).

There is no way for a producer to eliminate ergot alkaloid contamination from fields. There is also no way to treat an animal that is suffering from ergot poisoning or fescue toxicosis other than to remove cattle from the field and provide them with supportive care until symptoms have subsided. Proper grazing management is key to maintaining fields that are susceptible to ergot alkaloid production. The endophyte is concentrated in the seed head, much like is seen with Ryegrass and Dallisgrass. This means that grazing management is going to be one of the most important tools that a producer can utilize to reduce the impact of fescue toxicosis on a herd (Randle and Griffin, 2013). The herd should not be grazing the plant to the ground nor when it has flowered. A producer could plant clover in the field along with fescue to try and ameliorate the effects of endophyte infection. If it is feasible and another type of grass is available on the farm, the herd could be removed from fescue for the summer season (Ball et al., 2015; Hoveland, 1993; Lipham et al., 1989).

In recent years, many areas of the country have had wetter springs with higher humidity and cooler conditions during the time when the plants have gone to flower. This has only increased the contamination of ergot alkaloids in the fields and required more intensive management of those fields (Murphy, 2014).

Ryegrass can become infected with the fungus *Neotyphodium lolii*. This can result in the production of the tremorgenic neurotoxin lolitrem B which is an indole diterpene alkaloid. Ergovaline may also be produced, which is responsible for the symptoms of fescue toxicosis. The amount of fungus present in the plant begins to increase in late spring and will start to decline as temperatures decrease and the season shifts to fall. Ryegrass staggers, both perennial and annual, is a big problem in New Zealand but can still be an issue in other countries including North America, Europe, South America and Australia (Simpson, 2016).

Lolitrem B affects all parts of the plant above the ground but is most concentrated in the leaf sheath, seed and flower stalk. An infected plant is not easily visualized so it is impossible to tell if a field is infected prior to animals showing symptoms (Simpson, 2016). This is in contrast Dallisgrass where the infected seeds are larger than normal ones and exhibit a color change (Nicholson, 2011). Infection of a Ryegrass plant with *Neotyphodium lolii* can only occur through an infected seed. Lolitrem B acts as a Calcium-activated potassium-channel inhibitor. It has activity in the brain of the animal at the level of the cerebellum because it interferes with neuronal transmission (Simpson, 2016). When potassium channels do not function properly, a whole host of negative symptoms can occur. Keeping the channels from opening can result in agitation and incoordination (Shieh et al., 2000). If approached quietly, animals affected with ryegrass staggers will show very slight head tremors and nodding. If quickly approached, the head nodding will be much more aggressive and uncoordinated. If the animal attempts to run, it will be stiff and uncoordinated and the animal may collapse. If collapse does occur, the animal may flail their limbs. It is important to note that animals that are experiencing heat-stress will exhibit a higher degree of symptoms (Simpson, 2016), much like fescue toxicosis.

Even in a herd of cattle on the same farm, not all animals that are grazing the same pasture will exhibit symptoms of Ryegrass staggers. This is because disease susceptibility is a highly heritable trait and there is genetic variability among animals. Additionally, while 80-90% of susceptible animals fall prey to Ryegrass staggers, mortality is extremely low. Any deaths that occur are typically accidental, such as an animal drowning while drinking in a pond (Simpson, 2016). Recovery is spontaneous and takes as little as 1 week or up to 4 weeks (Agriculture Victoria, 2016). To prevent future cases from occurring, grazing

management is very important. Cattle should not be allowed to overgraze the pastures nor should pasture be allowed to overgrow to the point that the plant is flowering before the animals are turned out onto it. Either of these will give the animals a chance to graze part of the plant that contains a heavier concentration of the toxin. The producer could also plant a secondary type of grass or a legume in the field with the Ryegrass to dilute the effect of the toxin (Simpson, 2016). The infection of Ryegrass seed decreases over time as the seed is stored in an ambient temperature environment at high humidity. In fact, as the seed reaches an age of 18-24 months, there is almost no infection remaining. If this seed was planted in the field, the grass would still grow healthy and there should no ill-effect on the herd from it (Simpson, 2016).

Ryegrass is associated with *Neotyphodium lolii* because it is beneficial to the grass. The fungus makes the grass more vigorous with hardier growth to withstand more grazing. It is also a more productive grass that produces more seeds (Agriculture Victoria, 2016). This however, is a negative in an infected population because infected seeds are just going to allow more infected seeds to be produced. Additionally, the grass is both more drought and insect-resistant, which is an appealing characteristic for any producer (Agriculture Victoria, 2016).

Dallisgrass can be infected with the fungus *Claviceps paspali* (Nicholson, 2011). This allows for the production of several variations of a tremorgenic alkaloid, including paspalitrem A, paspalitrem B and papalinine (Poore, 2000). The fungus infects the flower of Dallisgrass and develops in the seed, the infected seed is called a sclerotia (Nicholson, 2011). If animals ingest the mature, infected seed heads, they can become susceptible to Dallisgrass Staggers (Poore, 2000). Symptoms include animals that are excitable and/or aggressive, have

tremors of the head, shoulder and flank, stiff gait, ataxia, collapse and possible death. If no treatment is provided, there is an estimate of up to 30% mortality from Dallisgrass staggers (Nicholson, 2011). Once symptoms present, it is important to remove animals from the pasture immediately and provide a quality pasture or hay source. It is also a good idea to remove animals from any pasture with hills or ponds to prevent stumbling down hills or drowning in a body of water while suffering from ataxia (Poore, 2000). This would be done for very similar reasons as would be done for an animal suffering from Ryegrass staggers, as accidental death might result. If an animal is down from tremors, they should be treated with supportive therapy and kept in a shaded area if possible. Full recovery from Dallisgrass Staggers could take up to 2 weeks (Nicholson, 2011). Claviceps paspali molecules are lipophilic and are rapidly absorbed from the gastrointestinal tract, can easily cross the blood-brain barrier and enter the central nervous system where claviceps paspali can have action. Effects are typically seen within a few days of ingestion but there is not a cumulative effect based on how much the animal ingested (Gupta, 2012).

To prevent future cases of Dallisgrass staggers, the producer could implement a rotational grazing model to make certain that any Dallisgrass pastures stay well grazed and that mature seedheads do not form. If the grass started to get too tall, it could be mowed and baled as hay before animals were allowed back on it but there is still the risk of infected seeds being on the ground when animals go back in that pasture. Infection of Dallisgrass with Claviceps paspali is the main disease that faces it. The infection occurs when the fungus replaces the caryopsis (seed) of the grass. The caryopsis initially ripen and fall to the ground in the fall and become mature in the spring, which is when the seeds also become toxic (Anderson, 1990).

## INVOLVEMENT OF ERGOT ALKALOIDS AND THE HYPOTHALAMIC-PITUITARY AXIS

**Catecholamines:** Epinephrine, norepinephrine and dopamine are classified as catecholamines. These are all biologic monoamines that are derived from L-tyrosine (Davis et al., 2009). Epinephrine and norepinephrine bind to the same type of receptor and those receptors are found on the cell membrane and can be found throughout the body (Calzada and Artiñano, 2001). There are two primary types of receptors, α and β adrenoreceptors, and each type of receptor has multiple subtypes (Guimarães and Moura, 2001).

The α-adrenoreceptors are typically associated with excitatory functions such as vasoconstriction and muscle contraction. There are both $\alpha_1$ and $\alpha_2$ subtypes of this adrenoreceptor, with multiple subtypes within them. $\alpha_1$-adrenoreceptor subtypes include $\alpha_{1A}$, $\alpha_{1B}$ and $\alpha_{1D}$. The $\alpha_2$-adrenoreceptor subtypes include $\alpha_{2A/D}$, $\alpha_{2B}$ and $\alpha_{2D}$ (Calzada and Artiñano, 2001). The $\alpha_1$-adrenorecptors are coupled to a $G_{q/11}$ proteins which stimulate Phospholipase C (PLC) activity within the activated cell. Once PLC has been activated, it catalyzes the hydrolysis of phosphatidylinositol 4,5-bisphosphate ($PIP_2$). Two different products can result, inositol trisphosphate ($IP_3$) which mediates the release of Calcium from mitochondria or diacylglycerol (DAG) which activates Protein Kinase C (PKC) (Guimarães and Moura, 2001). The $\alpha_2$-adrenoreceptor subtypes are typically coupled to $G_i$ proteins which inhibits the activation of adenylate cyclase and opening of Calcium channels while activating intracellular Potassium channels (Guimarães and Moura, 2001).

The β-adrenoreceptors have 3 definite receptor subtypes, $\beta_1$, $\beta_2$ and $\beta_3$. A fourth subtype, $\beta_4$, has been proposed and has been identified only in brown adipose tissue so far. $\beta_1$- and $\beta_2$-adrenoreceptor subtypes are coupled to $G_s$-proteins. Upon receptor activation,

adenylate cyclase is activated which generates cyclic AMP (cAMP) production. This activates Protein Kinase A (PKA) which phosphorylates intracellular Calcium channels and allows the influx of additional Calcium (Chen-Izu et al., 2000). The $\beta_3$-adrenoreceptor subtype is typically coupled to a $G_i$-protein and acts in the same manner as previously discussed in relation to the $\alpha_2$-adrenoreceptor, though it can be found coupled to a $G_s$-protein (Guimarães and Moura, 2001).

The steps that led to the eventual discovery of the adrenoreceptors occurred because it was determined that the effects of adrenaline were reversed by what was then called ergotoxine. It was found in 1958 that in fact, ergotamine is a selective agonist of the α-adrenoreceptor (Barger and Dale, 1907; Guimarães and Moura, 2001). Both the $\alpha_1$ and $\alpha_2$-adrenoreceptor subtypes can be involved in vasoconstriction, specifically $\alpha_{2A/D}$ (Calzada and Artiñano, 2001). As vasoconstriction is a significant symptom associated with fescue toxicosis and receptors for the catecholamines are located throughout the body, this is a very important target to consider when investigating the mode of action for ergot alkaloids in beef cattle (Calzada and Artiñano, 2001; Klotz, 2015).

**Dopamine:** Dopamine is a neurotransmitter that has many functions throughout the body. Receptors for dopamine are classified as D1-like and D2-like. The D1-like family includes D1 and D5. The D2-like families include D2, D3 and D4 (Civelli et al., 2003). Dopamine functions to aid in neuroendocrine regulation, cardiovascular regulation and general motor control (Gingrich and Caron, 2003). All dopamine receptors are G-protein coupled receptors (GPCR). The D1-like family associates with a G-stimulatory ($G_s$) protein and acts to increase intracellular adenylate cyclase and cyclic AMP (cAMP). The D2-like family

associates with the G-inhibitory ($G_i$) protein and inhibits intracellular cAMP release (Civelli, et al., 2003).

D2 receptor mRNA is most abundant in brain tissue which has led to the assumption that is the receptor associated with dopamine receptor at the level of the brain. Additionally, D2 receptors are more quickly desensitized than the other receptor types (Civelli et al., 2003; Gingrich and Caron, 2003). Dopamine and receptor agonists or antagonists can bind to the receptor either pre- or post-synaptically. Where the binding occurs alters the response. If binding occurs post-synaptically, a 2$^{nd}$ messenger system is initiated. Which 2$^{nd}$ messenger system cascade begins depends on which G-protein is associated with the receptor. If binding occurs pre-synaptically, dopamine release is altered (Civelli et al., 2003). The ergot alkaloids that are present in tall fescue bind to the D2 receptor pre-synaptically and acts as an agonist (Larson et al., 1999; Strickland et al., 2011).

Ergot alkaloids have a high affinity for the D2 receptor and their binding to the receptor has been shown to decrease adenylyl cyclase activity and alter downstream prolactin release. When cells were transfected with a rat D2 receptor, ergovaline inhibited the binding of radiolabeled dopamine. Ergovaline also inhibited induced cAMP production which is further evidence that it acts in an agonistic manner. To have further certainty that ergovaline was acting specifically at the D2 receptor, an antagonist to D2 was added to the cell culture. Once the D2 receptor antagonist was added to culture, the rat cells were able to resume cAMP production, even in the presence of ergovaline (Larson et al., 1995). Subsequent studies using a similar model illustrated that ergot alkaloid binding to D2 receptors decrease receptor density (Larson et al., 1999).

Decreased serum prolactin concentrations are a hallmark of fescue toxicosis and this is regulated via the D2 receptor (Larson et al., 1999). Binding of ergot alkaloids to the D2 receptor correlates with decreased prolactin concentrations (Larson et al., 1995). When rat pituitary cells were cultured in the presence of extracts of endophyte-infected tall fescue seed, prolactin secretion was suppressed. This effect was not seen when the extract was taken from endophyte-free tall fescue seed (Strickland et al., 1992). When a single-nucleotide polymorphism (SNP) occurred on chromosome 16, where bovine D2 is coded, the decreased serum prolactin associated with fescue toxicosis was alleviated when steers grazed endophyte-infected tall fescue. The small change in receptor coding could alter the affinity of ergovaline for the receptor and possibly explain why some animals can tolerate higher levels of ergot alkaloids (Campbell et al., 2014).

**Prolactin:** Prolactin is a polypeptide hormone that is approximately 200 amino acids in size that is released by the anterior pituitary gland (Brooks, 2012; Uddin et al., 2013). It is released from lactotrophs, a specific cell type in the anterior pituitary, in a form of calcium-dependent exocytosis (Ben-Jonathan et al., 2008). Secretion is affected by photoperiod and peaks during the summer when there is increased daylength. Secretion of prolactin is minimal in the months with reduced daylength. While there are no true, specific factors that cause the release of prolactin from the lactotrophs, several factors are thought to be involved. They include vasoactive intestinal peptide (VIP), thyrotropin-releasing hormone (TRH), and opiate peptides (Hard et al., 2001; Hashizume et al., 2010).

The main hormone involved in regulating the decrease of prolactin concentrations is dopamine and it does so through tonic, or sustained inhibition. Dopamine is carried to the anterior pituitary through the long portal vessels (Ben-Jonathan et al., 2008). When a group

of cattle had the hypophyseal stalk transected, prolactin concentrations in the portal blood showed an immediate increase which was followed by an increase in peripheral blood concentrations of dopamine. Prolactin concentrations did not show any further increases once dopamine increased and these researchers hypothesized that the prolactin was stimulating dopamine release, allowing the two hormones to work in a feedback loop together (Hard et al., 2001).

Once prolactin is released into the circulation, it must bind to the prolactin receptor (PRLR) to have activity. The PRLR is membrane bound and can be found in many tissues and cells including mammary, ovarian, prostate, adipocytes, liver and immune cells. Binding to the receptor activates the Janus-2 kinases (JAK2) which act as a signal transducer and phosphorylates signal transducer and activator of transcription 5 (STAT5) (Brooks, 2012; Uddin et al., 2013). When investigating a herd of Senepol crossbred cows in New Zealand that had either slick or rough coats, it was found that the slick coated cows had a mutation on chromosome 23 0.5 Mbp away from where prolactin is coded for. The cows also had a mutation of chromosome 20, which codes for PRLR. Interestingly, when their pituitary glands were examined the animals showed no difference in prolactin expression and when stimulated, secreted the same amounts of prolactin. The researchers hypothesized that the slick-coated animals with slightly altered gene expression might have a more efficient or higher-functioning 2$^{nd}$ messenger system once prolactin binds to PRLR (Littlejohn et al., 2014).

It has been well documented that animals suffering from fescue toxicosis have reduced serum prolactin concentrations (Bond et al., 1984; Browning, 2000; Klotz, 2015; Neal and Schmidt, 1985). When a group of 12-14 month old steers were grazed on either

endophyte-infected or endophyte-free tall fescue for approximately 3 months, prolactin concentrations were severely decreased in the group grazing the infected pasture when compared to the control (82 ng/mL versus 194 ng/mL). It is important to note that when animals are removed from endophyte-infected tall fescue, that prolactin concentrations will rapidly improve and return to normal concentrations (Aiken et al., 2013).

**Growth Hormone and Insulin-Like Growth Factor 1:** Growth Hormone (GH), also known as somatotropin, is a 191 amino acid peptide hormone that is secreted from the anterior pituitary. Secretion is driven in a positive manner by growth hormone releasing hormone (GHRH) that is being released from the hypothalamus. Negative feedback is exerted on GH release by somatropin (also called growth hormone inhibiting hormone), also released from the hypothalamus. There are 4 different varieties of GH that can be released, and they only vary from one another by a single amino acid substitution. This single difference in amino acid can change the activity of the GH being released and may alter the strength of the hormone (Etherton and Bauman, 1998).

Once released, GH binds to Growth Hormone Receptor (GHR). The GHR has a single transmembrane domain which spans across the cell membrane and an extracellular domain containing 6 cysteines with disulfide linkage along with a single free cysteine. Once GH binds to the receptor, JAK2 is activated leading to phosphorylation of the mitogen activated protein kinases (MAP kinases), ERK1 & ERK2. This allows for the activation of proteins including phospholipase A2 (PLA2) and various other intracellular transcription factors such as Stat proteins (Argetsinger and Carter-Su, 1996). GHR is found mostly on the liver, but can be found throughout the body though concentrations vary widely. Receptors have been identified in many tissues including lymphatic, adipose, intestine, heart, kidney,

lung, pancreas, brain, skeletal muscle and the testis. Feeding state can alter the level of GHR present on the liver as well. When rats were fasted for 4 days, it caused a 50% decrease in the amount of GHR present. The rats could rapidly recover, however, because when they were refed for 3 days GHR numbers recovered to normal. Decreased GH secretion has also been associated with decreased GHR numbers (Kelly et al. 1,991).

Changes in circulating concentrations of GH may not be completely related to secretion from the anterior pituitary. The kinetics of metabolic clearance may also play a role, particularly when looking at food intake of an animal. As food intake of an animal increases, the rate at which GH is cleared from the circulation also increases. In contrast, as food intake decreases, there is a linear decrease in the circulating concentration of GH. This has led researchers to believe that it may be more important to look at the hepatic clearance rate of GH from an animal to establish a relationship to average daily gain rather than trying to relate it strictly to GH concentration (Lapierre et al., 2000).

Growth hormone does have many direct effects on the animal. It is able to effect the growth of an animal by altering how nutrients are partitioned so that growth moves towards lean tissue accumulation (Etherton and Bauman, 1998). It can also increase the uptake of amino acids, protein synthesis and lipid oxidation (Argetsinger and Carter-Su, 1996). Growth hormone also directly stimulates cytochrome $P450_{15\beta}$ activity in the liver, which is involved in arachidonic acid metabolism and glucose metabolism and transport in adipocytes (Kelly et al., 1991).

There are also many indirect effects of GH and those are mediated by insulin-like growth factor 1 (IGF-1). IGF-1 is a 700 amino acid linear peptide that is mostly secreted from the liver. This secretion is highly controlled by GH. The gene encoding IGF-1 is

found on chromosome 12 and has 6 transcription start sites. The nutritional status of the animal at the time of transcription will determine which start site is utilized. In circulation, IGF-1 is typically found bound to one of 6 IGF binding proteins (IGFBP). Being bound to one of these binding proteins helps to increase the half-life of IGF-1 in circulation (Bentov and Werner, 2013).

The receptor for IGF-1 is a cell membrane receptor (IGF1R) that has 2 extracellular α subunits that are involved in binding the IGF-1/IGFBP complex and 2 transmembrane β subunits. These β subunits contain a tyrosine domain on the cytoplasmic side that, upon phosphorylation, activate the ras-raf-MAP kinase, involved in transcription and phosphatidylinositol-4,5-bisphosphate 3-kinase, which is active in cell proliferation (Bentov and Werner, 2013).

Fescue toxicosis has been found to have an impact on the circulating concentrations of GH and IGF-1. When cows were given injections of ergotamine, IGF-1 was significantly decreased by 2 hours post-injection but returned to pre-injection values by 3 hours post-injection (60 ng/mL versus 140 ng/mL, respectively) (Browning, 2003). When beef cows were grazed on endophyte-infected tall fescue versus common Bermuda grass, those grazing the endophyte-infected tall fescue had decreased IGF-1 concentrations once the study began when compared to controls (Looper et al., 2010). When groups of steers were grazed on pastures with either high or low endophyte-infection, serum GH concentrations were reduced in the group on the high-infection pasture (7.9 ng/mL versus 6.2 ng/mL) (Thompson et al., 1987). This is not always the case with GH, however, as some studies have seen that it does not change when animals are exposes to ergot alkaloids ((Browning et al., 1997). These

hormonal effects could potentially be important when trying to elucidate the decreased weight gain and feed intake associated with animals suffering from fescue toxicosis.

Prolactin and GH are also closely related. They help to form a family of polypeptide hormones that, through sequence homology, have been found to be closely related and are thought to have come about through duplication of the same gene. Both hormones are produced by the cells of the anterior pituitary and can be found in all vertebrates (Kelly et al., 1991). Functionally, both are part of a cascade of hormones that assist all mammals in developing body hair and regulating body temperature via homeostasis (Littlejohn et al., 2014). In rats, GH administration increases PRLR expression in the liver. This same study also found that both GH and PRL administration were able to upregulate the expression of PRLR (Kelly et al., 1991).

**Cortisol:** Cortisol is a glucocorticoid hormone that is released by the adrenal cortex. To lead to the release of cortisol, hypothalamic paraventricular nuclei release corticotropin releasing hormone (CRH) which goes to the anterior pituitary gland. There it triggers the release of adrenocorticotrophic-releasing hormone (ACTH) which travels through the bloodstream to the adrenal cortex and causes cortisol release. Cortisol is able to regulate its own negative feedback via binding to specific receptors that are located in the central nervous system (Dedovic et al., 2009; Ogino et al., 2014).

Cortisol is a hydrophobic molecule, so for it to remain soluble in blood it is typically bound to a binding protein known as transcortin (AKA: corticosteroid binding protein). In typical situations when an animal is unstressed, only 5% of cortisol will by unbound in circulation. This is because only unbound cortisol is able to cross the cell membrane and bind to the glucocorticoid receptor (GR) and elicit a response (Perogamvros et al., 2012).

The ligand binding domain (LBD) of the GR is located on the carboxyl terminal and the GR also contains a centrally located zinc-finger that acts as a DNA binding domain. The amino terminal is negatively charged and is home to an activation factor (AF-1) which is important for regulation of transcription. There is also a 2nd activation factor found in the LBD that is activated upon cortisol binding. Once the receptor and cortisol have dimerized and translocated to the nucleus, they are able to associate with glucocorticoid response elements (GRE) and exert control over genes involved in processes such as amino acid mobilization, gluconeogenesis, and blood glucose control (Heitzer et al., 2007).

Cortisol is secreted in a circadian rhythm and in cattle, concentrations peak in the morning when the animals first become active and decrease from there. It is also essential for the maintenance of homeostasis (Dedovic et al., 2009; Ogino et al., 2014). In addition to basal secretion of cortisol, it is also released as part of the body's reaction to stress and it acts along the hypothalamic pituitary axis in the same manner. In fact, it has been found that when humans had poor early life experiences, they were found to have increased cortisol in response to stressful experiences later in life (Dedovic et al., 2009).

Fescue toxicosis is known to be a stress-inducing situation in cattle. When cattle were given IV injections of ergotamine and then either restrained in a squeeze-chute or tethered for an extended period for blood sampling, it was found that serum cortisol was elevated when compared to control animals in both cows and steers. Cortisol concentrations did return to baseline concentrations within 3 hours in both studies (Browning, 2000; Filipov et al., 2000). When heifers were placed in environmental chambers and fed endophyte-infected or endophyte-free fescue seed with ergovaline as the toxin, cortisol concentrations were not different between the treatment and control groups (15.8 versus 16.7 ng/mL)

(Aldrich et al., 1993). In a group of Angus crossbred steers that were in the backgrounding stage that were either grazed on Kentucky-31 or one of two varieties of novel endophyte tall fescue (MaxQ or HiMag4), the animals on the infected tall fescue had the lowest serum cortisol of all 3 groups (Sales et al., 2010).

**Luteinizing Hormone:** Luteinizing hormone (LH) is a glycoprotein hormone composed of an α and a β subunit. It is released along the hypothalamic-pituitary-gonadal axis and this axis begins with the release of gonadotropin-releasing hormone (GnRH) from the hypothalamus. GnRH moves through the hypophyseal portal system and then binds to the GnRH receptor found on the gonadotrope cell, located on the anterior pituitary. The gonadotrope releases LH in a pulsatile manner and it travels through the circulation to the testes. Once there, LH binds to the LH receptor (LHR) which is found on the leydig cells and is involved in the synthesis and secretion of testosterone (Bliss et al., 2010; Desjardins, 1981; Segaloff, 2010). The LHR is a GPCR and is associated with $G_s$ (Segaloff, 2010). Upon activation of the $G_s$ protein, adenylate cyclase is activated which generates the production of cyclic AMP. This activates intracellular protein kinase A and permits the further actions that occurs in the leydig cells (Chen-Izu et al., 2000).

Both GnRH and LH are released in a pulsatile manner and this helps to keep the cells that respond to them from becoming unresponsive (Desjardins, 1981). Additionally, along this signaling pathway the amount of hormone in circulation is not what determines the cellular response. In fact, it is the duration and intensity of the hormone pulse as well as the amount of time between pulses. Negative feedback in this pathway is elicited by the concentration of testosterone in circulation. It acts indirectly at the level of the hypothalamus to decrease the release of GnRH, and subsequently LH (Bliss et al., 2010; Desjardins, 1981).

However, in castrated males this negative feedback is not exerted. It has been shown that systemic testosterone concentrations drop significantly following castration and in rats, within 3 hours of the procedure testosterone production was only at 5% of what it was prior and remained at the concentration (Potter et al., 2006).

There are varying reports on the effects of ergot alkaloids on LH concentrations in cattle. When ergonovine and ergotamine were administered to cows via IV administration, it was found to decrease serum LH concentrations for up to 4 hours (Browning et al., 1998). Similar results were found when steers were administered IV ergotamine (Browning et al., 1997). However, when groups of both steers and heifers were grazed on an endophyte-infected tall fescue pastures, it was found that there was no difference in serum LH concentrations when compared to controls that were not exposed to ergot alkaloids (Thompson and Stuedemann, 1993). When 16 Angus cows were pair-fed either endophyte-infected or endophyte-free tall fescue seed with ergovaline as the primary ergot alkaloid for 75 days, it was again found that LH secretion was not impacted. These researchers stated that this was in agreement with their previous research, although it was not using pair-fed or seed models and it was not comparing similar results (Mizinga et al., 1992).

## CONCLUSION

The consumption of ergot alkaloids initiates a complex cascade of physiological and hormonal responses in cattle that are grazing endophyte-infected tall fescue. Cattle exhibit reduced weight gain, poor feed intake, increased respiration rate and body temperature and decreased serum prolactin to name just a few symptoms. There is a great deal of information that is not known about fescue toxicosis and exactly how ergot alkaloids are functioning in the body, however. One of the biggest holes in the literature currently is the fact that

individual ergot alkaloids have not been investigated and until research reveals how the individual toxins are functioning, it will be difficult to reduce the symptoms of fescue toxicosis.

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# CHAPTER 2

## Effect of chronic endophyte exposure on growth and performance in steers

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## INTRODUCTION

Tall fescue (*Lolium arunddinaceum* Schreb. Darbysh) is the most predominant grass growing in the eastern United States due to its drought tolerance and ability to withstand the environmental conditions present in the area. However, it is estimated that the roots of 95% of the tall fescue in the United States are associated with the endophyte *Neotyphodium coenophialum* (Hoveland, 1993). While this symbiotic relationship between the grass and the endophyte has been observed to support hardiness, the endophyte also produces ergot alkaloids which have been shown to elicit the signs of a disease known as fescue toxicosis (Browning et al., 1998).

Fescue toxicosis, initially known as summer syndrome, is extremely costly to the beef industry with an estimated loss of $609 million annually. Most of these losses are attributable to reduced weight gains (Hoveland, 1993). Animals grazing tall fescue exhibit a rough haircoat, elevated rectal temperature, increased respiration rate, decreased weight gain and increased blood pressure. These animals are intolerant of the heat, likely because of their longer haircoats (Browning et al., 1998; Hoveland, 1993; Paterson et al., 1995; Porter et al., 1985; Porter and Thompson, 1992; Strickland et al., 2011). Experiments have revealed that when cattle were exposed to controlled amounts of known ergot alkaloids (ergotamine and ergovaline), decreased skin temperature and heart rate were observed. This finding led researchers to believe that the animals were also suffering from vasoconstriction, with less blood getting out to the peripheral areas of the body (Paterson et al., 1995).

These controlled studies were all performed with intravenous injections of the ergot alkaloid in question. The physiological characteristic changes were observed both prior to and within two hours of administration. While this information is extremely useful, it does

not answer questions about the long-term effect of ergot alkaloids on cattle grazing tall fescue. Many producers continue to utilize pastures of endophyte-infected tall fescue over the much more expensive and less hardy endophyte-free variety (Hoveland, 1993). This study investigated the effects of chronic exposure of steers to a specific ergot alkaloid, ergovaline, and considered the specific effects that it might have on the physiological characteristics of those. It is hypothesized that ergovaline alone will not elicit a full-blown case of fescue toxicosis without the other ergot alkaloids that are typically present in endophyte-infected tall fescue, as the toxins act in concert with one another to cause the disease. The design of this study will allow determination of which specific physiological characteristics are being caused by exposure to ergovaline.

## MATERIALS AND METHODS

**Animals:** Under an approved protocol by North Carolina State University's Institutional Animal Care and Use Committee (Protocol #13-95-A), 8 crossbred Angus x Simmental steers were purchased and placed in a freestall barn at Butner Field Research Laboratory in Butner, NC. The barn was equipped with Calan gates (American Calan, Northwood, NH) which ensured proper treatment application to individual animals, as well as to determine how much feed each animal consumed. Animals were allowed a 7-day transition period to become acclimated to the Calan gates. For the duration of the study, the steers were maintained under a covered barn to help minimize any effect of heat stress on the study.

**Fescue Seed:** Animals were randomly assigned to either the treatment (n=4) or control (n=4) groups. Treatment animals received a total mixed ration (TMR) containing endophyte-infected fescue seed (EI, Piedmont Tall Fescue) and the control group was fed a

TMR containing non-infected fescue seed (EF, Kentucky-32). The TMR was fed at 4.6% of the animal's body weight and TMR composition is presented in Table 1.2. Treatment was applied for 63 days and animals had unlimited access to fresh water.

Every 14 days, feed samples were taken and analyzed for alkaloid concentration and nutritional composition. The amount of ergot alkaloid that was found in the fescue seed was examined via high performance liquid chromatography (HPLC) at the University of Missouri Veterinary Medical Diagnostic Laboratory (Columbia, MO) (Rottinghaus et al., 1993). The data for overall alkaloid concentration during the study is presented in table 2.2. Data related to the nutrition content provided by the TMR is provided in table 3.2.

**Animal Measurements:** Beginning at day 0, animals were run through the chute every 7 days as a group at approximately 1:00 P.M. The whole process took approximately 1 hour, or 10 minutes per animal. Prior to entering the squeeze chute, each animal was weighed and was assessed for behavior on a scale of 1-5, with 1 being very calm and 5 being very excited. Once in the chute, 2 blood samples were taken via jugular venipuncture with a 20-gauge needle, one collected into a sterile silicone coated glass vacutainer serum tube without additive (for hormone analysis) and the other into a sterile glass vacutainer serum tube with liquid $K_3$EDTA added (for hematocrit). Rectal temperature was taken using a digital thermometer. To assess temperature via thermal camera (Fluke Ti45FT IR Flexcam®, Fluke Corporation, Everett, WA), the animals had an 18x20 cm square clipped behind the left shoulder using a 10 blade. Each week, images were taken of that square with the camera and the highest, lowest and average temperature were recorded (SmartView 3.5 Thermal Imager Software). Respiration rate was determined by visually watching the animal's diaphragm for 15 seconds. Heart rate and caudal blood pressure were measured

using a 16-24 cm blood pressure cuff (LifeSource A&D Engineering Inc., San Jose, CA). The tail was held steady during these measurements to minimize variation. Additionally, 3 measurements were taken for accuracy and if a measurement varied more than 15%, it was discarded. Caudal artery and vein diameter were measured using doppler ultrasonography (M-Turbo, SonoSite Inc, Bothell, WA). After exit from the chute, haircoat and hair shedding scores were assessed by the same, blinded observer on a scale of 1-5. See figure 1.2 for a study of the schematic.

**Necropsy:** At the completion of the 60-day study, all 8 animals were transported to the NCSU College of Veterinary Medicine (NCSU CVM). Two animals were provided anesthesia at the same time (1/treatment group) and were then euthanized individually in the necropsy lab. All organs were weighed and samples were taken of them. The various tissues were minced to the size of playing dice and were either: immediately frozen in liquid nitrogen, fixed in OCT and then frozen in liquid nitrogen, fixed in formalin and left at room temperature or fixed with RNAlater and left at room temperature. See Table 4.2 for a list of all tissues sampled and weight at time of necropsy.

**Statistics:** All statistical analyses were performed using Proc Mixed with repeated measures in SAS version 9.3. The experimental unit was the individual steer and the model statement included the date and treatment. A P-value of $\leq 0.05$ was considered statistically significant and a P-value between 0.05 and 0.10 was considered a trend towards statistical significance.

## RESULTS AND DISCUSSION

**Body Weight and Body Condition Score:** There was no direct impact of ingesting ergot alkaloids on the body weight of steers over the duration of the study. It is important to note, however that the EI steers started out at a significantly heavier weight and there was a

date by treatment interaction present (P=0.0499, Figure 2.2). This leads into the fact that there was a trend towards a reduced weekly weight gain and average daily gain in the EI group throughout the study (P=0.0902, Figure 3.2 and 4.2 respectively). Even though these animals started out at a heavier weight, gain was not as efficient as that seen in control steers, while there were no differences in feed intake as all provided feed was consumed. Additionally, there was a trend towards a date by treatment interaction effect for body condition score. The EI animals had a slightly reduced BCS over the course of the study when compared to control animals (5.3963 vs 5.4625, respectively; figure 5.2).

**Hematocrit:** Normal hematocrit is approximately 30% (Peiró et al., 2010). Hematocrit represents the percentage of red blood cells present in the blood and is an indication of the ability of the oxygen-carrying ability of the blood (Sejian et al., 2012). Average hematocrit levels were not different between the EI and EF groups in this study (32.9042% vs 32.4472% respectively; Figure 6.2). The average hematocrit ranges exhibited in this group of steers was consistent with data collected on a group of heifers at the same time at the same research facility (Lyons, 2015).

**Respiration Rate:** There was no impact of ingesting ergot alkaloids on respiration rate in this study (63.75 vs 66.9 breaths/minute for EI vs EF group P>0.05, Figure 7.2). This is not in agreement with most other studies that saw increased respiration rates in cattle suffering from fescue toxicosis (Bond et al., 1984; Porter and Thompson, 1992). However, this study had 4 animals per treatment group and if a study was performed with larger numbers a difference might have been seen. Additionally, these animals were kept in the shade for the duration of the study and were not subject to heat stress, which could have led to a reduced incidence of increased respiration rate.

**Heart Rate and Blood Pressure:** Average heart rate was reduced in the EI group compared to the control group (67.4252 beats/min vs 73.7168 beats/min respectively, P=0.0049; Figure 8.2). This is in agreement with findings that animals suffering from fescue toxicosis exhibit reduced heart rate when compared to controls (Klotz, 2015; Neal and Schmidt, 1985; Strickland et al., 2011) and could potentially implicate ergovaline as being one of the ergot alkaloids involved in causing this symptom.

Systolic blood pressure did not differ between the groups (124.21 mmHg vs 126.42 mmHg P>0.05 EF vs EI, respectively). The same can be said for diastolic blood pressure (68.2003 mmHg vs 70.2745 mmHg P>0.05 EF vs EI, respectively). Changes in both variables over the course of the study can be viewed in Figure 9.2. Blood pressure was highly variable over the course of the study in both groups.

**Rectal and Thermal Temperature:** Two types of temperature were taken on the animals and while the actual temperatures were different, values did not differ between treatment groups for the same variable (35.99°C vs 39.27°C for EI versus 36.01°C vs 39.14°C for EF; thermal vs rectal temperature; Figure 10.2). Animals were being handled in a shaded chute and were in a predominantly shaded area for the duration of this study. This could potentially have had some effect the similarity in body and surface temperature that was observed. This part of the study was designed specifically, however, as the aim of this study was to evaluate the effects of ergovaline and the housing attempted to remove the effect of heat stress. While one of the hallmarks of animals that are suffering from fescue toxicosis is elevated body temperature which leads to the animal spending increased time in the shade and decreased food intake (Strickland et al., 2009), this is a variable that was not focused on in this particular study.

**Haircoat and Shedding Scores:** When evaluating the quality of the steers haircoats, it was visually apparent that the EI group had a slightly rougher coat when compared to the EF controls (3.14 vs 2.64; P>0.05). There was only a numerical difference however, and this could be attributed to the fact that only a small number of animals was present in each group. The numerical difference observed is consistent with the findings of other researchers that animals suffering from fescue toxicosis have a rougher haircoat. The haircoat is longer in length which makes it more difficult for the animal to dissipate heat and causes expenditure of more energy through sweating and panting (Parish et al., 2013). Cattle suffering from fescue toxicosis spend a higher amount of time in the shade or near a source of water and because of this, a reduced amount of time grazing. This leads to reduced average daily gain and overall poor growth when compared to animals of the same age that are not grazing endophyte-infected grass (McClanahan et al., 2008). In this study when evaluated as an entire 63-day block, a significantly rougher haircoat was not present in the EI animals, however, the score was numerically higher and there was a trend towards reduced average daily gain (P=0.09, Figure 4.2) which follows along with the trend that is observed in the literature.

When looked at over the course of the entire study, shedding scores followed a similar trend that haircoat scores did. The EI steers were slower to shed out the winter coat (3.075) when compared to the EF steers (2.475). However, this was only a numerical difference and no statistical difference was observed (P>0.05). Animals with a lower shedding score have a shorter, sleeker coat and thus can dissipate heat more efficiently. This allows the cattle to utilize energy efficiently and spend a maximum amount of time grazing (McClanahan et al., 2008). There was a general trend for both haircoat and shedding scores to decrease throughout the duration of the study (Figures 11.2 and 12.2 respectively).

As it takes approximately 28 days to see the negative impact of ergovaline on cattle (Schmidt and Osborn, 1993), the data were analyzed looking at the first 4 weeks of the study versus the last 5 weeks of the study. It was found that there was a trend to have a reduced haircoat score in the EF steers when compared to those consuming the treatment diet in the 2nd half of the study (2.1 vs. 2.8, P=0.09). The same trend was apparent when looking at shedding scores. The steers started out similarly at the beginning of the study (3.5 vs. 3.1 for EI vs. EF steers, respectively) but the haircoat shedding scores became increasingly different as the study went deeper into the summer with animals on the treatment diet maintaining a longer, shaggier coat (shedding score of 2.8 vs. 1.9 for EI vs. EF steers, P=0.09). This could also implicate ergovaline as the ergot alkaloid that has activity to alter hair growth in animals that are consuming endophyte-infected tall fescue. As ergovaline has agonistic activity at the D2 dopamine receptor and alters prolactin release which is ultimately involved in hair growth and shedding, this is a potential pathway that is being altered (Aiken et al., 2011, 2013).

**Temperament:** Temperament scores were no different between treatment groups (1.4 vs 1.25 for EI vs EF, respectively P>0.05; figure 13.2). Ergot alkaloids disrupt the function of neurotransmitters, particularly norepinephrine and epinephrine (Paterson et al., 1995). With increased levels of either one of these circulating, it could make the animal more excitable and lead to an elevated temperament score. This was not seen in the current study and could be attributed to the fact that the animals were handled on a regular basis for sampling and weighing.

**Organ Weight:** At the time necropsy was performed, the heart, liver, spleen, pancreas and kidneys of each animal were weighed. There were no statistical differences in the weights of any organs (P>0.05) and organ weights are presented in table 4.2. While there

are very limited data available concerning alterations to organ weight caused by fescue toxicosis in cattle, there is some inconsistent data in rats. All studies involve feeding the rats seeds that have been infected with an ergot alkaloid (the most common being ergovaline). One study found reduced liver weight in rats that had been on a diet of ergovaline (Settivari et al., 2006). However, a previous study did not specify which ergot alkaloid was utilized in the seed and the liver, kidney, spleen and thymus were all similar across all 4 groups. Only the adrenal glands were significantly lower in weight in the treatment groups when compared to controls (Neal and Schmidt, 1985).

## SUMMARY

Tall fescue is a hardy, efficient forage when it is associated with an endophyte; and the endophyte-free variety lacks drought tolerance, attracts insects, must be intensively managed, aside from being expensive (Ball et al., 2015). For these reasons, producers are not likely to adopt endophyte-free tall fescue into regular practice. Persistent use of endophyte-infected tall fescue; however, will continue to expose cattle to ergot alkaloids including ergovaline and cause economic losses to the producer (Strickland et al., 2011). This study chronically exposed steers to ergovaline over a 63-day period and evaluated physiological responses on a weekly basis. The trends towards a loss in both weekly weight gain and average daily gain in the EI group are consistent with what has been observed by producers. Those same animals also tended to have a lower body condition score. Research suggests that for every 10% increase in ergovaline in the plant, there is a 10% kilogram decrease in average daily gain. These losses can be alleviated quickly by removing the animal from the endophyte-infected tall fescue with compensatory gains being the best about 7 weeks post-removal (Paterson et al., 1995).

Haircoat scores and shedding scores were also increased in the EI group during the second half of the study (weeks 5-9) when compared to the EF group. It takes approximately 28 days for ergot alkaloids to have an impact on cattle that are exposed to them (Schmidt and Osborn, 1993). Ergovaline was the particular ergot alkaloid investigated in this study and it has specific activity at the D2 dopamine receptor where it subsequently decreases the release of prolactin (Larson et al., 1995). Prolactin is involved in the appropriate timing of hair shedding and growth (Aiken et al., 2011, 2013). As it has been illustrated that the EI animals did not shed out winter coats as quickly as the EF group, it could be inferred from this study that ergovaline specifically is acting in an agonistic manner at the D2 receptor and altering prolactin release which is causing the treatment steers to have a poor haircoat when compared to control animals.

This is where any similarities to the symptoms associated with fescue toxicosis end in this study though, however fully eliciting those symptoms was never the true aim of this study. The aim of this study was always to determine how physiological response was altered in steers when the animals were chronically exposed to ergovaline. The animals were specifically maintained in a shaded barn for the 63-day study so that the effects of heat stress were not allowed to confound upon the effects of ergovaline. Keeping that in mind, no effect of toxin was observed on either body or surface temperature. There was additionally no difference between the two study groups in respiration rate. These are two key variables where an animal that is grazing endophyte-infected tall fescue will have elevated body temperature and increased respiration rate. However, this study was designed to attempt to control for this and appears to have been successful.

There were also no differences in blood pressure observed between the EI and EF group observed in this study. This is in disagreement with much of the literature which has found that many animals suffering from fescue toxicosis have elevated systolic and diastolic blood pressure (Browning and Leite-Browning, 1997; Browning, 2000). This could be because the study looked specifically at ergovaline versus looking at other ergot alkaloids such as ergotamine or ergonovine. Ergotamine has been shown induce contractions in the lateral saphenous vein of cattle, specifically at the α2-adrenergic receptor (Oliver et al., 1998). Because a specific toxin (ergovaline) was utilized in this study versus grazing pasture or feeding endophyte-infected tall fescue which contains a mixture of ergot alkaloids, it is possible that ergovaline is not the toxin responsible for increasing blood pressure in animals suffering from fescue toxicosis. This could be because it is not active at the α2-adrenergic receptor, but a current review of the literature does not reveal this information.

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## TABLES

Table 1.2 Total Mixed Ration composition, presented on DM basis

| | | %DM |
|---|---|---|
| **TMR Ingredient** | | |
| | Corn Silage | 57% |
| | Fescue Seed | 18% |
| | Concentrate | 25% |
| **Concentrate Composition** | | |
| | Corn | 15.2 |
| | Soybean Meal | 8.5 |
| | Limestone | 1.0 |
| | Trace Mineral Salt | 0.5 |
| | Rumensin 90 (g/kg)[1] | 1.8 |

[1]: Fed as part of diet to control for coccidiosis

Table 2.2 Alkaloid Content of Tall Fescue Seed

| **Seed Type[1]** | **EI** | **EF** | **EI On DM Basis** | **EF on DM Basis** |
|---|---|---|---|---|
| Ergonosine (ppb) | 0 | 0 | 0 | 0 |
| Ergotamine (ppb) | 0 | 0 | 0 | 0 |
| Ergocornine (ppb) | 0 | 0 | 0 | 0 |
| Ergocryptine (ppb) | 0 | 0 | 0 | 0 |
| Ergocristine (ppb) | 0 | 0 | 0 | 0 |
| Ergovaline (ppb) | 2,830 | 0 | 509.4 | 0 |
| Total Ergot Alkaloids | 2,830 | 0 | 509.4 | 0 |

[1]: EI (endophyte-infected) seed is Piedmont Tall Fescue. EF (endophyte-free) is Kentucky-32 (KY-32)
Sub-sampling of seed lots was performed in April, prior to trial initiation and analyzed using HPLC at the University of Missouri Veterinary Medical Diagnostic Laboratory

Table 3.2 Nutritional value provided by the total mixed ration

| Item | Fescue Seed | Corn Silage | Ground Corn | Soybean Meal | TM Salt | Limestone | Requirements |
|---|---|---|---|---|---|---|---|
| DM (%) | 18 | 57 | 15.2 | 8.5 | 0.3 | 1 | |
| TDN (%) | 12.6 | 39 | 13.2 | 7.4 | 0.0 | 0.0 | 72% |
| CP (%) | 2.6 | 3.9 | 1.4 | 4.6 | 0.0 | 0.0 | 14% |
| Ca (%) | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.4 | 0.35% |
| P (%) | 0.0 | 0.1 | 0.0 | 0.1 | 0.0 | 0.0 | 0.22% |

Table 4.2 Organ weights of steers consuming TMR with ground fescue seed from May to July

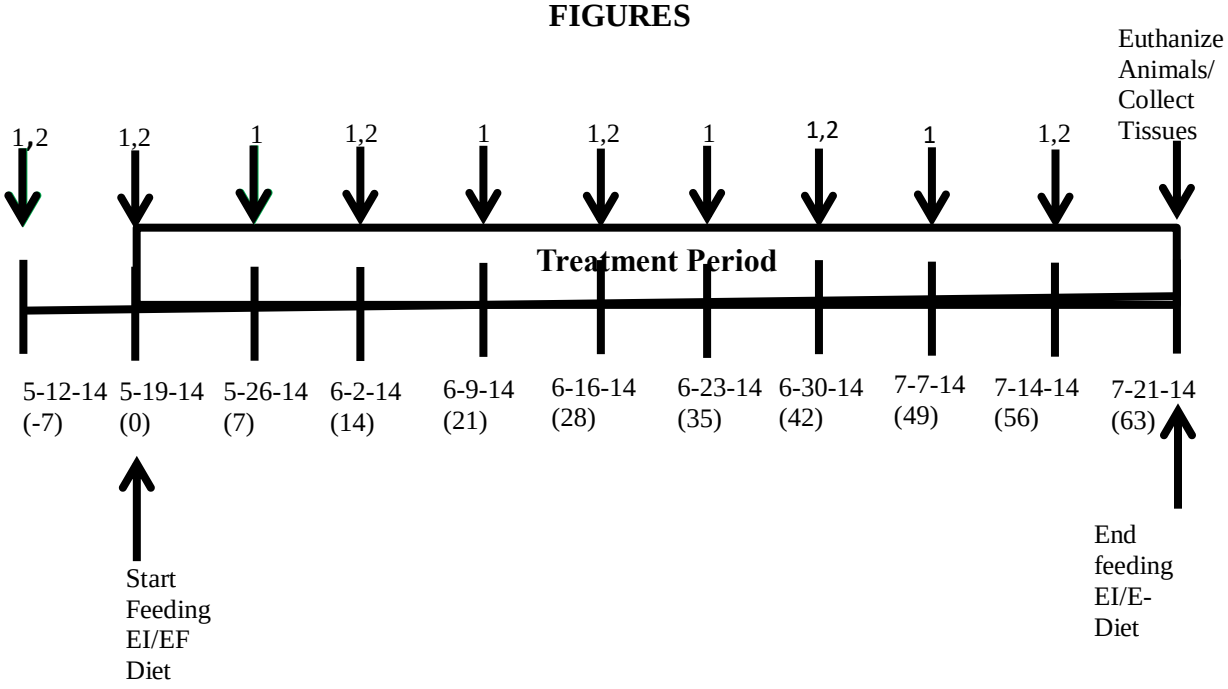
| Organ | EI Weight (kg) | EF Weight (kg) | SE | P-Value |
|---|---|---|---|---|
| Heart | 4.3125 | 4.0625 | 0.1731 | 0.3464 |
| Liver | 10.75 | 10.75 | 0.1443 | 1.00 |
| Spleen | 4.75 | 5.725 | 0.6271 | 0.3619 |
| Kidney | 2.125 | 2 | 0.08839 | 0.3559 |
| Pancreas | 0.65 | 0.6 | 0.1458 | 0.8164 |

Table 5.2 General data summary for steers consuming TMR with ground fescue seed from May to July

| Variable | EI | EF | SEM | Treatment P-Value | Date P-Value | Interaction P-Value |
|---|---|---|---|---|---|---|
| **Weight (kg)** | 425.82 | 409.49 | 7.9193 | 0.1951 | <0.0001 | 0.0499* |
| **Weekly Gain (kg)** | 6.9175 | 7.9508 | 0.6216 | 0.2844 | <0.0001 | 0.0902 |
| **Average Daily Gain (kg)** | 0.9882 | 1.1358 | 0.08881 | 0.2844 | <0.0001 | 0.0902 |
| **BCS** | 5.3863 | 5.4625 | 0.09035 | 0.5725 | <0.0001 | 0.0737 |
| **Haircoat** | 3.1375 | 2.6375 | 0.3896 | 0.3992 | <0.0001 | 0.6495 |
| **Shedding** | 3.075 | 2.475 | 0.4245 | 0.3562 | <0.0001 | 0.4417 |
| **Thermal Camera Temperature (°C)** | 35.9875 | 36.0128 | 0.2485 | 0.9451 | <0.0001 | 0.3523 |
| **Rectal Temperature (°C)** | 39.272 | 39.1418 | 0.07195 | 0.2478 | 0.3959 | 0.1761 |
| **Respiration Rate (breaths/min)** | 63.75 | 66.9 | 1.6871 | 0.2349 | 0.0003 | 0.3249 |
| **Average Heart Rate (Beats/Min)** | 67.4252 | 73.7168 | 3.6373 | 0.2672 | <0.0001 | 0.0049* |
| **Systolic Blood Pressure** | 124.21 | 126.42 | 4.236 | 0.7241 | 0.03 | 0.2729 |
| **Diastolic Blood Pressure** | 68.2003 | 70.2745 | 2.3609 | 0.5573 | 0.0094 | 0.2349 |
| **Caudal Artery Diameter (mm)** | 35.575 | 34.375 | 0.8107 | 0.3356 | 0.0008 | 0.0037* |
| **Caudal Vein Diameter (mm)** | 44.575 | 40.875 | 1.4384 | 0.1188 | 0.0272 | 0.7244 |
| **Average Hematocrit** | 32.9042 | 32.4472 | 1.1175 | 0.7822 | 0.0547 | 0.6022 |
| **Temperament** | 1.4 | 1.25 | 0.1568 | 0.5239 | 0.0427 | 0.3915 |

* indicates statistical significance

## FIGURES




1: Physical exam on animals & blood collection
2: Fescue seed collection & feed sampling

*Figure 2.2 Schematic of study for steers consuming TMR containing fescue seed from May to July*

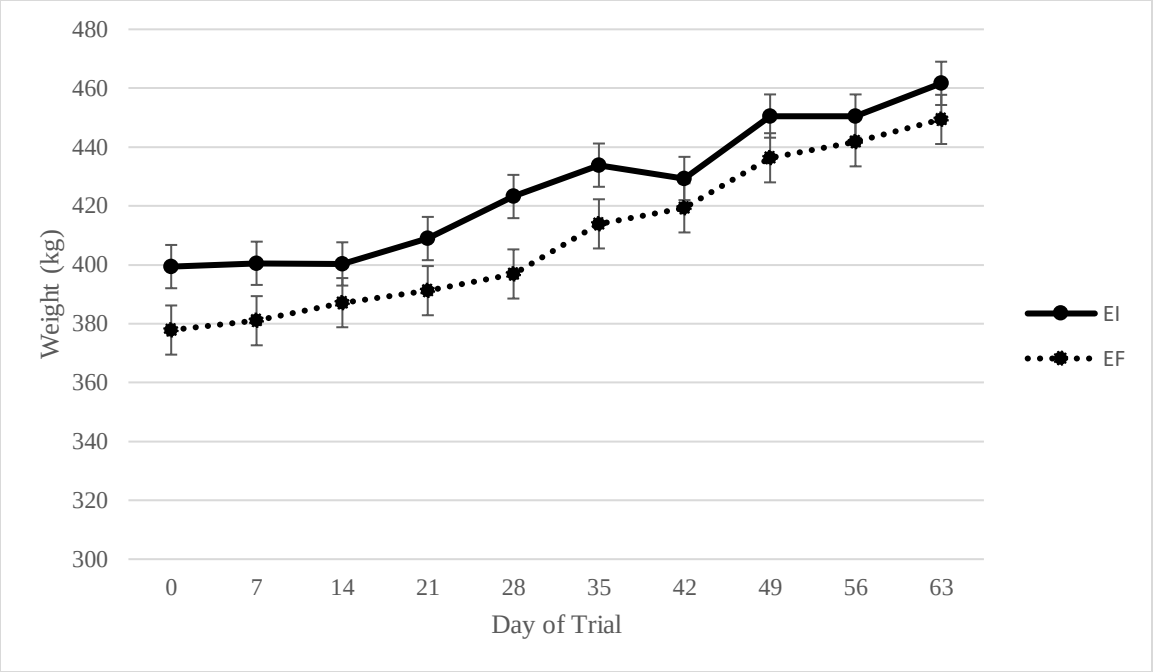



*Figure 3.2 Body Weight of steers consuming TMR with ground fescue seed from May to July, P=0.05*

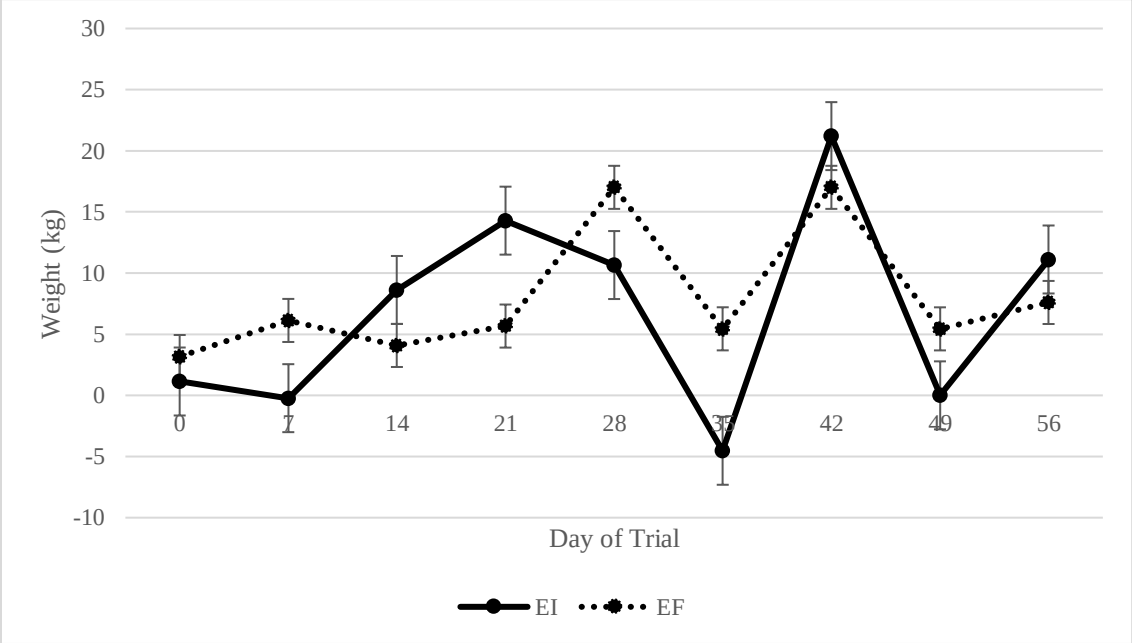



*Figure 4.2 Weekly weight gain of steers consuming TMR with ground fescue seed from May to July, P=0.09*

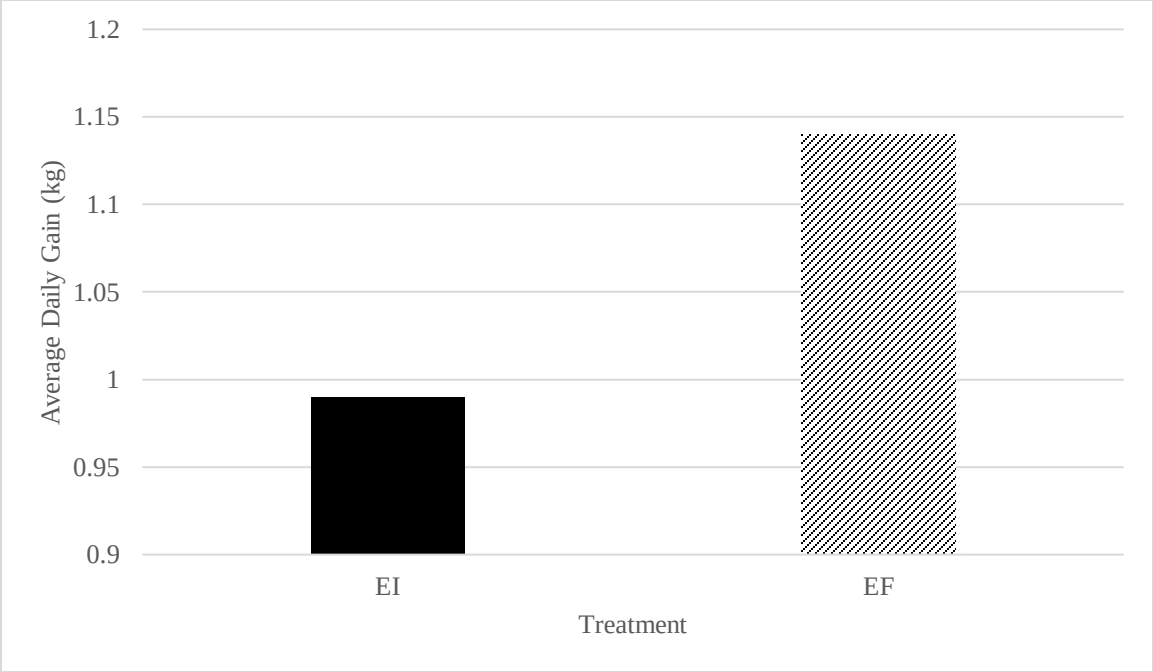



*Figure 5.2 Average daily gain of steers consuming TMR with fescue seed from May to July, P=0.09*

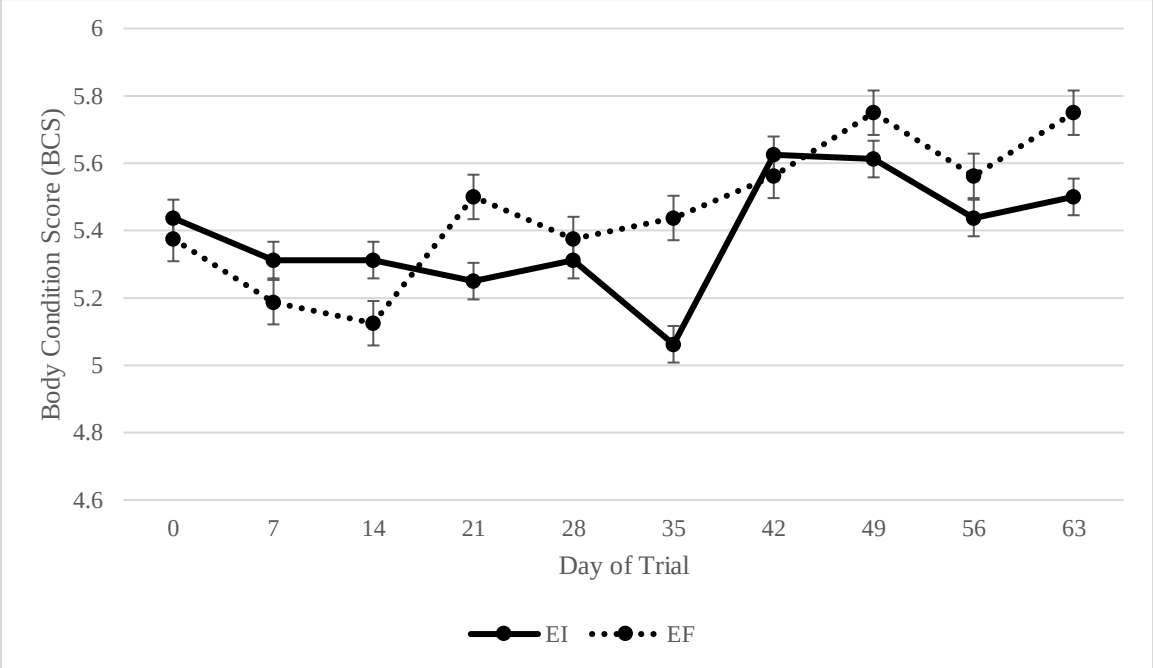



*Figure 6.2 Body condition score of steers consuming TMR with fescue seed from May to July, P=0.07*

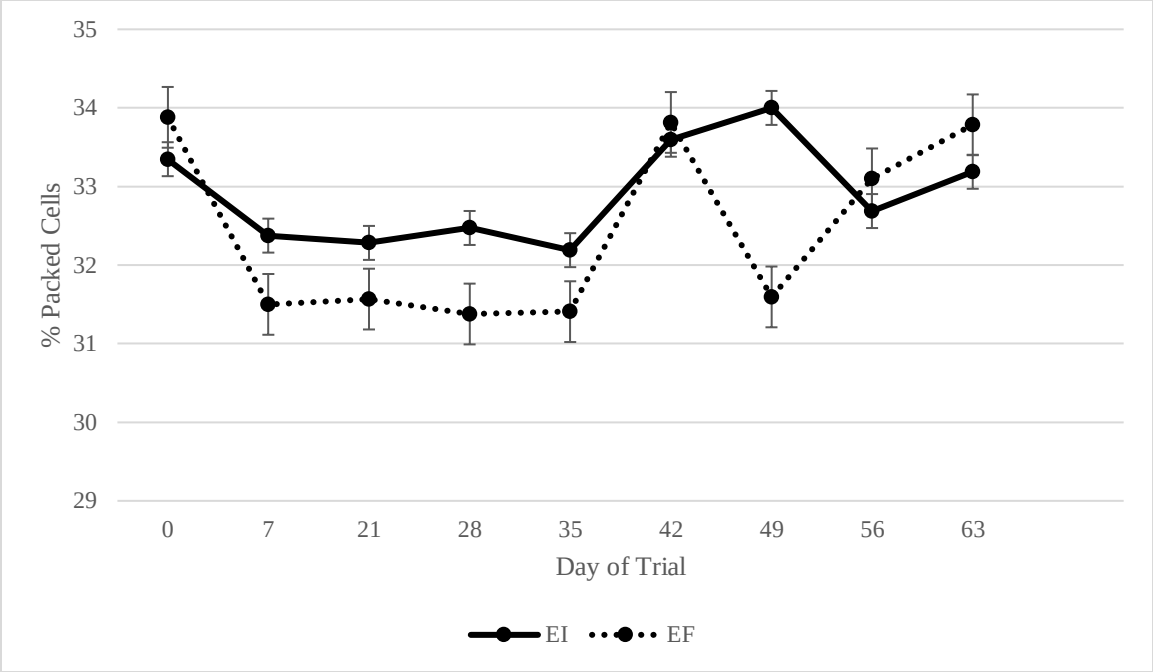



*Figure 7.2 Average hematocrit of steers consuming TMR with fescue seed from May to July, P>0.05*

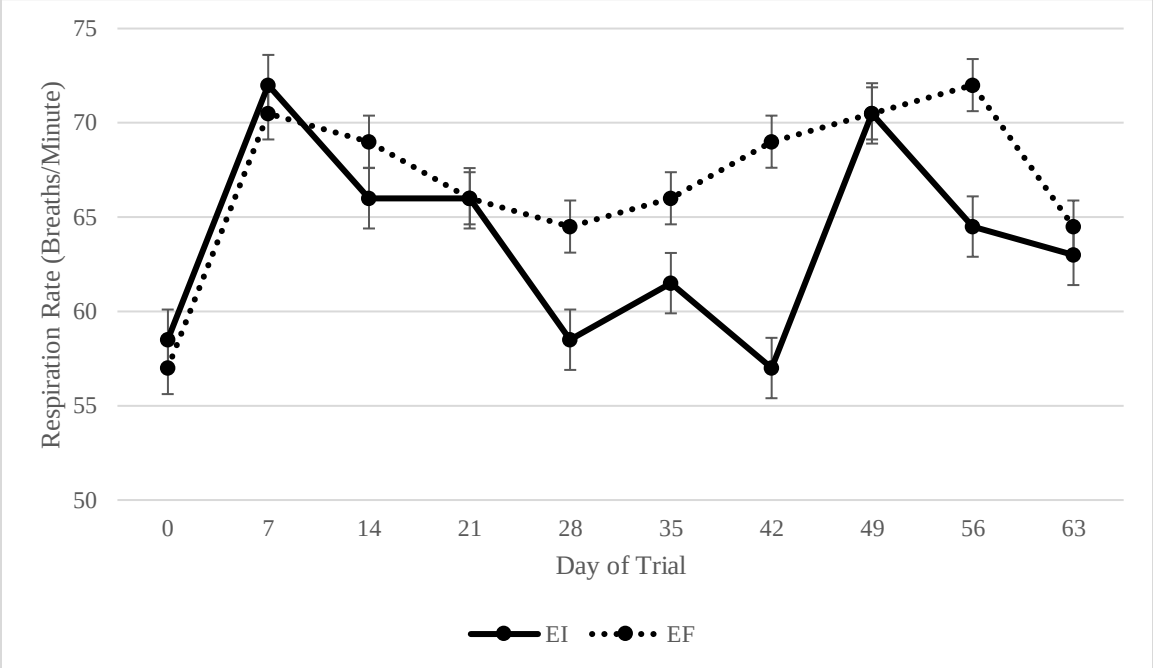



*Figure 8.2 Respiration rate of steers consuming TMR with fescue seed from May to July, P=0.05*

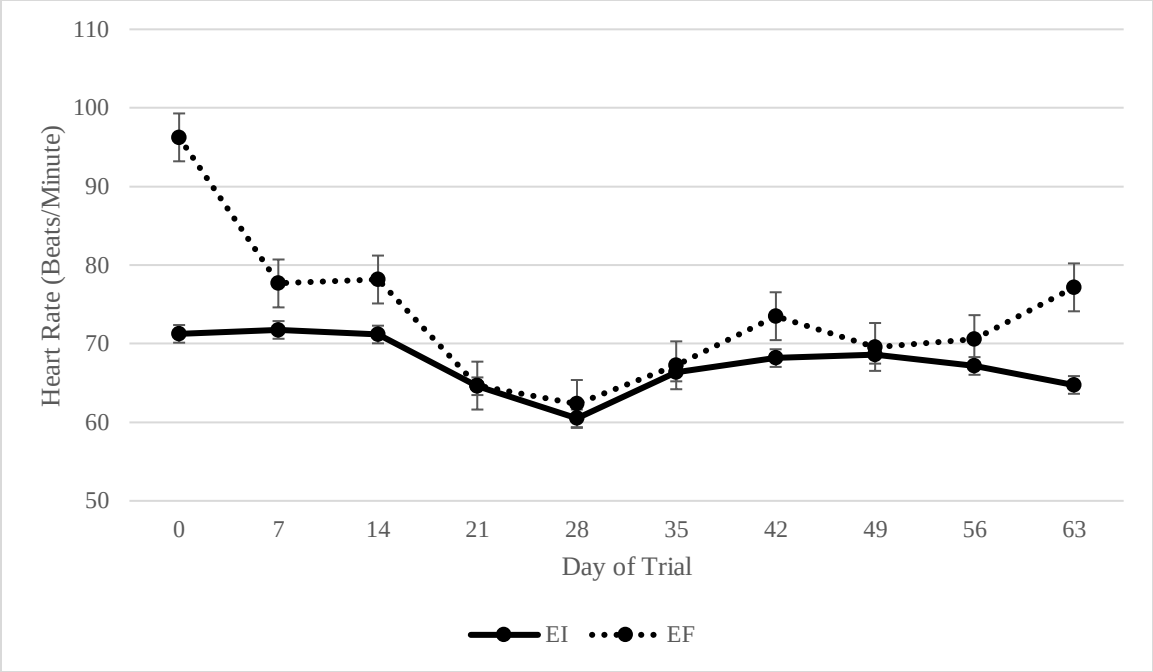



*Figure 9.2 Heart rate of steers consuming TMR with fescue seed from May to July, P=0.005*

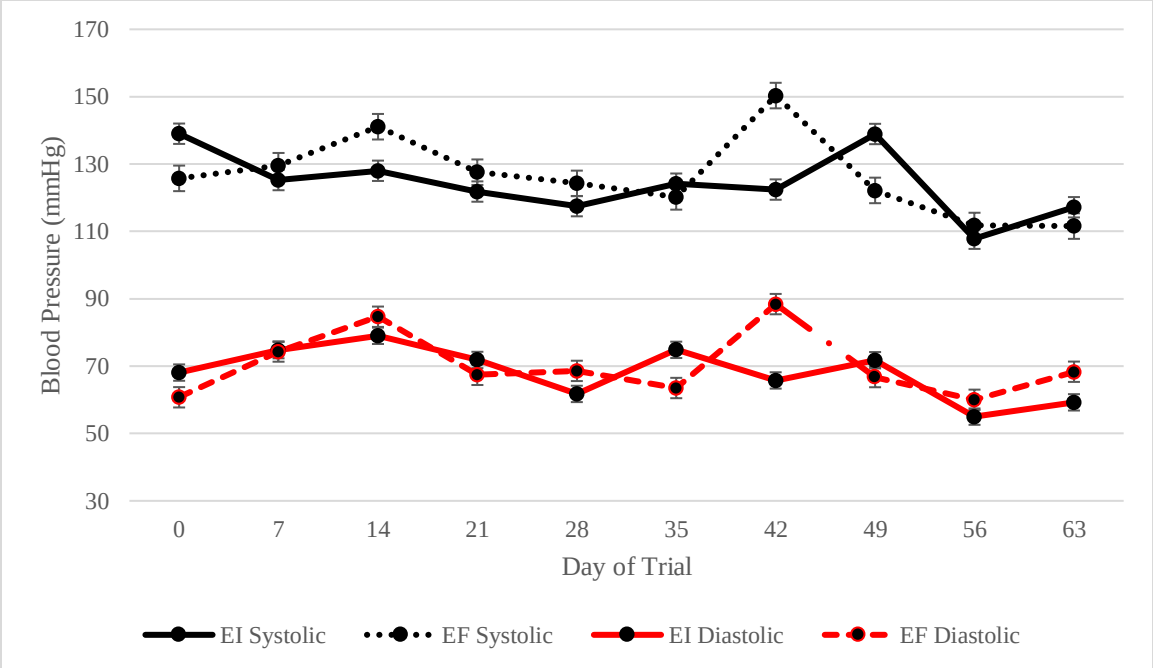



*Figure 10.2 Blood pressure of steers consuming TMR with fescue seed from May to July, P>0.05*

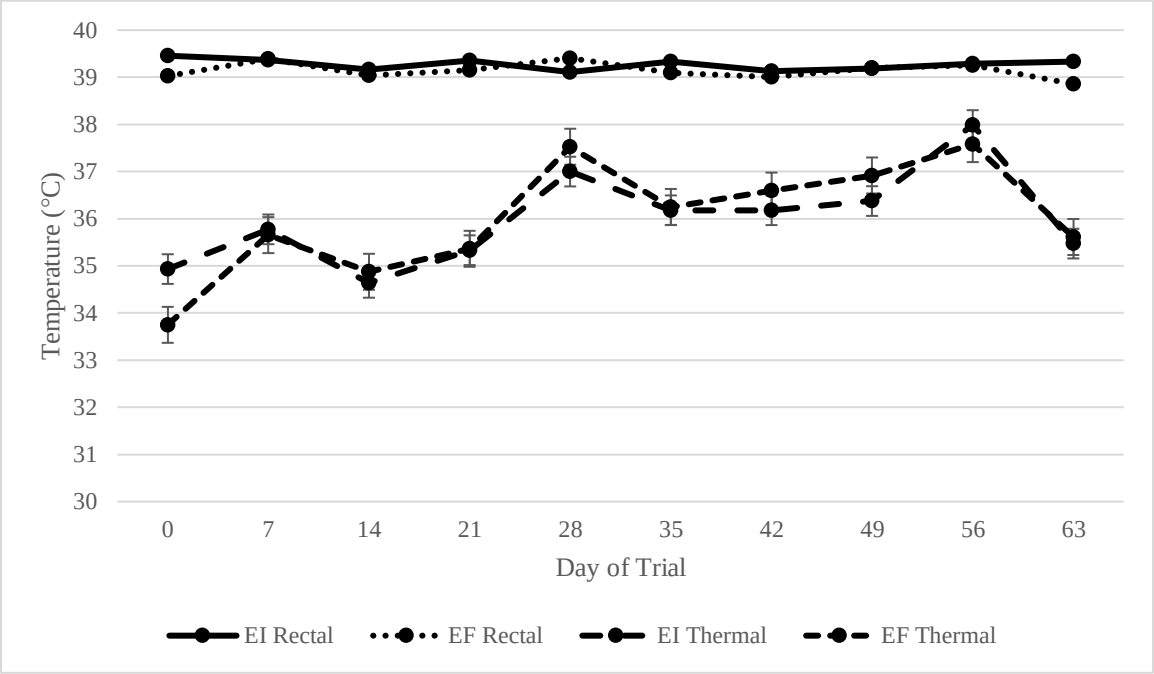



*Figure 11.2 Body temperature of steers consuming TMR containing fescue seed from May to July, P>0.05*

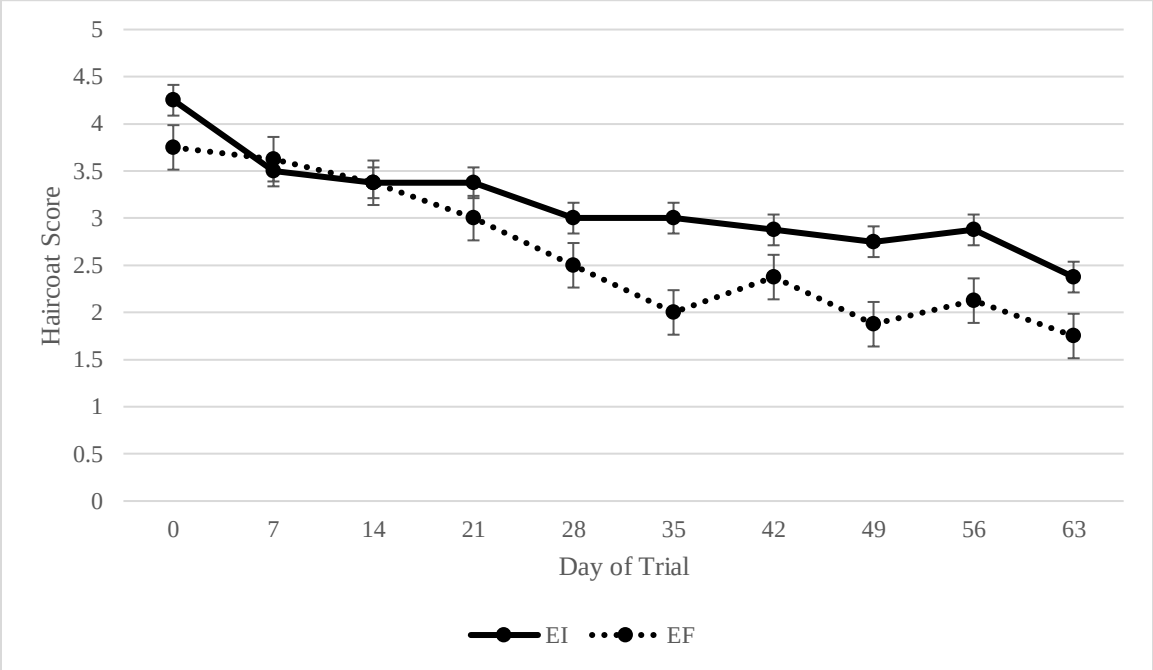



*Figure 12.2 Haircoat score of steers consuming TMR containing fescue seed from May to July, P>0.05*

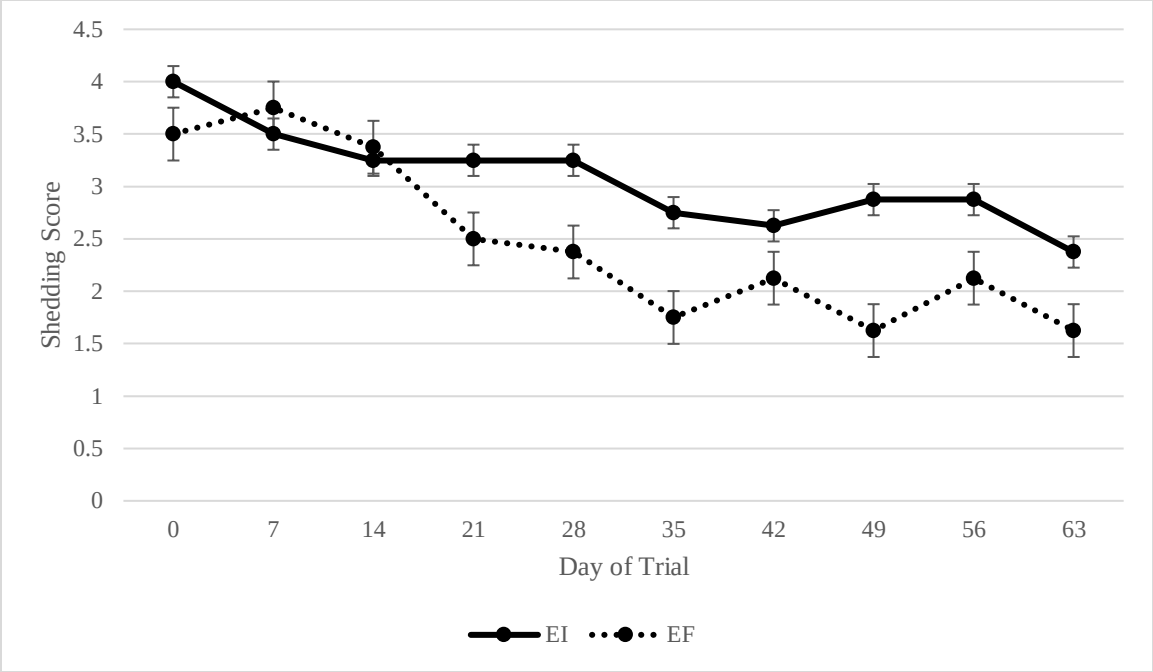



*Figure 13.2 Haircoat shedding score of steers consuming TMR containing fescue seed from May to July, P>0.05*

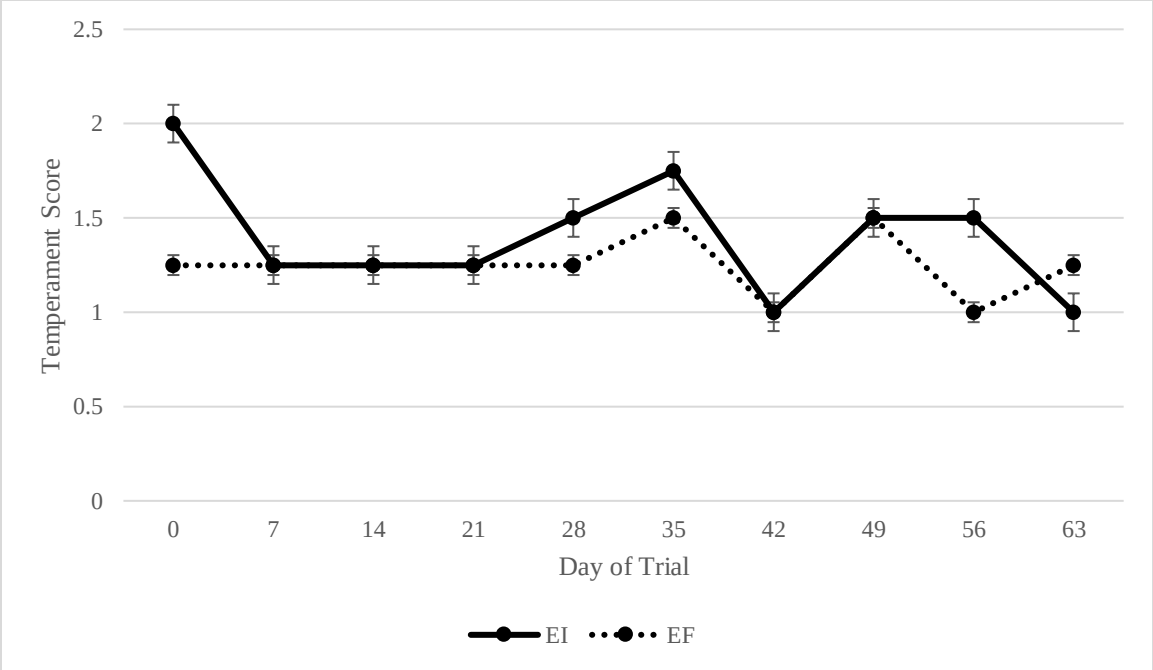



*Figure 14.2 Temperament score for steers consuming TMR containing fescue seed from May to July, P>0.05*

# CHAPTER 3

## Effect of chronic endophyte exposure on the hypothalamic-pituitary axis in steers

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## INTRODUCTION

Tall fescue (*Lolium arunddinaceum* Schreb. Darbysh) is the most predominant grass grown in the eastern United States due to its drought tolerance and ability to withstand the environmental conditions present in the area. However, it is estimated that the roots of 95% of the tall fescue in the United States are associated with the endophyte *Neotyphodium coenophialum* (Hoveland, 1993). While this symbiotic relationship between the grass and the endophyte has been observed to support hardiness, the endophyte also produces ergot alkaloids which have been shown to elicit the signs of a disease known as fescue toxicosis upon ingestion by cattle (Browning et al., 1998). Animals suffering from fescue toxicosis suffer from reduced weight gain, decreased feed intake, reduced serum prolactin and reduced body condition score to name a few of the symptoms (Klotz, 2015).

While the symptoms are well known, the actual mechanisms causing them are not. What also is not known is what specific ergot alkaloids are causing these symptoms as there is a combination of alkaloids present in all endophyte-infected tall fescue. With that in mind, a group of 8 steers were fed a diet containing either fescue seed that was either infected specifically with ergovaline or was endophyte-free. Blood samples were collected weekly over a 63-day period. This study looked at the effect of ergovaline on circulating concentrations of luteinizing hormone, growth hormone, insulin-like growth factor 1 and cortisol. It was hypothesized that luteinizing hormone concentrations would not be different between the treatment groups as there is not a complete feedback loop in place in steers that would alter luteinizing hormone production. It was further hypothesized that both growth hormone and insulin-like growth factor 1 concentrations would be reduced in the group consuming ergovaline-infected seed, as preliminary data collected revealed reduced average

daily gain and reduced body condition score even when feed consumption was the same in this group. It was finally hypothesized that circulating cortisol concentrations would be decreased or the same in the treatment group as ergovaline would not be expected to alter catecholamine function, as could be the case with some other ergot alkaloids found in tall fescue.

## MATERIALS AND METHODS

**Sample collection:** Treatment was applied and animals were processed as per Figure 1.2 between May and July 2014. Blood samples were taken via jugular venipuncture with a 20-gauge needle and collected into a sterile silicone coated glass vacutainer serum tube without additive. Samples were stored on ice and immediately spun down in the centrifuge upon return to campus. Serum was removed and stored in 1.5 mL microcentrifuge tubes at -80°C until Enzyme-Linked Immunosorbent Assay (ELISA) was performed.

**ELISA:** All ELISA's were performed using commercially available, bovine-specific kits purchased from Cloud-Clone Corporation (Houston, TX) following the manufacturers protocol. For all plates, standards were run in duplicate at both the top and bottom of the plate to provide a total of 4 values for each point on the standard curve. All samples were run in triplicate and a random sample, varying by hormone being analyzed, was run on each plate to account for inter-assay variation. All plate incubations were performed at 37°C in a warming oven (Boekel Scientific, Feasterville, PA). All plates were read immediately after the addition of stop solution using a microplate reader with a 450-nm filter (BioTek Synergy HT, Winooski, VT). There was no deviation from the company provided protocol, but a brief synopsis is provided for each hormone that was analyzed.

*Luteinizing Hormone (Cloud-Clone Corp, CEA441Bo):* The standard covers 6 concentrations that range from 0 pg/mL to 30,000 pg/mL. None of the samples in this study required dilution to fall into the detection range of 370.4 to 30,000 ng/mL. A monoclonal antibody specific to bovine LH is precoated onto the plate wells. After all required reagents were prepared for this competitive inhibition immunoassay, a reaction was initiated between biotin-labeled LH present in the first reagent and the unlabeled LH in the sample. Washing is performed and any LH present will remain bound to the well. Avidin bound to Horseradish Peroxidase (HRP) is added to each well and another incubation occurs. The amount of HRP that is bound to the well is in reverse proportion to the concentration of LH in the sample. TMB substrate solution is added which oxidizes and turns the solution blue and after a brief incubation, a Stop Solution composed of Sulfuric Acid is added to the well which changes the solution to a yellow color. The intensity of the color is reverse proportional to the LH concentration present in the sample.

*Growth Hormone (Cloud-Clone Corp, SEA044Bo):* The standard covers 8 concentrations that range from 0 ng/mL to 20 ng/mL. None of the samples in this study required dilution to fall into the detection range of 0.312 to 20 ng/mL. After all required reagents were prepared, sample was added to the well of the plate that is pre-coated with an antibody that is specific to GH. The first reagent contains biotin-conjugated antibody specific for GH and binds any GH present in the well during the first incubation. Washing is performed and any GH bound to biotin-conjugated antibody remains bound to the well. Avidin bound to HRP is added to each well and another incubation occurs. TMB substrate solution is added which oxidizes and turns the solution blue. After a brief incubation, a Sulfuric Acid solution is added to the well which changes the solution to a yellow color.

*Cortisol (Cloud Clone Corp, CEA462Ge):* The standard covers 6 concentrations that range from 0 ng/mL to 1,000 ng/mL. None of the samples in this study required dilution to fall into the detection range of 12.35 to 1,000 ng/mL. This test utilized a non-bovine specific, pan-species antibody. After all required reagents were prepared, sample was added to the well of the plate that is pre-coated with an antibody that is specific to Cortisol. The first reagent contains biotin conjugated antibody that is specific for Cortisol and will bind any Cortisol present in the well during the first incubation which starts a competitive inhibition immunoassay. Washing is performed and any Cortisol bound to biotin conjugated antibody will remain bound to the well. Avidin bound to HRP is added to each well and another incubation occurs. The amount of HRP that is bound to the well is in reverse proportion to the concentration of Cortisol in the sample. TMB substrate solution is added which oxidizes and turns the solution blue. After a brief incubation, a Stop Solution composed of Sulfuric Acid is added to the well which changes the solution to a yellow color. The intensity of the color is reverse proportional to the Cortisol concentration present in the sample.

*IGF-1 (Cloud-Clone Corp, SEA050Bo):* The standard covers 9 concentrations that range from 0 ng/mL to 200 ng/mL. None of the samples in this study required dilution to fall into the detection range of 1.56 to 100 ng/mL. After all required reagents were prepared, sample was added to the well of the plate that is pre-coated with an antibody that is specific to IGF-1. The first reagent contains biotin conjugated antibody that is specific to IGF-1 and will bind any present IGF-1 during the first incubation. Washing is performed and any IGF-1 bound to biotin conjugated antibody will remain bound to the well. Avidin bound to HRP is added to each well and another incubation occurs. TMB substrate solution is added which

oxidizes and turns the solution blue. After a brief incubation, a Stop Solution composed of Sulfuric Acid is added to the well which changes the solution to a yellow color.

**Statistics:** All statistical analyses were performed using Proc Mixed with repeated measures in SAS version 9.3. The experimental unit was the individual steer and the model statement included the date and treatment. A P-value of $\leq 0.05$ was considered statistically significant and a P-value between 0.05 and 0.10 was considered a trend towards statistical significance.

## RESULTS AND DISCUSSION

**Luteinizing Hormone:** Luteinizing hormone (LH) concentrations did not differ between steers consuming endophyte-infected (EI) or endophyte-free (EF) fescue seed (3.28 ng/mL vs. 3.22 ng/mL, respectively P=0.7). Intra-assay variation was 7.46% and inter-assay variation was 0.99%. There was also no date or date by treatment impact on circulating LH concentrations. The data for the date by treatment interaction is provided in Figure 3.1. This data agrees with several previous studies where animals were exposed to ergot alkaloids in various methods. One study placed a group of cows in environmental chambers and used a seed model similar to the one utilized in this experiment. Ergovaline had no impact on circulating LH concentrations over the 75-day course of the study (Mizinga et al., 1992). When cattle were grazed on endophyte-infected tall fescue, LH concentrations were not impacted in groups of steers or heifers when compared to controls (Thompson and Stuedemann, 1993).

There are studies that have found that exposure to ergot alkaloids does in fact, decrease LH. These studies utilize a model where ergotamine was given to the animal via IV injection and the hormonal response was studied over the course of several hours. Over the course of 4 hours, the researchers found that LH decreased in steers that received a bolus

injection of ergotamine when compared to saline-injected controls (2.12 ng/mL vs. 2.83 ng/mL). A subsequent study using the same model saw similar results, though LH concentrations began rising again 3 hours post-ergotamine injection (Browning et al. 1997, 1998). The results seen here could just be an acute effect of the bolus injection, or the fact that sampling was done every 30 minutes over the course of several hours versus looking at the long-term impact of the ergot alkaloids on the animals.

In an intact animal, LH is part of a feedback loop where its production is stimulated from the pituitary by hypothalamic Gonadotropin Releasing Hormone (GnRH). LH travels to the testes, binds to Leydig cells and aids in the production and secretion of testosterone. That testosterone is what acts as the negative feedback, at the level of hypothalamus to decrease GnRH production and subsequently decreases LH production (Desjardins, 1981). However, a steer model was utilized for this project and the feedback loop cannot be completed because testosterone is not present as the animals have been castrated. With this kept in mind, it is not surprising that LH concentrations are not different between the EI and EF treatment groups as neither group has a proper feedback loop in which LH can function. The concentrations are not going to be appropriately, negatively regulated so even if ergovaline was causing some type of negative impact on LH production it might not be relevant in this model.

**Growth Hormone:** There was a date by treatment effect of ergovaline on circulating GH concentrations in steers (P=0.05, Figure 3.2). The GH concentrations observed for the steers in the EI group were significantly lower than those of the steers in the EF group for all days of the experiment, except for day 63. There were no direct treatment or date effects observed (P=0.17 and P=0.79, respectively) on GH for steers consuming a TMR containing fescue

seed. This correlates well with the decreased average daily gain and reduced body condition score (Figures 4.2 and 5.2, respectively) that were observed in the treatment animals. In the animals consuming the EI diet, average growth hormone was 1.3 ng/mL. For those on the EF diet, it was 7.2 ng/mL. Intra-assay variation was 16% and inter-assay variation was 9%.

When ergotamine and ergonovine were administered to steers via IV bolus, it was found that GH concentrations increased compared to saline injected controls (5.53 ng/mL, 4.35 ng/mL and 5.02 ng/mL, respectively) at the end of a 4-hour observation period (Browning et al., 1997). When 12 steers were grazed on either high or low infection rate tall fescue pasture for approximately 6 months, an elevated GH was also observed in the high infection rate pasture when compared to the control group (7.9 vs. 6.2 ng/mL). However, the researchers in this study attributed the possible differences due to decreased intake in the group grazing the highly-infected pasture (Thompson et al., 1987) and infection rate does not correlate with level of ergot alkaloid infection. In contrast, when a group of steers were grazed on either highly-infected or low-infection rate tall fescue for 14 weeks, there was no difference in circulating GH concentrations between the steers grazing the two different pastures (Lipham et al., 1989). These results do not directly correlate with what was observed in this trial but that is not unexpected. These studies either looked at pastures where only infection rate was analyzed or looked at different ergot alkaloids which would have different biological activity in the animal. This study chose to specifically focus on ergovaline and looks at its impact on hormones along the hypothalamic-pituitary axis.

Decreased serum prolactin is assumed to be one of the hallmarks of an animal suffering from fescue toxicosis (Strickland et al., 2011). Prolactin and GH are both secreted from the anterior pituitary, are similar in structure and act via similar mechanisms. The two

hormones are also both involved in the growth and development of animals (Brooks, 2012). While it is not currently proven, if GH production is altered in the same manner that prolactin is in fescue toxicosis, this could be one potential pathway that is causing decreased growth and feed efficiency. Additionally, the preliminary data collected in this study revealed that steers consuming ergovaline had reduced average daily gain without exhibiting the most common symptoms of fescue toxicosis (i.e. elevated rectal temperature and increased respiration rate). Ergovaline may not be acting to elicit the physiological symptoms associated with fescue toxicosis but the data found here indicate that it may be a likely candidate to be the toxin that is acting along the hypothalamic-pituitary axis to alter growth and performance when animals are chronically exposure to endophyte-infected tall fescue.

**Insulin-like Growth Factor 1:** There was a trend towards steers consuming a diet with ergovaline-infected fescue seed to have reduced IGF-1 when compared to the animals consuming the control diet (28 ng/mL vs. 95.7 ng/mL, P=0.10, Figure 3.4). There was also a trend for an interaction by date effect on circulating IGF-1 concentrations with concentrations being significantly reduced for steers on the EI diet versus those on the EF diet from days 21-42 of the experiment (P=0.07, Figure 3.3). A trend was revealed towards a date effect on circulating IGF-1 concentrations with concentrations peaking between days 21 and 35 of the trial and then reducing again to be similar to levels at the beginning of the study (P=0.06). Intra-assay variation was 18% and inter-assay variation was 1%.

Hormone concentrations started out similar at the beginning of the trial, however for the steers that were on the EI treatment, IGF-1 and GH concentrations failed to increase at the same rate as what was observed in the EF group in the weekly blood samples that were collected. Typically, concentrations increased for steers consuming the control diet. If GH

secretion is being altered by the activity of ergot alkaloids in the anterior pituitary, this would affect downstream IGF-1 production. While IGF-1 production in the EI group when compared to controls, it did exhibit a peak at day 21 of the study, which was not observed with GH. Growth hormone secretion tightly controls the how much IGF-1 is released from the liver. Insulin-like growth factor 1 is also very involved in growth and cell proliferation and this could be a part of how fescue toxicosis, acting via ergovaline, is utilizing the hypothalamic-pituitary axis to alter growth in animals (Bentov and Werner, 2013).

When serum was analyzed from previous studies where ergotamine had been given to both steers and heifers via intravenous injection, it was found that plasma IGF-1 concentrations were significantly decreased compared to those animals receiving a saline injection by 2 hours' post-injection. Serum IGF-1 concentrations had recovered to pre-injection concentrations by 4-hours post-injection which lead researchers to hypothesize that this might be something that the animals recover quickly from but no potential pathway was proposed (Browning, 2003). It has additionally been found that serum concentrations of IGF-1 for cattle grazing endophyte-infected tall fescue was were reduced when compared to those grazing non-infected pasture (Strickland et al., 2009). These findings are all consistent with what was observed in this study and the steers in this study were exposed to a steady dose of ergovaline over a 63-day period.

**Cortisol:** There were no date or treatment effects on the concentration of circulating cortisol for steers consuming either an EI or EF diet (P=0.61 and P=0.82, respectively). There was a slight numeric reduction in cortisol concentrations for steers consuming the EI diet when compared to EF controls (489.2 ng/mL vs. 511.4 ng/mL, SE=66.2). There was a trend towards a significant date by treatment effect to see a reduction of cortisol concentrations in

the animals consuming the EI diet on days 28. 35, 42 and 63 of the study (P=0.11, Figure 3.5). Intra-assay variation was 9.5%, inter-assay variation was 13%.

Cortisol is released in cattle in a circadian rhythm and peaks in the morning, which is not when samples were being drawn for this study (animals were processed at approximately 1:00 P.M.). Basal cortisol secretion is used for maintenance of homeostasis but it is also released in response to stress along the hypothalamic-pituitary axis (Dedovic et al., 2009; Ogino et al., 2014). In circulation, cortisol is found associated with transcortin, a binding protein. In the unstressed animal, 95% of cortisol will be bound. This is because when bound, cortisol cannot cross the cell membrane and bind to its receptor to initiate transcription and cause further cellular responses. Once an animal becomes stressed, cortisol is released from transcortin and is able to become active (Perogamvros et al., 2012).

Stress is a condition that is typically associated with animals that are suffering from fescue toxicosis. However, the data that investigates the concentrations of cortisol in those animals are variable. When animals were given IV injections of ergotamine and kept restrained in either a squeeze chute or a tie-barn for an extended period of time (from 4-12 hours depending on the study), it was found that serum cortisol was elevated when compared to saline-injected controls (Browning, 2000; Filipov et al., 2000). In Angus heifers administered ergovaline via a seed model, it was found that cortisol concentrations were no different when compared to those animals consuming non-infected seed (15.8 vs. 16.7 ng/mL) (Aldrich et al., 1993). When steers in the backgrounding phase were allowed to graze either a KY-31 pasture, or one of 2 novel-endophyte pastures (MaxQ or HiMag4), the steers grazing the KY-31 pasture were found to have the lowest serum cortisol of all 3 groups (Sales et al., 2010).

The data collected in this study show a trend to reduce serum cortisol in animals that were exposed to ergovaline but there were fluctuations for both groups throughout the study. Non-stress related cortisol is involved in protein-building, fat breakdown and even gluconeogenesis (Ogino et al., 2014). These processes need to be functioning well to have an animal that grows well and has a good lean to fat ratio. In groups of steers with low serum cortisol, it was also observed that the animals had the lowest ADG when compared to other animals in the same study (Sales et al., 2010). This also correlates with what was observed in this study. Additionally, this study only examined the effects of ergovaline versus looking at all of the ergot alkaloids that are present in endophyte-infected tall fescue. Ergovaline is not as active at the adrenergic receptors as other ergot alkaloids and thus will not alter catecholamine release. The animals are not as excitable and thus may not be as stressed as an animal could potentially be if consuming a combination of ergot alkaloids versus just ergovaline.

## SUMMARY

Fescue toxicosis is a disease that is very detrimental to the beef industry, costing producers an estimated $1 billion annually in losses that are chiefly related to reproductive and growth losses (Strickland et al., 2009). Animals are susceptible to decreased weight gain, reduced feed intake, reduced time grazing and poor body condition score when grazing endophyte-infected tall fescue (Klotz, 2015). Much of this is known but not much is known about the actual hormonal mechanisms involved. Decreased serum prolactin is a symptom that is commonly observed in animals suffering from fescue toxicosis. However, prolactin is closely related with growth hormone in its structure and the signaling pathway that it utilizes to cause effects throughout the body. These hormones are involved in regulating growth and

performance in animals so alterations to the production of one by ergot alkaloids could certainly alter production of the other (Brooks, 2012; Kelly et al., 1991).

This study was specifically designed to determine the sole effect of chronic exposure to ergovaline on the hypothalaminc pituitary axis in steers. Many previous studies have attempted to get near this question by looking at the general effects of ergot alkaloids or endophyte-infected tall fescue pasture on various hormone levels in cattle, but many have had other confounding factors that make it difficult to tease out a specific ergot alkaloid impact. Inducing any actual symptoms of fescue toxicosis was a byproduct of this study and was simply an effect of administering treatment to the steers.

Ergovaline does have a role in the fescue toxicosis pathway, but it does not seem to be the major player. With the specific limitations of this study design, it did not induce the classic symptoms associated with fescue toxicosis even though the EI group were fed a sufficient amount of toxin to do so. This does not mean that ergovaline was not doing anything though. It appears to have a great deal of activity along the hypothalamic-pituitary axis to alter the activity of both growth hormone and insulin-like growth factor 1, and subsequently decrease average daily gain and reduce body condition score in animals consuming the treatment diet.

The drastic effect of ergovaline on GH and IGF-1 also leave an avenue to investigate for follow-up studies to this one. The next logical avenue to look into would be to examine the GH receptor density and function of those receptors on the liver. GH binding to its receptor on the liver causes the release of IGF-1 into circulation and mediates a majority of the effects of GH (Bentov and Werner, 2013). This initial experiment has shown that consumption of ergovaline decreases concentration of both GH and IGF-1 and with the liver

tissue processed and frozen from necropsy, this experiment would be easily designed and could provide useful information.

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# FIGURES

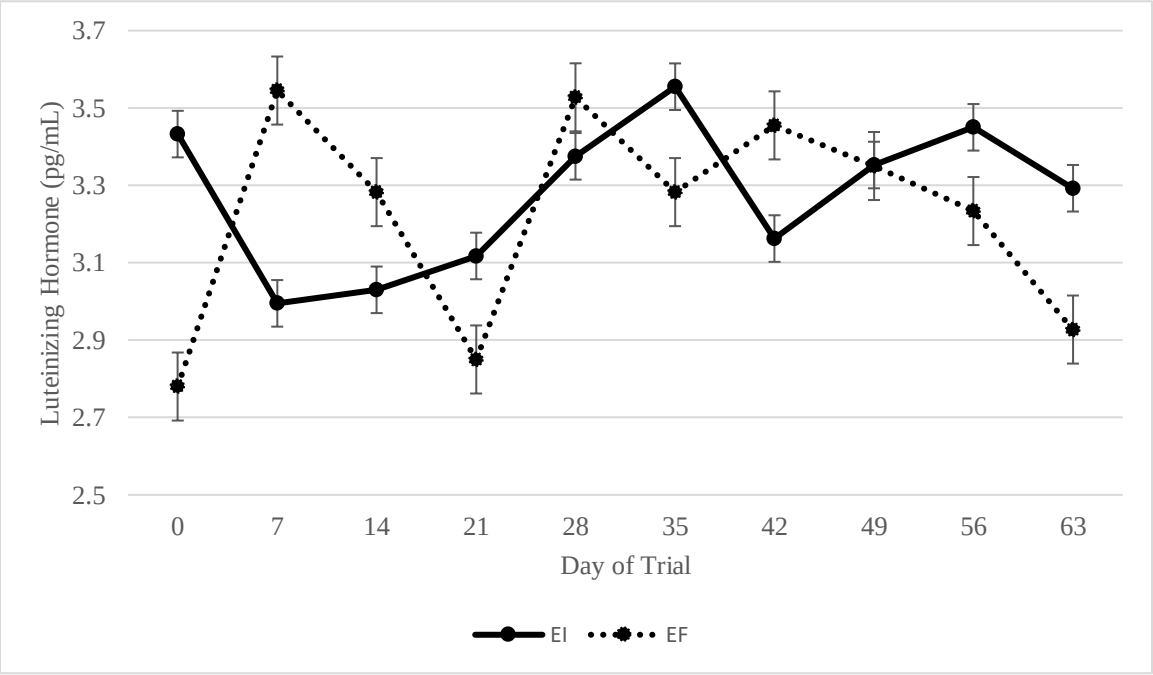


*Figure 3.1 Luteinizing Hormone concentration of steers consuming TMR with fescue seed from May to July*

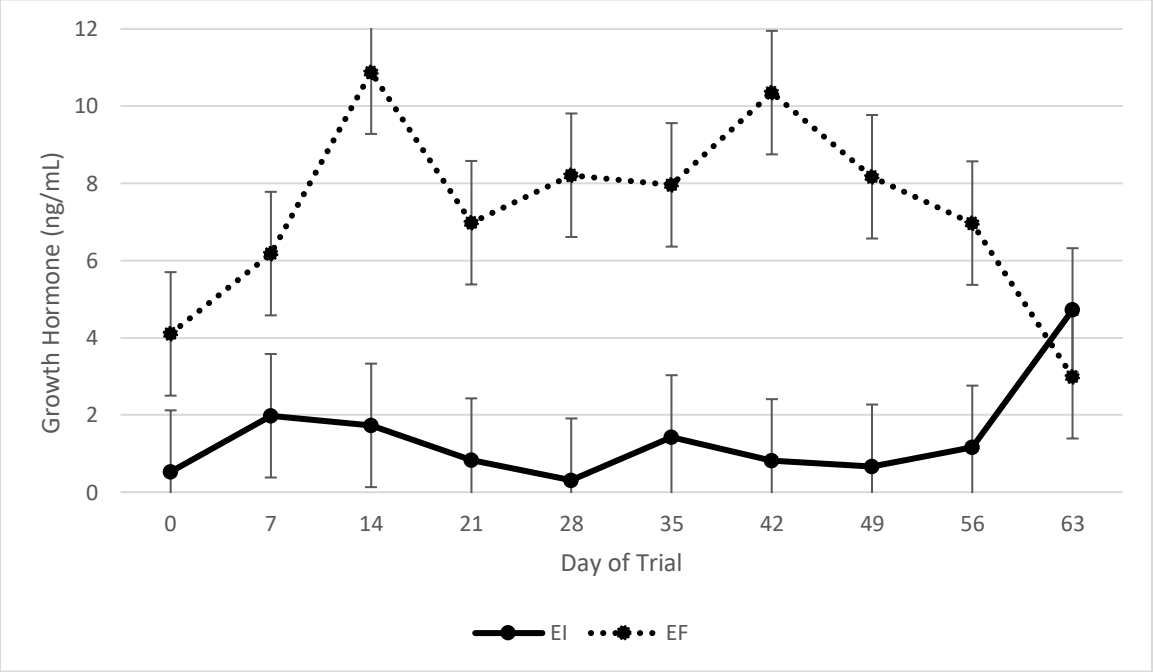


*Figure 3.2 Growth Hormone concentration of steers consuming TMR with fescue seed from May to July*

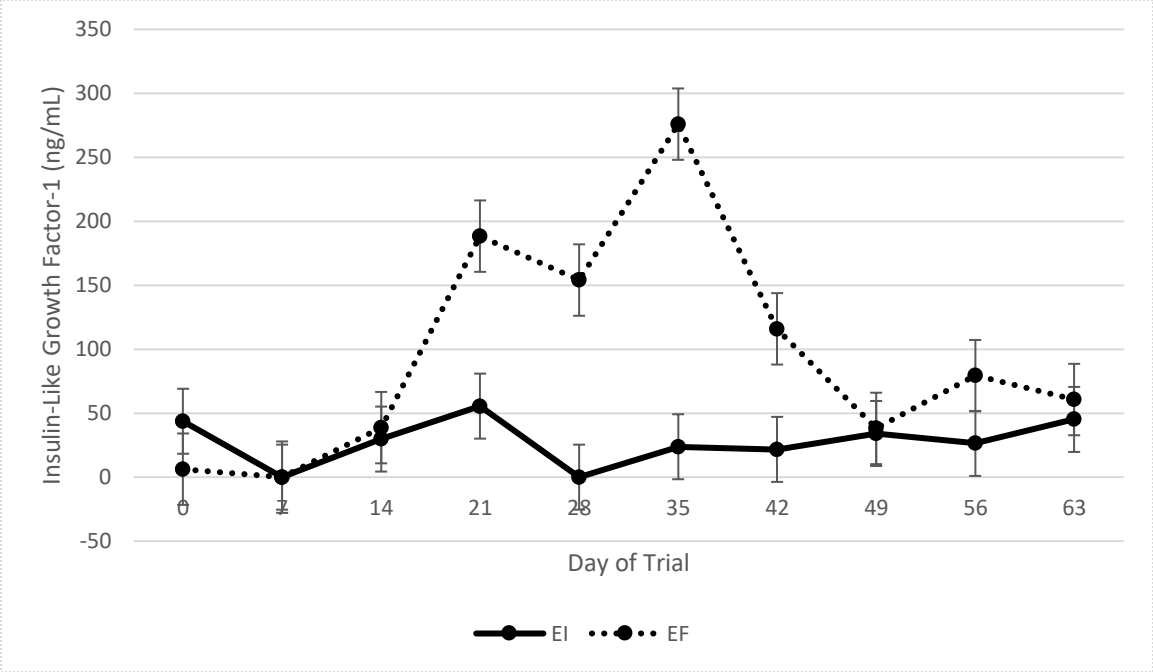


*Figure 3.3 Insulin-Like Growth Factor-1 concentration of steers consuming TMR with fescue seed from May to July*

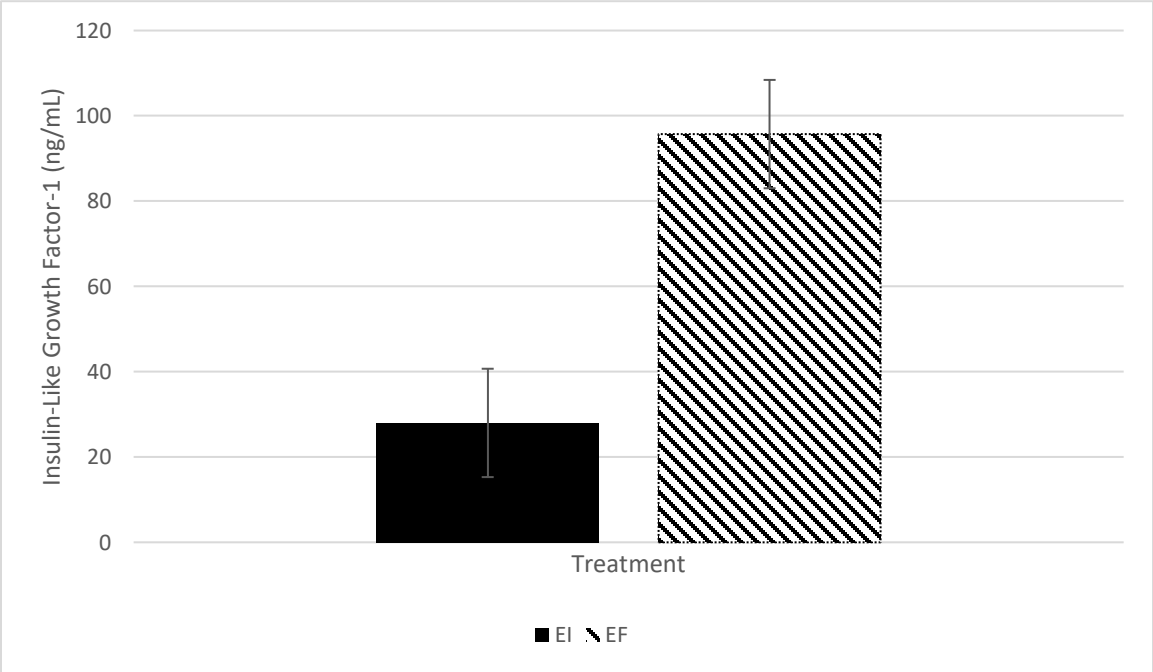


*Figure 3.4 Effect of treatment on Insulin-Like Growth Factor-1 concentration of steers consuming TMR with fescue seed*

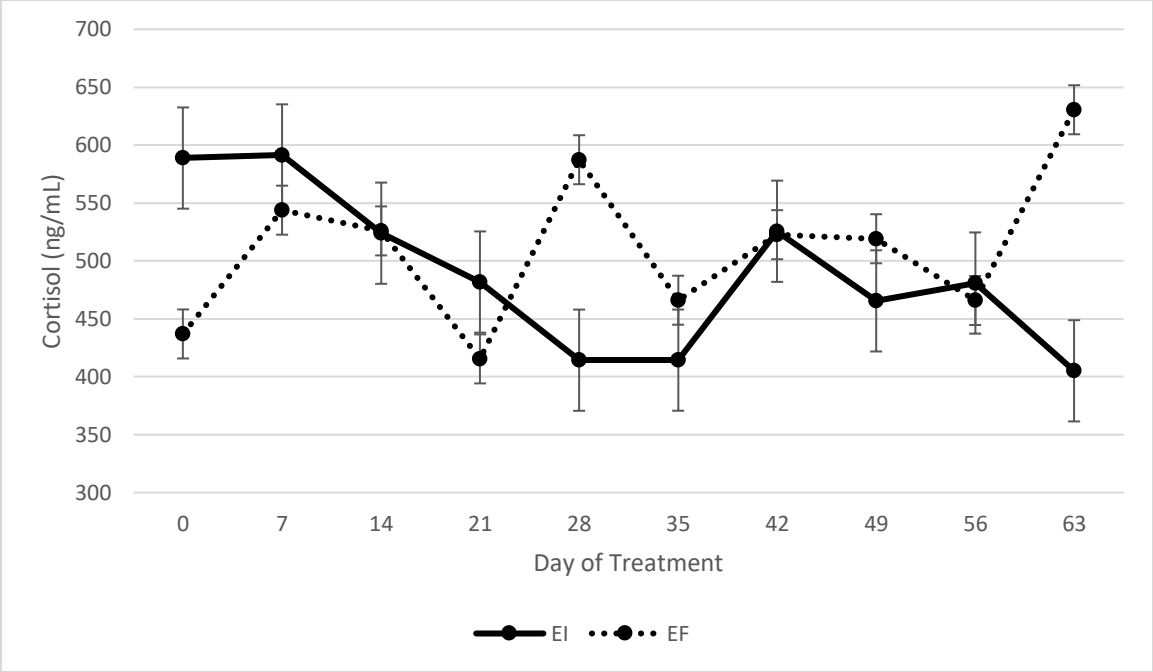


*Figure 3.5 Cortisol concentration of steers consuming TMR with fescue seed from May to July*