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THE EFFECT OF ENDOPHYTE-INFECTED TALL FESCUE SEED CONSUMPTION ON GUT AND SATIETY HORMONES RELATED TO INTAKE REGULATION IN HOLSTEIN STEERS

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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 at the University of Kentucky

By

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2021

ABSTRACT OF THESIS

THE EFFECT OF ENDOPHYTE-INFECTED TALL FESCUE SEED CONSUMPTION ON GUT AND SATIETY HORMONES RELATED TO INTAKE REGULATION IN HOLSTEIN STEERS

Cattle consuming endophyte-infected tall fescue (E+) typically experience a syndrome termed fescue toxicosis which is thought to be caused by ergot alkaloids produced by the endophyte. The most abundant alkaloid, and considered the most likely cause of the syndrome, is ergovaline (ERV). During fescue toxicosis, a decrease in ADG is observed which is likely due to the decrease in DMI commonly observed in animals consuming E+ compared to animals consuming non-endophyte-infected tall fescue (E-). However, the cause of the decrease in intake is not well elucidated. Many physiological responses control feed intake including, but not limited to, physical, neural, metabolic, and hormonal factors. The present study focused on investigating the impact of E+ consumption on hormonal factors related to intake regulation as well as investigating the effects on nonesterified fatty acid (NEFA) and β-hydroxybutyrate (BHB). Twelve growing Holsteins steers were assigned to one of three treatments (n=4 per treatment): 0 ppm ERV, 1.8 ppm ERV, and 2.7 ppm ERV. Animals were adapted to the treatment diets for 7 days followed by a 7-day treatment period.Cattle were catheterized to facilitate blood sampling. Blood samples (25mL) were collected every 20 minutes for 8 hours, beginning 1-hour before feeding, on day 7 of the treatment period. Samples were centrifuged for 30 minutes at 5000 x g at 4 C, and plasma was aliquoted for hormone, NEFA, and BHB analysis. DMI intake decreased linearly ($p<0.0001$) with increasing intake of ERV. Plasma insulin and leptin concentrations both displayed a quadratic response. Plasma active ghrelin exhibited a linear response ($p=0.0431$) where concentrations decreased as ERV concentration increased. NEFA concentrations produced a significant treatment x time interaction ($p<0.0001$). BHB concentrations exhibited a quadratic response where concentrations were lowest for the 2.7 ppm ERV treatment ($p=0.0286$). Glucose concentrations were shown to increase linearly with increasing ERV intake ($p=0.0456$). These results indicate that consumption of E+ decreases intake which may be possible through alteration of hormones related to intake regulation and potentially alter postabsorptive metabolism.

KEYWORDS: cattle, fescue toxicosis, hormones, intake regulation, ruminant

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July 18, 2021
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FREQUENTLY USED ABBREVIATIONS

ADG	Average Daily Gain
ARC	Arcuate Nucleus
BHB	ß-hydroxybutyrate
CNS	Central Nervous System
CV	Coefficient of variation
d	Day
DM	Dry matter
DMI	Dry Matter Intake
E-	Nonendophyte-infected tall fescue
E+	Endophyte-infected tall fescue
ERV	Ergovaline
GIT	Gastrointestinal tract
LCFA	Long-chain fatty acid
min	Minute
NDF	Neutral Detergent Fiber
NEFA	Non-esterified fatty acids
NTS	Nucleus Tractus Solitarii
ppm	Parts per million
RDP	Rumen Degradable Protein
RIA	Radioimmunoassay

CHAPTER 1. INTRODUCTION

Tall fescue, or *Lolium arundinaceum,* is a cool-season perennial grass that is commonly utilized in the southeast United States and became widely popular after "Kentucky 31" was released in the 1940s (Paterson et al., 1995; Ball et al., 1991). Unfortunately, this forage has resulted in negative performance characteristics as well as negative health issues for grazing livestock. Animals consuming tall fescue are at risk for a syndrome called fescue toxicosis. This is a result of consumption of the toxic endophyte (*Epichloë coenophiala*)which produces ergot alkaloids, the primary cause of the disorder. Negative impacts observed following consumption of tall fescue are decreased average daily gain (ADG) accompanied by a decrease in dry matter intake (DMI) as well as vasoconstriction and hyperthermia (Paterson et al., 1995; Rhodes et al., 1991, Aiken et al., 2007).

The characteristic decrease in DMI has been considered to be the cause of the decreased ADG following evaluation of effects of consumption of tall fescue on foregut blood flow, digestion and metabolism, and ruminal DM contents (Klotz, 2017), and thus, a large contributor to the economic losses associated with fescue toxicosis. Therefore, one of the next steps in fescue toxicosis research is to determine the cause of the decreased DMI.

This study focused on examining three essential hormones related to intake regulation, insulin, leptin, and ghrelin as well as investigating potential changes in postabsorptive metabolism. Insulin is a hypoglycemic hormone produced by the β cells of the islet of Langerhans in the pancreas and has the important role of maintaining

glucose concentrations in the blood as well as being essential for the regulation of metabolic function (Browning and Thompson, 2002; Wilcox, 2005). Excess insulin concentrations in the blood have been associated with a decrease in intake (Porte and Woods, 1981; Deetz and Wangness, 1981). Through regulation in the form of negative feedback, insulin can induce a hypophagic effect on the animal. Cattle undergoing heat stress conditions have exhibited increases in insulin concentrations although DMI is decreased (Baumgard and Rhodes, 2013a) which may alert to changes in postabsorptive metabolism particularly a shift from lipid mobilization to carbohydrate metabolism. Non-esterified fatty acids (NEFA) and ß-hydroxybutyrate (BHB) play an important role in lipid mobilization, and thus, may provide evidence for changes in this process.

Leptin is a peptide secreted by adipose tissue and plays an important role in the regulation of whole-body energy metabolism (Nkruman et al., 2005). The mechanism by which leptin acts in appetite regulation occurs mainly in the hypothalamus through interactions with the arcuate nucleus (ARC). Here, leptin can stimulate the release of appetite-stimulating or inhibiting neuropeptides which aid in the regulation of intake (Ahima et al., 1999).

Ghrelin is a gut peptide produced from endocrine cells in the gastrointestinal tract and is known to stimulate appetite (Sakata and Sakai, 2010). In plasma, ghrelin is present in an active and an inactive form. The active form is acylated and can stimulate growth hormone release by binding to the necessary receptor (Kojimia and Kangawa, 2002). Additionally, active ghrelin has been positively associated with DMI in cattle (Foote et al., 2014).

Analysis of insulin, leptin, and active ghrelin, though not the only hormones related to intake regulation, may serve as an important step in evaluating the effects of endophyte-infected tall fescue consumption on intake regulation, and analyzing NEFA and BHB may provide insight on postabsorptive metabolism changes. Therefore, the objective of this study was to investigate changes in gut and satiety hormones related to intake regulation through analysis of insulin, leptin, and active ghrelin concentrations, and to examine potential changes in postabsorptive metabolism following consumption of endophyte-infected tall fescue seed.

CHAPTER 2. LITERATURE REVIEW

History of Tall Fescue

Tall fescue (*Lolium arundinaceum*) is a widely abundant forage in the United States and is a commonly utilized cool-season perennial grass in the southeast (Paterson et al., 1995). This versatile grass became popular in the 1940s following the release of "Kentucky 31" and was subsequently planted throughout the 1940s and '50s. As this grass became a staple in livestock production, negative aspects began to be noticed. Producers noticed tall fescue was not as palatable as other cool-season grasses, and they witnessed inconsistencies in cattle performance. Additionally, health issues such as fescue foot, fat necrosis, and fescue toxicosis, also known as summer slump, were observed (Ball et al., 1991).

During the 1970s the endophyte was discovered which was determined to be the cause of the negative animal health issues. Researchers for the USDA were able to associate an endophytic fungus with decreased ADG in beef cattle which was confirmed by scientists at Auburn University. Two important practical characteristics were also discovered. Scientists discovered that the endophyte acted in a symbiotic relationship with the plant and did not seem to affect the plant's appearance or growth. Furthermore, they found that the endophyte also resided in the seed portion of the plant (Ball et al., 1991).

Fescue Toxicosis

Overview

Fescue toxicosis is a complication associated with the consumption of endophyte-infected tall fescue. Typically, it leads to inhibition of animal production through a decrease in intake and ADG (Thompson and Stuedemann, 1993). Cattle experiencing fescue toxicosis present a myriad of symptoms including, but not limited to, increases in rectal temperature, excess salivation, increase in respiratory rate, lameness, rough hair coat, and decreased grazing time (Strickland et al., 2011). Tall fescue inhabits around 14 million hectares in the United States (Casler and Kallenbach, 2007) and is one of the most costly animal health-related issues for the grazing livestock industry (Strickland et al., 2011) with around $2 billion lost to the beef industry in the US annually (Kallenbach, 2015).

Causative Agent

Much research has been directed at determining the causative agent(s) of fescue toxicosis. Of that research, much has been done investigating ergot alkaloids as a primary cause of the disorder. Ergot alkaloids are produced by the endophyte (*Epichloë coenophiala*) present in endophyte-infected tall fescue. Unfortunately, the specific mechanisms of action of these alkaloids are ill-defined.

Ergot alkaloids can be divided into two main classes, ergopeptines and ergolines, and they likely work in conjunction with one another, either additively or coactively, to elicit fescue toxicosis symptoms (Foote, 2013). These alkaloids produce varied effects on biological processes which seem to be independent of the dosage of the alkaloids and

more so related to the structure of the alkaloids. While the exact structures of the ergot alkaloids are not identical, they do typically share a characteristic tetracyclic ergoline ring. The characteristic ring allows the ergot alkaloids to bind to various receptors due to the structural similarity they share with norepinephrine, dopamine, and serotonin. Additionally, the structural similarity allows ergot alkaloids to acts as agonists, partial agonists, or antagonists at the receptors of the neurotransmitters (Pertz and Eich, 1999).

Impact on Animal Performance

Negative impacts on DMI and ADG are characteristic of fescue toxicosis in cattle and have significant economic importance for producers. The decrease in gain is likely due to the decrease in intake (Klotz, 2015). Research has shown that cattle consuming endophyte-infected tall fescue consume less dry matter than cattle fed noninfected tall fescue (Beers and Piper, 1987; Paterson et al., 1995; Matthews et al., 2005). Additionally, it has been shown that not only is intake decreased, but a decrease in ADG is observed as well (Schmidt et al., 1983; Paterson et al., 1995). The decrease in ADG in cattle consuming endophyte-infected tall fescue can range from 30-100% of cattle consuming nonendophyte-infected tall fescue (Paterson et al., 1995).

Aside from a decrease in DMI and ADG, cattle impacted by fescue toxicosis also undergo vasoconstriction and hyperthermia. Vasoconstriction in cattle can result in serious health problems such as gangrenous ergotism as well as affect nutrient availability and uptake. Gangrenous ergotism is caused by blood vessel dysfunction which has been attributed to ergot alkaloid consumption (Rhodes et al., 1991; Aiken et al., 2007). Additionally, this disorder can lead to issues with bovine extremities including

tissue necrosis of the tail, ears, and hooves (Klotz, 2015). Vasoconstriction of the right ruminal artery and vein are also symptoms of fescue toxicosis. Ergot alkaloids have been found to constrict both the right ruminal artery and vein, and this can alter blood supply and drainage from the foregut as well as compromise the absorption of nutrients and fermentative end products (Foote et al., 2011).

Hyperthermia also presents as an issue in cattle consuming endophyte-infected tall fescue, but it is arduous to distinguish between heat stress and ergot alkaloid consumption (Klotz, 2015). Cattle undergoing fescue toxicosis cannot regulate body temperature effectively (Spiers et al., 2012), and these animals can exhibit negative effects on the cardiovascular system which can exacerbate the hyperthermia (Browning Jr and Leite-Browning, 1997; Eisemann et al., 2014).

Intake Regulation

Physical Factors Regulating Intake

Physical constraints on voluntary intake in ruminants are largely a function of fill capacity and volume, and typically involve increased gastrointestinal distension that results in the decreased intake. These constraints are closely associated with diet composition with complications arising when feeding high forage diets. In ruminants consuming high forage diets, DMI is decreased with decreased flow of digesta through the gastrointestinal tract resulting in distension which in turn, causes a decrease in DMI (Allen, 1996). Grovum (1979) found that when water-filled balloons were inserted into the rumen, reticulum, and abomasum of sheep consuming alfalfa pellets increased

distension resulting in decreased intake which provides evidence that gut fill and distension can impact intake.

Neutral detergent fiber (NDF) includes the cell wall fraction present in a particular feedstuff which includes lignin, cellulose, and hemicellulose. NDF is commonly referred to as the best single chemical predictor of DMI as it stays in the rumen longer with less rapid fermentation and contributes to the filling effect in the rumen. Particularly, the indigestible fraction which is not available for microbial use and relies on passage to escape the rumen which results in a larger retention time that contributes to decreased intake (Poppi et al., 1981b, a). In sheep fed a wide range of roughage materials, cell wall constituent intakes were not different, and thus, provided evidence that DMI can be highly related to NDF (Van Soest, 1965).

Passage rate can also influence intake. Typically, fractional passage rate increases as DMI increases (Riewe and Lippke, 1970). Additionally, intake and retention time of the reticulorumen are inversely related (Allen, 1996) which means that as retention time is increased, passage rate is decreased resulting in decreased intake.

Neural Factors Regulating Intake

Neural regulation of intake encompasses communication within the central nervous system (CNS) to mount an appropriate intake response to afferent peripheral signals. The hypothalamus, which is a primary control center of intake in the neuronal system, receives afferent signals and responds by sending an appropriate efferent signal to modify food intake through a variety of neuronal pathways. Within the hypothalamus,

there are interconnecting nuclei that have orexigenic or anorexigenic functions and work in concert to aid in the formation of the necessary intake response. The nuclei have the capability for metabolic sensing to help maintain overall energy balance. The arcuate (ARC), paraventricular, ventromedial and dorsomedial nuclei and the lateral hypothalamic area represent the interconnecting nuclei present in the hypothalamus (Simpson et al., 2009).

The nucleus tractus solitarii (NTS), located in the caudal brainstem, serves as a key commencing point for vagal afferent nerve signals from the periphery. It also aids in the mitigation of integration of multiple signals. These peripheral signals originate from the GIT, nutrient chemicals, and gut peptides and aid in negative feedback control of intake (Schwartz, 2006). Additionally, the NTS is responsible for the integration of these signals (D'Agostino et al., 2016).

The ARC, located at the site of the incomplete blood-brain barrier, is the primary hypothalamic area involved in regulating intake and contains neuropeptide Y, agouti-related protein, cocaine and amphetamine-related transcript, and pro-opiomelanocortin which are commonly referred to as “first-order” neurons (Valassi et al., 2008). Neuropeptide Y and agouti-related protein are known to increase food intake whereas cocaine and amphetamine-related transcript and pro-opiomelanocortin decrease intake (Simpson et al., 2009). Additionally, projections from the ARC allow the axons of these neurons to interact with other areas of the hypothalamus containing “second-order” neurons, such as the paraventricular nucleus and lateral hypothalamic area. The paraventricular nucleus contains the anorexigenic substances thyrotropic-releasing hormone and corticotropin-releasing hormone, and the lateral hypothalamic area contains

primarily orexins and melanin-concentrating hormone. Signals related to adiposity and satiety control which peptides are released to inhibit intake. When adiposity signals are high, primarily in the form of circulating leptin and insulin, the signals are delivered from the peripheral tissues, mainly via the NTS, and anorexigenic peptides are released. In contrast, when adiposity signals are low in the peripheral tissues, orexigenic peptides will be released to stimulate intake (Valassi et al., 2008).

Metabolic Factors Regulating Intake

Metabolic feedback for regulating intake occurs primarily through nutrient-sensing via the CNS. As described above, various portions of the hypothalamus participate in receiving and sending signals to properly adjust feeding behavior and energy expenditure as well as to optimize glucose utilization and production. Carbohydrates, proteins, and lipids are the primary macronutrients involved in regulating feed intake which will be further explored below.

Carbohydrates

In ruminants, the majority of dietary carbohydrates are metabolized in the rumen to volatile fatty acids (VFA) following the breakdown of glucose monomers to pyruvate. The primary VFAs produced include acetate, butyrate, and propionate. Acetate and butyrate are commonly associated with fatty acid synthesis whereas propionate is most commonly associated with the production of glucose. Research has provided evidence that these VFAs have the potential to modulate intake. Acetate, propionate, and butyrate have all been shown to decrease intake following injection into the rumen (Montgomery et al., 1963). Intraruminal infusion of VFA in sheep consuming either high or low forage

diets has been shown to decrease digestible energy intake (Bhattacharya and Alulu, 1975). Although acetate, propionate, and butyrate have all been seen to decrease intake in ruminants, research suggests that propionate is a more potent regulator of intake over acetate and butyrate (Phillipson, 1970; Anil and Forbes, 1988). Additionally, research into the impact of propionate on intake concluded that after intraruminal infusion of propionate, energy intake and DMI were decreased with the degree of decrease increasing as propionate concentration increased (Oba and Allen, 2003b). This impact of propionate on intake is likely due to the increased glucose production associated with the increase.

Research also suggests that amounts of rapidly degraded starch available to the animal can affect intake. Notably, the effects of increased starch can cause digestive issues such as ruminal acidosis which will ultimately decrease intake, so it is important to ensure the cause of decreased intake is attributed appropriately. The addition of more rapidly fermenting starch typically results in decreased intake (Allen et al., 2009). A decrease in intake was observed as ruminally degraded starch, as a % of DM, increased in lactating cows (Oliveira et al., 1995; Knowlton et al., 1998). However, research suggests that using high concentrate diets can alter feed intake without causing digestive upsets. In lactating dairy cows fed high-corn rations, the increase in fermentable starch caused a decrease in meal size % and a decrease in overall intake (Oba and Allen, 2003a). In feedlot cattle, intake may be regulated by metabolic signals, such as ruminally degraded starch, as opposed to gut fill (Allen et al., 2009). Steers fed an all concentrate diet had a lower DMI compared to steers fed diets containing roughages (Shain et al., 1999), and

similar results were reported in steers fed increasing amounts of roughages in the diet where DMI was decreased linearly (Gill et al., 1981).

Lipids

Lipids are provided to the ruminant through de novo synthesis, feedstuffs, and dietary supplements. Fatty acids are considered the most important lipid fraction in ruminants and are regarded as high-energy substrates with the potential to impact intake. These fatty acids can be oxidized which involves the breakdown of fatty acids into acetyl CoA units which can ultimately be used to generate energy and are thought to serve as a post-absorptive satiety signal. Increases in fatty acid oxidation have been shown to cause a hypophagic intake response whereas data suggests inhibition of fatty acid oxidation stimulated intake (Allen et al., 2009). In cows postruminally infused with vegetable oil containing a mixture of various fatty acids with long-chain fatty acids (LCFA) constituting the majority, DMI was significantly decreased (Benson et al., 2001). Similarly, cows fed high-fat diets with increasing amounts of LCFA exhibited decreased DMI (Choi and Palmquist, 1996).

Nonesterified fatty acids (NEFA) are also known to cause hypophagia in ruminant animals (Allen, 2000). Endogenously, NEFA are a major source of fatty acids oxidized in the liver (Emery et al., 1992), and supplementing fat increased NEFA in lactating cows which was followed by decreased intake (Choi et al., 1997). Overall, fatty acids are capable of serving as a satiety signal for ruminant animals and inducing a hypophagic response.

Protein

Metabolism of dietary protein can result in high-energy substrates that have the ability to influence intake. Dietary protein has two major fates upon entry to the rumen: degradation or escape through the reticulo-omasal orifice where it will be metabolized in the small intestine. In the rumen, dietary protein can be broken down into small peptides and amino acids ultimately to form ammonia and microbial crude protein, which is the primary source of amino acids in the ruminant. Protein that escapes the rumen will be utilized in the small intestine where amino acids are formed and subsequently absorbed. Absorbed amino acids will be used for various processes including tissue synthesis and glucose synthesis (Stern et al., 1994).

Amounts of dietary protein can influence voluntary intake in animals, and increasing amounts of protein are typically associated with increases in intake. Research suggests that crude protein has a positive effect on DMI in lactating cows (Roffler et al., 1986), and increasing amounts of supplemented protein resulted in increased intake of lambs (Cheema et al., 1991). Rumen degradable protein (RDP) provided to the animal is also positively associated with intake in ruminants which at least partly explains the associated positive effect with crude protein and supplemented protein. In feedlot steers consuming increased amounts of RDP, DMI tended to be increased (Wagner et al., 2010), and similar results were observed in cows (Köster et al., 1994). However, as demands were met, the increase in DMI plateaued (Köster et al., 1994) which indicates that there may be a maximum threshold for the effect of RDP on intake. Although positive effects on DMI were observed with dietary protein, abomasal and duodenal infusion of protein has resulted in little to no effect on DMI in cows (Clark et al., 1977; Dhiman et al., 1993) which indicates that postruminal supply of protein may not influence intake. Dietary

protein can induce a hyperphagic response in ruminants and is likely associated with the amount of RDP supplied to the animal. This likely results because RDP provides the main source of amino acids in ruminants (microbial crude protein) and increases can result in increased amounts of high energy substrates.

Hormonal Factors Regulating Intake

Many hormones are involved in intake regulation. See Table 1 for a partial list; however, for this review, we will be focusing on insulin, leptin, and ghrelin.

Insulin

Insulin is a primary hormone involved in intake regulation and is important for the regulation of metabolic function (Browning and Thompson, 2002). It is a hypoglycemic peptide hormone that is released from the B cells of the islet of Langerhans located in the pancreas and functions to maintain normal blood glucose concentrations (Wilcox, 2005). Insulin achieves maintenance of blood glucose primarily by increasing glucose uptake in peripheral tissues (Woods et al., 2006) and has been regarded as a potential peripheral feedback signal for intake regulation.

Increased blood glucose concentrations promote insulin release into the blood. This hypoglycemic effect is most commonly associated with increases in intake (Brockman, 1978), but research suggests that insulin can induce a hypophagic effect as well. In animals with cerebrospinal fluid infusions of insulin, intake was decreased (Porte and Woods, 1981), and similar results were observed in wethers administered insulin through the jugular vein (Deetz and Wangsness, 1981). When excess insulin is present in the bloodstream, such as animals with excess amounts of adipose tissue, it can enter the

brain through insulin-receptor facilitated transport and result in negative feedback (Woods et al., 2006). This negative feedback will induce hypophagia in the animal causing decreases in voluntary intake.

Leptin

Leptin is a peptide mainly present and secreted by adipose tissue. It is associated with various biological mechanisms such as body weight, feed intake, energy expenditure, reproduction, and immune functions. This hormone also has a major role in regulating whole-body energy metabolism (Nkrumah et al., 2005) and is directly proportional to the amount of body fat which allows it to be reflective of long-term energy storage status (Park and Ahima, 2015).

By binding to specific leptin receptors found in the CNS, leptin can employ various effects. Negative feedback can be initiated through binding to receptors and initiating signaling pathways. Additionally, intake can be regulated by leptin as it interacts with the ARC. Upon interaction, the synthesis of pro-opiomelanocortin and cocaine-and-amphetamine-regulated transcript are activated while the synthesis of agouti-related protein and neuropeptide Y are inhibited. These functions are reversed when leptin concentrations decrease to stimulate feed intake (Ahima et al., 1999). Although research investigating the direct effects of leptin on intake in ruminants is limiting, mice models have been paramount in determining possible effects on intake. When mice of the *ob*/*ob* genetic line were injected with leptin, feed intake was reduced (Campfield et al., 1995). Intracerebroventricular injections of leptin in mice have been shown to decrease intake (Stephens et al., 1995) with similar results being seen in mice induced with

hyperleptinemia via gene therapy (Chen et al., 1996). Leptin is involved in many biological processes and likely plays a major role in intake regulation.

Ghrelin

Ghrelin is an orexigenic peptide known to stimulate intake and is mainly secreted from the endocrine cells located in the gastrointestinal mucosa (Sakata and Sakai, 2010). It is involved in the short-term regulation of food intake in addition to involvement in long-term regulation of body weight (Castaneda et al., 2010). Ghrelin is an important regulator for nutrient sensing, meal initiation, appetite, and it also can stimulate growth hormone release (Pradhan et al., 2013).

Upon secretion into the plasma, ghrelin is found in two forms: acylated (active) or unacylated (inactive). The acylated form of ghrelin can stimulate the release of growth hormone by binding to the growth hormone secretagogue receptor (Kojima and Kangawa, 2002; Asakawa et al., 2005).

The role of ghrelin in nutrient sensing and DMI has been investigated in cattle. In steers injected with either bovine ghrelin or saline, steers injected with bovine ghrelin spent more time feeding and tended to have a greater DMI than those injected with saline. Subsequently, in steers who were fed or fasted, fed steers had elevated plasma ghrelin concentrations pre-feeding compared to fasted steers (Wertz-Lutz et al., 2006). In cattle participating in a finishing study, DMI was found to be positively associated with active ghrelin concentrations (Foote et al., 2014). The results of these studies suggest that ghrelin concentrations, when increased, stimulate DMI and confirm that ghrelin, particularly active ghrelin, is a key component of the intake regulation system.

Ghrelin is known as an appetite-stimulating hormone, and before meal initiation, plasma ghrelin concentrations are increased and subsequently fall after a meal (Sato et al., 2012). While the specific mechanisms for ghrelin regulation have yet to be elucidated, it most likely has some interaction with the CNS. Ghrelin-containing neurons are located in the ARC and can send signals to neuropeptide Y and agouti-related protein-expressing neurons to stimulate the release of orexigenic peptides (Abdalla, 2015). Additionally, ghrelin receptors have been found on vagal afferent neurons in rats which may indicate that transmission occurs from the gastrointestinal tract to the brain via the vagus nerve (Date, 2012). Although much is not yet known about the specific mechanisms involved in the regulation of ghrelin, it is likely regulated by communication of the gastrointestinal tract with the CNS with feeding playing a major role as well.

Fescue Toxicosis and Intake Regulation

Fescue toxicosis is known to decrease DMI as well as ADG. After an evaluation of the effects of fescue toxicosis on foregut blood flow, digestion and metabolism, and ruminal DM contents, it has been concluded a likely cause of the decrease in gain is due to the characteristic decrease in intake (Klotz, 2017). Therefore, the importance of understanding the cause of the decrease in intake is vital to potentially improving animal gain. Gastrointestinal fill and distention can lead to decreased DMI (Allen, 1996), and therefore, has the potential to play a role in the reduction of DMI in animals consuming endophyte-infected tall fescue. Steers consuming endophyte-infected tall fescue had higher ruminal DM contents (Foote et al., 2013; Koontz et al., 2013; Ahn et al., 2020). Additionally, ruminal contractions were found to decrease in frequency and amplitude in endophyte-infected fescue consuming steers (Ahn et al., 2020). Although gastrointestinal

distention due to an increase in ruminal DM contents likely plays a role in altering intake, there is potential that fescue toxicosis may elicit changes to the animal's intake regulation systems through modification of hormone secretion.

Hormones Related to Intake Regulation

Limited research regarding hormones related to intake regulation has focused on insulin, glucagon, and triiodothyronine with brief regard to leptin. In cows injected with ergotamine, a commercially available ergot alkaloid, plasma was used to analyze insulin, glucagon, and triiodothyronine concentrations. Upon analysis, insulin was found to decrease while glucagon and triiodothyronine were found to increase (Browning et al., 2000). A subsequent study using the same experimental approach in steers found similar results with insulin being decreased with an increase in glucagon (Browning and Thompson, 2002). Results for both insulin and glucagon are consistent with their roles in maintaining metabolic function and may impact intake. Triiodothyronine is a thyroid hormone involved with assisting metabolic processes and nutrient utilization (Browning et al., 2000). The increase in triiodothyronine may indicate an increase in basal metabolic rate resulting in increased maintenance requirements signifying potential nutritional stress associated with fescue toxicosis (Hurley et al., 1980; Huszenicza et al., 2002).

Research concerning the response of leptin to fescue toxicosis has been met with inconsistent results. A study involving cows and ewes consuming endophyte-infected tall fescue determined that serum leptin concentrations were decreased in cows during the first trial but remained unchanged during the second. Additionally, ewes showed no change in leptin concentrations (Burke et al., 2006). The inconsistency in these results makes a conclusion difficult; however, leptin is an important hormone involved in intake

regulation and could potentially be affected by fescue toxicosis. At this point, no studies regarding effects of fescue toxicosis on ghrelin concentrations have been published, but the impact of ghrelin on intake regulation establishes the possibility that it may be impacted by fescue toxicosis.

Research regarding the impact of fescue toxicosis on hormones related to intake regulation is severely lacking. The existing research has focused on insulin, glucagon, and thyroid hormones with some emphasis on leptin. However, the limited amount of research investigating potential effects on key hormones related to intake regulation leaves little possibility of forming a reliable conclusion. Many important hormones exist that play a role in intake regulation, and research evaluating the effect of fescue toxicosis on each of these hormones will be vital to determine the impact of fescue toxicosis on intake regulation.

Conclusion

Tall fescue remains a widely utilized forage in much of the United States. Fescue toxicosis is known to limit performance in animals mainly through a decrease in ADG which results from a decrease in DMI. This makes investigating the potential causes behind the decrease in DMI incredibly important to understanding how the effects of fescue toxicosis can be mitigated. Intake can be regulated through a myriad of ways involving physical, neural, metabolic, and hormonal regulation. Insulin, leptin, and ghrelin all have a role in intake regulation, and thus, may serve as an important investigative step in elucidating the mechanisms by which fescue toxicosis decreases intake.

Table 2.1. Partial list of hormones and peptides related to intake regulation.

Increase Intake	Decrease Intake
B-Endorphin	Anorectin
Dynorphin	Amylin
Ghrelin	CCK 8 and 33
Growth hormone-releasing hormone	Dopamine
Neuropeptide Y	Estrogen
Melanin-concentrating hormone	Glucagon
Melanocyte stimulating hormone	Glucagon-like-peptide 1
Opiods	Insulin
Orexin A and B	Leptin
Progesterone	Somatostatin
Peptide YY	Thyrotropin-Releasing Hormone

Adapted from: (Ingvartsen and Andersen, 2000; Sakata and Sakai, 2010).

CHAPTER 3. THE EFFECT OF ENDOPHYTE-INFECTED TALL FESCUE SEED CONSUMPTION ON GUT AND SATIETY HORMONES RELATED TO INTAKE REGULATION IN HOLSTEIN STEERS

Introduction

Fescue toxicosis is a consequence of the consumption of endophyte-infected tall fescue that is infested with the fungal endophyte *Epichloë coenophiala* which produces ergot alkaloids, the causative agent (Strickland et al., 2011). Of these alkaloids, ergovaline is the major alkaloid produced and is thought to be the main cause of the toxicity (Lyons et al., 1986; Klotz et al., 2007). The syndrome results in decreased animal growth characterized by a decrease in ADG likely through the characteristic decreased intake (Klotz, 2015). Additionally, fescue toxicosis has been attributed to economic losses to the beef industry of up to $2 billion annually (Kallenbach et al., 2015).

The specific mechanisms by which fescue toxicosis decreases intake have not been well elucidated. One potential mechanism is that various hormones related to the physiological regulation of intake may be involved in the observed decrease in intake. Many hormones are involved in intake regulation, but the focus of this experiment will be leptin, insulin, and active ghrelin.

Leptin is an adipokine that is present in adipose tissue and is responsible for the regulation of many biological functions such as body weight and energy expenditure (Nkrumah et al., 2005). Intake can be regulated by leptin through its interactions with the ARC where the appetite-stimulating, neuropeptide Y and agouti-related protein, or the appetite inhibiting, POMC and CART neuropeptides can be released and further interact with the hypothalamus and central nervous system. Research evaluating the role of leptin in fescue toxicosis is limited and inconsistent. In mature cows and ewes, serum leptin

concentrations of cows consuming the endophyte-infected tall fescue were decreased in comparison to those receiving the non-endophyte infected tall fescue. However, in a second experiment in the same study, leptin concentrations were found to be similar among both groups of animals (Burke et al., 2006).

Insulin is a hormone that is commonly associated with the regulation of metabolic function (Browning and Thompson, 2002) and is released from B cells of the islet of Langerhans in the pancreas (Wilcox, 2005). Insulin would be expected to decrease during fescue toxicosis due to the decrease in intake. Cows that were exposed to an ergotamine challenge as a model of fescue toxicosis had decreased insulin concentrations; however, intakes were not reported (Browning et al., 2000). Although little research has been completed examining insulin in relation to fescue toxicosis and intake, insulin response has been documented in heat-stressed cattle. In cattle undergoing heat stress, insulin concentrations were increased although intake was decreased which may signify a shift in postabsorptive metabolism, most specifically lipid mobilization (Baumgard and Rhodes, 2013a). NEFA and BHB are key metabolites for lipid mobilization and may serve as signals for changes in this process. Glucose, an important energy source for ruminant tissues, is provided to the animal primarily via gluconeogenic pathways utilizing propionate, amino acids, glycerol, and lactate (Church, 1993).

Ghrelin is an appetite-stimulating hormone produced from endocrine cells located in the gastrointestinal tract (Sakata and Sakai, 2010). Two forms of ghrelin exist in plasma, the acylated, or active, form and the unacylated, or inactive, form. The active form can stimulate growth hormone release by binding to the growth hormone secretagogue receptor (Kojima and Kangawa, 2002). Exogenous administration of

ghrelin has resulted in increases in intake (Nakazato et al., 2001; Wren et al., 2001) which confirms its role in the stimulation of feed intake.

The hypothesis of this study was that consumption of endophyte-infected tall fescue seed would result in changes of circulating insulin, leptin, and active ghrelin concentrations as well as eliciting changes in markers for postabsorptive metabolism. Therefore, the objective of this study was to investigate potential changes in insulin, leptin, and active ghrelin concentrations after consumption of endophyte-infected tall fescue seed as a model to induce fescue toxicosis, and to investigate potential changes in postabsorptive metabolism by analyzing NEFA, BHB, and glucose.

Materials and Methods

All procedures involved in this experiment were approved by the University of Kentucky Institutional Animal Care and Use Committee. Research was conducted at the University of Kentucky C. Oran Little Research Center, Beef Unit, located in Versailles, KY.

Animals and Experimental Design

12 Holstein steers (initial BW 260 ± 16.0 kg) were used in a 21 day randomized complete-block design experiment consisting of a 4 day environmental adaption and a 17 day treatment period. Animals were fed varying amounts of endophyte-infected (E+) or non-endophyte-infected (E-) fescue seed. Animals were stratified by body weight and randomly assigned to 1 of 3 dietary treatments (n=4 per treatment) in two blocks (sampling day). Steers were housed indoors in individual stalls and had free-choice access to water and were adapted to the environment for 4 days before treatment. For this experiment, summer conditions were mimicked by maintaining a 16:8 h light:dark cycle

and cycling the room from above thermoneutral (~26.7-32.2°C) during the light period to thermoneutral (~ 21.1°C) during the dark. Treatments were a total dietary ergovaline/ergovalinine (ERV) concentration of 0 ppm, a total dietary ERV concentration of 1.8 ppm, and a total dietary ERV concentration of 2.7 ppm. Percentages of E- and E+ seed were fed to balance seed intake across treatments (Table 3.1).

Feeding and Treatment Diets

During the adaption period, a silage-based basal diet was offered to all animals with a standard supplement added. For the duration of the experiment, animals were given *ad libitum* access to all diets.

Both the E+ seed (KY31 Tall Fescue, Shawneetown Feed and Seed, Jackson, MO) and the E- seed (KY32 Bull Fescue, Caudill Seed, Louisville, KY) were ground through a 3mm screen in a hammer mill before inclusion in the diet. The E+ seed was tested for ERV and its stereoisomer, ergovalinine concentrations as described previously (Ji et al., 2014).

Orts were collected at 600 with animals being fed at 700 each morning. To ensure ad libitum access to basal and treatment diets, amounts fed were adjusted daily to achieve an excess of 10-20% orts. To calculate daily DMI, 250 g samples were collected from orts and dried at 55°C in a forced-air oven overnight. DMI from days 7-14 were used for analysis.

Blood Sampling

Due to the volume and frequency of blood collection required for sampling, steers were randomly assigned to 1 of 2 blocks, with one block sampled on d 16 and the remaining block sampled on d 17. To facilitate blood collection, indwelling jugular catheters (Medical Instruments for Animals, DayCath, 14 gauge, 5.25 inches) were placed the evening before sampling. Briefly, hair was clipped from the area of the neck over the jugular vein and scrubbed with betadine. The catheter was inserted with the extension line (Medical Instruments for Animals, #8573M) attached immediately following insertion. Heparinized saline was used to lock the extension line to ensure patency. The catheter and extension line was secured using Braunamid sutures placed cutaneously. Following securing of the catheter, the neck was wrapped (Rural365 Self Adhesive Bandage Vet Wrap), and a hernia belt (F.L.A. Orthopedics Inc. Universal).

On days 16 and 17, blood samples were collected every 20 minutes beginning 1 hour before feeding (600). Steers were tied in the stall for the duration of the sampling period with free access to water and feed. A 25mL sample was collected into a heparinized syringe at each time interval. At the time of sampling, a 7-8 mL waste syringe was drawn to ensure no heparinized saline was present in the blood sample. The sample was collected with subsequent flushing of the extension line and catheter with 5-10mL of heparinized saline. Samples were transferred to a 50mL conical tube and placed on ice immediately. Plasma samples were collected by centrifuging the collected blood at 5000 x g for 30 min at 4°C. Following centrifugation, samples were divided into 1mL aliquots and stored at -80°C until hormone analysis.

Hormone and Metabolite Analyses

Plasma samples were analyzed for insulin using a radioimmunoassay kit (MP Biomedicals, Porcine Insulin #PI-12K) that has been validated for bovine insulin and had a mean inter- and intra-assay CV of 4.1% and 2.9%, respectively. Leptin samples were analyzed via a commercial RIA kit (Multi-Species Leptin, EMD Millipore Corporation, St. Charles, MO) and had a mean inter- and intra-assay CV of 3.2% and 6.7%, respectively. Plasma aliquots for active ghrelin determination had 10 μL phenylmethylsulfonyl fluoride (PMSF; Sigma Lot# BCBQ7649V) and 50 μL of 1 N hydrochloric acid added to protect the acyl group. To prepare the PMSF solution, 0.05g of PMSF was added to 50 mL of 100% methanol. Aprotinin solution was prepared by incorporating aprotinin (LEE Biosolutions, Cat No: 125-10, Lot: W144576, Activity: 4.5 TIU/mL) at its solubility level (2mg/mL) with ethylenediaminetetraacetic acid. To achieve a desired activity of 0.6 TIU/mL, 67 μL of aprotinin solution was added to 1mL of plasma. Active ghrelin samples were analyzed using a commercial RIA kit (MP Biomedicals, Ghrelin (Active), #GHRA-88HK) and had a mean inter- and intra-assay CV of 2.8% and 2.0%, respectively.

Samples drawn at each hour interval (600, 700, etc.) were used for NEFA and BHB analysis. NEFA (Dole and Meinertz, 1960) and BHB (Koch and Feldbruegge, 1987) samples were analyzed using a Konelab Analyzer with a CV of 1.22% and 1.16%, respectively. Hourly samples were also used for glucose concentration determination. Plasma glucose concentrations were analyzed using a YSI 2700 SELECT Biochemistry Analyzer and had a CV of 0.78 %.

Statistical Analysis

The normality of the residuals was first tested using the UNIVARIATE procedure in SAS 9.4 (SAS Inst. Inc., Cary, NC). All data met normality assumptions. The experimental unit for all data was animal, and block was included as a random effect in all analyses. The DMI data were analyzed using the MIXED procedure in SAS with the effects of treatment, day of treatment period, and the resulting interaction being included as fixed effects. All hormones and metabolites were analyzed using the MIXED procedure in SAS including the fixed effects of treatment, collection time, and the interaction. Collection time was also included as a repeated measure. Orthogonal contrasts for treatment were analyzed to determine linear and quadratic relationships of ERV intake. Given that treatments were not evenly spaced, coefficients for contrasts were determined using PROC IML in SAS. Differences between treatments were assessed using the DIFF option in SAS to determine significant interactions. Effects were considered significant when $p \leq 0.05$ and considered a tendency when $0.05 < p \leq 0.10$.

Results

As expected with increased consumption of ERV, DMI decreased linearly ($p<0.0001$) as the dietary ERV concentration increased. However, there were no day or treatment-by-day interactions. Increasing the dietary concentration of ERV caused insulin to be greatest at the highest dietary ERV concentrations; however, insulin was similar for the control and low ERV diets (quadratic $p<0.0001$). Conversely, leptin increased at the low dietary ERV concentration before decreasing at the high concentration (quadratic $p<0.0001$). No significant time or treatment-by-time interactions occurred for either

insulin or leptin (Figures 3.1 and 3.2). Active ghrelin had a significant linear response where active ghrelin concentrations decreased as ERV concentration increased (Table 3.3). For NEFA, steers consuming the lowest ERV concentration had the highest NEFA concentration. A significant treatment-by-time interaction was observed for NEFA as well. One hour prior to feeding (-60 min), the 1.8 ppm ERV treatment had the greatest NEFA concentration. At the time of feeding (0 min), both the 1.8 ppm ERV and 2.7 ppm ERV treatments had higher NEFA concentrations than the 0 ppm ERV treatment. 300 minutes post-feeding, the 2.7 ppm ERV treatment had a higher NEFA concentration than the 0 ppm ERV treatment, and 360 minutes post-feeding, the 2.7 ppm ERV treatment had a higher NEFA concentration than both the 0 and 1.8 ppm ERV treatments (Figure 3.4). A quadratic response was observed for BHB concentrations (Table 3.2), and concentrations were lowest for steers consuming the highest ERV concentrations (Figure 3.5). A significant linear response was observed for glucose where as ERV concentrations increased, glucose concentrations also increased (Table 3.2; Figure 3.6)

Discussion

DMI

Endophyte-infected tall fescue consumption is known to reduce intake in cattle. In steers ruminally-dosed with endophyte-infected tall fescue seed, DMI was shown to decrease (Koontz et al., 2012). Similarly, steers being ruminally-dosed with endophyte-infected tall fescue seed at ERV dosages of 0, 5,10, 15, or 20 µg/kg BW exhibited decreased DMI when the ERV reached 15 or 20 µg/kg BW (Ahn et al., 2019). Baldwin et al. (2016) conducted a study utilizing lactating cows consuming endophyte-infected tall fescue seed in the diet to provide 7.7-9.9 µg/kg BW ERV per day and observed

dramatic decreases in DMI. In the present study, steers consuming the 1.8 ppm and 2.7 ppm treatments consumed an average of 46 µg/kg BW and 59 µg/kg BW of ERV, respectively. When comparing the relationships between DMI and ERV intake from each study (Figure 3.6), intake for steers in the present study decreased by 0.01387 percent for each µg of ERV consumed compared to Ahn et al. (2019) where intake was decreased by 0.00915 percent for each µg of ERV and Baldwin et al. (2016) where cows had a reduction of 0.1254 percent per µg ERV consumed.

The ERV intake during the present study was higher than typical for previous fescue toxicosis studies. The high ERV concentration in the seed used in the present study, 9.33 ppm, the method of seed inclusion (incorporated into a high corn silage diet as opposed to ruminal dosing), and the *ad libitum* intake by the animals likely contributed to the higher than average ERV consumption. Nonetheless, the higher ERV intake did not result in decreases in DMI more than that of previous studies. This could be due to a variety of reasons including differences in diet, differences in dosing, and physiological state of the animal. Steers dosed with ERV in Ahn et al. (2019) consumed a diet of alfalfa cubes while steers in the present study consumed a diet mainly consisting of corn silage. Steers in the present study were provided endophyte-infected tall fescue seed as a proportion of the diet while those in Ahn et al. (2019) were dosed ruminally. When ruminally-dosing, cattle consume an exact amount of ERV; however, since steers in the present study were permitted to consume feed *ad libitum*, there was no method to guarantee a specific ERV intake which may explain the differences in reduction of DMI.

The calculated ERV consumption of the present study is under the assumption that the steers consumed an exact proportion of seed. During the study, it was noticed that steers,

among all treatments, did sort seed from the total mixed ration resulting in excess seed in the refusals, but no samples were collected for ERV analysis. Thus, it is likely that the calculated ERV consumption is an overestimation.

Although steers in this study consumed more ERV than previous studies, no abnormal consequences were observed, and intake did not decrease anymore drastically than previous studies. However, it is apparent that endophyte-infected tall fescue consumption decreases DMI and is a proponent to production losses associated with endophyte-infected tall fescue consumption.

Insulin and Glucose

The presence of increased insulin concentrations are generally associated with decreases in feed intake. In wethers administered insulin via the jugular vein, feed intake decreased (Deetz and Wangsness, 1981). Mice subjected to ventricular administration of exogenous insulin also exhibited a decrease in intake (Air et al., 2002). Presently, animals consuming the 2.7 ppm treatment experienced the highest concentrations of insulin even though they had the greatest reduction in DMI. Interestingly, a similar response is observed in animals experiencing heat stress. Although nutrient intake is decreased during periods of heat stress, insulin concentrations are increased which may be the result of a shift towards carbohydrate metabolism and away from lipid mobilization (Baumgard and Rhoads Jr, 2013a). NEFA and BHB are good indicators of postabsorptive metabolism changes due to their involvement in the lipid mobilization process which is why we chose to further this study by including these metabolites in our analysis. This study did not evaluate the effects of heat stress. However, physiological responses associated with heat stress are very similar to those of cattle experiencing fescue

toxicosis, and environmental temperature was elevated during the study. Cattle experiencing heat stress or fescue toxicosis commonly have increased body temperature, increased respiratory rate, and increases in salivation (Strickland et al., 2011; Dash et al., 2016). Although the present study did not measure physiological responses, steers consuming the highest ERV concentration appeared to have excess salivation and more labored breathing compared to those consuming the lower concentration of ERV and the control group. The similarity of responses between heat stress and fescue toxicosis may provide an avenue for further research particularly when analyzing effects on postabsorptive metabolism. Additionally, the similar insulin concentration-response coupled with reduced DMI may warrant more detailed research on postabsorptive metabolism after consumption of endophyte-infected tall fescue seed, specifically when consuming ERV at this magnitude.

Glucose is a primary energy source for various tissues in the ruminant and is mainly provided to the animal by gluconeogenic pathways. Propionate, amino acids, glycerol, and lactate can all be precursors to glucose synthesis with propionate being the most important (Church, 1993). Insulin promotes uptake of glucose into peripheral tissues and is considered a major regulator of glucose homeostasis (Brockman, 1978; Sasaki, 2002). The present study demonstrated a linear relationship between ERV concentration and glucose concentration where as ERV concentration increased, glucose concentration also increased. Previous studies have demonstrated no significant changes in glucose concentration with endophyte-infected tall fescue consumption (Oliver et al, 2000; Eisemann et al., 2020) which is conflicting with the results of the present study. This may be due to differences in ERV amounts. Oliver et al. (2000) conducted a grazing study

where specific ERV concentrations were not recorded, and Eisemann et al. (2020) had an average intake of 7.5 µg/kg BW which was much lower than the present study.

Typically, gluconeogenesis is greatest after consumption of a meal, and propionate available for gluconeogenesis is directly related to the amount of diet consumed (Church, 1993). However, the 0 ppm ERV treatment, which had the highest DMI, had the lowest glucose concentrations. The present study did not evaluate VFA concentrations, but it is possible that other gluconeogenic substrates may have contributed to the increasing glucose concentrations for the 1.8 and 2.7 ppm ERV treatments. This study did not evaluate changes in gluconeogenic substrates, but this may be an avenue to research further particularly by investigating changes in propionate in response to higher ERV concentrations.

Insulin stimulates uptake of glucose into peripheral tissues and inhibits gluconeogenesis (Church, 1993). In the present study, the 2.7 ppm ERV treatment had the highest insulin concentration coupled with the highest glucose concentration which was unexpected considering insulin typically promotes glucose uptake into peripheral tissues resulting in a higher insulin and lower glucose concentrations. This may indicate the occurrence of insulin resistance which is described as occurring when normal concentrations of insulin do not produce a normal biologic response (Kahn, 1978). Although insulin's ability to stimulate glucose uptake is less in a ruminant than a nonruminant (Sasaki, 1990), steers fed the 0 ppm ERV treatment did have the lowest glucose concentration along with the second highest insulin concentration which may indicate an increased responsiveness to insulin for the 0 ppm ERV treatment compared to the 2.7 ppm ERV treatment. Additionally, steers in the 1.8 ppm ERV treatment group had

the lowest insulin concentration and the second highest glucose concentration which is an expected response. This may indicate that responsiveness to insulin may be related, either directly or indirectly, to concentration of ERV in the diet. Further research should include providing a wider range of ERV concentrations in the diet to better describe the relationship between ERV and response to insulin.

Non-esterified fatty acids and β-hydroxybutyrate

To investigate potential changes to postabsorptive metabolism in the present study, NEFA and BHB concentrations were analyzed. NEFA are important metabolic fuel and are released during times of increased energy demands through lipolysis of triglycerides by hormone sensitive lipase which is stimulated by various hormones, like glucagon, and inhibited by insulin (eClinpath, Cornell University). In the present study, there was a significant treatment-by-time interaction for NEFA concentrations. 60 min prior to feeding, 1.8 ppm ERV treatment had the highest NEFA concentration. At the time of feeding, 0 ppm ERV treatment had the lowest NEFA concentrations. At 300 min post-feeding, 2.7 ppm ERV treatment had a greater NEFA concentration than the 0 ppm ERV treatment, and a similar patter occurred at 360 min post-feeding where 2.7 ppm ERV treatment had the highest NEFA concentration overall. Upon comparison to insulin concentrations at these time points, the 2.7 ppm ERV treatment had the highest insulin concentrations for all time points excluding at the time of feeding. As stated previously, NEFA concentrations are typically lower when insulin concentrations are increased; however, that pattern is not consistently observed in the results of this study. This may signify a change in postabsorptive metabolism and result in changes in NEFA concentrations.

BHB is a ketone that, in ruminants, is produced from metabolism of NEFA and volatile fatty acids, mainly butyrate (eClinpath, Cornell University). In the present study, the 2.7 ppm ERV treatment had the lowest BHB concentration combined with the lowest DMI. Though VFAs were not measured in this study, it is possible that the decrease in DMI resulted in a decrease in butyrate production resulting in decreased BHB. At various time points, NEFA concentrations were increased; however, BHB was consistently lower for the 2.7 ppm ERV treatment across all time points. BHB would be expected to be increased with the increased NEFA concentrations, but this is not what is described in the present study. It is possible that this is due primarily to a potential decrease in butyrate production, but that cannot be concluded due to butyrate not being measured. This does, however, provide an avenue for further research into this area.

Leptin

Increased leptin concentrations have been associated with decreases in DMI. In *ob/ob* mice, which are leptin deficient, subjected to leptin injections, DMI was decreased (Campfield et al., 1995). An additional study using mice undergoing hyperleptinemia via gene therapy had similar results in which the mice experience a decrease in intake (Chen et al., 1996). The steers consuming the low level of ERV had decreased intakes and increased leptin. However, the 2.7 ppm ERV steers had the lowest leptin concentration even though their DMI was also the least. This may suggest a direct effect of ergots on leptin as the response was potentially uncoupled from intake. However, steers in the present study were given *ad libitum* access to treatment diets which makes it difficult to truly know if the response of leptin was unrelated to intake. Further research involving restriction of intake is necessary to determine the exact relationship of leptin

concentrations in response to ERV intake. In a similar study involving mature cows fed endophyte-infected tall fescue, leptin concentrations were decreased for those exposed to the toxic fescue. However, a second experiment of that same study reported no changes in leptin concentrations (Burke et al., 2006). Although increased leptin is usually correlated with decreased intake, leptin also has a larger role in long-term intake than short-term. The steers in this study were only fed their respective treatment for 17 days, and therefore, may not have been exposed to the toxic endophyte long enough to exhibit increases in leptin. Additionally, the results from Burke et al. (2006) may suggest that if leptin is impacted, it may not follow its typical behaviors. The results in the present experiment suggest that at low ERV intakes that leptin may be associated with intake but at high concentrations there may be a direct inhibition. Furthermore, there is potential for ergot alkaloids to significantly impact adipose tissue in the animal.

McLean et al. (2020) described a study investigating the effects of a synthetic ergot alkaloid, bromocriptine, on gene expression related to mesenteric adipose. Steers injected with bromocriptine had more differentially expressed genes in the mesenteric adipose tissue than those injected with saline. A downregulation for genes related to enzymes, transporters, ion channel, cytokines, and immune responses was observed with upregulation of genes related to enzyme activity. The pathways most affected by bromocriptine were inflammation, the immune response, and lipid metabolism. The present study investigated changes in leptin concentrations. Leptin is an important adipokine produced in adipose tissue, and McLean et al. (2020) demonstrated that bromocriptine, which mimics the binding effects of ERV, greatly impacts the gene expression in mesenteric adipose tissue. These findings suggest that ERV may cause

significant changes to adipose tissue and maybe a partial mechanism by which intake is decreased following consumption of ERV. Further research is warranted to further elucidate the mechanism by which leptin concentrations are affected upon consumption of ERV and to further investigate changes in adipose tissue in ruminants.

Active Ghrelin

Active ghrelin concentrations, when increased, are typically known to stimulate intake. In mice undergoing intracerebroventricular injection of acylated ghrelin, feed intake was increased (Nakazato et al., 2001). Similarly, steers injected with bovine ghrelin spent more time feeding along with tending to have higher intakes (Wertz-Lutz et al., 2006). In this study, no treatment effects were observed. It has been shown that ghrelin stimulates appetite, and as DMI intake of steers in the present study decreased with increasing ERV concentrations, active ghrelin concentrations also decreased. This may provide evidence that active ghrelin concentrations are related to ERV consumption as well as . However, future research should include total ghrelin analyses to determine if there is a change in active ghrelin alone, or if total ghrelin concentrations also change with increasing ERV concentrations. It is also possible that active ghrelin concentrations are more so related to intake as opposed to ERV consumption.

Conclusion

This study investigated the effects of endophyte-infected tall fescue seed consumption and the resulting ERV consumption on DMI and hormones related to intake regulation. DMI decreased with increasing amounts of ERV in the diet which was expected. Insulin concentrations were lowest for animals consuming the 1.8 ppm ERV diet with no reduction observed in steers consuming the 2.7 ppm ERV treatment, and

leptin concentrations were decreased for those consuming the 2.7 ppm ERV diet and increased at 1.8 ppm ERV. Active ghrelin concentrations decreased with increasing ERV concentration. NEFA concentrations were highest for the 1.8 ppm ERV treatment, and BHB concentrations were lowest for steers consuming the 2.7 ppm ERV treatment. Additionally, glucose concentrations increased linearly with increasing ERV concentrations. These results indicate a possible effect on hormones associated with intake regulation as well as a possible effect on postabsorptive metabolism following consumption of ERV. More research is warranted to fully investigate the effect endophyte-infected tall fescue consumption has on hormones related to intake regulation as well as effects on metabolites related to postabsorptive metabolism, and this may prove an avenue to elucidate the mechanisms by which endophyte-infected tall fescue alters feed intake. Further research should include intake control as the present involved allowing *ad libitum* access to feed which may have confounded variables of this study and investigate changes in VFA concentrations with high ERV concentrations.

Table 3.1. Experimental diets for steers consuming endophyte-infected tall fescue seed providing dosages of 0, 1.8, and 2.7ppm total ergovaline (ERV)

	Basal Diet	0 ppm ERV	1.8 ppm ERV	2.7 ppm ERV
Ingredient	% of diet DM			
Corn Silage	86.7	57.7	57.7	57.7
Soybean meal	10.2	10.2	10.2	10.2
Ground corn	1.28	1.28	1.28	1.28
Limestone	0.97	0.97	0.97	0.97
Mineral Mix[1]	0.67	0.67	0.67	0.67
A,D,E Premix[2]	0.03	0.03	0.03	0.03
Fat	0.13	0.13	0.13	0.13
Deccox (6%; 12.36 g Dq/kg)	0.02	0.02	0.02	0.02
E+ Seed	0	0	19.3	29
E- Seed	0	29	9.7	0

Mineral Mix[1] contents: 92.7% salt, 0.02% cobalt sulfate, 0.7% copper sulfate, 0.13% iodine, 0.2% selenium, 1.55% zinc sulfate, 3.0% iron sulfate, and 1.7% manganese sulfate.

A,D,E Premix[2]: 1,818,182IU/kg Vitamin A, 363,636 IU/kg Vitamin D_3, 227 IU/kg Vitamin E

The E+ seed was determined to contain 9.33 ppm of ergovaline and ergovalinine, combined.

Table 3.2. Least square mean estimates for dry matter intake (DMI), insulin, leptin, active ghrelin, β-hydroxybutyrate (BHB), and glucose following consumption of endophyte-infected tall fescue seed with dosages of 0, 1.8, and 2.7 ppm total ergovaline (ERV).

	Treatments				P Values	
Variable	**0 ppm**	**1.8 ppm**	**2.7 ppm**	**SEM[1]**	**Linear**	**Quadratic**
DMI, kg	7.81	6.56	5.77	0.247	<0.0001	0.6744
Insulin, ng/mL	0.232	0.205	0.310	0.0382	<0.0001	<0.0001
Leptin, ng/mL	7.449	10.710	4.882	1.864	0.0006	<0.0001
Active Ghrelin, pg/mL	79.285	73.084	67.564	19.130	0.0471	0.7701
BHB, mmol/L	0.3313	0.3312	0.2487	0.0197	0.0117	0.0286
Glucose, mmol/L	4.568	5.132	5.222	0.2625	0.0456	0.1317

SEM[1]: Standard Error of the Mean, n=4 steers per treatment

Figure 3.1. Plasma Insulin concentrations following consumption of endophyte-infected tall fescue seed with dosages of 0, 1.8, and 2.7 ppm total ergovaline (ERV)

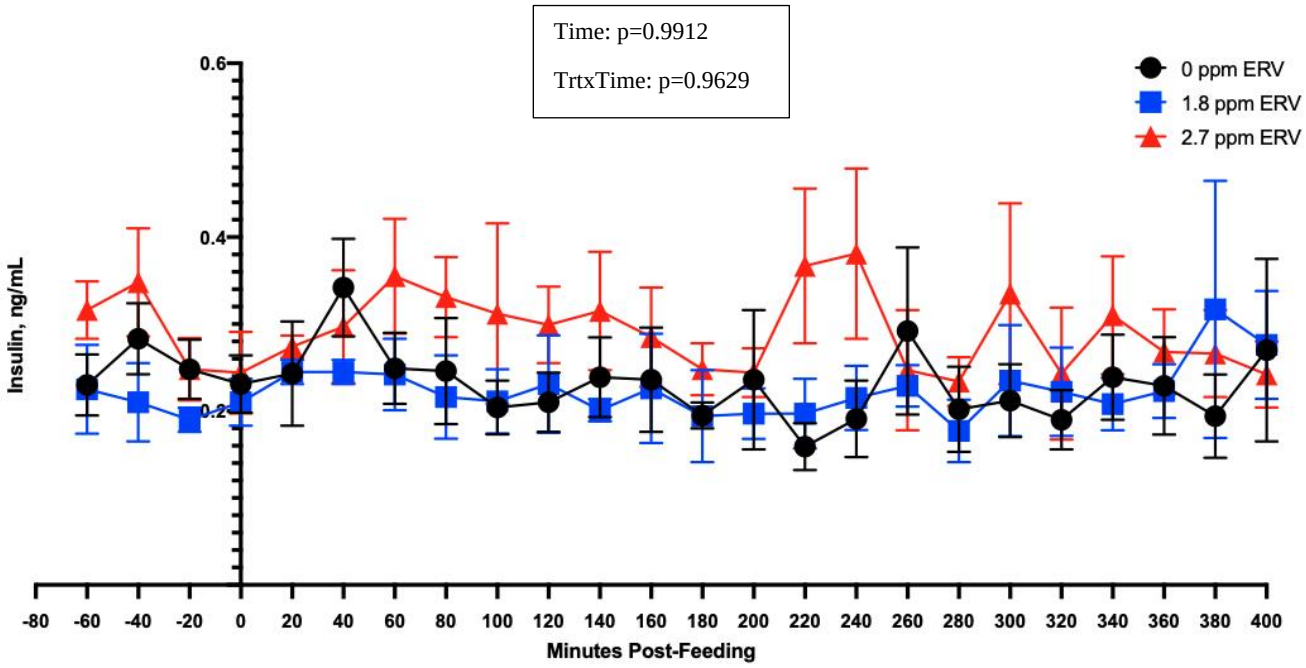


Figure 3.2. Plasma leptin concentrations following consumption of endophyte-infected tall fescue seed with dosages of 0, 1.8, and 2.7 ppm total ergovaline (ERV)

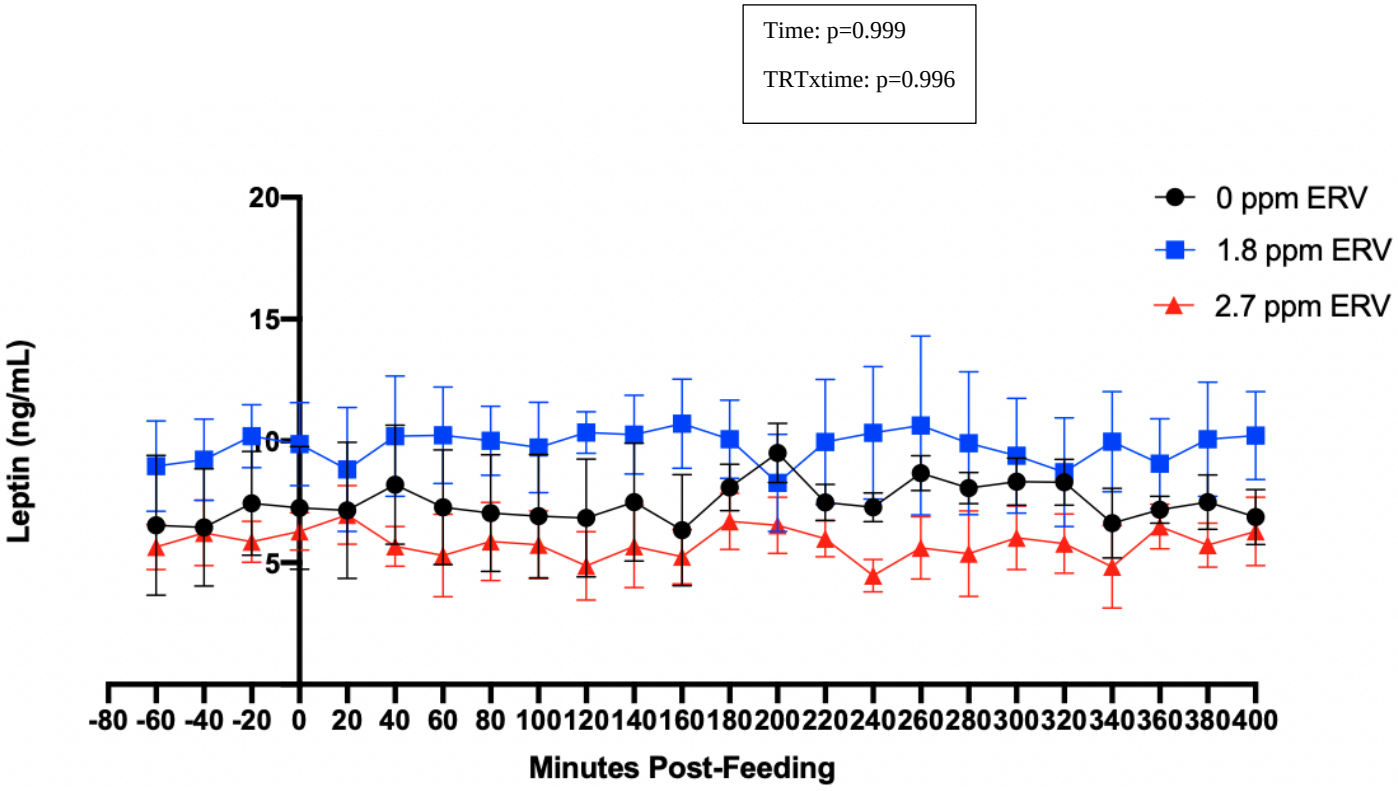


Figure 3.3. Plasma active ghrelin concentrations following consumption of endophyte-infected tall fescue seed with dosages of 0, 1.8, and 2.7 ppm total ergovaline (ERV)

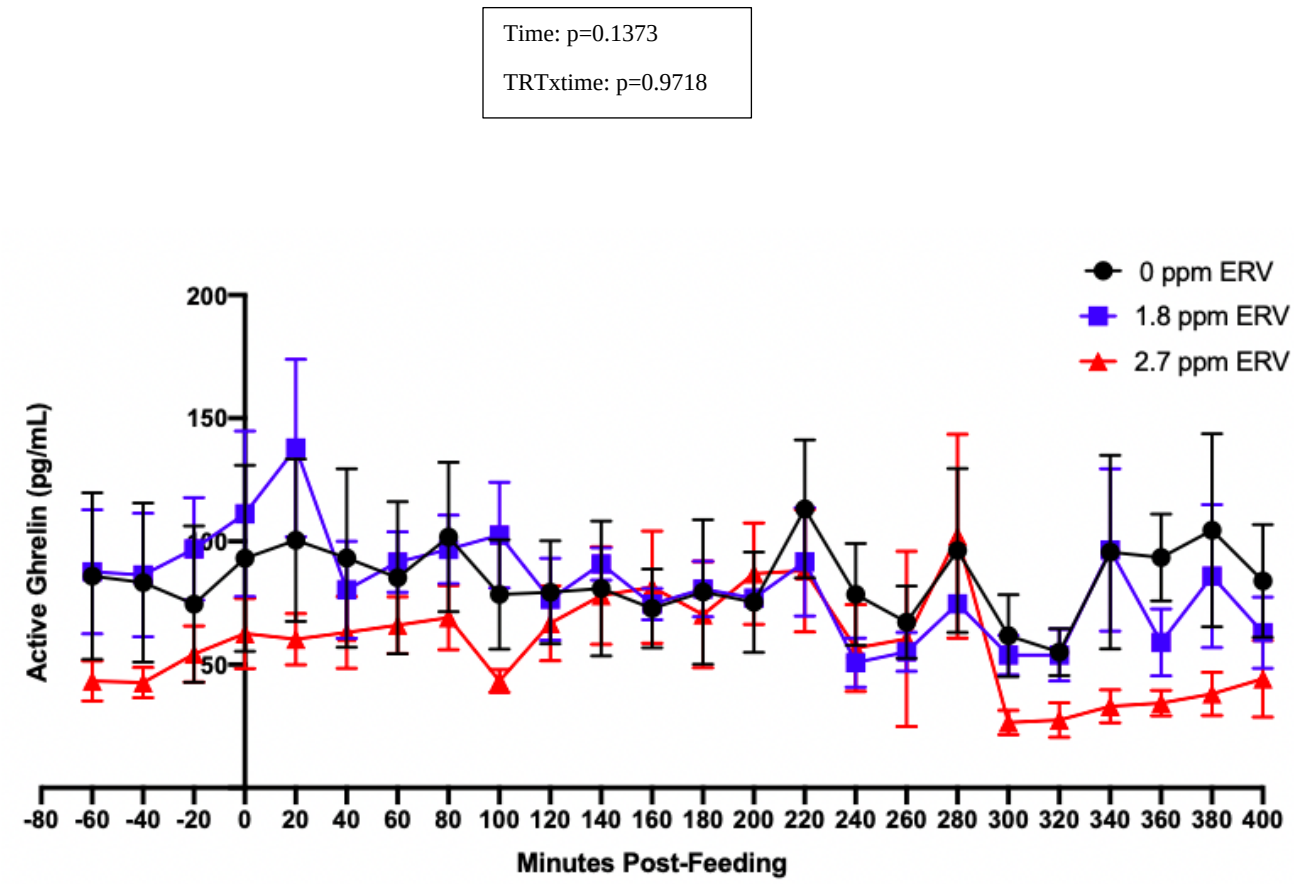
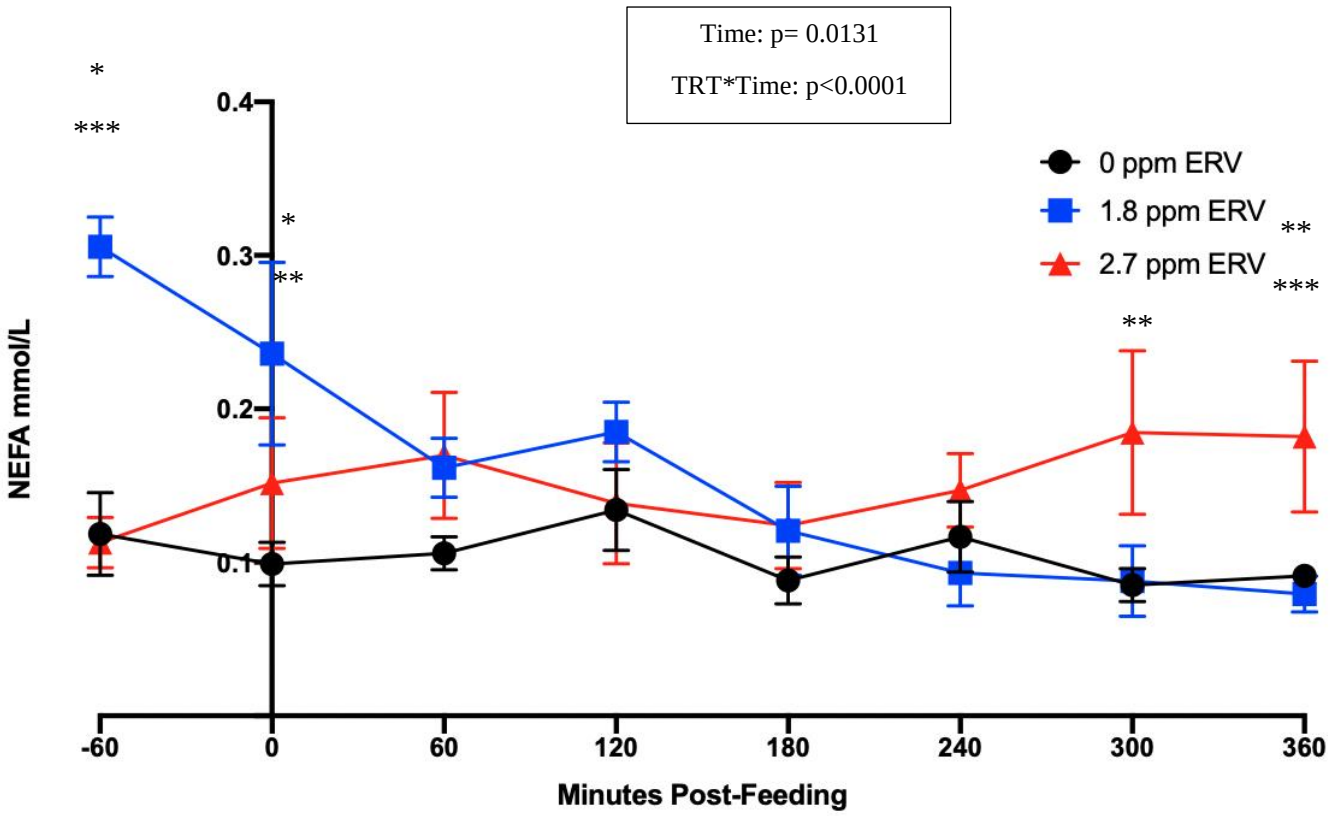


Figure 3.4. Plasma non-esterified fatty acid (NEFA) concentrations following consumption of endophyte-infected tall fescue seed with dosages of 0, 1.8, and 2.7 ppm total ergovaline (ERV)



*: significant difference between 0 and 1.8 ppm ERV treatments ($p<0.05$)
**: significant difference between 0 and 2.7 ppm ERV treatments ($p<0.05$)
***: significant difference between 1.8 and 2.7 ppm ERV treatments ($p<0.05$)

Figure 3.5 Plasma β-hydroxybutyrate (BHB) concentrations following consumption of endophyte-infected tall fescue seed with dosages of 0, 1.8, and 2.7 ppm total ergovaline (ERV)

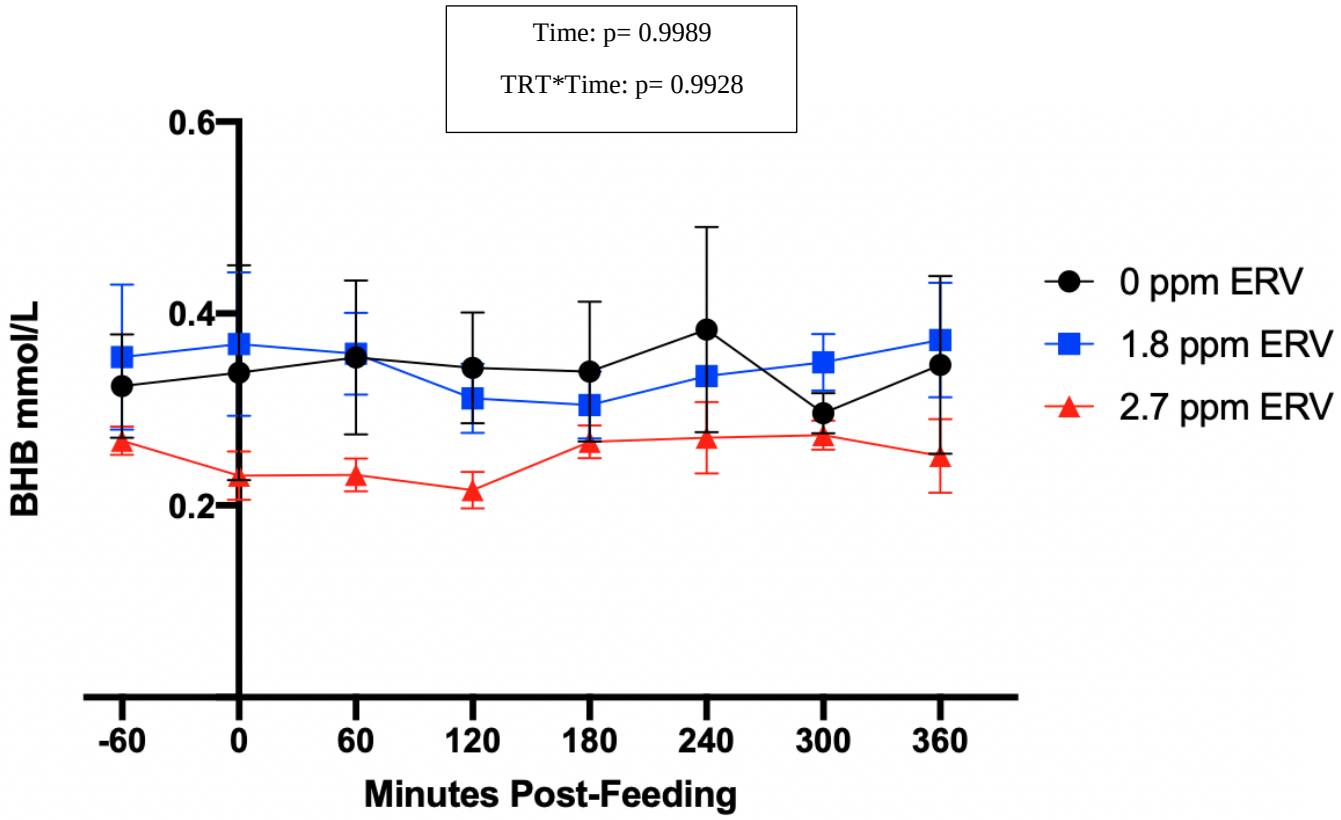


Figure 3.6. Plasma Glucose concentrations following consumption of endophyte-infected tall fescue seed with dosages of 0, 1.8, and 2.7 ppm total ergovaline (ERV)

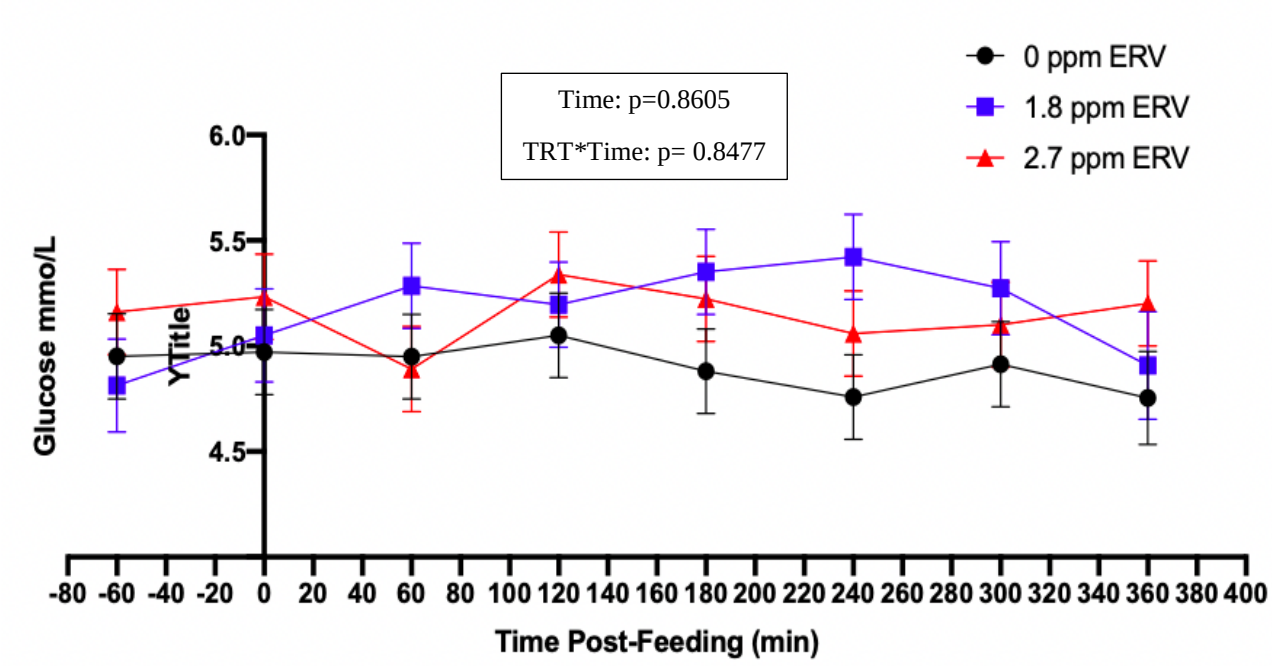
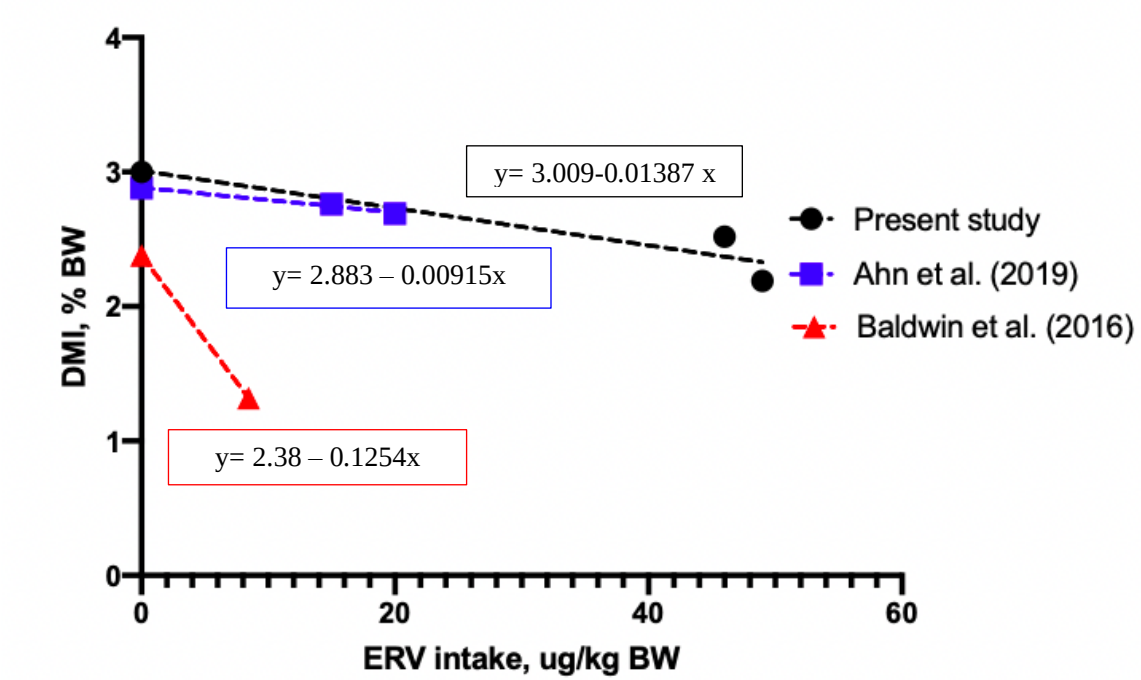


Figure 3.7 DMI data comparisons of present study to Ahn et al. (2019) and Baldwin et al. (2016)



CHAPTER 4. SUMMARY AND CONCLUSIONS

The symbiotic relationship between tall fescue, *Lolium arundinaceum,* and the endophyte *Epichloë coenophiala* increases stress tolerance for the forage and aids in resistance to unfavorable conditions (Bacon, 1993). Consumption of this forage causes a syndrome called fescue toxicosis that results in multiple negative consequences. Production losses are often observed after consumption of tall fescue and costs associated with those losses exceed $2 billion annually (Kallenbach, 2015). Ergot alkaloids are produced by the endophyte and considered to be the causative agent of fescue toxicosis, and since ergovaline (ERV) is produced in the most abundance within the plant, it is regarded as the primary toxicant (Lyons et al., 1986).

Production losses experienced during fescue toxicosis include decreased ADG, as well as reduced DMI (Thompson and Stuedemann, 1993; Paterson et al., 1995). The decrease in ADG is likely a result of the DMI reduction (Klotz, 2015) which makes elucidating the cause of the reduce DMI important. Multiple factors are involved in intake regulation including physical, neural, metabolic, and hormonal factors. This study focused on hormonal factors related to intake by analyzing changes in insulin, leptin, and active ghrelin concentrations following consumption of endophyte-infected tall fescue seed as well as investigated potential effect on postabsorptive metabolism through the analysis of NEFA and BHB.

As hypothesized, DMI was decreased in the present study for steers consuming 1.8 and 2.7 ppm ERV. Insulin is hypophagic when administered exogenously (Deetz and

Wangsness, 1981; Air et al., 2002), and this occurred in the 2.7 ppm ERV treatment. The 1.8 ppm ERV did not exhibit this same pattern and had decreased insulin concentrations compared to control. Glucose concentrations were also analyzed in the present study where glucose increased linearly with increasing ERV concentration. Concentrations of glucose were highest for the 2.7 ppm ERV treatment which also had the highest insulin concentration. This may signify potential insulin resistance given circulating glucose concentrations are remaining elevated despite elevated insulin concentrations. Additionally, it is possible that more gluconeogenic precursors, such as propionate, are available resulting in increased glucose concentrations. Further research into this area should also include evaluation of VFA concentrations to better describe the glucose response in relation to ERV concentration.

Similar insulin responses as seen in the present study have also been observed in cattle experiencing heat stress where insulin concentrations remained elevated despite a reduction in DMI (Baumgard and Rhoads Jr, 2013b). Cattle experiencing both fescue toxicosis and heat stress often exhibit similar symptoms such as hyperthermia, increased respiratory rate, and increased salivation along with the decrease in DMI (Strickland et al., 2011; Dash et al., 2016). Baumgard and Rhodes (2013a) suggested that in heat-stressed animals the increased insulin concentration may suggest a change in postabsorptive metabolism, specifically lipid mobilization. In the present study, we analyzed NEFA and BHB to examine the possible effect on postabsorptive metabolism. NEFA concentrations were highest for the 1.8 ppm treatment one hour prior to feeding and was highest for the 1.8 ppm ERV treatment compared to the 0 ppm treatment at the time of feeding. The 2.7 ppm ERV treatment was also higher than that of the 0 ppm

treatment at the time of feeding as well as at 300 minutes post-feeding. Also, the 2.7 ppm ERV treatment had the highest NEFA concentration 360 minutes post-feeding. At 300 and 360 minutes post-feeding, the increased NEFA concentrations paired with increased insulin concentrations. This was surprising given that NEFA is usually decreased with increased insulin. The 2.7 ppm ERV steers had the lowest BHB concentrations overall, including at time points where NEFA concentrations were highest for the 2.7 ppm ERV treatment. This was unexpected since increases in NEFA are generally associated with increases in BHB, but this could be the result of decreased ruminal butyrate production. These results coupled with the insulin results suggest a potential change in postabsorptive metabolism, and this provides an additional area to further research concerning changes in VFA concentrations with high ERV concentrations.

Leptin concentrations were highest for the 1.8 ppm ERV treatment with no increase being observed for the 2.7 ppm ERV treatment. Increased leptin concentrations have been associated with intake inhibition (Campfield et al., 1995; Chen et al., 1996). The increase in leptin concentrations for the 1.8 ppm ERV treatment was expected; however, the lack of increase in leptin concentrations for the 2.7 ppm ERV treatment was an unexpected result. This may indicate that when ERV concentrations are low, leptin concentrations are modulated, but at high ERV concentrations, a direct inhibition effect may be present. Ghrelin, while known to stimulate intake, increased linearly with increasing ERV concentrations in the present study. This suggests that active ghrelin may be associated with ERV concentrations, but further research is needed to separate the effects of ERV from those of DMI.

Moving forward, continued research examining the effects of endophyte-infected tall fescue consumption, and ERV concentration, on hormones related to DMI, is warranted to continue to elucidate the mechanisms by which DMI is reduced during consumption. Additional research should also include investigating changes in VFA concentrations in response to high ERV intakes. The present study has provided insight on potential hormonal changes related to intake regulation. Expansion on the current study may be beneficial to include other hormones and peptides related to intake regulation as well as to further investigate the changes seen for leptin and insulin concentrations. Specifically, analyzing peptides such as neuropeptide Y and agouti-related protein may be valuable given their important role in central nervous system intake regulation. Examining changes in hormones such as glucagon and thyroid hormones may also provide an important next step in determining how consumption of endophyte-infected tall fescue affects intake regulation. Additional research should include restriction of intake to better separate the effects of intake and ERV concentration on variables.

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VITA

EDUCATION

University of Kentucky; Lexington, KY

Master of Science in Animal Science: Ruminant Nutrition; August 2018-Present

- GPA: 3.594

University of Tennessee at Martin (UTM); Martin, TN

Bachelor of Science in Agriculture; Concentration: Animal Science and Veterinary Science; May 2018

- GPA: 3.43
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EXPERIENCE

University of Kentucky; Lexington, KY
Graduate Research Assistant; August 2018-Present

University of Kentucky, Lexington, KY
Animal Lab Manager, August 2018-Present

UTM Ned Ray McWherter Institute; Martin, TN

Innovator; September 2015 – May 2018

Otis L. Floyd Nursery Research Center; McMinnville, TN
Chemical Ecology Intern; May – July 2015

TEACHING EXPERIENCE

University of Kentucky; Lexington, KY
Graduate Teaching Assistant; August 2018-May 2019

UTM First Year Initiative; Martin, TN
Peer Enabling Program (PEP); August 2015 – December 2017

AWARDS

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PROFESSIONAL PUBLICATIONS

Eriton E L Valente, James L Klotz, Gyuchul Ahn, Kyle R McLeod, Hannah M Herzing, **Mindy King**, David L Harmon; "Ergot alkaloids reduce circulating serotonin in the bovine".