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Impact of Mineral Supplementation on Intake Behavior and Blood Flow in Growing Beef Cattle Exposed to Fescue-Derived Alkaloids

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IMPACT OF MINERAL SUPPLEMENTATION ON INTAKE BEHAVIOR AND BLOOD FLOW IN GROWING BEEF CATTLE EXPOSED TO FESCUE-DERIVED ALKALOIDS

THESIS

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science in the
College of Agriculture, Food and Environment
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By

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2021

ABSTRACT OF THESIS

IMPACT OF MINERAL SUPPLEMENTATION ON INTAKE BEHAVIOR AND BLOOD FLOW IN GROWING BEEF CATTLE EXPOSED TO FESCUE-DERIVED ALKALOIDS

Consumption of endophyte-infected tall fescue by cattle has repeatedly been shown to negatively impact dry matter intake (DMI), growth rate, and circulating prolactin concentrations. The objective of the current study was to determine if a mineral supplementation would mitigate the negative effects of fescue-derived alkaloid consumption on feed intake, circulating prolactin, and the vascular system. Twelve Angus crossbred steers were used in a triplicated Latin Square design consisting of three 28 d experimental periods. Treatments consisted of three top-dressed mineral supplements (142 g/head/d): a non-medicated control (CON), commercially available Fescue EMT® Mineral Defense (EMT), and a test prototype (EMT 2).

Each period was composed of an adaptation/washout subperiod (d 1-14), a step-up sub-period (d 15-21), and a sampling subperiod (d 22-28). During the adaptation/ washout sub-period, all steers received CON and were fed a basal diet, composed primarily of alfalfa haylage and corn silage, 1.5 x NE_m. During both the step-up and sampling subperiods, steers received their appropriate supplemental treatment and were fed the basal diet containing 15% KY-31 endophyte-infected seed on an as-fed basis. The amount of feed offered was increased incrementally during the step-up period to allow for ad-libitum intake by d 19 and throughout the sampling period.

Steers were housed indoors in individual pens and the room temperature was cycled above thermoneutral (~29.4°C) 16 h from 0600-2200 and thermoneutral (~21.1°C) 8 h from 2200 to 0600 to mimic summer conditions. A light:dark cycle was set corresponding to the room temperature cycle. Steers were fed each d at 0830 and the amount offered was recorded. DMI was determined daily based on the DM of feed delivered and the corresponding ort. Meal feeding behavior (i.e., meal size, meal number, and meal duration) was monitored using feed bunks suspended from load cells with communications to a data handler. Daily water consumption was determined using in-line meters. Rectal temperature and respiration rate were recorded daily at 1200h during the treatment sub-period. The arterial luminal cross-sectional area of the caudal artery was measured using a Doppler ultrasound on d 14, d 21, and d 28 of each period. Blood samples were collected on d 14 and d 28 of each period for the determination of circulating prolactin.

The EMT treatments reduced ($P \leq 0.04$) DMI during the last 7 days of the treatment sub-period. The EMT treatments did not affect water intake ($P = 0.67$) during the treatment sub-period. The EMT treatments tended ($P = 0.07$) to decrease meal frequency but did not affect ($P \geq 0.37$) meal size or duration.

Prior to treatment on d 14, the arterial luminal cross-sectional area of the caudal artery was smaller for steers ($P \leq 0.008$) assigned to EMT treatments compared with those assigned to CON. On d 21, the arterial luminal cross-sectional area was greater for EMT ($P = 0.007$) and EMT 2 ($P = 0.10$) compared with control. On d 28, arterial luminal cross-sectional area tended ($P = 0.06$) to be greater for EMT compared with control. EMT ($P = 0.0004$) and EMT 2 ($P = 0.02$) reduced endophyte-induced vasoconstriction on d 21. Endophyte-induced vasoconstriction on d 21 tended ($P = 0.09$) to be less for EMT compared with EMT 2. On d 28, endophyte-induced vasoconstriction was lower for EMT compared with CON ($P = 0.002$) and EMT 2 ($P = 0.07$), with no difference ($P = 0.11$) between CON and EMT 2.

Prolactin concentration decreased following the addition of endophyte-infected seed, but concentration was unaffected ($P \geq 0.50$) by EMT treatments. Rectal temperature and respiration rate were unaffected ($P = 0.12$) by EMT treatments. Overall, induction of fescue toxicosis was achieved throughout the experiment. The EMT 2 formulation was not as effective in reducing vascular constriction and did not benefit the EMT formulation. This data suggests EMT formulation is effective in partially alleviating the negative impacts of fescue-derived alkaloids when consumed by cattle.

KEYWORDS: Cattle, Ergot Alkaloids, *Neotyphodium coenophialum,*
Tall Fescue

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DEDICATION

To my grandparents, James and Jean Herzing and Melvin and Louise Kabat, who are the beginning of my passion for education and agriculture. They sacrificed for each other, their kids, and their grandkids so that everyone would have the opportunity to achieve their goals in education. They taught me how through the power of God, hard work, and dedication, agriculture truly does feed the world.

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CHAPTER 1. INTRODUCTION

Tall Fescue *(Lolium arundinaceum)* is a cool-season grass that is consumed by cattle primarily in the Pacific Northwest and Southeastern region of the United States (Bacon, and Siegal, 1988). After the release of the Kentucky-31 cultivar in the 1940s, tall fescue was widely incorporated into beef cattle grazing systems because of its impressive growth rate, persistence, erosion control, and production in good forage yields (Ball et al., 1991,). Unfortunately, however, producers noticed an inconsistency in the performance of cattle grazing the Kentucky-31 variety of fescue grass. To identify the cause of this inconsistency, researchers discovered the presence of an endophytic fungus, i.e., *Neotyphodium coenophialum*, on the grass. Subsequently, it was determined that a symbiotic relationship exists between the fungus and the associated grass. The fungus gains protection, nutrients, and means of reproduction from the plant, while the tall fescue is more persistent and drought tolerant when infected with the endophyte (Arachevaleta et al., 1989; Bacon et al., 1993). Unfortunately, the fungus also produces ergot alkaloids that are toxic to cattle, which are determined to be the cause for the inconsistent cattle performance.

Consumption of endophyte-infected tall fescue has been shown to reduce dry matter intake, weight gain, and reproductive efficiency, and to cause vasoconstriction (Klotz and Nicol, 2016). Downstream effects of vasoconstriction can then lead to heat stress and poor nutrient transport in cattle. Other notable symptoms of fescue toxicosis include elevated respiration rates, increased heart rates, extensive salivation, gangrene of extremities, and decreased prolactin concentrations (Siegel et al., 1984).

Fescue toxicosis is a concerning problem in the beef cattle industry as it is the largest animal health-related production cost with a combined annual loss exceeding $2 billion (Kallenbach, 2015). As such, a variety of research efforts have been aimed at alleviating the impact of fescue toxicosis on cattle by way of pasture management, utilizing pharmacological compounds, and providing feed additives.

The addition of supplements is a practical method to combat the negative physiological effects of cattle consuming endophyte-infected tall fescue. The addition of 1 g of thiamin was observed to improve alfalfa intake in cows consuming endophyte-infected KY-31 seed exposed to heat-stress conditions in comparison to cows that did not receive thiamin treatment (Dougherty et al., 1991). Likewise, oral thiamin supplementation increased the rate of herbage intake where cows given 1 g of thiamin per day consumed 1.57 kg/h and cows that did not receive thiamin supplementation consumed herbage at 1.19 kg/h (Lauriault et al., 1990). Smith et al. (1984) found a 0.14 kg increase in average daily gain (ADG) for cattle exposed to infected tall fescue when given Se injections (15 mg every 28 d). Similarly, Thompson (1996) showed that an injection of 120 mg Cu increased ADG by 0.16 kg/d over an 84-d period in calves consuming endophyte-infected tall fescue. Jia et al. (2018) did not observe a difference in ADG when steers were subject to summer-long grazing endophyte-infected fescue and fed vitamin-mineral mixes that contained 3mg/d of Se as sodium selenite (Ise), SELPLEX (OSe), or a mix of ISe:OSe at a 1:1 ratio. Although ADG was not improved with the addition of Se treatments, metabolic parameters such as whole blood Se content, serum prolactin, and liver glutamine synthetase were increased for steers provided the OSe and the mix of Ise:OSe forms. In another experiment conducted by Jia et al. (2019), researchers found steers grazing endophyte-infected tall fescue pasture had increased serum albumin concentration and alkaline phosphatase activity when fed 3mg of Se/d that was provided in a vitamin-mineral mix. These studies suggest that the addition of Se in vitamin-mineral mixes could be beneficial in improving metabolic parameters in steers under the condition of fescue toxicosis by resulting in greater whole-body Se assimilation, alkaline phosphatase, and hepatic glutamine synthetase activity, and mitigating serum prolactin and serum albumin concentration reduction.

The objective of this study was to utilize free choice mineral prototype commercial products supplemented to steers consuming endophyte-infected tall fescue seed and determine their effectiveness for alleviating negative physiologic responses commonly associated with fescue toxicosis.

CHAPTER 2. LITERATURE REVIEW

Introduction

Livestock and equine industries have heavily relied on tall fescue as a source of nutrients for their grazing herds. Tall fescue is an attractive forage source that is known for its ease of cultivation and survivability in warmer climates. While it has positive attributes, this grass embraces a symbiotic relationship with an endophytic fungus, *Neotyphodium coenophialum.* The endophytic fungus produces ergot alkaloids that scientists acknowledge are the cause of poor animal health and performance. It is well documented when consuming infected tall fescue, cattle can experience a reduction in dry matter intake, changes in intake behavior, reduction of heat dispersion, and undergo vascular constriction. Although ergot alkaloids are causative, the exact compound(s) and mechanism(s) responsible for the negative effects on animal health and performance remains unknown. As such, many agronomic, pharmaceutical, and dietary approaches are being investigated as a means of overcoming the negative impact of fescue-derived alkaloids on grazing animals.

Tall Fescue

Tall Fescue *(Lolium arundiaceum)* is a perennial, cool-season grass that was introduced to the United States from Europe (Hoveland, 2009). Originally, meadow fescue seed was introduced to the United States in the early 1800s. Soon after this forage was widely sown, it is believed a contaminant of meadow fescue seed was imported from England (Vinall, 1909). This contaminant is known as tall fescue. Grass trials conducted in Kentucky (Garman, 1900) and Virginia (Kennedy, 1900) reported that tall fescue performed superiorly in comparison to the meadow fescue in terms of its growth rate and drought tolerance. There was an exponential increase in the cultivation of tall fescue as it expanded from 16,000 hectares (ha) in the 1940s and grew to 15 million ha in the 1970s Tall fescue originally covered the transition region of the United States, but has now spread from Canada down to Florida, exhibiting an extreme tolerance to climate diversity (Siegel et al., 1984).

Recognition of tall fescue's effectiveness dramatically grew upon the release of two cultivars in the mid-1900s. The first ecotype, Alta, found in the Pacific Northwest and western parts of the United States was released by Oregon Agricultural Experiment Station and the USDA (Cowan, 1956). Alta was well accepted due to its winter hardiness, resistance, and drought tolerance (Cowan, 1956).

The second ecotype, Kentucky 31 (KY-31), was collected from a mountain sheep pasture located in Menifee County, Kentucky in 1931. Local farmers were impressed with this ecotype's pasture and erosion control and brought it to the attention of Dr. E.N. Fergus. Dr. Fergus, a professor at the University of Kentucky then publicly released *Festuca arundinacea* Schreb, commonly known as KY-31, in 1942. After many tests by The Kentucky Agricultural Experiment Station, KY-31 was observed to stay green under harsh conditions, provided a longer grazing period to livestock, and was adaptable in many different soil varieties (Buckner et al., 1979).

The KY-31 cultivar is of medium maturity. Physical traits of KY-31 include leaves that are narrow-medium width, rolled, and have an outer wax covering which leaves a vibrant, matte appearance (Fergus, 1952; Fergus and Buckner, 1972; Rottinghaus et al., 1991). Tall fescue is favored for its ability to avoid drought over other cool-season grasses such as perennial ryegrass or Kentucky bluegrass (Sheffer et al., 1987). When determining the effectiveness of drought tolerance, water uptake efficiency is often examined. Water uptake efficiency is based on root size, activity, spatial distribution, and depth of the roots. Of the tall fescue cultivars, KY-31 was observed to have the greatest root length, dry mass, and water uptake rates under drought stress in comparison to other tall fescue cultivars. It also exhibited less root mortality when put under extreme drying conditions compared with the other cultivars (Huang and Gao, 2000).

Fescue Toxicosis

Characterization of Condition

The utilization of tall fescue grew in popularity and became a staple grass in livestock pastures. However, as livestock producers put their herds on the tall fescue pastures to graze, they noticed an increase in numerous health issues. In the summer months, cattle consuming tall fescue suffered the inability to dissipate heat. The cattle spent more time wading in ponds, seeking out shade, and exhibited increased respiration rates and excessive panting. In contrast, as winter months came, cattle got frostbite from the cessation of circulation causing gangrene in their extremities (i.e., ears, tail switches, and hooves) (Jacobson et al., 1963). These clinical symptoms together became known as fescue foot.

In addition to fescue foot, another syndrome, fat necrosis, was observed in cattle consuming tall fescue. When high rates of N fertilizer are applied to tall fescue pastures, cattle can be subject to fat necrosis (Hoveland, 2009). Fat necrosis is characterized by masses of fat formed in various shapes and sizes in the mesentery of the abdominal cavity. The fat masses grow to put pressure on the gastrointestinal and urinary tracts, which can lead to obstruction of organs (Smith et al., 2004). Early detection of fat necrosis can be difficult; however, cattle experiencing this syndrome have been reported to have trouble calving as well as digestive problems (Bush et al., 1979; Stuedemann et al., 1975).

Summer slump is the third syndrome resulting from cattle consuming tall fescue. This syndrome was termed “summer slump” because it primarily occurs during months when temperatures are highest. This syndrome is characterized by reduced dry matter intake, ADG, decreased circulating prolactin concentrations (Hoveland et al., 1983). Other clinical symptoms include increased respiration rate and rectal temperature, excessive salivation, and rough hair coat (Siegal et al., 1987). Cows experiencing summer slump exhibit reduced milk production and low conception rates (Hoveland et al., 1983).

Although tall fescue has impressive pasture management qualities for the producer, there is the potential economic loss that could arise when cattle consume the grass and experience some form of fescue toxicosis. Over 8.5 million cattle graze on tall fescue pastures in the United States. Of the 15 million hectares of tall fescue in the United States about 90% is infected (Siegal et al., 1987). It has been reported that cattle consuming tall fescue has cost producers in the United States upwards of $609 million annually due to reduced conception rates and decreased calf weight gains (Hoveland, 1993). Combining equine, bovine, and small ruminant industries, it is likely that fescue toxicosis has grown to over $2 billion in cost to production (Kallenbach, 2015). The negative economic impact motivated research to determine the cause of the fescue toxicity. After years of researching the causative agent, scientists came to understand the culprits responsible for fescue toxicosis are ergot alkaloids, produced within endophyte-infected tall fescue (Hemken et al., 1981).

Endophyte Symbiotic Relationship

Taxonomy

Endophytes are organisms that live within plants (defined such that endo meaning, within, and phyte meaning, plantlike organism). *Epichloë coenophiala* is an endophytic fungus that acts in symbiosis with tall fescue (Bacon et al., 1977). After the original classification of *Epichloë typhina*, Morgan-Jones and Games (1982) renamed the specific fungus found in tall fescue to *Acremonium coenophialum* as they are related to Balansiea of the Clavicipitaceous group but are not able to form sexual spores (Siegal et al., 1987). Glenn et al. (1996), then renamed the fungus *Neotyphodium coenophialum*.

Symbiosis

Neotyphodium coenophialum is the anamorphic species of Clavicipitaceae of which is a symbiont of grasses (Leuchtmann, et al., 2014). The endophytic fungus survives intercellularly in leaf sheaths and stems of the grass. The effect on the plant is mutualistic and leaves the grass with no symptoms (Bacon and Siegal, 1988). The spreading of the endophyte is due to the seed-borne nature of the grass through vertical transmission (Leuchtmann, et al., 2014). Once the infected seeds grow to mature grass, tall fescue is characterized to better withstand severe climate changes.

Both tall fescue and the endophyte fungus benefit from mutualistic symbiosis. The fungus, *Neotyphodium coenophialum*, can gain nutrients, protection within the plant's cells, and dissemination (Arachevaleta et al., 1989). The symbiotic grasses develop low osmotic potential observed in meristematic and elongated leaves which allows tall fescue to endure severe drought (Elmi and West, 1989; West et al., 1989, 1993). The endophyte allows the plant to have greater persistence and hardiness (Bacon et al., 1993). Arachevaleta et al. (1989), evaluated endophyte-free and endophyte-infected clones of the Kentucky 31 cultivar. Morphological, anatomical, and physiological responses to flooding, N fertilizer rates, and drought stress were analyzed in response to the endophyte-free and endophyte-infected treatments. Results showed that although plant ultrastructure was not altered between endophytes, the endophyte-infected plants exhibited a 50% greater growth rate than the endophyte-free plants especially when nitrogen levels were higher. This suggested that endophyte-infected plants had greater herbage production rates and better-utilized N from the soil. When the plants were placed under mild water stress (-0.05 MPa), the endophyte-infected plants out produced the endophyte-free plants. Physically, the leaf morphology for the endophyte-infected grass resulted in a greater proportion of rolled leaves. Also, the leaves of the endophyte-infected plants were thicker and narrower than the endophyte-free plants. Finally, the endophyte-infected plants had a much greater regrowth with the addition of watering to drought-stressed plants (where 75% of the endophyte-free plants died during the study due to the inability to survive the heat stress). Overall, it was concluded that the endophyte-infected Kentucky 31 was more productive than the endophyte-free tall fescue (Arachevaleta et al., 1989).

This symbiotic relationship also enhances the pest tolerance attributes of tall fescue. Parasitic nematode resistance was observed in endophyte-infected tall fescue pastures (Pedersen and Rodriguez-Kabana, 1984). In a comparison between endophyte-free and endophyte-infected tall fescue, a lesion nematode named *P. scribneri* was found to be in greater abundance in endophyte-free tall fescue roots than in endophyte-infected tall fescue roots (Kimmons et al., 1990; West et al., 1988). The ability for the tall fescue to have such pest resistance allows the plant to be more persistent and maintain greater root systems.

Ergot Alkaloids

Alkaloids, primarily produced in plants, are organic nitrogen-containing compounds. Ergot alkaloids are mycotoxins that are produced by a variety of fungi, one endophytic species being *Neotyphodium coenophialum,* which belongs in the Clavicipitaceae family (Bush and Fannin, 2009) They are considered the causative agents for poor animal performance when animals consume tall fescue. These endophytes are systemic symbionts that survive and reproduce through vertical transmission in the host plant. The endophytes are endobiotic meaning they grow within cells of plant tissues (Florea et al., 2017). The concentration of ergot alkaloids produced by the endophyte in tall fescue fluctuates, depending on temperature and N-fertilization (Arechavaleta et al., 1992; Bush and Fannin, 2009).

Ergot alkaloids are categorized into four subclasses which include clavines, lysergic acid, lysergic acid amides, and ergopeptines (Gerhards, 2014). Ergopeptine makes up as much up as 50% of total alkaloids (Lyons et al., 1986). Ergovaline is an end product of ergopeptine and is the largest and most complex of ergot alkaloids and comprises up to 57-73% of ergopeptides present in tall fescue (Rhodes et al., 1991; Yates et al., 1985; Bacon et al., 1986). It has received the most attention and is considered to be the primary toxin responsible for fescue toxicosis symptoms (Bacon et al., 1986).

Structure and Biogenic Amine Receptor Interactions

Ergot alkaloids produced from the *Claviceps purpurea* species belong to the peptide alkaloid class containing a tetracyclic ring structure (Strickland et al., 2011). Ergot alkaloids are synthesized by the precursors mevalonic acid derivative dimethylallyl diphosphate and L-tryptophan which forms dimethylallyl-tryptophan (DMAT). DMAT synthase catalyzes this reaction and is most likely the rate-limiting step into forming clavines, lysergic acid, derivatives of lysergic acid, and ergoline alcohols (Bush et al., 1997). The ergoline ring is the core structure for all ergot alkaloids. Ergopeptides are amides that have a proline residue attached at the third amino acid position. The amino acids vary at positions one and two of the cyclic tripeptide dependent on what alkaloid is formed (Schardl et al., 2006, Peyronneau, et al., 1994).

Ergot alkaloid structures are similar to biogenic amine neurotransmitters such as dopamine, serotonin, norepinephrine, and epinephrine which enables the ergot alkaloids to bind to their receptors (Strickland et al., 2011). The biogenic amines are associated with receptors of G protein-coupled membrane proteins located across various tissues of the animal (Goddard, et al., 2007). Ergot alkaloids can act as antagonists and agonists at the neurotransmitter receptors. Furthermore, they have the capability of acting as antagonists, partial agonists, or agonists at the same receptor (Klotz, 2015). The heterogeneity of ergot alkaloids and the combination of specificity and selectively to associated receptors' binding sites are very complex. Thus far, it is reported that there are at least 14 subtypes of serotonin receptors, 5 subtypes of dopamine receptors, and at least

10 subtypes of adrenoceptors. These subtypes have been classified based on their structural, transductional, and operational characteristics (Pertz and Eich, 1999).

The interactions between diverse ergot alkaloids and the biogenic amine receptors can produce a variety of biological and physiological responses in the central nervous system and blood vessels. For instance, if ergovaline binds to a receptor in vascular smooth muscle it will act agonistically (stimulating), but if ergocrostine binds to the same receptor along the vascular system, it would act in a partial agonist way, only partially stimulating the receptor (Klotz, 2015). Ergopeptine alkaloids can also act as antagonists where if bound to biogenic amine receptors such as serotonin receptors, vascular responses are blocked (Berde and Stürmer, 1978). This raises concern if ergot alkaloids are continuously consumed by cattle because if, for example, ergovaline concentration is persistently present, then the antagonistic mechanism of the ergot alkaloid will not allow the receptor to perform its function. This can then cause desensitization of receptors and decrease signal transduction (Millan et al., 2008). Furthermore, an increased rate of receptor internalization occurs due to agonist occupancy. This can lead to increased accumulation of ergot alkaloids that exceed the rate of receptor recycling which could enhance the impact on the biological response (Klotz, 2015). The biological responses can vary tremendously depending on the number of consumed ergot alkaloids, the structure and type of ergot alkaloid, and the type of biogenic amine receptor and its location in the body.

Absorption

Ergot alkaloids are absorbed through the gastrointestinal tract through passive and/or facilitated transport across the gastrointestinal epithelium (Eckert et al., 1978). The rate and magnitude of the absorption of ergot alkaloids are dependent on their solubility within the gastrointestinal tract, as well as the extent of ionization which determines the partitioning of the alkaloid between water and lipid phases. Ergot alkaloids are typically weak bases and have both polar and nonpolar components. Because of this, the pH of the environment will have a great effect on the absorption site of the ergot alkaloid (Strickland et al., 2011). In ruminants, ergopeptine alkaloids are thought to be absorbed in the forestomach and small intestine (Westendorf et al. 1993; Hill et al., 2001). The rumen has a reasonably neutral environment (~6.8), thus, there is an optimal opportunity for nutrients to be transported across the epithelium (Russell and Tychlik, 2001). Likewise, the small intestine has a higher pH, which allows the ergot alkaloids to be absorbed across the epithelium. In contrast, the abomasum acts as the gastric stomach of the ruminant and has a very low pH. While the mucosal layer is present to protect tissues from digesta, ergot alkaloids are inhibited from passing across the lining for absorption (Strickland et al., 2011).

In vitro studies have been conducted to provide evidence for the absorption of ergot alkaloids across ruminal gastric tissues. Hill et al. (2001) compared ergot alkaloid transport between reticular, ruminal, and omasal tissues that were surgically removed from sheep and placed in parabiotic chambers (Hill et al., 2001). Equimolar concentrations of lysergol, lysergic acid, and ergonovine, ergocryptine, and ergotamine tartrate were added to Kreb's Ringer phosphate solution on the mucosal side of the tissue. Tissues were incubated for 240 min and samples were collected at allotted times from the serosal side and analyzed for ergot alkaloids to examine potential transport across the gastric tissues. It was concluded that the rumen had a greater transport of alkaloids than the omasal and reticular tissue. This was suspected to be due to the rumen having a larger surface area. The greatest potential for alkaloid transport was located at the posterior dorsal sac. While it has been mentioned through various studies that alkaloid transport can be passive or active, this study exhibited alkaloid transport across the tissue to be active. The rumen exhibited the capability of transporting ~25% more alkaloids than the omasum and ~600% as much as the reticulum. When deciphering which of the alkaloids could better be transported in the greatest quantity, it differed between the rumen the omasal tissues. The rumen had a greater transport for lysergic acid and lysergol while the omasal tissues had a greater abundance of ergopeptine transport. This provides evidence that with a variety of epimers, there is a strong difference in the transport systems across the ruminant gastrointestinal tract. In support of Hill et al. (2001), Ayers et al. (2009), investigated ergot alkaloid transport using ruminal and omasal tissues from steers grazing endophyte-infected and endophyte-free tall fescue. Parabiotic chambers were used for analysis. Researchers found that ~60% of lysergic acid was transported across the ruminal epithelium and 45% of lysergic acid was transported across omasal tissue. No ergovaline was detected in the serosal chamber.

Ergovaline is presumed to have three major potential paths of absorption once in the small intestine of ruminants (Klotz and Nicol, 2016). First, intact ergovaline passes through the small intestine and progresses towards the large intestine where it can be metabolized by microbes and excreted in feces. Secondly, ergovaline can enter the bloodstream directly and the absorbed molecules are transported through the mesenteric veins towards the liver for detoxification and are likely converted to lysergic acid. It is also suggested that ergovaline may be absorbed in the small intestine and transported through the lymphatic system through the thoracic duct. If absorbed through the lymphatic system, ergovaline enters the blood outside of the portal blood and does not undergo initial hepatic detoxification. The metabolic compounds are then circulated in peripheral tissues and are thought to accumulate in adipose tissue (Klotz and Nicol, 2016). Therefore, when examining ergot alkaloid transport, both ruminal (for lysergic acid) and intestinal transport (for ergopeptines) should be considered.

Metabolic Mechanisms

Ergot alkaloid metabolism can occur in several different sites through biotransformation (Eckert et al., 1978). Metabolism of ergot alkaloids is mediated by the CYP3A subfamily of cytochrome P450 enzyme systems (Ball et al., 1992). These enzymes are responsible for catalyzing primary oxidizing endogenous substrates such as steroids, fatty acids, and prostaglandins. They also aid in the metabolism of exogenous compounds such as drugs, solvents, and environmental pollutants. Peyronneau et al. (1994) observed ergopeptine, bromocriptine, ergocryptine, and dihydroergotamine exhibited a high affinity for P450s of the 3A subfamily in human and rats liver microsomes. Bromocriptine was hydroxylated by P450s 3A at the proline ring of the cyclopeptide moiety. Researchers found that the recognition of the tripeptide portion of ergopeptines was necessary to bind to CYP3A. This suggests P450s 3A could play a role in oxidizing peptides and pseudo peptides. Further findings of involvement of cytochrome P450 3A on the metabolism of the ergot alkaloid, ergotamine, in the bovine liver were observed by Moubarak and Rosenkrans, (2000). Researchers incubated ergotamine in cattle liver microsomes. Once hydroxylated, ergotamine was converted to hydrophilic metabolites (M1 and M2). After longer periods in incubation, M1 and M2 were again hydroxylated to metabolites M3 and M4 (8,9-dihydroxy derivatives). After an hour, all the ergotamine was completely converted to metabolites. This work suggested the liver is an active site for the metabolism of ergot alkaloids in cattle.

As previously mentioned, the rumen is a prominent absorption site for ergot alkaloids. In addition, the ergot alkaloids are also metabolized via rumen microbial fermentation (De Lorme et al., 2007). Microorganisms in the rumen catabolize substrates and release compounds not suitable for absorption. Ergovaline undergoes biotransformation to lysergic acid before being absorbed into the ruminal epithelium. In an in vitro study, Moyer et al. (1993) observed that when endophyte-infected tall fescue was incubated with microbes, the concentration of ergovaline decreased to 35% of the original concentration at 24 h exposure in ruminal fluid. Ayers et al. (2009) found that total ergot alkaloid concentration increased after 48 h of incubation in ruminal fluid. It was reported that as microbial fermentation progressed, the concentration of other ergot alkaloids, primarily lysergic acid, increased in the rumen liquor. This suggests that microbial fermentation increases the liberation of ergot alkaloids from the infected-tall fescue in the rumen allowing greater absorption rates.

Excretion

Pathways of excretion for ergot alkaloids include urinary, fecal, and biliary systems. Ergovaline is excreted in the form of lysergic acid through the renal system and urine (Stuedemann et al., 1998). Absorbed ergovaline can also accumulate in bile for re-entry into the gastrointestinal tract and be excreted in feces (Klotz et al., 2016). Stuedemann et al. (1998) examined urinary and biliary excretion patterns to determine the bioavailability of ergot alkaloids. Steers were randomly assigned to endophyte-infected or endophyte-free tall fescue pastures. Steers grazed for 169 d before being sampled for bile via percutaneous puncture into the gall bladder. Steers were returned to their assigned pasture for 7 d before urine was collected. Bile output was determined using a biliary excretion rate of 100 mL of bile per steer per hour and total biliary alkaloid excretion was calculated by multiplying the concentration of alkaloids in bile by total bile output. The urinary alkaloid concentration was determined as nanograms (ng) of alkaloid per milligram (mg) of creatinine. The daily urinary alkaloid excretion was calculated based on a constant rate of 20 mg of creatinine per kilogram (kg) of live weight per day. Ergot alkaloid analyses were determined using an ELISA kit. Urinary excretion of ergot alkaloids was ~20 fold more than detected in the bile of steers grazing infected tall fescue. Researchers found that of the endophyte-infected tall fescue consumed, it was reported that 96% of the ingested ergot alkaloids were excreted in the urine.

Researchers also tested the rate of appearance or clearance of ergot alkaloids in urine that were taken from steers grazing endophyte-infected and non-infected tall fescue used in the previously mentioned study. Steers were denied access to water on October 22 (37 d after urine collection from the former experiment at 1400). The next d (October 23) at 0800 urine samples were collected. Two steers were randomly switched from endophyte-free to endophyte-infected tall fescue pastures and vice versa. Two additional steers remained in their original pastures (either endophyte-free or endophyte-infected) to serve as controls during the duration of the experiment. Urine samples were then collected on October 25, 28, and 30. After the sampling on October 30, steers were returned to their original pastures and urine samples were collected again on November 1, 4, and 6. Results showed that within 2 d of switching from endophyte-free to endophyte-infected pastures, ergot alkaloid concentrations reached comparable concentrations to steers that had continuously stayed on endophyte-infected pastures. Likewise, urinary ergot alkaloid concentrations of steers that were switched from endophyte-infected to endophyte-free decreased to concentrations comparable to the endophyte-free control steers when returned to their original pasture. This suggests that lysergic acid amides and ergopeptine alkaloids were rapidly absorbed and excreted (Stuedemann et al., 1998).

Vasoconstriction

Ergot alkaloid interaction with biogenic amine neurotransmitter receptors throughout the animal's body allows for numerous response variables. One of these responses is vasoconstriction. Ergot alkaloids bind to adrenergic receptors in the peripheral vasculature (Solomons et al., 1989). The binding of ergot alkaloids to serotonin receptors has caused the bovine lateral saphenous vein to contract (Klotz et al., 2012) as well as bovine uterine and umbilical arteries (Dyer, 1993). In addition, ergovaline has been largely responsible for inducing vasoconstriction in the ruminal artery and vein (Foote et al., 2011). The specific mechanisms of vasoconstriction in cattle are not fully understood; however, studies have shown that there is a relationship between cattle consuming ergot alkaloids and induced vasoconstriction.

Proper and consistent blood flow is essential to maintain homeostasis. When cattle grazed endophyte-infected fescue, lateral saphenous veins of steers were smaller in diameter compared to steers grazing endophyte-free tall fescue (Klotz et al., 2012). If cattle are grazing endophyte-infected tall fescue in hot temperatures, lack of sufficient blood flow to peripheral tissues and skin could inhibit cattle from being able to dissipate heat. Cattle then have the potential to undergo heat stress. To relieve the lack of heat dissipation, cattle are likely to be found wading in nearby water or mud to cool down their bodies, even in cooler temperatures. If cattle are consuming ergot alkaloids in cold temperatures, lack of blood flow to the extremities results in gangrenous ergotism. Gangrenous ergotism has been referred to as St. Anthony's fire or fescue foot (Strickland et al., 2011) and this form of ergotism has been shown to affect humans as well. Failure of proper blood supply causes the cells of the tissues to die and dry gangrene sets in. There is damage to the vessels' endothelial lining, edema, and thrombosis can occur (Vuong and Berry, 2002). Consequentially, cattle have had swollen fetlocks and hooves leading to lameness (Klotz, 2015). In extreme cases, these extremities can fall off.

New technologies are being developed to measure the physiological response of vasoconstriction due to cattle grazing endophyte-infected tall fescue. In a study conducted by Aiken et al. (2007), color doppler ultrasonography was used to analyze the caudal artery of eighteen crossbred heifers that were fed chopped alfalfa hay and concentrate mix containing either endophyte-infected or non-infected tall fescue seed. **Figure 2.1** displays the ultrasound scan at a 2-cm depth of the cross-section of the caudal artery that allows the flow signal of the area to be analyzed. During an 11 d experimental period, three baseline color doppler ultrasound measurements were taken of the caudal artery which was then compared to responses at 4, 28, 52, 76, 100, 172, and 268 hours after initial feeding of endophyte-infected seed. These measurements included caudal artery area, peak systolic velocity, end-diastolic velocity, mean velocity, heart rate, stroke volume, and flow rate. Results from the experiment exhibited that as soon as 4 hours post-feeding the caudal artery area in the heifers fed endophyte-infected tall fescue was reduced in comparison to the baseline. The caudal artery areas remained reduced throughout the rest of the experiment. The blood flow rate was also decreased in heifers consuming the endophyte-infected seed in comparison to their baseline taken before treatment. In contrast to these results, measurements in heifers consuming the non-infected seed remained similar to baseline measurements (Aiken et al., 2007).

Decreased Prolactin Concentrations

Understanding mechanisms that are critical to the growth and development of animals is of utmost importance in research to better the efficiency of production. One hormone that is known to regulate several physiological functions via its effects on cellular processes such as proliferation, differentiation, cell survival, and immune function is prolactin (Yu-Lee et al., 2002). Prolactin is a polypeptide hormone that is synthesized in and secreted from lactotrophs of the anterior pituitary gland (Freeman et al., 2000). Prolactin is part of the prolactin/growth hormone/placental lactogen family based on its genetic, structural, binding, and functional properties (Horseman and Yu-Lee, 1994). Prolactin is made up of 199 amino acids with a molecular mass of ~23,000 Da in cattle (Wallis, 1974); Freeman et al., 2000). The regulation of prolactin secretion is heavily influenced by a variety of environments and endogenous (i.e., stress, suckling, estrogen). This affects the hypothalamus which includes prolactin-releasing factors and prolactin-inhibiting factors. Prolactin inhibiting factors include dopamine, somatostatin, and γ-aminobutyric acid (Freeman et al., 2000).

Mechanistically, dopamine directly affects lactotrophs through the stimulation of dopamine type 2 receptors (D2R) on the cell surface. The binding of dopamine to the D2R stimulates the inhibitory G protein cascade (Gαi2, Gβγ), which in turn inhibits phospholipase C and adenylate cyclase activities. Consequently, both protein kinase C – mediated and cAMP level-dependent PKA stimulation of Pit-1, which binds and stimulates the prolactin gene, are not stimulated, which results in reduced prolactin synthesis. This occurs because there is no activation of CREB (cAMP response element protein) which prevents Pit-1 from binding. **Figure 2.2** displays the mechanism behind prolactin synthesis.

Ergovaline, the predominant ergot alkaloid in fescue, is a D2R agonist. When cattle graze tall fescue and consume ergovaline, there is a suppression in prolactin release (Larson et al., 1995). Referencing **Figure 2.2** in short, the agonist binds to D2R, thus blocking cAMP and the driving force that allows Pit-1 to bind to the prolactin gene leading to inhibition of prolactin secretion.

The first group to connect the relationship between inhibited prolactin secretion and tall fescue was Hurley et al. (1980). They demonstrated that calves that were fed low ergot alkaloid had higher prolactin concentrations (6.0 ng/ml) than those fed highly toxic fescue that had a prolactin concentration of 1.8 ng/ml. Also, as temperatures increased, prolactin concentrations of calves fed less toxic fescue had increasing prolactin concentrations, and calves fed toxic fescue had even lower prolactin concentrations (Hurley et al., 1980).

As days grow longer, prolactin concentrations tend to increase, which signals cattle to shed their winter hair coat. Cattle that undergo ergotism, however, often do not have high enough prolactin secretion to do so. Thus, the hair coat remains long and shaggy which alters the animal's ability to disperse heat, increasing body temperature. This aids in heat stress to the animal in addition to a reduction in blood flow due to vascular constriction caused by the ergot alkaloids (Aiken et al., 2011). In a study conducted by Niklowitz and Hoffman (1988), hamsters that underwent hypophysectomy were put under long photoperiods which allowed change in hair coat color and texture. Within 35 days of the operation, there was a decrease in growth, change in hair coat color, and the hamsters' testicles were involuted. Scientists then substituted prolactin through implants of anterior pituitaries under kidney capsules and the inhibitory effects of the hypophysectomy were reversed. This provided evidence that changes in prolactin secretion exhibit changes in weight and hair coat (Niklowitz and Hoffman, 1988).

With much of the tall fescue belt being grazed by cow-calf pairs, studies exemplifying the effect of fescue toxicosis in this production model have become more of a focus. In a study conducted by the University of Tennessee (UT), 34 Holstein cows were assigned to infected KY-31 tall fescue (E+) or non-infected tall fescue (E-) for 28 days to determine the effect of prepartum consumption of E+ hay on prolactin concentrations. In cattle, there is a surge in prolactin secretion at parturition. At the peak of the surge of prolactin, cows that were assigned KY-31 infected tall fescue secreted half as much prolactin compared to cows that had been assigned to the non-infected tall fescue (Bernard, 1993).

While the University of Tennessee study demonstrated a significant decrease in prolactin due to E+ treatment, Baldwin et al. (2016) also fed endophyte-infected tall fescue (E+) and non-infected tall fescue (E-) with the addition of bromocriptine to 24 cows. Bromocriptine is a synthetic ergot alkaloid that is structurally similar to ergovaline. In that experiment, prolactin was reduced in the E+ and bromocriptine groups compared to the control (E-) group. These results exemplified that there was a significant difference in the milk yield for the control versus the E+ and bromocriptine groups. The cattle were fed treatment throughout the dry period and this reduced the surge of prolactin as well as lactogenesis in the subsequent lactation.

Decreased prolactin concentration in growing heifers consuming endophyte-infected tall fescue is also concerning. Aldrich et al., (1993) found Angus heifers consuming endophyte-free fescue had prolactin concentrations that were higher in comparison to Angus heifers consuming endophyte-infected tall fescue diets. In agreement with these findings, Emile et al. (2000) observed 20 Holstein heifers in a 97 d feeding study and found that both average daily gain and prolactin concentrations were significantly less for heifers fed E+ compared to E- fescue. More research is warranted to determine the long-term impact of heifers' performance potential when consuming endophyte-infected tall fescue at an early age.

Vitals in Response to Ergot Alkaloids

Ergot alkaloid ingestion has repeatedly impacted vital symptoms of cattle. Cattle have exhibited increased respiratory rate, heart rate, salivation, and rectal temperature when consuming ergot alkaloids (Thompson and Stuedemann, 1993, Hemken et al. 197l, Browning and Leite-browning, 1997). Osborn (1988) reported that steers in a controlled environment fed infected-tall fescue had increased rectal temperature and respiration rates than did steers fed non-infected tall fescue. Likewise, when researchers increased the temperature in the room, rectal temperature and respiration also increased. Zanzalarie et al., (1989) observed ewes' respiration rates to increase 2.6-fold after 11 d on endophyte-infected hay (26 to 68). Rectal temperatures were increased by 1.1°C after 8 d on endophyte-infected hay. Burke et al., (2001) reported that when studying the interaction between environmental temperature (either thermoneutral or heat-stressed) and feeding heifers endophyte-infected tall fescue versus non-endophyte tall fescue, rectal temperatures, and respiration rate were increased only for those fed endophyte-infected seed under the heat-stressed environment.

Browning and Leite-Browning (1997), examined physiological responses of fescue toxicosis detected from individual ergot alkaloids. Ergotamine tartrate, ergonovine maleate, and saline vehicle were injected in a crossover design using nine Angus heifers consuming a fescue-free diet. Heifers received each treatment (one per week). Vital measurements (respiration rate, rectal, and skin temperatures) were taken 15 minutes before administration of treatment, and 30, 60, and 90 minutes after treatment. Blood was drawn approximately 5 minutes before ergot alkaloid administration and 105 minutes after to analyze prolactin concentrations. The ambient temperature recorded during the study was 35°C. Respiration rate and blood pressure were significantly higher for those under influence of ergot alkaloids. Researchers concluded that the ergot alkaloids themselves were the viable cause of fescue toxicosis symptoms.

Collectively, three disorders describe physiological responses to ergot alkaloids. Some of the physiological responses provided by ergot alkaloids are known as "summer slump”, which is one of the three (summer slump, fescue foot, and fat necrosis) previously mentioned disorders that arise from cattle having fescue toxicosis. The term summer slump is given due to its increased appearance during increased environmental temperatures. Cattle experience increased respiration, rectal temperatures, blood pressure, salivation, and steer away from consuming feed. Summer slump is the most well-known disorder that producers tend to acknowledge as their production losses tend to rise (Schmidt and Osborn, 1993).

Intake Behavior

The main goals in beef cattle production efficiency are increasing growth rate, average daily gain, and reducing the feed:gain ratio. However, one of the most concerning symptoms associated with fescue toxicosis is the decrease of dry matter intake (DMI). Reduction in dry matter intake is considered the cause of the reduced weight gain in cattle (Klotz, 2017).

Heifers consuming endophyte-infected tall fescue had reduced intake in comparison to those consuming endophyte-free fescue (Burke et al., 2001). Research has shown that when cattle are under extreme conditions and consume endophyte-infected tall fescue, weight gain decreases. Paterson et al. (1995), reported that average daily gain (ADG) decreased from 30% to 100% for cattle fed endophyte-infected tall fescue in comparison to cattle consuming endophyte-free tall fescue. The reduction in weight gain is attributed to less desirable palatability and dry matter intake when cattle are fed endophyte-infected versus endophyte-free fescue (Fribourg et al., 1991). Fribourg et al., (1991) noted that when given a choice between fescue or clover, fescue was grazed by steers over clover if the fescue was endophyte-free; however, if placed in a field where endophyte-infected tall fescue was sewn with clover, the steers chose to graze the clover. This suggests that tall fescue is desired by cattle, thus they will eat well on it if endophyte-free; however, if the tall fescue is endophyte-infected, cattle may choose not to consume as much in situations where other options are available.

Cattle consuming endophyte-infected fescue has been shown to have altered grazing and eating behavior. The grazing behavior of Angus and Holstein steers consuming fescue varieties was observed over a three-year study (Bond et al., 1984). All steers, regardless of the tall fescue variety, began to increase time spent grazing as the temperatures progressively decreased (September to October). It was also reported that steers grazing endophyte-infected fescue grazed more in the night than in daylight where those consuming endophyte-free primarily grazed in daylight. Steers consuming varieties with the greatest concentration of alkaloids exhibited rough hair coats, had increased temperature and respiration rate, increased salivation, and had lower gains (Bond et al., 1984).

Likewise, steers were given the choice between monocultures of tall fescue or alfalfa or a 1:3 alfalfa to tall fescue ratio, or 2:3 alfalfa to tall fescue ratio. Utilizing vibracorders, (which were placed on random steers in the paddock), researchers were able to monitor grazing time. Analysis showed steers that consumed the monoculture alfalfa and 2:3 alfalfa to tall fescue ratio had several short meals throughout the day, but those consuming the monoculture tall fescue or 1:3 alfalfa to tall fescue ratio consumed few meals for longer periods of the day. Thus, eating behavior could be dependent on forages consumed by steers (Seman et al., 1999).

Koontz et al. (2012), effectively induced fescue toxicosis by ruminally dosing 0.5 kg of ground endophyte-infected KY-31 seed (5.2 mg/kg of ergovaline; 3.3 mg/kg of ergovalinine). Feeding behavior was monitored utilizing feed bunks attached to load cells which allowed for continuous measurement of feed intake, number of meals consumed, and rate of intake. Under controlled temperatures (22°C d 1 to 3 and 32°C d 4 to 7), steers that were dosed with endophyte-infected tall fescue seed had reduced feed intake, increased respiration rate, and increased core temperatures. However, there was an endophyte x temperature interaction for total feed intake data, with steers being dosed with endophyte-infected tall fescue seed having a slight reduction of intake at 22°C and a great reduction of intake at 32°C. It was also reported that steers receiving endophyte-free seed had the greatest rate of intake among steers at 22°C. Finally, as the temperature was raised, steers reduced the number of meals consumed per day, regardless of whether they were ruminally dosed with endophyte-free or endophyte-infected ground fescue seed (Koontz et al., 2012).

Alleviating Fescue Toxicosis

Tall fescue is an attractive cool-season grass for use by grazing livestock across the Mid-Atlantic and Southeastern United States due to its hardiness, persistence, and drought-tolerant characteristics. Despite the benefits from the endophytic symbiotic relationship in tall fescue, there are financial costs associated with fescue toxicosis. Researchers have spent much time and effort exploring potential solutions. Pasture management practices have been put in place to alleviate fescue toxicity in cattle. Some of these include but are not limited to, increasing biodiversity by incorporating other grass and legume species, sowing pastures with grasses that contain novel-endophyte cultivars, and rotating cattle away from pastures that have high endophyte concentration during warmer seasons (Strickland et al., 2011). Moving cattle away from pastures that have high endophyte concentration is especially important during May to June when seed heads that have the highest alkaloid concentrations are present (Rottinghaus et al., 1991).

Feed additives have also been evaluated as a potential solution. Producers have tried to make up for the loss of weight gain in their herds by providing concentrate supplements. Other approaches have included but are not limited to, adding growth stimulants, anthelmintics, and binders (Schmidt and Osborn, 1993). Although some supplements have been shown to alleviate effects, additional experiments repeating the methods demonstrated inconsistent responses. The body has various forms of protection from toxins, one being the excretion of the ergot alkaloids themselves. It was reported that mycotoxin binders can adsorb mycotoxins, prevent their absorption in the gastrointestinal tract, and increase amounts of ergot alkaloids excreted to decrease concentrations in the animal's system (Stanford et al., 2018). In this study, lambs were fed a commercial product mycotoxin binder called, Biomin II (BB), which contained diatomaceous earth, kaolin clay, yeast, and plant extracts, and an enzyme targeted toward the degradation of zearalenones and trichothecenes. The test treatments included a barley-based diet control treatment (no additive), control with the addition of BB, solely ergot alkaloid-based diet, and ergot alkaloid-based diet with the addition of BB. Results showed that with the addition of the BB with the ergot alkaloids, there was a 38.5% increase in ergot alkaloid concentration in feces compared to lambs strictly consuming an ergot alkaloid-based diet (Stanford et al., 2018). This study suggests that further experimental design could aid in increasing the amount of ergot alkaloid extraction from the body and decreasing the amount that is maintained in the circulatory system that has downstream negative impacts on the animal.

Conclusion

While the endophyte, *N. coenophiala*, provides persistence and adaptability in a symbiotic relationship with tall fescue, the impact that the production of ergot alkaloids has when consumed by cattle can be extremely costly to producers across the United States because of the many symptoms associated with the condition of fescue toxicosis. Ergot alkaloids' similarity in the chemical structure of the tetracyclic ring with biogenic amine neurotransmitters increases the opportunity for adrenergic, dopamine, and serotonin alterations in the cattle's metabolic and neuronal systems. This can negatively affect cattle's performance in a variety of ways including decreased dry matter intake, increased temperature and respiration rates, vasoconstriction, and decreased prolactin circulation. Collectively, a reduction in nutrient transport and heat dispersion affects the feed intake of cattle and their growth performance. Therefore, it is of utmost importance to find solutions that limit the detrimental impact ergot alkaloids have when consumed. The objective of the current study was to determine if an ergot alkaloid binding product could relieve symptoms associated with fescue toxicosis.

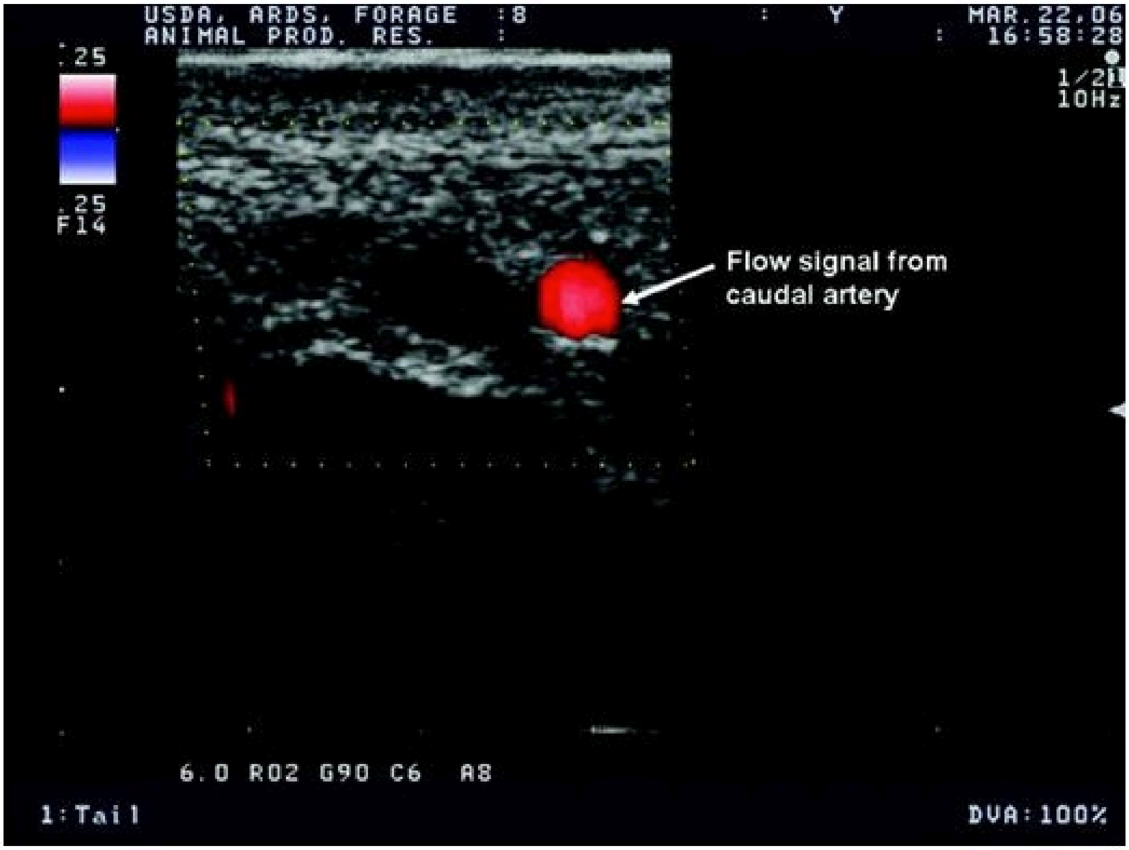

Figure 2.1. Ultrasound scan at a 2-cm depth of the cross-section of the caudal artery taken at the fourth coccygeal vertebra. Red Doppler flow signal delineates the area of flow through the artery (Aiken et al., 2007).

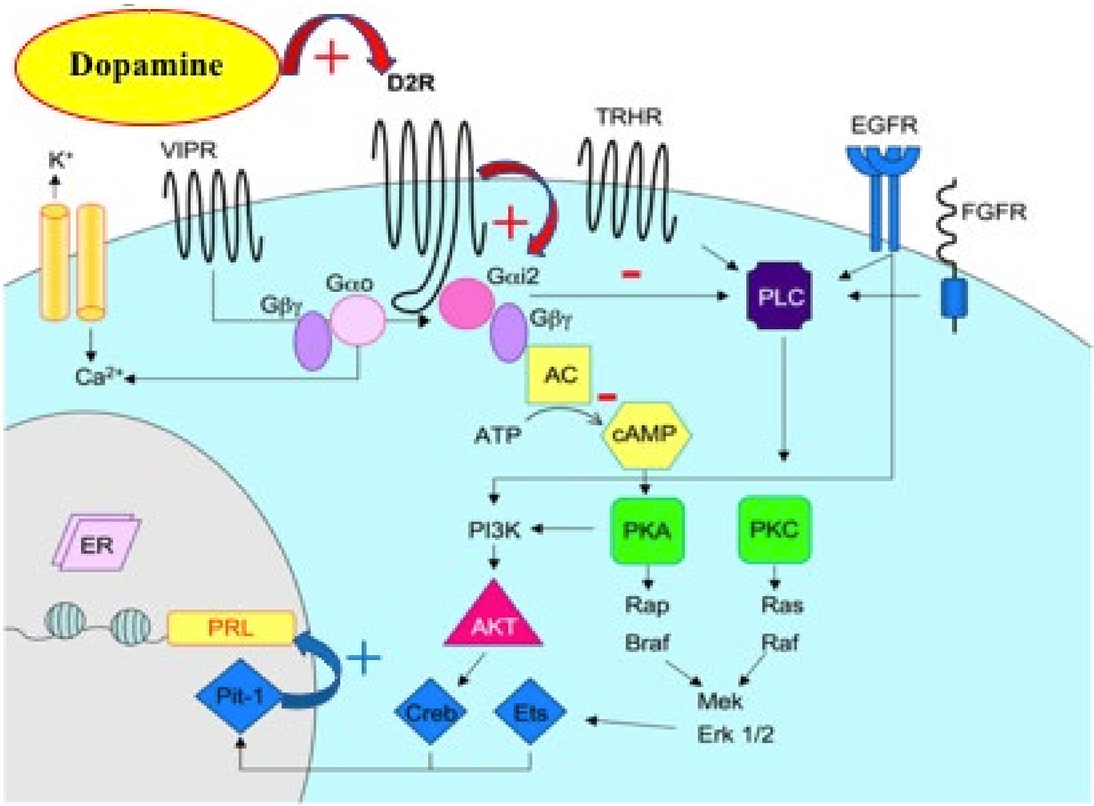

Figure 2.2. Dopamine (and other D2R agonists) Control Pituitary Pit-1 Expression and Blood Prolactin. Adapted from PLOS ONE 12:e0184612.

CHAPTER 3. IMPACT OF MINERAL SUPPLEMENTATION ON INTAKE BEHAVIOR AND BLOOD FLOW IN GROWING BEEF CATTLE EXPOSED TO FESCUE-DERIVED ALKALOIDS

Introduction

Tall fescue (*Lolium arundinaceum*) has exhibited attractive agronomic qualities including high performance under continuous grazing environments. A mutualistic relationship between tall fescue grass, and the endophytic fungus, *Epichloë coenophiala,* has contributed to the prevalence of this forage, which occupies millions of acres in the United States, primarily in the Southeast region. While the advantages and benefits of tall fescue are well documented, there are also well-documented negative effects on livestock performance. *E. coenophiala* produces ergot alkaloids that have been shown to be the causative agent in an abundance of symptoms that are collectively known as fescue toxicosis (Strickland et al., 2011).

When ergot alkaloids, particularly ergovaline, are consumed by livestock (i.e., bovine, equine, and ovine species) performance has drastically decreased (Lyons et al., 1986). Such results have included but are not limited to, decreased intake, weight gains, and circulating prolactin concentrations. In addition, results have included increases in the skin and rectal temperatures as well as respiration rate (Klotz, 2015). The poor performance resulting from intake of endophyte-infected tall fescue has been suggested to represent over a $2 billion production loss to livestock producers across the United States (Kallenbach, 2015).

Ergot alkaloids contain an ergoline ring that is similar to the structures of the neurotransmitters dopamine, serotonin, norepinephrine, and epinephrine. This structural similarity enables ergot alkaloids to bind to the respective biogenic amine receptors (Freeman et al., 2000). The binding of ergot alkaloids to receptors and their antagonistic effects results in negative impacts such as vasoconstriction that could lead to impaired thermoregulation (Strickland et al., 2011). Moreover, ergovaline, the predominant ergot alkaloid in tall fescue, is a dopamine agonist. When it binds to D2 dopamine receptors, there is a significant reduction in circulating prolactin (Hurley et al., 1981). Therefore, prolactin concentrations have been used as a hallmark indicator of fescue toxicity.

Numerous studies have shown that feed intake and growth are sensitive to alkaloid intake. Burke et al. (2011) observed heifers consuming endophyte-infected tall fescue consumed less than those consuming endophyte-free tall fescue under heat-stressed conditions. Likewise, ADG decreased from 30% to 100% for cattle fed endophyte-infected tall fescue compared to cattle fed endophyte-free tall fescue due to reduced dry matter intake (Paterson et al, 1995).

The feeding behavior for cattle consuming tall fescue has also been investigated. Seman et al., (1999) reported that steers grazing monocultures of tall fescue and a 2:3 tall fescue:alfalfa ration consumed few meals for longer periods of the day, whereas steers consuming monoculture alfalfa and a 2:3 alfalfa:tall fescue ration had several short meals throughout the day. Furthermore, feeding behavior has been altered for steers grazing endophyte-infected compared with non-infected fescue. Steers grazing endophyte-infected fescue experienced elevated temperatures and respiration rates and were observed to graze more at night than in daylight in contrast to steers consuming endophyte-free which grazed primarily in daylight (Bond et al., 1984). Koontz et al. (2012) utilized feed bunks attached to load cells to determine feed intake, the number of meals consumed, and the rate of intake for cattle ruminally dosed with ground endophyte-infected KY-31 seed. As the temperature was increased, the number of meals consumed per day and the rate of intake were reduced for those dosed with the infected seed.

One attempt to alleviate the negative effects of cattle consuming endophyte-infected tall fescue is through the incorporation of feed additives. Approaches towards utilization of feed additives include the inclusion of supplements, growth stimulants, anthelmintics, and binders (Schmidt and Osborn, 1993). Mycotoxin binders have been evaluated for the potential to prevent ergot alkaloid absorption in the gastrointestinal tract (Binder, 2007). Stanford et al., (2018) fed lambs diets containing ergot alkaloids, along with mycotoxin binders, and a reported 38.5% increase in ergot alkaloid concentration in the lamb's feces.

The objective of this study was to evaluate an alkaloid-binding mineral supplement for beef cattle consuming endophyte-infected fescue to determine the potential to mitigate or prevent the negative effects of alkaloid consumption on feed intake and feeding behavior, circulating prolactin, vasoconstriction, rectal temperatures, and respiration rates.

Materials and Methods

The procedures used in this study involving live animals were approved by the University of Kentucky Institutional Animal Care and Use Committee. The research was conducted at the University of Kentucky C. Oran Little Research Center, Beef Unit, located in Woodford Co. KY.

Animals and Environment

Twelve Angus crossbred steers (BW = 310 $\pm$ 27 kg) were used in a 3x3 Replicated Latin square design consisting of three 28-d experimental periods. Steers were housed indoors in individual pens (10x10 ft) and had free-choice access to water. Steers were weighed upon arrival at the facility and adapted to the environment for 3 d prior to initiation of the experiment. Room temperature was cycled above thermoneutral (~29.4°C) for 16 h each day (0600-2200) and at thermoneutral (~21.1°C) for 8 h daily (2200 to 0600) to mimic summer conditions. The light:dark cycle was maintained to 16 h light and 8 h dark corresponding to the room temperature cycle.

Experimental Design and Feeding

Treatments included three top-dressed mineral supplements ($142 g \cdot hd^{-1} \cdot d^{-1}$): a non-medicated control (CON), commercially available Fescue EMT® Mineral Defense (EMT), and a test prototype mineral (EMT 2) provided by Cargill Animal Nutrition, Minneapolis, MN, USA (Table 3.1). Each period consisted of an adaptation/washout sub-period (d 1-14), a step-up sub-period (d 15-21), and a sampling sub-period (d 22-28). Steers were fed once daily at 0830 and the amounts offered were recorded.

During the adaptation/washout sub-period, all steers received CON and were fed a basal diet, composed primarily of alfalfa haylage and corn silage, at 1.5 x NE_m (**Table 3.2**). During both the step-up and sampling sub-periods, steers received their appropriate supplemental treatment and were fed the basal diet containing 15% KY-31 endophyte-infected seed on an as-fed basis. The chemical composition on a percent dry matter basis of both diets is shown in Table 3.3. The amount of feed offered was increased incrementally during the step-up period to allow for *ad-libitum* intake by d 19 and throughout the sampling period. A diagram of the experimental model is shown in **Fig. 3.1**.

To ensure *ad-libitum* access to treatment diets, amounts fed were adjusted so that 10-15% of orts were recovered daily. Orts were collected, weighed, and recorded for each animal before feeding (0730). Approximately 250g samples were collected from orts and placed in a forced-air oven and dried overnight at 55 ºC to calculate dry matter intake. Daily water consumption was measured using water meters installed in individual pens.

Tall fescue seed used throughout the study was endophyte-infected Kentucky-31 (KY-31 Tall Fescue, Shawneetown Feed and Seed, Jackson, MO). Fescue seed was ground to pass through a 3-mm Hammer Mill before being mixed in the diet. The endophyte-infected seed was incorporated at a set percentage of the diet at 15% on an as-fed basis. With this approach the actual ergovaline dose did change with intake, however, this was intended to simulate the dynamics of alkaloid effects on intake in grazing animals. Laboratory analysis (University of Kentucky, Lexington, KY) of the infected seed indicated that concentrations of ergovaline and ergovalinine (an isomer of ergovaline) were 5.32 ppm and 4.01 ppm, respectively: providing a total "ergovaline" equivalent of 9.33 ppm. The total dietary ergovaline equivalent concentration was 2.62 ppm.

Feeding behavior was determined using individual feeders attached to load cells with communication to a data handler (e.g., to permit determination of meal size, meal number, and meal duration). Data were downloaded each d and analyzed using MATLAB and previously published algorithms (Egert-McLean et al., 2019).

Vital Measurements

Rectal temperature and respiration rate were measured and recorded daily at a maximum of 3 hours post apex of ambient temperature (1200) d22-27 of the treatment sub-period. All vital measurement recordings were recorded at the time of observation. Respiration rate was determined by observing the rib expand on the inspiration of the steers. At the start of the assessment, a timer was set at 30 seconds. During those 30 seconds, the number of times the steer inspired was documented and multiplied by two to achieve the number of breaths per minute. Immediately following recordings of respiration, rectal temperatures were obtained utilizing an AG-Medix, LLC animal thermometer.

Vascularization Measurements

The arterial luminal cross-sectional area of the caudal artery (a measure of vasoconstriction) was measured using a Doppler ultrasound on d 14, 21, and 28 of each period. Ultrasounds were obtained after moving steers from their individual pens to the chute at 0730 before feeding.

Doppler ultrasound was used to measure the caudal artery luminal area according to the method described by Aiken et al. (2009). In brief, color doppler ultrasound scans were taken of the caudal artery at the 4th coccygeal (Cd4) vertebrae with a Classic Medical TeraVet 3,000 Ultrasound Unit (Classic Universal Ultrasound, Tequesta, FL) with a 12L5-VET (12 MHz) linear array transducer set to a 4-cm depth. Cross-sectional images were collected using a frequency of 6.0 MHz and a pulse repetitive frequency of 2.0 to 3.0 kHz. Following the freezing of individual scans for each animal, frames stored in the cine memory of the unit were searched to store the image exhibiting maximum flow signal which was assumed to be at peak systolic phase.

Blood Collection and Plasma Prolactin Analysis

Blood samples were collected from the jugular vein of steers before feeding (0730) on d 14 and 28 of each period for the determination of circulating prolactin concentrations. Samples were collected before feeding and blood (10 mL) was collected in heparinized test tubes using a vacutainer needle per steer. The collected sample was immediately placed on ice. Immediately after blood had been sampled from all steers, collected tubes of blood were placed into a centrifuge and spun at 5,000 x g for 30 minutes at 4°C. Harvested plasma was placed into microfuge tubes (~2mL per tube) and frozen at -80°C until analysis.

Plasma prolactin concentrations were measured in duplicate using a double-antibody radioimmunoassay as previously described (Forrest et al., 1980). Purified bovine prolactin (AFP-6432B0) and bovine prolactin antiserum raised in rabbits (AFP-753180) were obtained from the National Hormone and Peptide Program (Dr. A.F. Parlow, Harbor-UCLA Medical Center, Torrance, CA). All plasma samples were run within a single assay. Assay sensitivity was 55 ng/mL, and the intra-assay coefficient of variation was 10.1%.

<u>Statistical Analysis</u>

Data were analyzed using the PROC MIXED procedure of SAS 9.4 (SAS Inst. Inc, Cary, NC, USA). The experimental unit was individual steer. The denominator of degrees of freedom for tests of fixed effects was determined using the Kenward-Roger method. Mineral supplement (CON, EMT, EMT 2) was considered a fixed effect. Response variables included dry matter intake, water intake, blood flow, prolactin concentration, rectal temperature, respiration rate, number of meals consumed, meal size, and meal duration. The period was used as the repeated measure term with animal specified as the subject and compound symmetry used as the covariance structure. Results are reported as the least square means. Proc Univariate was used to evaluate the distribution of studentized residuals for each response variable. Outliers were indicated by skewed distributions combined with large studentized residual values (>3); in which case they were removed. There were three outliers removed from water intake data, all of which represented unreasonably large estimates of water intake. Additionally, one erroneous serum prolactin value was detected for day 28, resulting in the removal of all prolactin values for that animal.

Results

Dry Matter and Water Intake

The EMT treatments tended ($P = 0.12$) to decrease dry matter intake during the step-up sub-period and reduced ($P \leq 0.04$) dry matter intake during the treatment sub-period. No difference ($P \geq 0.61$) in dry matter intake was observed between the two EMT treatments **(Table 3.4)**. EMT treatment did not affect ($P = 0.67$) water intake during the treatment sub-period **(Table 3.4)**.

Dry matter intake for the adaptation and step-up phases was lower ($P \leq 0.05$) for the first period of the Latin square compared with the subsequent two periods. Dry matter intake for adaptation and step-up periods as per design were limit-fed based on body weight. Dry matter intake tended ($P = 0.07$) to be lower during the treatment sub-period for periods 1 and 2 compared with period 3 **(Table 3.5)**. Dry matter intake during the treatment sub-period were 17.5, 16.4, and 16.3 g DMI/kg BW for periods 1, 2, and 3 respectively. Water intake was higher (P = 0.003) during periods 2 and 3 compared with the first period.

Intake Behavior

EMT treatments tended (P = 0.07) to decrease the number of meals but did not affect (P ≥ 0.37) meal size or length **(Table 3.6)**. The effect of period on intake behavior during the treatment period is exhibited in **Table 3.7**. Meal frequency tended (P = 0.07) to be greater for PER 1 compared with PER 3. Meal size was greatest (P ≤ 0.006) for PER 3 compared with PER 1 and 2. Period had no effect (P = 0.46) on meal duration.

Arterial Luminal Cross-sectional Area

The effects of the supplement on the arterial luminal cross-sectional area of the caudal artery are shown in **Table 3.8**. Prior to treatment on d 14, the luminal cross-sectional area was smaller for steers (P ≤ 0.008) assigned to EMT treatments compared with those assigned to CON. On d 21, arterial luminal cross-sectional area was greater for EMT (P = 0.007) and EMT 2 (P = 0.10) compared with CON. On d 28, arterial luminal cross-sectional area tended (P = 0.06) to be greater for EMT compared with CON. Although the luminal cross-sectional area was numerically greater for EMT on d 21 and 28, there were no differences between EMT treatments.

For this study, vasoconstriction was measured as the reduction in arterial luminal cross-sectional area. It was calculated by subtraction of d 21 and d 28 arterial area measurements (cm^2) from d 14 arterial area measurements. EMT (P = 0.0004) and EMT 2 (P = 0.02) reduced endophyte-induced vasoconstriction on d 21. Endophyte-induced vasoconstriction on d 21 tended (P = 0.09) to be less for EMT compared with EMT 2. On d 28, endophyte-induced vasoconstriction was lower for EMT compared with CON ($P = 0.002$) and tended to be lower for EMT 2 ($P = 0.07$), with no difference ($P = 0.11$) between CON and EMT 2. These results are exhibited in **Table 3.8**. Period had no effect ($P \geq 0.81$) on the arterial luminal cross-sectional area or endophyte-induced vasoconstriction (**Table 3.9)**.

Prolactin Concentration

Prolactin concentration **(Table 3.10)** decreased dramatically following the addition of endophyte-infected seed, but concentration was unaffected ($P \geq 0.50$) by EMT treatments. **Table 3.11** shows plasma prolactin concentration on d 14 was greater ($P = 0.005$) for PER 1 compared with PER 2 and 3, but no differences ($P = 0.21$) were detected between PER on d 28.

Vital Measures

Vital measurements are found in **Table 3.12**. Rectal temperature and respiration rate were unaffected ($P = 0.12$) by EMT treatments. Rectal temperature was unaffected ($P = 0.67$) by PER; however, respiration rate was greater ($P \leq 0.01$) for PER 2 and 3 compared with PER 1 which is shown in **Table 3.13**.

Discussion

The purpose of this study was to determine if the negative impact of fescue-derived alkaloids on cattle could be alleviated by top-dressing a commercial mineral binding supplement to diets containing KY-31 endophyte-infected seed. Analyzed parameters throughout the study included feed intake and feeding behavior, circulating prolactin concentrations, vasoconstriction, rectal temperatures, and respiration rates.

Dry Matter and Intake Behavior

Diets that were used throughout the study were designed to meet the recommended nutrient requirement for growing steers. During the 14-d adaptation/washout phase of each period, steers were limit fed a basal diet plus control mineral treatment at 1.5x maintenance energy requirements. Because ergot alkaloid consumption has been shown to reduce dry matter intake in cattle (Baldwin et al., 2016), the basal diet was limit-fed to equalize intake across treatments and to minimize potential carryover effects of ergot alkaloid and treatment on intake. During the treatment period, all steers received the same ergot alkaloid concentration as it was adjusted to 15% of the diet on an as-fed basis. Although this allowed for the actual intake of ergot alkaloids to vary with dry matter intake, it enabled the comparison of treatments under typical conditions encountered by grazing cattle. The amount of endophyte-infected fescue seed incorporated into the diet was based on previous work which had shown comparable alkaloid levels to reduce dry matter intake in cattle (Baldwin et al., 2016). Steers were initially limit fed the treatment diet and then "stepped up" progressively to ad libitum intake. During the study three mineral supplements were top-dressed each day at feeding. The mineral supplements included CON, EMT, and EMT 2 where EMT and EMT 2 supplements included bentonite. Bentonite is a clay mineral composed of montmorillonite that is commonly present in feed additives (Santos et al., 2011; WHO, 2005). The addition of bentonite was included as a mycotoxin adsorbent with the intent to alleviate negative symptoms associated with fescue toxicity.

Koontz et al. (2012) found that for steers ruminally dosed with endophyte-infected tall fescue seed, total dry matter intake, rate of intake, and the number of meals was reduced. The inclusion of endophyte-infected seed did reduce dry matter intake in the current study. Unexpectedly, the mineral supplements that included the clay binder bentonite, (both EMT treatments), reduced dry matter intake and meal frequency in comparison to the CON treatment. The rate of intake and number of meals were similar across treatments. Chestnut et al. (1992) supplemented mineral mix containing hydrate sodium calcium aluminosilicate to sheep fed endophyte-infected tall fescue seed diets. Hydrate sodium calcium aluminosilicate is another type of clay (zeolite) that binds mycotoxins to prevent absorption (Ramos and Hernandez, 1997). Researchers found the mineral supplement did not eliminate the negative effects of endophyte-infected seed on intake.

During the adaptation and step-up phases, dry matter intake was lower in PER 1 than PER 2 and PER 3. The steers were fed at 1.5% of body weight during the adaptation subperiod. During this period no orts were observed. Throughout the step-up subperiod, the steers consumed all of the diet offered until d 3 when they were progressed to 2.0% of body weight. Thus, prior to *ad-libitum* consumption, dry matter intake was strictly a function of body weight. Dry matter intake was numerically greater in PER 2 and PER 3; reflective of greater average body weight during these periods compared with PER 1. Based on dry matter intake as a percentage of body weight (1.75%, 1.63%, and 1.63% for PER 1, 2, and 3, respectively), steers were consuming less in PER 2 and PER 3 in comparison to PER 1. Meal size was greatest for PER 3 compared to PER 1 and 2. As mentioned previously, steers numerically consumed more, and increased in weight, as the study progressed. Therefore, it is not surprising that they had greater meal size in PER 3. However, based on a percentage of body weight, the steers were consuming less as age progressed.

Arterial Luminal Cross-sectional Area

Specific mechanisms of vasoconstriction in cattle are not fully elucidated; however, studies have shown an apparent relationship between cattle consuming ergot alkaloids and induced vasoconstriction. Ergot alkaloids' interactions with biogenic amine neurotransmitter receptors throughout the animal's body induce responses that cause vasoconstriction. Ergot alkaloids bind to adrenergic receptors in the peripheral vasculature (Solomons et al., 1989). The binding of ergot alkaloids to serotonin receptors has been shown to cause the bovine lateral saphenous vein to contract (Klotz et al., 2012) as well as bovine uterine and umbilical arteries (Dyer, 1993). In addition, ergovaline has been shown to be largely responsible for inducing vasoconstriction in the ruminal artery and vein (Foote et al., 2011). Vasoconstriction caused by the consumption of ergot alkaloids may be responsible for many of the symptoms associated with fescue toxicosis (Klotz and Nicol, 2016). Aiken (2007) saw a tendency for vasoconstriction of the caudal artery of heifers fed endophyte-infected seed as early as 4 h post-feeding and continually found further constriction up until 28 h. Reduction in the arterial luminal cross-sectional area of the caudal artery is an indication of fescue toxicosis induction (Aiken et al., 2007). In the present study, the arterial luminal cross-sectional area was measured on d 14 before the introduction of endophyte-infected seed or treatments. Results of the initial measurement showed the arterial luminal cross-sectional area was smaller for steers assigned to EMT and EMT 2 treatments compared to the CON. On d 21, steers had consumed diets containing endophyte-infected seed and supplement treatment for 7 d. Measurements of the arterial luminal cross-sectional area on d 21 were greater for both EMT treatments compared with the CON. The overall reduction in the arterial luminal cross-sectional area across treatments is in agreeance with Aiken et al. (2007) and Klotz et al. (2016) which both indicated that consumption of endophyte-infected seed induced vasoconstriction of the caudal artery. After 14 d of treatment (d 28), the arterial luminal cross-sectional area tended to be greater for EMT compared with the CON. There were no differences between EMT treatments on d 21 and 28. Because the arterial luminal cross-sectional area was smaller for steers assigned to EMT treatments prior to the introduction of seed or treatment on d 14, changes in arterial luminal cross-sectional area were also calculated to determine the degree of vasoconstriction. Changes in the arterial luminal cross-sectional area were measured by the difference in area between d 21 and d 14 as well as the difference in area between d 28 and d 14. Both EMT and EMT 2 reduced endophyte-induced vasoconstriction on d 21. Endophyte-induced vasoconstriction on d 21 tended to be less for EMT compared with EMT 2. This suggests that within the first 7 d, EMT treatments potentially mitigated some of the ergot alkaloid-induced vasoconstriction; however, the EMT formulation tended to be more effective in relieving this vasoconstriction than the EMT 2 formulation after 7 d exposure to alkaloids. On d 28, endophyte-induced vasoconstriction was lower for EMT compared with CON and EMT 2, and there was no difference between CON and EMT 2 treatments. This indicates that EMT was more effective in reducing vasoconstriction than the EMT 2 formulation.

Because bentonite has been shown to bind mycotoxins, and EMT treatments included bentonite, these findings suggest bentonite could be effective in binding ergot alkaloids and reducing the absorption of the toxins so that overall vasoconstriction caused by ergot alkaloids was partially alleviated. The differences in EMT treatments are difficult to explain because the exact formulations are proprietary. Both EMT treatments include bentonite; however exact amounts in each are unknown. Other differences between the EMT treatments could be attributed to differences in NaCl concentration and oil sources. In contrast to feed additives mitigating ergot-alkaloid induced vasoconstriction, Jia et al. (2018) reported supplementation of Se vitamin-mineral mix was not effective in alleviating vasoconstriction of the caudal artery. The mechanism behind ergot binding and vasoconstriction is not fully understood. However, the reduction in vasoconstriction when supplemented EMT treatments did provide evidence that these free-choice mineral prototype commercial products could be possible methods to combat negative effects of fescue toxicosis, with the EMT blend being more advantageous. Expansion of this work may benefit from including measurements such as heart rate and blood flow rate to better represent the sensitivity of ergot alkaloids on vasoconstriction.

There was no period effect on the arterial luminal cross-sectional area of the caudal artery throughout the experiment. This indicates evidence that there were no carry-over effects of ergot alkaloid consumption. These findings are instrumental in showing that 14 d is sufficient to reverse ergot alkaloid-induced vasoconstriction under the current conditions. This is consistent with Aiken et al. (2009) which observed the arterial luminal cross-sectional area of the caudal artery of heifers consuming ergot alkaloids were able to return to the initial baseline arterial luminal cross-sectional area after 8 d. This contrasts with Kloz et al. (2016) which observed that 35 to 42 d minimum is needed for vascular recovery. This may be due to the length of alkaloid exposure where steers grazed endophyte-infected grass for 88 d in comparison to the current study where steers consumed endophyte-infected seed for 14 d.

Prolactin Concentrations

Reduction in prolactin concentration is a hallmark indication of fescue toxicosis in cattle. Ergot alkaloids, particularly ergovaline, act agonistically at the dopamine type 2 receptors, causing inhibition of prolactin secretion from the anterior pituitary (Larson et al., 1995). Cows consuming KY-31 endophyte-infected tall fescue secreted 50% as much prolactin at the peak of the surge of secretion in comparison to cows that consumed endophyte-free tall fescue (Bernard, 1993). Baldwin et al. (2016) also fed endophyte-infected tall fescue and endophyte-free tall fescue with the addition of bromocriptine to cows. Bromocriptine is a synthetic ergot alkaloid that is structurally similar to ergovaline. These researchers found prolactin was reduced in the endophyte-infected group and the bromocriptine group compared to the endophyte-free group, coupled with a significant decrease in the milk yield. The cattle were continually exposed to treatments throughout the dry period, and this reduced the surge of prolactin as well as the lactogenesis of the subsequent lactation.

In the current study, the introduction of endophyte-infected seed drastically reduced prolactin concentrations, though treatments had no effect in alleviating the decrease in prolactin secretion. Between d 14 and 28 prolactin concentrations drastically decreased by 96.5% for the control supplement, 96.3% with the addition of EMT supplement, and 95.2% with the addition of EMT 2 supplement. This magnitude of reduction in prolactin concentration is similar to that reported by other groups (Jia et al. 2018 and Brown et al. 2009). The overall decrease in prolactin concentration indicates that induction of fescue toxicosis was achieved.

Prolactin concentrations were higher on d 14 for PER 1 in comparison to PER 2 and 3. This could suggest possible carry-over effects despite the 14 d washout period in between treatment periods in the cross-over design. This contrasts with the arterial diameter of the caudal artery data that showed no period effect throughout the study. There were no differences detected between periods on d 28 across the three periods.

Vital Measurements

Various studies have reported an interaction between cattle consuming endophyte-infected tall fescue and vital responses. Cattle have been shown to exhibit increased respiratory rate and rectal temperature when consuming ergot alkaloids (Thompson and Stuedemann, 1993, Hemken et al. 197l, Browning and Leite-Browning, 1997). Increased respiration rate provides a method to aid in dissipating heat (Hahn, 1999; Koontz et al., 2012). Osborn (1988) reported that steers in a controlled environment fed endophyte-infected tall fescue had increased rectal temperature and respiration rates in comparison to steers fed non-infected tall fescue. Likewise, when researchers increased the temperature in the room, rectal temperature and respiration also increased. Burke et al., (2001) observed an interaction between environmental temperature (either thermoneutral or heat-stressed) and feeding heifers endophyte-infected tall fescue versus endophyte-free tall fescue. Both rectal temperatures and respiration rate were increased for those fed endophyte-infected seed under the heat-stressed environment.

In the present study, steers were subject to a controlled environment with temperatures above thermoneutral (~29.4°C) for 16 h and 8 h at thermoneutral (~21.1°C). Results showed that rectal temperature tended to be lower for the EMT 2 treatment compared to the CON. As previously mentioned, cattle consuming ergot alkaloids typically have increased rectal temperature. The average rectal temperature for growing steers is 36.7-39.1°C (Robertshaw, 2004) and the steers in the present study averaged 38.6°C across treatments. This result is unexpected as the steers were provided a diet that was composed of 15% ergot alkaloids on an as-fed basis which provided a total dietary ergovaline equivalent of 2.62 ppm. In addition to ergot alkaloid consumption, rectal temperatures would be expected to increase due to the hot environment as the room temperature was designed to mimic summer conditions. Koontz et al. (2012) reported that both core temperature and respiration rates increased at 32°C for steers consuming endophyte-free and endophyte-infected tall fescue seed. The normal range for resting respiratory rates in cattle is 26-50 breaths/minute (Reece and Swenson, 2004). In the current study, respiration rate tended to be reduced for both EMT and EMT 2 treatments when the room temperature was ~29.4°C. Respiration rates on average were 50.1, 45.7, and 45.1 breaths per minute for the CON, EMT, and EMT 2 treatments, respectively. Therefore, results indicate respiration rates were on the higher end range; however, rates were lowered as a result of EMT treatments. This may suggest that EMT treatments aided in alleviating symptoms of fescue toxicosis as the need for dissipating heat and increased respiration rates were eliminated.

Conclusion

This study was conducted to evaluate an alkaloid binding mineral supplement for use in cattle consuming endophyte-infected fescue. The inclusion of endophyte-infected seed in the diet was effective in generating changes associated with endophyte consumption as evident by the decrease dry matter intake and plasma prolactin concentration and induction of vascular constriction. Although both EMT treatments reduced meal frequency and dry matter intake they exhibited effectiveness at reducing vascular constriction and numerically decreasing respiration rate. EMT 2 formulation was less effective in reducing vascular constriction and offered no clear positive advantages to the EMT formulation. Although period effects were present for dry matter intake and water intake, only the apparent indication of carryover effects from exposure to endophyte-infected seed is the observed reduction in plasma prolactin concentration at d 14 for PER 2 and 3. The potential for carryover effects of treatment are unknown but could explain differences in vascular diameter area between steers on d 14.

Table 3.1. Chemical composition (DM basis) of mineral supplement[1] fed to steers.

Ingredient	Diet		
	Control	EMT	EMT 2
Ca, %	27.7	27.7	27.7
P, %	5.0	5.0	5.0
NaCl, %	28.6	28.6	34.5
Mg, %	2.0	2.0	1.0
Cu, ppm	2,000	2,000	1,600
Se, ppm	21.1	21.1	21.1
Zn, ppm	5,000	5,000	6,000
Vitamin A, IU/lb	239,000	239,000	239,000
Vitamin D_3, IU/lb	12,000	12,000	12,000
Vitamin E, IU/lb	110	110	120

[1]Mineral Mix contents: provided by Cargill Animal Nutrition, Minneapolis, MN, USA

Table 3.2. Dietary ingredient composition (% DM basis) fed to steers.

Ingredient	Diet	
	Basal	Treatment
Alfalfa Haylage, %	34.0	24.4
Corn Silage, %	53.2	38.3
Distillers Grains, %	12.8	9.2
KY-31 Seed, % (15% As-fed Basis)	---	28.1

The KY-31 endophyte-infected tall fescue seed was determined to contain 9.33ppm of ergovaline and ergovaline combined.

Table 3.3. Chemical composition (DM basis) of experimental diet fed to steers.

	Diet	
Chemical Component	Basal	Treatment
TDN,%	67.5	66.8
MP,%	8.9	8.8
CP, %	15.5	15.6
DIP, % of CP	66.5	67.5
UIP, % of CP	33.5	32.5
Ca, %	0.73	0.58
P, %	0.53	0.47
Fat, %	3.6	2.6
NE_m, Mcal/kg	1.54	1.52
NE_g, Mcal/kg	0.95	0.93

Table 3.4. Effect of mineral supplement on DM and water intake

	Supplement				*P-value*[b]			
Item	CON	EMT	EMT 2	SEM[a]	TRT	CON v EMT	CON v EMT 2	EMT v EMT 2
DMI, kg/d								
d 1 to 14	6.51	6.42	6.49	0.14	0.35	0.18	0.79	0.27
d 15 to 21	6.76	6.39	6.41	0.23	0.12	0.07	0.08	0.93
d 22 to 28	6.18	5.74	5.64	0.20	0.03	0.04	0.01	0.61
Water Intake, L/d								
d 22-28	28.7	27.3	30.4	2.98	0.6659	0.6907	0.62	0.38

Data are presented as least square means

[a]SEM: Standard Error of the Mean; DMI: n=12/treatment; Water intake: n=11/treatment

[b]Contrast based on Fischer Protected test

Table 3.5. Effect of Latin square period on DM and water intake.

	Period				*P-value*[b]			
Item	PER 1	PER 2	PER 3	SEM[a]	PER	PER 1 v PER 2	PER 1 v PER 3	PER 2 v PER 3
DMI, kg/d								
d 1 to 14	6.10	6.63	6.70	0.14	<.0001	<.0001	<.0001	0.25
d 15 to 21	6.21	6.60	6.75	0.23	0.03	0.05	0.009	0.46
d 22 to 28	5.70	5.72	6.13	0.20	0.07	0.92	0.04	0.05
Water Intake, L/d								
d 22-28	20.83	32.54	32.92	2.98	0.003	0.002	0.003	0.91

Data are presented as least square means
[a]SEM: Standard Error of the Mean; DMI: n=12; Water intake: Period 1 n=11, EMT n= 11, EMT 2 n=11
[b]Contrasts are based on Fischer Protected test

Table 3.6. Effect of mineral supplement on intake behavior during the treatment period (d 22-28)

	Supplement				*P-value*[b]			
Item	CON	EMT	EMT 2	SEM[a]	TRT	CON v EMT	CON v EMT 2	EMT v EMT 2
Meals Per Day	8.4	7.8	7.6	0.47	0.07	0.08	0.03	0.59
Meal Size, kg	1.4	1.5	1.5	0.11	0.61	0.80	0.34	0.48
Meal Length, min	18.5	19.8	19.9	2.14	0.37	0.23	0.21	0.96

Data are presented as least square means
[a]SEM: Standard Error of the Mean; n=12
[b]Contrasts are based on Fischer Protected test

Table 3.7. Effect of Latin square period on intake behavior during the treatment period (d22-28)

Item	Period				*P-value*[b]			
	PER 1	PER 2	PER 3	SEM[a]	PER	PER 1 v PER 2	PER 1 v PER 3	PER 2 v PER 3
Meals Per Day	8.3	7.8	7.5	0.47	0.07	0.16	0.02	0.33
Meal Size, kg	1.3	1.4	1.7	0.11	0.0003	0.08	<.0001	0.01
Meal Length, min	19.3	18.7	20.1	2.14	0.50	0.61	0.47	0.22

Data are presented as least square means
[a]SEM: Standard Error of the Mean; n=12
[b]Contrast are based on Fischer Protected test

Table 3.8. Effect of mineral supplement on the arterial luminal cross-sectional area of the caudal artery

	Supplement				*P-value*[b]			
Item	CON	EMT	EMT 2	SEM[a]	TRT	CON v EMT	CON v EMT 2	EMT v EMT 2
Arterial Luminal Cross-sectional Area, cm^2								
d 14	16.0	15.0	15.4	0.67	0.0004	<.0001	0.008	0.07
d 21	6.5	8.6	7.7	0.55	0.02	0.007	0.10	0.22
d 28	6.3	8.6	7.2	0.84	0.06	0.02	0.34	0.15
Change from d 14, cm^2								
d 21	-9.6	-6.4	-7.7	0.80	0.001	0.0004	0.02	0.09
d 28	-9.7	-6.4	-8.2	1.10	0.007	0.002	0.11	0.07

Data are presented as least square means
[a]SEM: Standard Error of the Mean; n=12
[b]Contrast are based on Fischer Protected test

Table 3.9. Effect of period within square on the arterial luminal cross-sectional area of the caudal artery

Item	Period				*P-value*[b]			
	PER 1	PER 2	PER 3	SEM[a]	PER	PER 1 v PER 2	PER 1 v PER 3	PER 2 v PER 3
Arterial Luminal Cross-sectional Area, cm^2								
d 14	15.5	15.5	15.4	0.67	0.99	---	---	---
d 21	7.3	7.7	7.7	0.55	0.81	---	---	---
d 28	7.0	7.5	7.5	0.84	0.83	---	---	---
Change from d 14, cm^2								
d 21	-8.2	-7.8	-7.7	0.80	0.81	---	---	---
d 28	-8.4	-8.0	-7.9	1.10	0.82	---	---	---

Data are presented as least square means
[a]SEM: Standard Error of the Mean; n=12
[b]Contrasts are based on Fischer Protected test

Table 3.10. Effect of mineral supplement on plasma prolactin concentration

Item	Supplement				*P-value*[b]			
	CON	EMT	EMT 2	SEM[a]	TRT	CON v EMT	CON v EMT 2	EMT v EMT 2
Prolactin, ng/mL								
d 14	350.9	385.2	422.5	82.47	0.71	---	---	---
d 28	12.2	14.1	20.3	6.07	0.50	---	---	---

Data are presented as least square means
[a]SEM: Standard Error of the Mean; Period 1: n=12, Period 2: n=11, Period 3: n=12
[b]Contrasts are based on Fischer Protected test

Table 3.11. Effect of Latin square period within square on plasma prolactin concentration

	Period				*P-value*[b]			
Item	PER 1	PER 2	PER 3	SEM[a]	PER	PER 1 v PER 2	PER 1 v PER 3	PER 2 v PER 3
Prolactin, ng/mL								
d 14	573.8	295.1	289.6	82.47	0.01	0.01	0.003	0.95
d 28	22.9	10.0	13.7	6.07	0.21	0.09	0.21	0.62

Data are presented as least square means
[a]SEM: Standard Error of the Mean; Period 1: n=12, Period 2: n=11, Period 3: n=12
[b]Contrasts are based on Fischer Protected test

Table 3.12. Effect of mineral supplement on vital measurements during the treatment period

Item	Supplement				*P-value*[b]			
	CON	EMT	EMT 2	SEM[a]	TRT	CON v EMT	CON v EMT 2	EMT v EMT 2
Rectal Temperature, °C								
d 22 to 27	38.7	38.6	38.5	0.07	0.12	0.43	0.04	0.19
Respiration rate, breaths/min								
d 22 to 27	50.1	45.7	45.1	3.48	0.12	0.09	0.06	0.83

Data are presented as least square means
[a]SEM: Standard Error of the Mean; n=12
[b]Contrasts are based on Fischer Protected test

Table 3.13. Effect of Latin square period within square on vital measurements during the treatment period

Item	Period				*P-value*[b]			
	PER 1	PER 2	PER 3	SEM[a]	PER	PER 1 v PER 2	PER 1 v PER 3	PER 2 v PER 3
Rectal Temperature, °C								
d 22 to 27	38.6	38.6	38.6	0.07	0.67	---	---	---
Respiration rate, breaths/min								
d 22 to 27	41.4	48.4	51.0	3.48	0.002	0.01	0.001	0.32

Data are presented as least square means
[a]SEM: Standard Error of the Mean; n=12
[b]Contrasts are based on Fischer Protected test

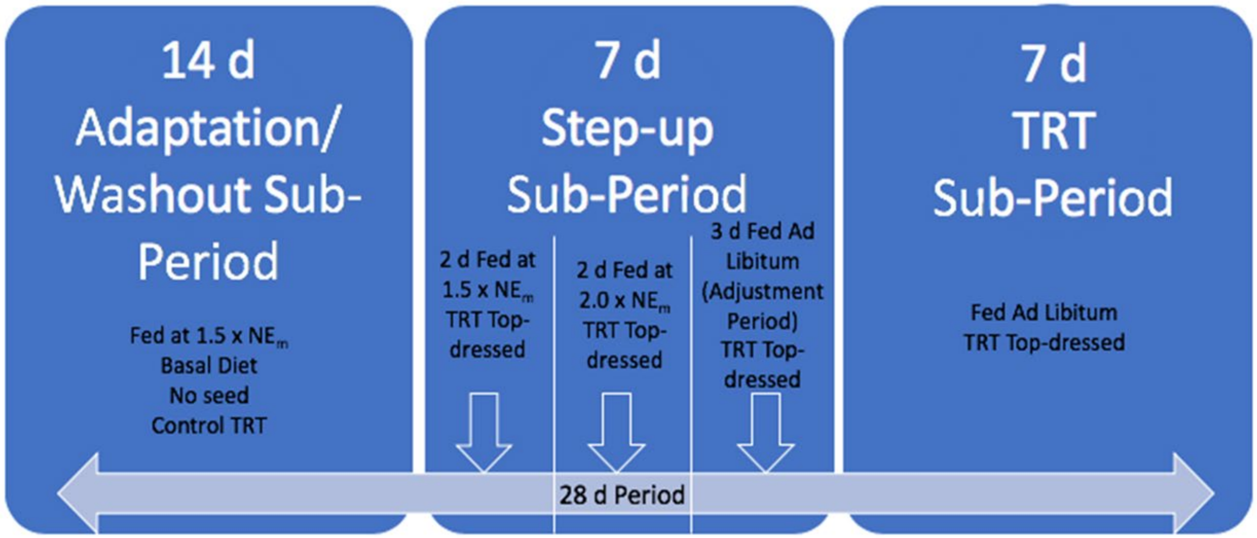

Figure 3.1. Experimental model for replicated Latin square design (three 28 d experimental periods).

CHAPTER 4. SUMMARY AND CONCLUSIONS

Tall Fescue (*Lolium arundinacea*) is a perennial forage grass that is spread across 12 to 14 million ha and is commonly utilized for grazing animals in the United States (Siegal, et al., 1985). The adaptability in soil varieties, growth hardiness and tolerance in diverse climate conditions, and persistence under heavy grazing are accredited to a symbiotic relationship with the ergot alkaloid producing endophytic fungus, *Neotyphodium coenophialum* (Bacon and Siegal, 1988). Although applauded for these characteristics, poor performance qualities were soon noticed in animals grazing tall fescue. Reductions in dry matter intake, weight gain, and milk production, and increased body temperature and respiration rate are just a few of the symptoms associated with consumption of endophytic tall fescue in herds (Hoveland, 2009; Strickland et al. 2011). Collectively these symptoms are recognized as the syndrome, fescue toxicosis. The negative consequences of fescue toxicosis have created a tremendous economic impact on the U.S. cattle industry. This syndrome has resulted in losses estimated at nearly $2 billion annually (Kallenbach, 2015).

The production of ergot alkaloids from the endophytic fungus, *N. coenophialum,* are deemed the causative agents for the poor animal performance when consuming endophyte-infected tall fescue. Ergot alkaloids are mycotoxins that contain a tetracyclic ring structure like that of biogenic amine neurotransmitter receptors: dopamine, serotonin, and norepinephrine. The similarity in chemical structures allows ergot alkaloids to bind to these receptors either agonistically or antagonistically causing a variety of physiological responses that can affect intake regulation, vascular function, endocrine activity, and thermoregulation (Berde and Stürmer, 1978; Strickland et al., 2011). The binding of ergot alkaloids, specifically ergovaline, to adrenergic and serotonin receptors have shown to cause vasoconstriction of peripheral blood vessels (Solomons et al., 1989), bovine lateral saphenous vein (Klotz et al., 2012), bovine uterine and umbilical artery (Dyer, 1993) and ruminal artery and vein (Foote et al., 2011). When ergot alkaloids bind to dopamine type 2 receptors (agonistically), there is a depression of prolactin secretion which has become a classic indication of fescue toxicosis. Prolactin is a polypeptide hormone that is synthesized and secreted from the anterior pituitary gland and regulates physiological functions such as milk production and immunity (Freeman et al., 2000).

Other production losses associated with steers consuming ergot alkaloids have excessive salivation, and increased respiration rate, heart rate, and rectal temperature (Thompson and Stuedemann, 1993; Browning and Leite-browning, 1997). Especially under elevated environmental temperatures, consumption of ergot alkaloids inhibits cattle from being able to dissipate heat. Cattle spend more time trying to cool off by wading in ponds, spending time in the shade, etc. which discourages grazing time. This contributes to reduced dry matter intake and subsequently, reduced weight gain (Paterson et al. 1995).

Supplementation of feed additives has been utilized to mitigate the symptoms associated with fescue toxicosis. The objective of this study was to evaluate if mineral prototype commercial supplement (EMT and EMT 2) would alleviate the negative impact of steers when fed diets that included 15% KY-31 endophyte-infected tall fescue seed. In the present study, KY-31 seed was effective in inducing expected changes that are typical for steers consuming ergot alkaloids such as decreased dry matter intake, arterial diameter, and prolactin concentrations between d 14 when KY-31 seed was introduced and d 28. Both EMT treatments reduced dry matter intake and meal frequency. The inclusion of KY-31 endophyte-infected tall fescue seed also resulted in vascular constriction in the steers' caudal artery. EMT treatments were effective in mitigating some adverse effects of ergot alkaloids by reducing the vascular constriction and numerically decreasing respiration rate. EMT was more effective in alleviating the reduction in vasoconstriction than EMT 2.

By using the 3x3 Replicated Latin Square experimental design, there was a concern for potential carry-over effects throughout the study. Period effects were analyzed between the three periods. There were period effects for dry matter intake and water intake; however, only the reduction in plasma prolactin concentration at d 14 for PER 2 and 3 appear to be caused by the inclusion of endophyte-infected tall fescue seed.

Moving forward, continued research examining potential feed additives for cattle consuming endophyte-infected tall fescue is warranted to alleviate the negative impact of fescue-derived ergot alkaloids. The present study has provided insight that the free choice mineral prototype commercial supplement EMT and EMT 2 aided in mitigating vascular constriction under fescue toxicosis conditions, with EMT being more advantageous than EMT 2. Expansion on this current study may benefit from including other varieties of mineral-vitamin mixes in diets to better aid in combating production losses by increasing dry matter intake and changes in intake behavior.

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