RESPONSE OF BEEF CATTLE SELECTED FOR TOLERANCE TO TALL FESCUE TOXICOSIS

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By

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DEDICATION

To my wife, Courtnie, and my parents, Steve and Jeanne Jones.

There is no way to express the appreciation I have for your influence in my life.

Thank you!

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ABSTRACT

Tall fescue (*Schedonorus arundinaceus* (Schreb.) Dumort.) infected with the endophytic fungus, *Epichloë coenophiala* [(Morgan-Jones & W. Gams) C.W. Bacon & Schardl, comb. nov.] produces ergot alkaloids that result in a livestock disorder known as tall fescue toxicosis. Tall fescue cultivars have been developed that do not contain the toxic endophyte. These novel endophyte cultivars have been used to alleviate tall fescue toxicosis. Recently, genetically testing cattle using the dopamine receptor D2 (DRD2) gene has been identified as a way to determine tolerance to tall fescue toxicosis. Results will be presented from three research projects involving cow-calf pairs on pasture, heifers in the GrowSafe feeding system, and rumen fluid in a continuous culture. Research on cow-calf pairs grazing toxic Kentucky 31 tall fescue pasture show that cow pre-calving weight and calf 205-d adjusted weaning weight were greater for animals with tolerant genotype. Evaluation of heifers in the GrowSafe feeding system showed that there was no difference in dry matter intake as a percent of body weight between the tolerant heifers fed Kentucky 31 and the tolerant heifers fed BarOptima. Research in the continuous culture showed that there were very few responses in fermenter characteristics due to genotype when toxic or novel endophyte-infected tall fescue was fed. These results indicate that providing animals with novel endophyte-infected tall fescue is the best way to prevent tall fescue toxicosis. However, the DRD2 gene shows promise as another possible way to mitigate the effects of tall fescue toxicosis in cattle.

CHAPTER 1. REVIEW OF LITERATURE

Tall fescue

Tall fescue [*Lolium arundinaceum* (Schreb.) Darbysh. = *Schedonorus arundinaceus* (Schreb.) Dumort., formerly *Festuca arundinacea* Schreb. var. arundinacea Schreb.] is a perennial cool-season grass introduced to the United States in the late 1800s (Ball et al., 1993). It is the predominant forage of the transition zone in the eastern portion of the United States between the temperate northeast and the subtropical southeast. Originally from Europe, it has also been successfully cultivated in many other countries including Argentina, Australia, Canada, Chile, Japan, Mexico, New Zealand, South Africa, and Uruguay (Burns and Chamblee, 1979; Fribourg et al., 2009). In the United States the latest estimates suggest that tall fescue occupies 14 million hectares across the humid-temperate region and is utilized by approximately 12 million beef cows (Casler and Kallenbach, 2007).

Widely used today as forage for beef operations, tall fescue's popularity in the United States traces back to Kentucky. According to Buckner et al. (1979), in 1931 a professor named E.N. Fergus from the University of Kentucky discovered a vigorous ecotype of tall fescue on a farm in Menifee County, Kentucky. Seeds from these plants were collected, evaluated, and released in 1943 as the cultivar 'Kentucky 31' (Fergus, 1952). Tall fescue became prevalent, and remains popular today, due to its easy establishment, long growth cycle, tolerance to overgrazing, pest resistance (Popay, 2009), persistence during drought (West et al., 1993), poor soil drainage, and low soil fertility (Ball et al., 1993; Burns and Chamblee, 1979; Hoveland, 2009; Stuedemann and Hoveland, 1988).

Tall fescue endophyte infection

Tall fescue has a mutualistic relationship with an endophytic fungus that has had multiple name classifications over the years. Originally this endophyte was classified as *Epichloë typhina* (Bacon et al., 1977). From that point it was later classified as an *Acremonium* species, and then moved to the genus *Neotyphodium*. Today the endophyte is classified as *Epichloë coenophiala* [(Morgan-Jones & W. Gams) C.W. Bacon & Schardl, comb. nov.].

The mutualistic relationship between the endophyte and the tall fescue plant results in benefits for both organisms. The endophyte utilizes the intercellular spaces of the leaf primordia, leaf sheath and blade, and inflorescence tissue of the tall fescue as a place to survive and asexually reproduce (Clay, 1990). The endophyte provides the tall fescue with the benefits of increased competitiveness with other plants (Hill et al., 1991), improved nutrient acquisition (Malinowski et al., 1999), persistence during drought (West et al., 1993), resistance to plant diseases and pathogens (Latch, 2009), reduced herbivory from insect (Popay, 2009) and nematodes (West et al., 1988), enhanced tillering (West et al., 1993), and increased seed production and germination (Clay, 1987; Rice et al., 1990).

As early as 1898, endophyte infection of tall fescue was reported by Vogl in Germany (White et al., 1993). Later, Neil (1941) reported that a fungal endophyte in tall fescue cultivars in New Zealand produced toxic compounds. These ergot alkaloids include clavines, lysergic acid and derivatives, and ergopeptide and ergopeptine alkaloids (Bush and Fannin, 2009). Of the ergopeptine alkaloids, Lyons et al. (1986) reported that five – ergovaline, ergosine, ergonine, ergoptine, and ergocornine – are present in samples of toxic endophyte-infected tall fescue. The predominant ergopeptine alkaloid is ergovaline, which accounts for 84 to 97 percent of the ergopeptide alkaloids (Lyons et al., 1986) and is identified as the principal cause of tall fescue toxicosis by many researchers. Tall fescue also contains loline alkaloids and peramine, which are not the principle cause of tall fescue toxicosis but act as an insect toxin or feeding deterrent (Bush and Fannin, 2009).

Early attempts at improving tall fescue focused on removing the endophyte (Pedersen and Sleper, 1988) in an effort to remove the toxins associated with them. These endophyte-free cultivars did not produce the ergot-like alkaloids associated with tall fescue toxicosis (Bouton et al., 2002). Livestock grazing endophyte-free cultivars performed well. However, Bouton et al. (1993) reported that endophyte-free stands showed significant stand deterioration under grazing conditions. Producers and scientists alike found that endophyte infection was important for drought tolerance (West et al., 1993), resistance to pests (Popay, 2009), and other stresses (Arachevaleta et al., 1989; Belesky and West, 2009).

After discovering that tall fescue was more persistent when endophyte was present, a new strategy was developed to overcome the negative effects of the harmful wild-type endophyte. Naturally occurring endophyte strains, which produced little or no ergot alkaloids, were used to re-infect the most productive cultivars of tall fescue (Bouton and Easton, 2005; Bouton and Hopkins, 2003). These new endophyte strains were called novel, non-toxic, beneficial, or introduced endophytes. These novel tall fescue varieties have become the focus of most tall fescue toxicosis research over the past 20 years and have been shown to assist in the elimination of tall fescue toxicosis symptoms and improve animal performance (Bouton et al., 2002; Nihsen et al., 2004).

A study conducted by Bouton et al. (2002) on toxic, nontoxic, and endophyte-free tall fescue compared the amount of ergot alkaloids contained in the tall fescue, as well as the average daily gain of the lambs being tested. In the study, the differing forage and endophyte combination showed substantial differences in ergot alkaloid concentrations. The tall fescue cultivar infected with the toxic endophyte contained a mean ergot alkaloid content of 1184 $\mu g\ g^{-1}$, compared to less than 40 $\mu g\ g^{-1}$ in the endophyte-free and novel endophyte-infected tall fescue cultivars. As a result, lambs grazing toxic tall fescue gained 78.5 $g\ d^{-1}$ while animals grazing endophyte-free tall fescue and novel endophyte tall fescue cultivars gained 115 to 130 $g\ d^{-1}$.

In addition, animal preference for tall fescue with different endophyte statuses has also been a focus for researchers. Fisher and Burns (2008) tested the differences in animal preference of hay infected with a wild-type endophyte, novel endophyte or endophyte-free tall fescue. The ergovaline content of the wild-type tall fescue was 221 $\mu g\ kg^{-1}$ compared to a mean of 33 $\mu g\ kg^{-1}$ for the novel and endophyte-free samples. Results showed that steers did not prefer hay based on endophyte status. They concluded intake preference was based on nutritive value and would not be affected until the onset of severe tall fescue toxicosis. But once the animals become affected by tall fescue toxicosis, it was found by Stuedemann and Hoveland (1988) that intake was 10 to 50 percent higher on low endophyte-infected hay or seed compared to hay or seed with high endophyte infection. Similar intake results were shown by Jackson et al. (1984) and Schmidt et al. (1982).

Tall fescue toxicosis

The term "tall fescue toxicosis" is used to describe a variety of negative symptoms that are exhibited by livestock consuming toxic endophyte infected tall fescue. The common symptoms can be broken down into two categories relating to how they affect the animal. The categories are animal production and animal health. The variety of symptoms associated with these two categories include poor weight gains, reduced intake, reproduction failure, reduced milk production, fescue foot, fat necrosis, vasoconstriction, retained winter hair coats into the summer, elevated core body temperatures, elevated respiration rates, altered hormone balance and a compromised immune system (Aiken and Strickland, 2013; Hoveland et al., 1983; Strickland et al., 2011; Strickland et al., 1993; Stuedemann and Hoveland, 1988; Waller, 2009).

There is some variation in the reported threshold level of ergovaline in livestock diets where symptoms of tall fescue toxicosis occur. Stamm et al. (1994) suggested that livestock experienced symptoms of tall fescue toxicosis when concentrations of ergovaline exceeding 150 $\mu g\ kg^{-1}$ during winter. Other research indicates that tall fescue diets containing an ergovaline concentration of 200 $\mu g\ kg^{-1}$ produced clinical signs of toxicity in heat stressed steers (Rottinghaus et al., 1991). Research conducted in Oregon suggests the threshold level of ergovaline in the diet to produce the clinical disease in cattle is 400-750 $\mu g\ kg^{-1}$ (Aldrich-Markham et al., 2007). The variability in the reported threshold level vary between location and time of year.

Animal production

Consumption of toxic tall fescue by cattle reduces weight gain. A collection of early projects on the endophyte effects on cattle were summarized by Schmidt and

Osborn (1993) and show the average daily gains of steers, average daily gains of cows, and average daily gain and 205-d adjusted weaning weight of calves (Table 1.1 & 1.2). Results were reported from multiple states across the fescue belt and indicate that cattle grazing toxic endophyte infected tall fescue have decreased gains (Table 1.1). Similarly, cows grazing toxic tall fescue lost more weight and raised calves that had reduced average daily gains and lower 205-d adjusted weaning weights (Table 1.2). A study conducted by Watson et al. (2004) compared the productivity of cow-calf pairs grazing tall fescue pastures infected with either a wild-type or non-ergot alkaloid producing endophyte strain. Results showed that calves from the cows consuming a non-toxic tall fescue cultivar had greater birth weights, ADG, and weaning weights.

It is common to see publications comparing tall fescue cultivars infected with novel endophyte to those with toxic endophytes and/or cultivars that are endophyte-free. Typically, research concludes that livestock consuming novel or endophyte-free tall fescue have greater gains and improved reproduction compared to the toxic endophyte infected tall fescue. Research on stocker cattle conducted in Georgia (Parish et al., 2003), Missouri (Nihsen et al., 2004), Oklahoma and Louisiana (Hopkins and Alison, 2006), Kentucky (Johnson et al., 2012), and Mississippi (Parish et al., 2013) show an increase of 25 to 50 percent in average daily gain when novel endophyte infected tall fescue is grazed compared to tall fescue infected with a toxic endophyte.

(Caldwell et al., 2013) examined the performance of both spring- and fall-calving cows grazing pasture systems, where animals had access to varying percentages of toxic endophyte and novel endophyte infected tall fescue. There were five treatments: spring calving cows grazing toxic tall fescue the entire season (S100), fall calving cows grazing toxic tall fescue the entire season (F100), spring calving cows grazing novel endophyte infected tall fescue the entire season (SNE100), spring calving cows grazing toxic tall fescue 75% of the grazing season and novel endophyte infected tall fescue the other 25% of the season (S75) and finally, fall calving cows grazing toxic tall fescue 75% of the grazing season and novel endophyte infected tall fescue 25% of the season (F75). In general, fall calving treatments were less affected by toxic tall fescue than were spring calving treatments. For instance, the calving rate in the SNE100 and S75 treatments averaged 80%, while the S100 treatment had only a 44% calving rate. In comparison, cows on treatments F100 and F75 had 90% and 95% calving rates, respectively. These results suggest that adjusting the breeding season can be an effective tool to combat the negative effects of toxic tall fescue on reproduction. Also, adjusted weaning weights of calves in the SNE100 treatment averaged 237 kg; this was equivalent to the 227 and 215 kg for the F75 and F100 treatments, respectively, but was 28 to 33 kg more than the other spring calving treatments (S75 and S100). There was no difference in forage mass produced between the treatments and the average total ergot alkaloid in the diet was 515 $\mu g\ kg^{-1}$ for the S100 and F100 treatments, 343 $\mu g\ kg^{-1}$ for the F75 and S75 treatments but only 104 $\mu g\ kg^{-1}$ for the SNE100 treatment (Caldwell et al., 2013). Similarly, Caldwell et al. (2013) found that weaning weights were over 30 kg greater for spring calves on novel endophyte infected tall fescue pasture compared to the toxic tall fescue pastures.

In Table 1.2, Schmidt and Osborn (1993) compiled data on reproductive rates which show that cows on toxic tall fescue conceive less often or have trouble reproducing. Reproductive problems are a common issue associated with breeding cattle exposed to toxic tall fescue and affect both male and female reproductive function (Siegel et al., 1987). Low pregnancy rates, delayed puberty, thickened or retained placenta, and dystocia are common reproductive complications associated with the consumption of toxic tall fescue (Gay et al., 1988). A reduction in fertility may also be caused by hormone imbalances or disruptions by ergot alkaloids being consumed (Porter and Thompson, 1992). Reproductive issues have also been reported in yearling bulls grazing endophyte-infected tall fescue pastures. Semen from bulls grazing toxic tall fescue had decreased cleavage rates in vitro compared to semen from bulls consuming novel endophyte infected tall fescue, which may lower reproductive performance because of reduced fertilization ability (Schuenemann et al., 2005).

Milk production decreases when lactating cows consume toxic endophyte infected tall fescue (Brown et al., 1993; Peters et al., 1992). In some severe cases, the animal exhibits a failure to produce milk, which is known as agalactia (Strickland et al., 1993). Peters et al. (1992) reported that milk production, and subsequent consumption, was significantly less for cows and calves grazing toxic tall fescue than cows grazing endophyte free tall fescue or orchardgrass pastures. Brown et al. (1993) compared milk production of Angus and Brahman cows consuming either toxic endophyte-infected tall fescue or common bermudagrass over 3 years. On average, cows grazing tall fescue produced approximately 1 kg less milk each day through the 199 day lactation period (Brown et al., 1993).

Body condition score has also been shown to be negatively affected by toxic endophyte-infected tall fescue. A study by Watson et al. (2004) showed that body condition scores of cows grazing non-ergot alkaloid producing tall fescue pastures were higher at the end of the grazing period compared to wild type endophyte-infected tall fescue.

Animal health

Tall fescue toxicosis, also described as “summer slump”, has an effect on animal weight gain, intake, and overall production that results in noticeable losses to the producer. However, there are also symptoms related to animal health that cause decreased performance. One of these symptoms has been coined fescue foot. Fescue foot is another term used for the lameness in cattle that are grazing toxic tall fescue. Fescue foot typically manifests in cooler months and can be identified by tenderness and swelling around the hoof, lameness, dry gangrene on tips of ears and tails, loss of tail switch, and in severe cases, having the hooves slough off (Oliver, 2005). Consumption of ergot alkaloids causes vasoconstriction, damaged blood vessels, and increase blood clotting, which results in the tissue necrosis and hoof loss associated with fescue foot (Oliver, 2005; Strickland et al., 1993).

Fat necrosis, also known lipomatosis, is another symptom associated with tall fescue toxicosis and is characterized by hard fat masses within the abdominal cavity of the animal that leads to discomfort and blockage of digesta (Hemken et al., 1984; Stuedemann et al., 1985). In some cases the masses can develop in the pelvic cavity and obstruct the birth canal and result in difficulty during birth or dystocia (Arnold et al., 2014). The development of fat necrosis in grazing cattle has been associated with the consumption of toxic tall fescue that have received high levels of N fertilization (Stuedemann et al., 1985).

Tall fescue toxicosis is also associated with vasoconstriction such that when animals consume toxic endophyte-infected tall fescue reduced blood flow to the peripheral and core body tissues limits the ability to dissipate heat (Rhodes et al., 1991). Consumption of ergot alkaloids in toxic tall fescue decreases blood flow to the skin (Rhodes et al., 1991; Solomons et al., 1989), reduces the evaporative cooling effect (Aldrich et al., 1993), and increases energy expenditure (Zanzalari et al., 1989). The alkaloids produced by toxic endophyte infected tall fescue are responsible for the vasoconstriction in cattle and the ergopeptide alkaloids are more venoconstrictive than loline and its derivative alkaloids (Solomons et al., 1989). Research on the vasculature of endophyte naïve heifers was shown to be sensitive to ergovaline within 27 hours of being fed diets containing 0.8 mg ergovaline/kg DM and within 51 hours for those fed 0.4 mg ergovaline kg^{-1} DM (Aiken et al., 2009). According to Klotz et al. (2008), ergovaline, ergonovine, ergocryptine, ergocristine, and ergocornine all caused a contractile response of the veins in naïve heifers. Ergovaline was determined to be the most potent vasoconstrictor but it was concluded that ergopeptines likely have additive effects, thus showing all of them likely contribute to tall fescue toxicosis.

Altered hormone balance is another symptom of tall fescue toxicosis. Hurley et al. (1981) was the first to associate the presence of toxins in tall fescue with a decrease in prolactin secretion. Following that publication, most of the researchers working with toxic endophyte infected tall fescue and livestock have reported the decline in serum prolactin (Hoveland et al., 1983; Parish et al., 2003; Schillo et al., 1988). Paterson et al. (1995) compiled the data from multiple projects involving other hormones associated with toxic endophyte infected tall fescue in different livestock types, but results varied because of differences in temperature and location.

Another symptom of tall fescue toxicosis that is not always readily apparent but can influence animal performance is behavioral change. The main change that is seen when grazing toxic tall fescue is a shift in the time of day cattle graze the pastures. Often when animals are suffering from symptoms of tall fescue toxicosis the grazing events will occur more at night than during daylight hours (Stuedemann et al., 1985).

Some additional symptoms that can be seen in animals suffering from tall fescue toxicosis are rough hair coats, elevated core body temperatures, and elevated respiration rates (Hoveland et al., 1983; Nihsen et al., 2004). Respiration rates recorded by Nihsen et al. (2004) showed a significantly higher respiration rate with steers grazing toxic tall fescue taking 24 more breaths per minute than animals grazing novel or endophyte-free tall fescue. Rectal temperature was 0.8°C greater for animals on toxic tall fescue and hair coat scores were 1.6 points (scored on a scale of 1 to 5) higher compared to the other treatments. Cattle grazing toxic tall fescue commonly retain their winter hair coat that results in long, rough, and bronze appearance, of the hair coat (Stuedemann and Hoveland, 1988).

Economic losses and costs

Estimates of annual economic losses associated with tall fescue toxicosis for the United States beef industry were $609 million in the early 1990’s (Hoveland, 1993). If the impact on small ruminants and equine were taking into consideration Strickland et al. (2011) suggested the losses could exceed $1 billion. Recent calculations by Kallenbach

(2015a) suggested that tall fescue toxicosis currently costs the U.S. beef industry nearly $2 billion annually.

Solutions for tall fescue toxicosis

Due to the costly effects of toxic tall fescue, research has been conducted to evaluate possible solutions or methods livestock producers can utilize to decrease the negative effects of tall fescue toxicosis. These solutions can be broken down into two categories, forage management and animal management. These remedies are not mutually exclusive and can be broken down further into multiple techniques, which could be used to decrease the negative effects of tall fescue toxicosis.

Forage management strategies

Endophyte-free tall fescue was one of the strategies developed to alleviate the negative effects tall fescue toxicosis. To remove the endophyte from the tall fescue the seeds were exposed to fungicide, heat, or storage (Siegel et al., 1987). Producers removed the wild-type tall fescue from the fields and reseeded with tall fescue that did not contain endophyte. In the absence of endophyte, the animals had improved performance and no symptoms of tall fescue toxicosis (Hoveland, 1993; Nihsen et al., 2004), but stand persistence reduced compared to endophyte-infected tall fescue (Read and Camp, 1986).

After evaluating the endophyte-free tall fescue and learning the importance of the endophyte for persistence, novel endophytes were discovered and selected for use (Bouton et al., 2002). The goal of novel endophyte was to prevent tall fescue toxicosis in the livestock while maintaining plant persistence in the field. There are now many novel endophyte-infected tall fescue varieties commercially available.

Another possible strategy to reduce the effects of the toxic tall fescue is dilution of the diet with other forages or supplementation. The addition of legumes in pastures reduces the amount of toxic tall fescue being consumed and reduces the negative effects of the ergot-like alkaloids. The addition of legumes, such as clover, to tall fescue pastures has been shown to provide steers with an additional gain of 0.07 kg d^{-1} (Thompson et al., 1993). Similarly to the addition of legumes, supplemental feeding can be used to reduce the effects of tall fescue toxicosis (Elizalde et al., 1998). The addition of supplemental feed often results in a reduction in forage intake and dilutes ergot alkaloids consumed.

Seedhead control is another form of pasture management that can be used to help reduce the effects of tall fescue toxicosis in grazing cattle. Reducing seedhead production of tall fescue is a reasonable way to reduce the amount of toxin the animal may consume. Rottinghaus et al. (1991) showed that seedheads contain five times more ergovaline than the leaves or stems. Clipping seedheads is an effective way to reduce the ergot alkaloids as well as maintaining forage quality (Roberts and Andrae, 2004). Another strategy that may be used to suppress seedhead emergence in tall fescue is the herbicide, Chaparral (Dow Agrosciences, Indianapolis, IN). Aiken et al. (2012) reported Chaparral herbicide suppresses seedhead emergence in tall fescue, which boosted weight gain in steers, and reduced the severity of tall fescue toxicosis. The herbicide was sprayed in the spring to provide 86.8 g active ingredient (aminopyralid and metsulfuron) ha^{-1} and resulted in seedhead suppression that substantially reduced the density of reproductive tillers, which contain a higher concentration of ergot alkaloids (Aiken et al., 2012).

Additionally, limiting the amount of nitrogen fertilizer applied can help to reduce the ergot alkaloid concentration in tall fescue. Nitrogen is part of the heterocyclic ring of ergot alkaloids and the concentrations increase quickly following N fertilizer application (Rottinghaus et al., 1991). Research by Stuedemann and Hoveland (1988) found that high application rates of N fertilizer or poultry litter are positively related to the severity of both fat necrosis and fescue foot.

Hay ammoniation, which is the process of treating low-quality hay with anhydrous ammonia, can partly detoxify toxic tall fescue hay. Lambs fed ammoniated toxic endophyte-infected tall fescue hay had increased dry matter intake, gain, and serum prolactin concentrations compared to lambs fed untreated toxic tall fescue hay (Chestnut et al., 1991a). Roberts et al. (2002) evaluated the difference between green chop (control), ensiled forage, hay, and ammoniated hay. Green chop contained 1240 $\mu g\ kg^{-1}$ ergot alkaloids and ensiled tall fescue contained 972 $\mu g\ kg^{-1}$, which were statistically similar. Ergot alkaloid concentrations in the ammoniated hay were 247 $\mu g\ kg^{-1}$ while non-ammoniated hay samples averaged 373 $\mu g\ kg^{-1}$ (Roberts et al., 2002). Even though non-ammoniated hay had 1.5 times the ergot alkaloid concentration the differences were not statistically significant.

Animal management strategies

Altering the calving season is another strategy that can be used to help decrease the effects of toxic tall fescue. It has been reported that a fall calving season would be more profitable than a spring calving season when grazing toxic tall fescue. Fall calving improved calving rate, and an increased adjusted weaning weight compared to the spring born calves (Caldwell et al., 2009). One possible reason for the improved performance of the fall calving cows is because they are bred between January and March, while consumption of toxic endophyte infected tall fescue pasture is low. The ration may include hay from those same pastures but the cow will not suffer from heat stress and likely receives a feed supplement. Ergovaline concentration can drop below the toxic level of 150 μg kg^{-1} in January or February when tall fescue is stockpiled (Kallenbach et al., 2003).

Grazing cattle commonly exposed to toxic tall fescue is another way to help reduce the likelihood of tall fescue toxicosis in a herd. When cattle are transported long distances it increases the probability that those animals are naïve to toxic endophyte-infected tall fescue. Introducing cattle that have not been exposed to toxic tall fescue will increase the chances of seeing the negative symptoms associated with tall fescue toxicosis. Research comparing Angus steers from Missouri and Oklahoma found that region of origin may alter the tall fescue toxicosis response in cattle and differences were most prevalent when animals experienced rapid changes in their environment (Johnson et al., 2015).

Ergot alkaloids in toxic tall fescue act as dopamine agonists. This means that consumption causes a decrease in the circulating serum prolactin concentration in the animal. Dopamine antagonists can be used to increase the circulating serum prolactin levels in animals consuming toxic tall fescue (Lipham et al., 1989). The increase in prolactin levels show that tall fescue toxicosis is reduced by administering dopamine. It has been reported that a treatment with domperidone helps maintain normal weight gain and circulating progesterone in heifers consuming toxic endophyte infected tall fescue (Jones et al., 2003).

Selecting animals that are genetically tolerant to tall fescue toxicosis is one of the most recent strategies developed and used to manage tall fescue toxicosis in cattle.

Campbell et al. (2014) identified a link between a single nucleotide polymorphism for a dopamine receptor D2 (DRD2) gene and prolactin responses in cattle grazing toxic tall fescue. Bastin (2013) identified additional phenotypes that are linked to the effects of tall fescue toxicosis. This research led to the investigation of the link between Kell blood group complex subunit-related family, member 4 (XKR4) gene and tall fescue toxicosis (Bastin et al., 2014).

Animal genotype

There are genetic tests that can be used to help determine the genotype of animals and ultimately help mitigate the negative effects of tall fescue toxicosis. Campbell et al. (2014) was among the first to identify putative resistance to tall fescue toxicosis in beef cattle. In the work, there were four separate survey-type experiments that found that angus-based cattle genotyped as “AA” on the dopamine receptor D2 (DRD2) gene were more likely to withstand the effects of tall fescue toxicosis. The DRD2 gene is located on bovine chromosome 15 and is important in the regulation of prolactin secretions in cattle (Civelli et al., 1993). The relationship between prolactin level and the DRD2 gene makes it a good candidate for containing a marker for resistance to tall fescue toxicosis (Campbell et al., 2014). A single nucleotide polymorphism (SNP) was discovered at position 534 within the DRD2 gene, where a guanine/adenine substitution exists (Campbell et al., 2014). The ‘A’ allele found at this position was determined to be related to increased serum prolactin concentrations and decreased hair coat scores in Angus cattle consuming toxic endophyte-infected tall fescue (Campbell et al., 2014).

Objectives and importance of this research

The overall objective of this research was to determine how beef cattle, genotyped using the DRD2 gene, respond when consuming novel ‘BarOptima plus E34’ (BarOptima) or toxic ‘Kentucky 31’ endophyte-infected tall fescue.

The objective of the first study was to determine how cow-calf pairs, genotyped as tolerant or susceptible to tall fescue toxicosis, respond while grazing tall fescue pastures infected with either a novel or toxic endophyte-infected tall fescue. This study examined the forage production and the performance of cows and their calves while grazing BarOptima or Kentucky 31 tall fescue.

The objective of the second study was to examine the effects of novel or toxic endophyte-infected tall fescue baleage diets on tolerant and susceptible heifers fed in the GrowSafe feeding system (Model 4000E, GrowSafe Systems Ltd., Airdrie, AB, Canada). The study examined the intake of individual heifers, using the automated GrowSafe feeding system, as well as weight gain, body condition score, hair coat score, body temperature and serum prolactin concentrations.

The objective of the third study was to evaluate the effects that novel or toxic endophyte-infected tall fescue and tolerant or susceptible genotyped cattle have on fermentation characteristics in the continuous culture. This research will help determine whether differences exist in the rumen ecosystem that may influence the production responses of genotyped cattle consuming BarOptima or Kentucky 31 tall fescue.

Table 1.1. Effects of endophyte level in tall fescue on daily gains of steers.[a]

	% Endophyte [b]		Daily gain, kg			Reference
Location	Low E	High E	Low E	High E	Diet	
AL	0	>90	0.66	0.28	Hay	(Schmidt et al., 1982)
AL	0	100	0.96	0.20	Seed	(Schmidt et al., 1982)
AL	2	>90	0.83	0.45	Pasture	(Hoveland et al., 1983)
AR	0	81	0.71	0.51	Pasture	(Goetsch et al., 1988)
GA	0	76	0.59	0.45	Pasture	(Stuedemann et al., 1986)
KY	<1	61	0.70	0.45	Pasture	(Boling, 1985)
MS	NR[c]	NR	0.68	0.46	Pasture	(Evans et al., 1989)
MO	3	83	0.62	0.21	Pasture	(Crawford et al., 1989)
OK	<1	76	0.85	0.62	Pasture	(McMurphy et al., 1990)
TN	2	71	0.67	0.48	Pasture	(Chestnut et al., 1991b)
TX	8	91	0.97	0.46	Pasture	(Read and Camp, 1986)
VA	0	77	0.65	0.41	Pasture	(Tulley et al., 1989)

[a] Adapted from Schmidt and Osborn (1993) and Paterson et al. (1995)
[b] Number of infected tillers per 100 tillers.
[c] Not reported.

Table 1.2. Effects of endophyte-infected versus endophyte-free tall fescue on cow-calf performance.[a]

Cows				Calves				Reference
Daily gain, kg d^{-1}		Pregnancy rate, %		Daily gain, kg d^{-1}		205-d Weaning weight, kg		
E-	E+	E-	E+	E-	E+	E-	E+	
0.46	-0.23	-[b]	-	1.16	0.85	236	197	(Schmidt et al., 1983)
0.21	-0.05	95	55	0.78	0.62	215	186	(Gay et al., 1988)
0.19	0.27	87	58	0.74	0.59	222	190	(Essig et al., 1989)
0.04	-0.11	89[c]	74	0.73	0.58	-	-	(Tucker et al., 1989)
0.36	0.21	78	49	1.02	0.86	240	209	(McDonald, 1989)
0.20	0.01	-	-	1.07	0.54	-	-	(Forcherio et al., 1992)
0.11	-0.12	-	-	1.00	1.00	-	-	(Forcherio et al., 1993)

[a] Adapted from (Schmidt and Osborn, 1993).
[b] Not determined or not reported.
[c] E- fescue was 21% infected vs 77% for E+.

CHAPTER 2. RESPONSE OF GENOTYPED COWS AND CALVES CONSUMING BAROPTIMA PLUS E34 OR KENTUCKY 31 TALL FESCUE

ABSTRACT

Livestock consuming the ergot-like alkaloids produced by tall fescue [*Schedonorus arundinaceus* (Schreb.) Dumort.] infected with wild-type *Epichloë coenophiala* suffer from tall fescue toxicosis, which costs U.S. producers an estimated $2 billion annually. A recently discovered dopamine receptor D2 (DRD2) gene in cattle may confer some tolerance to these alkaloids. The objective of this experiment was to determine the response of cow-calf pairs when cows were genotyped for tolerance or susceptibility to toxicosis when grazing Kentucky 31 tall fescue (toxic) or BarOptima plus E34 tall fescue (non-toxic). Five cow-calf pairs were rotationally grazed on each of the tall fescue-endophyte cultivars, which were replicated three times (n=30 cow-calf pairs each year). Body weight of cows and calves was recorded along with forage accumulation and pasture quality data. Ergovaline concentration of Kentucky 31 tall fescue was greater (P<0.01) than for BarOptima. When cows grazed toxic Kentucky 31, tolerant cows weaned calves that were 25 kg heavier (P<0.01) than calves weaned by susceptible cows. When cows grazed non-toxic BarOptima, 205-d adjusted weaning weight of calves did not differ (P=0.76) between tolerant and susceptible cows. There was no difference (P=0.06) in forage accumulation between BarOptima and Kentucky 31. Animal grazing days were greater (P<0.01) for Kentucky 31 compared to BarOptima.

Based on these results, the DRD2 gene shows promise as a way to help mitigate tall fescue toxicosis.

INTRODUCTION

Since ‘Kentucky 31’ (KY31) was released in 1943, tall fescue [*Schedonorus arundinaceus* (Schreb.) Dumort.] has become the most popular perennial cool-season grass in the United States. Tall fescue has a symbiotic relationship with the endophyte fungus, *Epichloë coenophiala* [(Morgan-Jones & W. Gams) C.W. Bacon & Schardl, comb. nov.], which helps the plant tolerate abiotic and biotic stresses. As a result, endophyte-infected tall fescue withstands a wide range of environmental conditions and management strategies.

The endophyte that improves tall fescue persistence also produces ergot-like alkaloids that are toxic to livestock. Known as “tall fescue toxicosis”, this syndrome costs the US livestock industry nearly $2 billion annually (Kallenbach, 2015a). The symptoms for tall fescue toxicosis include vasoconstriction, elevated core body temperature, increased respiration rate, reduced heart rate, altered fat metabolism, low serum prolactin, agalactia, suppression of the immune system, reduced forage intake and low rate of weight gain (Roberts and Andrae, 2010; Strickland et al., 1993).

In response to the negative effects of the toxic endophyte-infected tall fescue, in the 1980’s endophyte-free varieties were released. These endophyte-free varieties improved livestock performance but did not persist well. Endophyte-free tall fescue were evaluated and revealed to be susceptible to overgrazing, drought, plant diseases, insects, nematodes and mineral-deficient soils (Elmi et al., 2000; Latch, 1993; Malinowski and Belesky, 2000; West et al., 1993).

In response novel endophyte-infected tall fescue was developed. Novel endophytes are strains of the endophyte that produce little or no ergot alkaloids (Bouton et al., 2002). The mycelia are physically injected into endophyte-free plant germplasm, thereby producing non-toxic or "novel" endophyte tall fescue plants (Latch and Christensen, 1985). One common cultivar of novel endophyte tall fescue is 'BarOptima plus E34' (henceforth "BarOptima") that produces a modest amount of ergot alkaloids (Green et al., 2013).

In addition to novel endophyte tall fescue to address toxicosis, a recently discovered option is the use of advanced genetic technologies to identify cattle tolerant to fescue toxicosis. Campbell et al. (2014) evaluated the dopamine receptor D2 (DRD2) gene for the ability to select animals that show tolerance to tall fescue toxicosis. Steers genotyped as susceptible had decreased serum prolactin concentration and increased hair coat scores compared to tolerant steers when grazing toxic tall fescue pastures (Campbell et al., 2014).

The objective of this research was to evaluate forage production and the performance of tolerant and susceptible genotyped cows and their calves while grazing novel endophyte-infected BarOptima and toxic endophyte-infected Kentucky 31 tall fescue pastures. The hypothesis was that tolerant genotyped cow-calf pairs grazing Kentucky 31 would perform similar to either genotype cow-calf pairs grazing BarOptima and better than susceptible cow-calf pairs grazing Kentucky 31.

MATERIALS AND METHODS

The University of Missouri Animal Care and Use Committee approved the use of animals in this experiment. Research was conducted over three consecutive years from 2011 to 2013 at the University of Missouri Southwest Research Center in Mount Vernon, MO.

Cow-calf pairs

Five Angus x Simmental cross cow-calf pairs were assigned to each pasture unit (six pastures; n=30 cow-calf pairs). Cows remained in their assigned group for the duration of the study unless they did not conceive, wean a calf, or demonstrate good disposition. Replacement cows were similar to animals removed from the study. The calves were born on pasture, in the fall, while the study was in progress. When not on study, cow-calf pairs were comingled on toxic endophyte-infected tall fescue pasture or fed hay.

Cows used for this trial were genotyped using the identification of the DRD2 single nucleotide polymorphism (SNP), as outlined by Campbell et al. (2014), to determine tolerance or susceptibility to tall fescue toxicosis. Genomic DNA was isolated and the DRD2 segment was amplified at the University of Tennessee. The SNP was discovered at position 534 within the DRD2 gene, where a guanine/adenine substitution exists. Cows with AA and GG alleles, which were both utilized in the research, determine tolerance or susceptibility, respectively. For this trial, calf genotype was not evaluated; results for calves were reported based on cow genotype.

Pasture design

A 22-ha area of Kentucky 31 tall fescue, which had been established for over 20 years, was identified and blocked by soil type, slope and aspect. The soil series for the pastures consisted predominantly of Creldon, Gerald, Hoberg, Viraton and Wilderness associations. These soils are mainly silt loams and represent common soils of the region.

The pasture treatments consisted of two tall fescue-endophyte cultivars, each replicated three times. The first treatment was a toxic endophyte-infected Kentucky 31 tall fescue pasture. The second treatment was novel endophyte-infected BarOptima tall fescue pasture. Each of the 6 pastures (2 cultivars x 3 replications) was subdivided into 8 paddocks of 0.46 (± 1%) hectares each (Figure 2.1).

Because the original land area for this experiment consisted of an established field of toxic Kentucky 31 tall fescue, the second cultivar treatment, BarOptima, had to be established into a renovated Kentucky 31 pasture. The renovation procedure began in May of 2009. Pasture units that would contain BarOptima were sprayed with glyphosate (GLY-4, Universal Crop Protection Alliance, LLC, Saint Paul, MN) plus ammonium sulfate (AMS) at a rate of 4.68 L ha^{-1}. Following the initial herbicide application, the pastures were no-till planted to a sorghum-sudangrass hybrid (*Sorghum* spp.), which was harvested for hay. Following the final hay harvest, an August application of glyphosate (Roundup Power Max, Monsanto, St. Louis, MO) plus AMS was applied at a rate of 1.61 L ha^{-1}. Then BarOptima seed was sown using a no-till drill (1590, John Deere, Moline, IL.) at a rate of 25 kg ha^{-1} in September 2009. Based on soil sample results, lime, nitrogen, phosphorus, and potassium were applied at the level recommended by the University of Missouri Soil Testing Laboratory (Brown and Rodriguez, 1983).

During the final research year, on October 3, 2013, pastures were tested for percent endophyte to confirm the toxic or nontoxic status of the treatments. Tillers were selected evenly and randomly from pasture areas. There were 81, 83, and 97 tillers selected from the three BarOptima pastures and 93 tillers from all of the Kentucky 31 pastures. Tillers were analyzed for the presence of endophyte mycelia using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Agronostics, Watkinsville, GA). The BarOptima pastures were determined to contain 82.0% infected tillers and Kentucky 31 pastures contained 91.8% infected tillers.

Pasture management and measurements

Cattle were rotationally stocked on pastures on which tall fescue height was measured weekly with an ultrasonic reader mounted to the front of an ATV. The height data collected was then used to make pasture rotation decisions. The target residual height was 75 ± 5 mm. Once the pastures were grazed to that height, the cattle rotated to the next paddock that had the most available forage. This rotation strategy was maintained through all three years of research.

Calibration of the ultrasonic reader for forage mass was determined four to six times per year using procedures outlined by (Kallenbach, 2015b). These calibrations occurred throughout the year when environmental factors resulted in differences in forage mass. To calibrate, a 5-m (± 2 m) strip of forage was measured twice with the ultrasonic reader. A 0.82-m wide strip, centered over that same area, was cut to a 3-cm height with a flail-type harvester. Approximately 25 (± 3) such strips were cut from paddocks, representing a range of forage masses. In addition, a subsample of forage [350 g (±50 g)] from the harvested strips was weighed fresh and again after oven drying at 50 ° C for 4 days, to determine dry matter. The forage mass values, along with their corresponding ultrasonic reader height measurements, were then used to develop a regression equation to estimate forage yield. After pre- and post-stocking forage mass values were calculated, the forage growth rate for each stocking period was determined by averaging the growth rates of the undisturbed paddocks within the same pasture unit. Forage growth while animals were stocked in an individual paddock was added to the pre-stocking forage mass to determine the amount of forage available for grazing.

Neural network was used to determine the growth rate of the forage during the year. The independent variables used for forage yield predictions in the neural network calculations were the forage height, date, year, temperature, precipitation, soil moisture, and grazed or non-grazed status. Those values were then used to calculate the growth rate and ultimately the forage accumulation of the tall fescue pastures (Kallenbach, 2015b).

Forage samples were collected each time livestock were moved into a new paddock. The samples were used to determine forage nutritive value and total ergot alkaloid concentrations. The samples were clipped to 3 cm from 25 different areas throughout the paddock. Samples were stored at –4 ° C immediately following clipping. Each sample was then freeze dried, ground to pass a 1-mm screen, and stored at –20°C until analysis.

Forage nutritive values for crude protein (CP), neutral detergent fiber (NDF), and in vitro true digestibility (IVTD) were determined by near-infrared (NIR) spectroscopy. The NIR spectrophotometer and software were FOSS NIR 6500 system with WinISI software version 1.02 (Foss Analytical, Hillerod, Denmark). For the forage nutritive value samples, prediction equations were determined using 70 randomly selected samples that were analyzed in the laboratory using standard chemical methods for reference values employed by Dairy One (Ithaca, NY). Chemical analyses for forage nutritive value were then used to create a prediction equation using modified partial least squares regression. NIR equations were selected based on the squared correlation coefficients of calibration (R^2), 1 minus the variance ratio, and standard errors of calibration and cross validation. All calibration and validation statistics for NIR spectroscopy determination of CP, NDF and IVTD are included in Table 2.2.

Total ergot alkaloid concentrations were determined by analyzing a subsample of the total forage nutritive value samples collected per month, per pasture, per year. A total of 142 samples were analyzed for total ergot alkaloid concentration (Hill and Agee, 1994) using the procedure from Agrinostics Ltd. Co. (Watkinsville, GA).

Each year the livestock were monitored on pasture during the grazing season. The grazing season for each pasture in 2011, 2012, and 2013 are listed in Table 2.1. The grazing period began each year when enough forage mass had developed to sustain grazing until summer or the end of grazing in the fall. Hay was harvested from individual paddocks in each of the pastures in the spring. The amount of hay harvested from each pasture was dependent on how much forage was available in each paddock at the time of cutting. In 2011 and 2012, grazing was discontinued in the summer due to the lack of tall fescue forage availablility. During these summer periods the animals were fed large round bale silage harvested from the respective pasture until it was no longer available, after which additional hay was supplied. Additional hay consisted of large round bale tall fescue silage harvested from toxic tall fescue pastures at the Southwest Research Center. The additional hay was fed until the pastures had grown back enough to sustain grazing again. Cattle were also provided access to American Stockman® Big 6® mineral blocks (Compass Minerals, Overland Park, KS) throughout the entire grazing period.

Nitrogen fertilizer applications were split in spring to manage forage growth. One-half of the paddocks in each pasture received 56 kg N ha^{-1} in March; the other half received the same amount of N in May. All pastures were fertilized again in the fall at a rate of 84 kg N ha^{-1}. This fertilizer application strategy was used to balance forage growth with livestock demands.

Hay balance was calculated using the amount of hay produced on each individual pasture minus the amount of purchased hay fed. A negative number indicates that all of the hay produced on a given pasture, in a given year, was fed back and additional hay was necessary. Animal grazing days was calculated by dividing the number of days livestock grazed by the stocking rate.

Animal growth and performance measurements

In this trial, cow body weight was measured every 28 days. A double weighing, which consisted of weighing animals on two consecutive days, was conducted at the initiation and conclusion of each trial year. The calves were weighed at birth and every 28 days until weaning. Average daily gains, 205-day adjusted weaning weight, and pounds of calf produced per hectare were calculated using collected body weights. The adjusted 205-day weaning weights were calculated on the basis of average daily gain from birth to weaning, using the equation:

$$\text{Adj. 205-day wean wt.} = \frac{\text{Wean wt.} - \text{Birth wt.}}{\text{Weaning age}} \times 205 + \text{Birth wt.} + \text{Age of dam adj.}$$

The equation and age of dam adjustments were based on those found in the Guidelines for Uniform Beef Improvement Programs (Beef Improvement Federation, 2010). Calculations for the kilograms of beef produced per hectare were evaluated by taking the total kilograms of weaned calf divided by the total hectares grazed by each group. Average daily gains were calculated as total weight gain divided by number of days. Hair coat scores (1 to 5 scale; Tucker and Ringer (1982)) were recorded on each cow at the conclusion of the trial each year.

Statistical analysis

This experiment was analyzed as a split plot, randomized complete block design, with pasture as the whole plot, animal genotype as the subplot, and individual animals as subsamples. Data were analyzed using the PROC GLIMMIX procedure of SAS 9.4 (SAS Inst. Inc., Cary, NC, USA). Treatments were designated as the combination of fescue cultivar (BarOptima and Kentucky 31) and cow genotype (tolerant and susceptible). Three blocks were designated as based on the pasture soil type, slope and aspect. The model for animal and forage performance contained treatment, block and the interaction of treatment and block. Year was a random effect. For animal data least squares means were estimated using the LSMEANS statement and when significant effects were detected in the model ($P \leq 0.10$) the LINES option was used for mean separation. For forage data least squares means were estimated using the LSMEANS statement and when significant effects were detected in the model ($P \leq 0.05$) the LINES option was used for mean separation.

RESULTS AND DISCUSSION

Forage nutritive value and production

Monthly forage nutritive value data are reported in Figures 2.2, 2.3, and 2.4. Over the timespan of this experiment, BarOptima tall fescue pastures averaged 200 g kg^{-1} crude protein, which was higher (P<0.01) than Kentucky 31 (187 g kg^{-1}). Also, BarOptima had 14 g kg^{-1} lower neutral detergent fiber than Kentucky 31 (P<0.01), as well as greater in vitro true digestibility (P<0.01; Table 2.7). BarOptima was developed using soft leaf tall fescue germplasm and is marketed to have improved digestibility. This data supports that claim with results showing improved crude protein, neutral detergent fiber and in vitro true digestibility values.

BarOptima contained lower total ergot alkaloid concentrations (P<0.01; Table 2.7) than Kentucky 31, as expected. BarOptima pastures averaged 172 μg kg^{-1} total ergot alkaloids compared to 2537 μg kg^{-1} for Kentucky 31; further, the concentrations for BarOptima remained consistent during the grazing season (Figure 2.5). Total ergot alkaloid concentration for Kentucky 31 exhibited a bimodal curve with peaks in May and October, as would be expected. Similarly, Greene et al. (2013) reported that BarOptima did not differ from endophyte-free tall fescue and also had lower ergovaline concentrations (P<0.01) compared to Kentucky 31.

Forage accumulation did not differ (P=0.06; Table 2.7) between BarOptima and Kentucky 31 over three years. However, Kentucky 31 pastures had a surplus of hay compared to a deficit for BarOptima (P<0.01). Forage accumulation values were calculated to include growth harvested as hay as well as growth that was grazed. BarOptima pastures also had fewer grazing days per hectare (P<0.01) compared to

Kentucky 31. It is likely the hay deficit for BarOptima as well as its reduced number of grazing days, resulted from uninhibited forage consumption compared to toxic Kentucky 31. Uninhibited consumption is likely due to the reduced concentrations of toxic ergot alkaloids (*P*<0.01). Results from Olson et al. (2009) indicated that BarOptima was a preferred tall fescue cultivar by cattle. Similarly, Kenyon (2017) reported that BarOptima was the preferred forage, based on time spent grazing and preferential selection. It is also possible that intake was decreased on the Kentucky 31 pastures compared to BarOptima. Schmidt et al. (1982) reported that Kentucky 31 hay and seed diets had feed intake reduced by 8 and 36 percent, respectively.

Cow performance

<u>Effect of Cultivar</u>

In the spring, cows grazing BarOptima gained 0.7 kg more body weight per day (*P*<0.01; Table 2.8) than cows grazing Kentucky 31. However in the summer, cows grazing Kentucky 31 and BarOptima did not differ (*P*=0.27) in average daily gain. Dry and hot conditions limited grazing during the summer of the first two years and thus increased the need to feed hay. During the autumn grazing period, cows grazing BarOptima and Kentucky 31 did not differ in average daily gain (*P*=0.11). Compared to Kentucky 31, cows grazing BarOptima weighed more (*P*<0.01) at pre-calving in the autumn than cows grazing Kentucky 31. Cows grazing BarOptima pastures weighed approximately 42 kg more (Table 2.8) than cows grazing Kentucky 31.

Also, cows grazing BarOptima pastures weighed more (*P*<0.01) at the end of the autumn grazing period than did cows grazing Kentucky 31. Cows grazing BarOptima were 66 kg heavier (Table 2.8) than cows grazing Kentucky 31 pastures at the end of the study each year. BarOptima and Kentucky 31 also differed in cow body weight at weaning (P<0.01). In the spring period, cows grazing BarOptima were 33 kg heavier (P<0.01) than those grazing Kentucky 31 (Table 2.8).Based on these results, it is apparent that BarOptima is the best forage choice to provide greater cow body weight throughout the year and improved average daily gains. BarOptima prevented weight loss over the course of the grazing seasons while Kentucky 31 had weight loss in the autumn. Watson et al. (2004) reported spring-calving cows grazing novel endophyte infected tall fescue pastures had greater ADG from March to September, than cows on toxic endophyte-infected tall fescue pastures. Although this research reports a negative average daily gain in the autumn Caldwell et al. (2009) concluded that a fall calving season is the most profitable option when cows are grazing toxic endophyte-infected tall fescue.

Effect of Genotype

Cow genotype for tolerance (AA alleles) or susceptibility (GG alleles) to tall fescue toxicosis was also evaluated for the parameters discussed above. Tolerant cows grazing Kentucky 31 had 19 kg greater (P<0.01; Table 2.9) pre-calving body weight compared to susceptible cows grazing Kentucky 31. Tolerant cows on the Kentucky 31 pastures also had hair coat scores that were not different than either genotype grazing BarOptima (P>0.11).

These results agree with the limited research evaluating the DRD2 genotype in cattle and the response to tall fescue toxicosis. Campbell et al. (2014) reported a relationship between the DRD2 gene and hair coat scores and serum prolactin concentrations. Susceptible genotyped steers were reported to have decreased serum prolactin concentrations and increased hair coat scores compared to tolerant genotyped steers when grazing toxic endophyte infected tall fescue. Ely (2014) evaluated differences in DRD2 genotype, along with another genotype, and found that tolerant or susceptible animals genotyped using the DRD2 gene only differed in hair coat score. Research evaluating bulls genotyped using the DRD2 gene found that susceptible bulls gained less weight regardless of the ergot alkaloid concentration of the tall fescue cultivar (Jennings, 2016).

Calf performance

Effect of Cultivar

Birth weight, calf weight in autumn, and autumn average daily gain did not differ ($P \geq 0.17$; Table 2.8) whether dams grazed BarOptima or Kentucky 31. The latter two responses are likely due to the age of the calves (fall calving) during this time. Curtis and Kallenbach (2007) evaluated cow-calf data from a fall-calving herd and found calf average daily gains and body weight were unaffected by toxic endophyte levels in stockpiled tall fescue during the winter. In similar research, Waller et al. (1988) reported that gains from fall-calving calves was not affected when cows grazed toxic endophyte-infected tall fescue during the winter.

During spring grazing, calves on BarOptima had 0.5 kg d^{-1} greater ADG than calves on Kentucky 31 ($P<0.01$; Table 2.3). In addition, grazing BarOptima pastures resulted in greater ($P<0.01$) 205-day adjusted weaning weights compared to calves on Kentucky 31. Calves weaned from the BarOptima pastures were approximately 35 kg heavier than calves weaned on the Kentucky 31 pastures (Table 2.9). Overall, BarOptima pastures produced an average of 57 more (*P*<0.01) kilograms of beef per hectare than the Kentucky 31 pastures.

Watson et al. (2004) reported calves raised on novel endophyte tall fescue pastures had higher ADG and weaning weights than calves on toxic pastures in Georgia. Parish et al. (2003) found that stocker cattle consuming novel endophyte infected tall fescue had higher ADG in spring and autumn.

Effect of Genotype

Cow genotype resulted in no differences in calf birth weight (*P*=0.64), calf autumn ADG (*P*=0.17), or end of grazing calf body weight (*P*=0.30). In addition, calves from tolerant and susceptible cows grazing Kentucky 31 did not differ in ADG (*P*>0.10) in the spring grazing period. However, 205-day adjusted weaning weights of calves from tolerant cows were 25 kg heavier than calves weaned from susceptible cows (*P*<0.01; Table 2.8) when grazing Kentucky 31. As expected, cow genotype had no effect (*P*>0.10) on 205-day adjusted weaning weight when grazing BarOptima pastures.

Tolerant cows grazing BarOptima produced 394 kg beef ha^{-1}, which was greater (*P*<0.01; Table 2.8) than beef produced on Kentucky 31 pastures, regardless of whether cows were tolerant or susceptible. Tolerant genotyped animals grazing Kentucky 31 were not significantly different from susceptible animals grazing BarOptima (*P*=0.20). Calves raised by susceptible genotyped cows grazing Kentucky 31 produced the lowest gains of all treatments with 306 kg beef ha^{-1} which differed (P=0.07) from the calves raised by tolerant genotyped cows grazing Kentucky 31.

The 205-day weaning weight and amount of beef produced per hectare is directly related to the profitability of the herd for producers. By using the DRD2 genotype on toxic endophyte-infected tall fescue pastures the producers has the potential to increase profits, if novel endophyte-infected tall fescue pastures are not an option. The results provided above are reported on calves prior to weaning compared to other research on the DRD2 genotype that has been conducted on stocker calves or bulls. Ely (2014) evaluated the effects of dam and calf genotype on adjusted birth weight and adjusted 205-day weight but the research utilized more than one genotype and cattle were grazing only toxic tall fescue. Results did not show differences in birth weight or 205-d weaning weight when comparing animals with different genotypic classifications for the DRD2 gene.

CONCLUSION

In general, cow-calf pairs grazing BarOptima pastures outperformed cow-calf pairs grazing toxic endophyte infected Kentucky 31. This is primarily due to the low toxin levels that resulted in greater intake of BarOptima. Under the conditions of this experiment, it appears that novel endophyte infected tall fescue is a preferred solution for tall fescue toxicosis but the DRD2 genotype affected performance when cattle grazed toxic Kentucky 31. Ultimately, if renovation of toxic tall fescue pastures into novel varieties is not an option the DRD2 genotype or a similar genetic test can help recover some of the losses due to the negative effects associated with tall fescue toxicosis.

Table 2.1. Start and end of grazing in each pasture each year of the experiment.[1]

		2011[2]		2012[2]		2013	
Pasture	Fescue	Start	End	Start	End	Start	End
1	BO	4/7/2011	11/27/2011	3/29/2012	11/13/2012	4/17/2013	11/25/2013
2	KY	4/7/2011	12/7/2011	3/29/2012	11/19/2012	4/17/2013	12/9/2013
3	BO	4/7/2011	11/4/2011	3/29/2012	11/15/2012	4/17/2013	11/21/2013
4	KY	4/7/2011	12/5/2011	3/29/2012	11/15/2012	4/17/2013	12/3/2013
5	BO	4/7/2011	11/13/2011	3/29/2012	11/20/2012	4/17/2013	11/25/2013
6	KY	4/7/2011	12/4/2011	3/29/2012	11/19/2012	4/17/2013	12/9/2013

[1] Cattle were comingled on toxic tall fescue pastures when not on study and fed hay when necessary.
[2] Years with a period of hay fed in the summer months.

Table 2.2. Calibration statistics for near-infrared spectrophotometric determination of nutritive value in BarOptima and Kentucky 31 tall fescue pastures.

Constituent	n	R^2	Mean	SEC‡	SECV‡‡	1 – VR†
			g kg^{-1} DM			
Crude protein	68	0.99	185	5.9	8.3	0.97
Neutral detergent fiber	69	0.96	536	15.3	17.9	0.94
In vitro true digestibility	67	0.91	814	21.7	25.5	0.88

†1 – VR = 1 minus the variance ratio (calculated in cross-validation in modified partial least squares regression).
‡SEC = SE of calibration calculated in modified partial least squares regression.
‡‡SECV = SE of cross-validation calculated in modified partial least squares regression.

Table 2.3. ANOVA for forage nutritive value and toxicity of BarOptima or Kentucky 31 tall fescue pasture.

Source	d.f.	Probability > F test			
		Total ergot alkaloids	Crude protein	Neutral detergent fiber	In vitro true digestibility
Cultivar	1	<0.0001	0.0002	0.0089	<0.0001
Block	2	0.9560	0.1957	0.7012	0.7319
Cultivar*Block	2	0.3911	0.8477	0.3365	0.2279

Table 2.4. ANOVA for forage performance of BarOptima or Kentucky 31 tall fescue pasture.

		Probability > F test		
Source	d.f.	Forage accumulation	Hay balance	Animal grazing days
Cultivar	1	0.0590	0.0011	0.0011
Block	2	0.0016	0.1032	0.2883
Cultivar*Block	2	0.8585	0.7833	0.1823

Table 2.5. ANOVA for tolerant or susceptible cow performance while grazing BarOptima or Kentucky 31 tall fescue pasture.

		Probability > F test						
Source	d.f.	Spring ADG	Summer ADG	Autumn ADG	Weaning weight	Pre-calving weight	End of grazing weight	Hair coat score
Treatment[1]	3	<0.0001	0.2709	0.1146	<0.0001	0.0001	<0.0001	0.0008
Block	2	0.2694	0.0690	0.1230	0.3677	0.0272	0.0282	0.7093
Treatment* Block	6	0.2844	0.1710	0.9266	0.2722	0.8606	0.9873	0.7344

[1] Treatment is the combination of tolerant or susceptible cows on BarOptima or Kentucky 31 tall fescue.

Table 2.6. ANOVA for calf performance when raised by tolerant or susceptible cows grazing BarOptima or Kentucky 31 tall fescue pasture.

Source	d.f.	Probability > F test					
		Birth weight	Autumn ADG	End of grazing weight	Spring ADG	205-d Adj. weaning weight	Beef produced
Treatment[1]	3	0.6389	0.1699	0.3023	<0.0001	0.0002	0.0050
Block	2	0.5387	0.7623	0.8784	0.2233	0.5018	0.7576
Treatment* Block	6	0.2706	0.1305	0.8046	0.1457	0.0075	0.1256

[1] Treatment is the combination of tolerant or susceptible cows on BarOptima or Kentucky 31 tall fescue.

Table 2.7. Least squares means for forage nutritive value, forage accumulation, hay balance, and animal grazing days for BarOptima or Kentucky 31 tall fescue pasture.

Item	Treatment		SEM	*P*-value
	BO	KY-31		
Forage nutritive value				
Crude protein, g kg^{-1}	200	187	4	<0.01
Neutral detergent fiber, g kg^{-1}	514	528	5	<0.01
In-vitro true digestibility, g kg^{-1}	837	817	5	<0.01
Total ergot alkaloids, µg kg^{-1}	172	2537	108	<0.01
Forage measurements				
Forage accumulation, kg ha^{-1}	8,559	9,082	245	0.06
Hay balance, kg ha^{-1}	-511	424	10	<0.01
Animal grazing days, d ha^{-1}	233	267	12	<0.01

[1] Forage nutritive value was determined using NIR and averaged over the entire year.
[2] Total ergot alkaloids were determined by analyzing a composite forage samples from each pasture in a month.
[3] Forage accumulation was calculated using forage height data collected with the ultrasonic reader and calibration samples.
[4] Hay balance is the amount of hay produced on the pasture minus purchased hay fed. A negative number means all hay produced was fed and additional hay was needed.
[5] Animal grazing days was calculated by dividing number of days livestock grazed by the stocking rate.

Table 2.8. Performance of susceptible and tolerant fall-calving cow-calf pairs grazing BarOptima or Kentucky 31 tall fescue pasture.

	Treatment					
Item	BO-T	BO-S	KY-T	KY-S	SEM	*P*-value
Cow measurements						
Weaning BW, kg	622^{a}	621^{a}	592^{b}	586^{b}	7.5	<0.01
Pre-calving BW, kg	694^{a}	697^{a}	663^{b}	644^{c}	10.5	<0.01
End of grazing BW, kg	692^{a}	698^{a}	634^{b}	624^{b}	9.1	<0.01
Spring ADG, kg d^{-1}	0.96^{a}	0.95^{a}	0.30^{b}	0.15^{b}	0.29	<0.01
Summer ADG, kg d^{-1}	0.79	0.78	0.76	0.63	0.11	0.27
Autumn ADG, kg d^{-1}	-0.04	0.00	-0.30	-0.18	0.14	0.11
Hair coat score	2.4^{a}	2.0^{a}	2.3^{a}	2.9^{b}	0.14	<0.01
Calf measurements						
Birth BW, kg	35.4	37.4	35.2	34.6	2.0	0.64
End of grazing BW, kg	108	103	117	97	7.3	0.30
205-d Adj. WW, kg	247^{a}	245^{a}	223^{b}	198^{c}	11.5	<0.01
Autumn ADG, kg d^{-1}	1.23	1.20	1.16	0.98	0.08	0.17
Spring ADG, kg d^{-1}	1.47^{a}	1.39^{a}	0.96^{b}	0.91^{b}	0.13	<0.01
Beef produced, kg ha^{-1}	394^{a}	369^{ab}	344^{b}	306^{c}	18.0	<0.01

$^{a-c}$ Least squares means within a row with different superscripts differ (P<0.10).
BO-T = tolerant genotyped cows grazing BarOptima, BO-S = susceptible genotyped cows grazing BarOptima,
KY-T = tolerant genotyped cows grazing Kentucky 31, KY-S = susceptible genotyped cows grazing Kentucky 31
ADG = average daily gain; BW = body weight; WW = weaning weight
[1] Autumn ADG was from birth to end of grazing in autumn.
[2] Spring ADG, 205-day weaning weight, and beef produced was calculated using data from 2012 and 2013.
[3] Cow weight was adjusted so all animals were equal at the start of the study across all three years. This eliminates year-to-year variations in starting weight. The adjusted cow weight values were then used to calculate average daily gain.
[4] Hair coat scores were evaluated on a scale from 1 to 5 (with 1 being a slick and 5 being a rough hair coat).

Figure 2.1. Layout of the research area with pastures of BarOptima or Kentucky 31 at the University of Missouri Southwest Research Center in Mt. Vernon, MO.

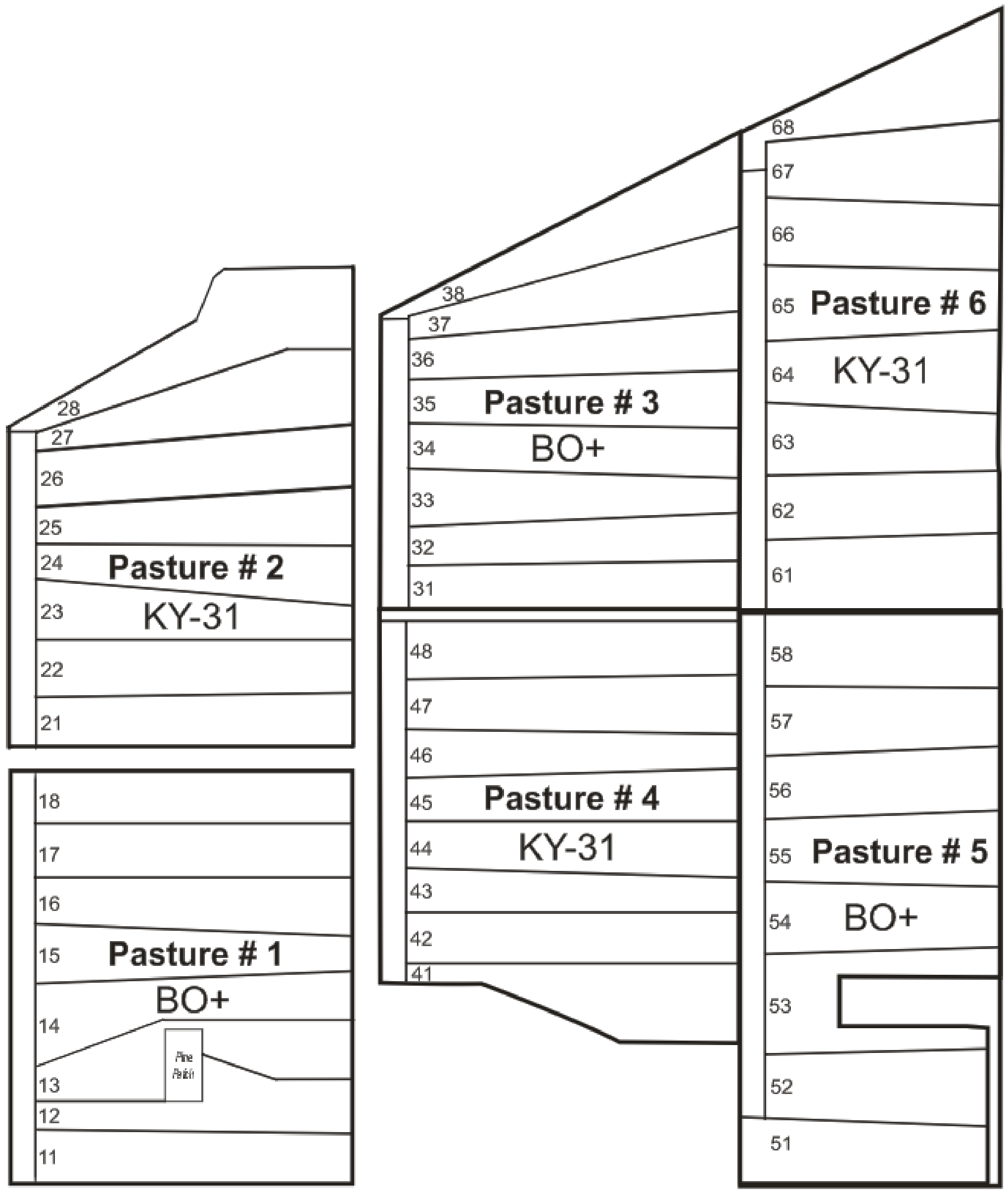


Figure 2.2. Three year averages for monthly crude protein of BarOptima and Kentucky 31 during the grazing season.

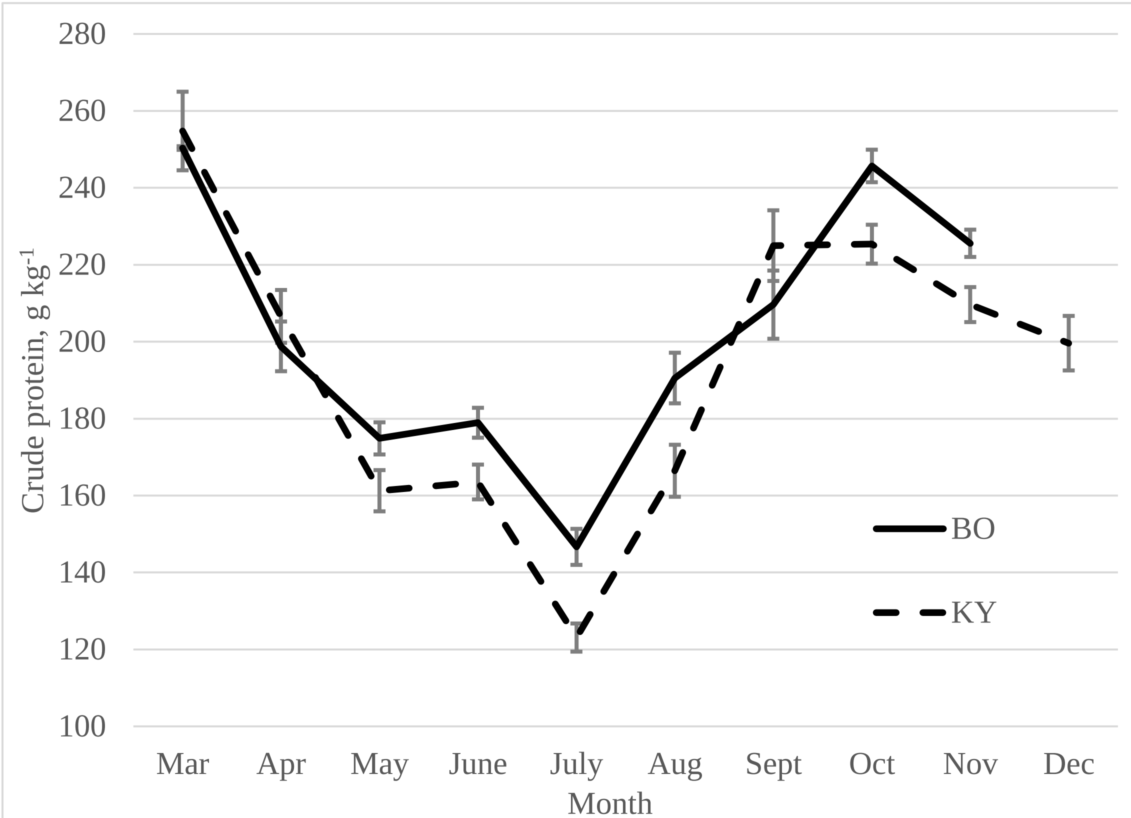


Figure 2.3. Three year averages for monthly neutral detergent fiber of BarOptima and Kentucky 31 during the grazing season.

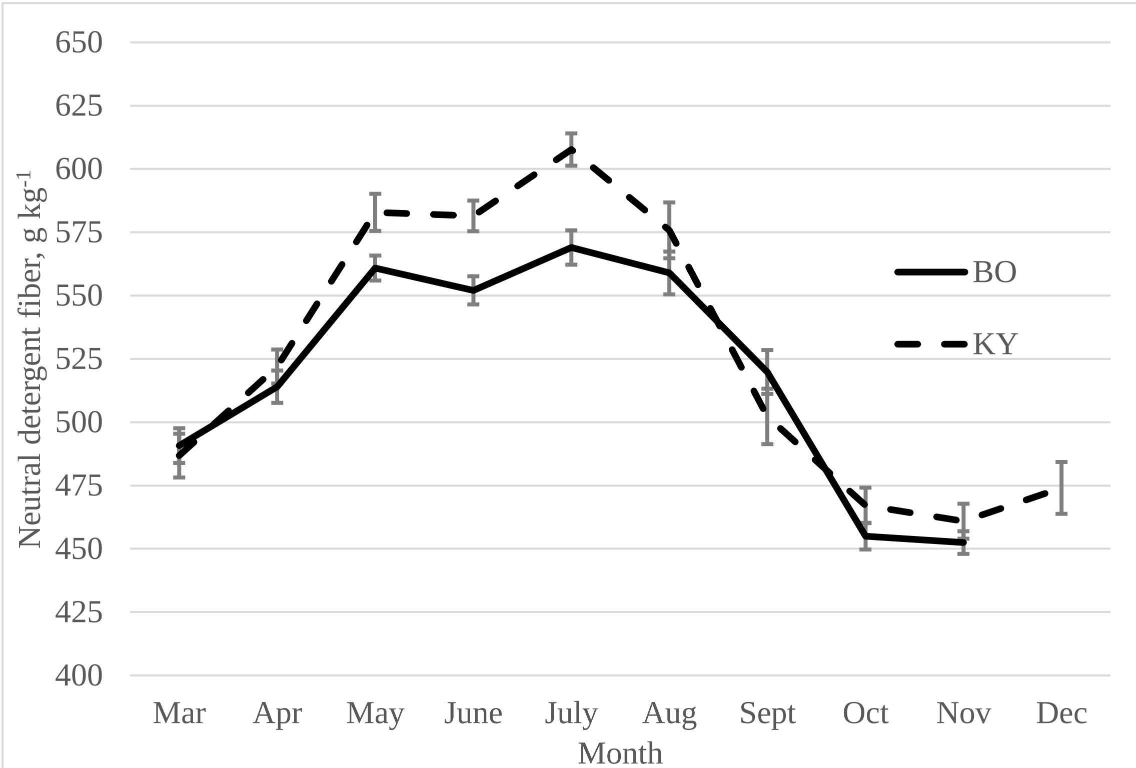


Figure 2.4. Three year averages for monthly in vitro true digestibility of BarOptima and Kentucky 31 during the grazing season.

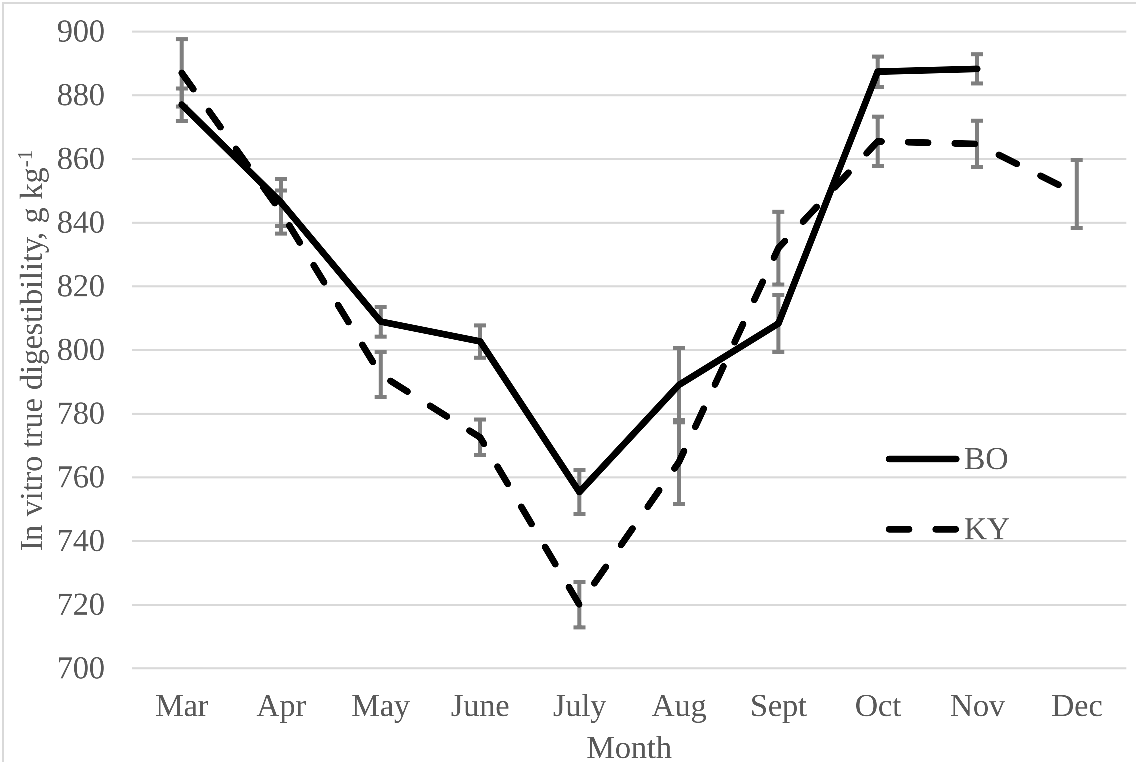
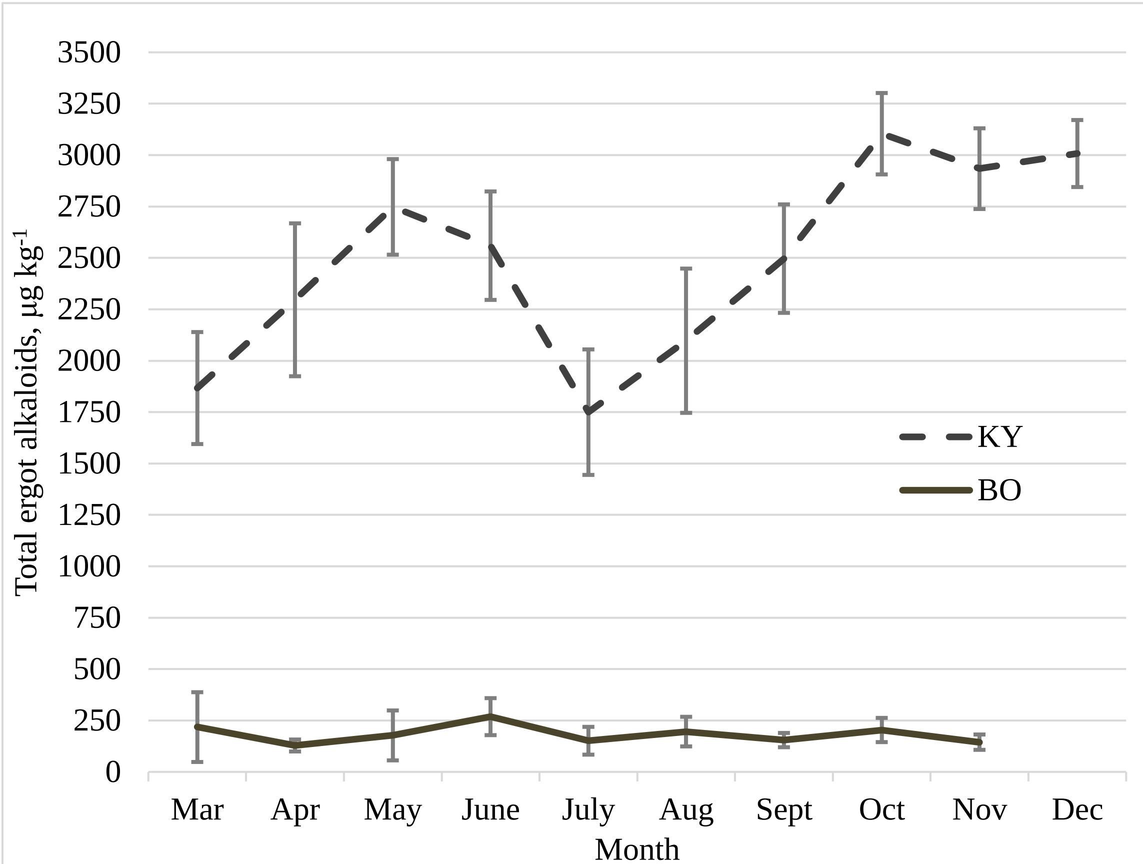


Figure 2.5. Three year averages for monthly total ergot alkaloids of BarOptima and Kentucky 31 tall fescue pastures.



CHAPTER 3. PERFORMANCE OF GENOTYPED FESCUE TOLERANT OR SUSCEPTIBLE BEEF HEIFERS FED BAROPTIMA OR KENTUCKY 31 TALL FESCUE

ABSTRACT

Cattle appear to have genetic adaptations at the dopamine receptor D2 (DRD2) gene that improve tolerance to ergot alkaloids produced by tall fescue (*Schedonorus arundinaceus* (Schreb.) Dumort.) infected with wild-type *Epichloë coenophiala* [(Morgan-Jones & W. Gams) C.W. Bacon & Schardl, comb. nov.]. The objective of this experiment was to determine the performance of heifers selected for tolerance or susceptibility to ergot alkaloids when fed toxic or non-toxic tall fescue baleage. Forage intake, body weight and physical characteristics were monitored on heifers (initial BW = 270 ± 37 kg) for 70 days while fed in a GrowSafe feeding system. Ergovaline levels averaged 52 ± 6 μg kg^{-1} in the novel and 283 ± 93 μg kg^{-1} in the toxic endophyte-infected tall fescue diets. Heifers consuming non-toxic tall fescue had increased (P<0.01) average daily gain, greater (P=0.03) feed intake, lower (P<0.01) feed:gain ratio, lower (P<0.01) hair coat score, and lower (P<0.01) serum prolactin levels when compared to heifers fed toxic tall fescue. There was no difference (P=0.13) in dry matter intake as a percent of body weight between the tolerant heifers fed toxic tall fescue and the tolerant heifers fed non-toxic tall fescue. Similarly, there was no difference (P=0.06) in body condition score between tolerant heifers fed toxic tall fescue and susceptible heifers on non-toxic tall fescue. These results show evidence that the DRD2 genotype for ergot alkaloid tolerance

is beneficial but consumption of tall fescue producing low levels of ergot alkaloids resulted in the greatest intake and average daily gains for beef heifers.

INTRODUCTION

Tall fescue [*Schedonorus arundinaceus* (Schreb.) Dumort.] is a popular perennial cool-season grass planted in improved pastures across the United States. Tall fescue is widely used because of its symbiotic relationship with the fungus *Epichloë coenophiala* [(Morgan-Jones & W. Gams) C.W. Bacon & Schardl, comb. nov.], which helps the plant tolerate abiotic and biotic stresses associated with a wide range of environmental and management conditions (Latch, 1993; Malinowski and Belesky, 2000; West et al., 1993). While the endophyte provides tall fescue with a variety of benefits related to plant persistence, it also produces ergot-like alkaloids that are harmful to livestock. The combination of negative effects in livestock associated with ergot alkaloid consumption is known as tall fescue toxicosis, which costs the US livestock industry nearly $2 billion annually (Hoveland, 1993; USDA Agricultural Marketing Service, 2015).

A diverse set of management solutions related to reducing or diluting ergot alkaloids in livestock diets exists. Renovation of pastures containing toxic fescue to novel endophyte-infected tall fescue results in little to no ergot alkaloid consumption and should be considered non-toxic to livestock (Bouton et al., 2002). Dilution of tall fescue with a feed supplement or clover has also been shown to help reduce the effects or tall fescue toxicosis (Elizalde et al., 1998; Thompson et al., 1993). Alkaloid management, which involves managing the pastures for alkaloids rather than yield, is another tool to reduce the effects of tall fescue toxicosis. Alkaloids contain nitrogen, and nitrogen fertilization has been found to increase alkaloid concentrations (Rottinghaus et al., 1991). By managing the alkaloids available to livestock, producers can reduce potential toxicity by two thirds and possibly eliminate it (Roberts and Andrae, 2010). However, livestock that are tolerant to ergot alkaloids could fill an important void, where the replacement of fescue or dilution of ergot alkaloids in the diet is not feasible or practical.

Recent genetic discoveries may allow genetic selection of cattle tolerant to tall fescue toxicosis. A single nucleotide polymorphism in the dopamine receptor D2 (DRD2) gene was evaluated by Campbell et al. (2014) for the ability to select cattle that show tolerance to tall fescue toxicosis. The DRD2 gene is located on the bovine chromosome 15 and has been found to be involved in prolactin secretions (Civelli et al., 1993). Steers genotyped as susceptible had decreased serum prolactin concentrations and increased hair coat scores compared to steers classified as tolerant when grazing toxic tall fescue pastures (Campbell et al., 2014). Ely (2014) also found the DRD2 genotype was associated with serum prolactin concentrations, hair coat score, and body condition score. Susceptible genotyped bulls have been shown to have nearly two-fold greater ergot alkaloid concentrations in urine samples compared to tolerant bulls consuming toxic endophyte-infected tall fescue (Jennings, 2016).

The following research compares genotyped heifers while grazing either novel or toxic endophyte-infected tall fescue, while animals in previous research consumed only toxic tall fescue. The objective of this research was to compare animal intake, weight gain, body condition score, hair coat score, body temperature, and serum prolactin levels of tolerant or susceptible genotyped heifers fed non-toxic BarOptima or toxic Kentucky 31 tall fescue diets. The hypothesis was that tolerant genotyped heifers consuming

Kentucky 31 would perform similar to either genotype heifer consuming BarOptima and better than susceptible heifers consuming Kentucky 31.

MATERIALS AND METHODS

The University of Missouri Animal Care and Use Committee approved protocol #7820 for the use of animals in these research experiments.

Experimental animals

This experiment utilized Angus-cross heifers. There were 48 heifers used in year one (initial BW = 266 ± 35 kg), 57 heifers in year two (initial BW = 281 ± 38 kg) and 56 heifers in year three (initial BW = 262 ± 35 kg). Heifers were raised at the University of Missouri Southwest Research Center in Mount Vernon, MO, Forage Systems Research Center in Linneus, MO, and Beef Research and Teaching Farm in Columbia, MO.

Heifers were genotyped prior to the start of research, and classified as tolerant or susceptible to tall fescue toxicosis using the single nucleotide polymorphism in the DRD2 gene as outlined by Campbell et al. (2014). Heifers with AA and GG alleles were used and determined to be tolerant or susceptible, respectively. In the first year there were 22 tolerant and 18 susceptible heifers. There were also 8 heifers which were later determined to be heterozygous (AG) for the DRD2 genotype and were not used in the data analysis. The second year had 24 tolerant and 33 susceptible heifers. The third year had 28 tolerant and 28 susceptible heifers.

Heifers were stratified by weight and previous diet. The stratification ensured that heifers previously grazing on toxic and non-toxic endophyte infected pastures were evenly distributed throughout the pens.

Diet and feeding management

The experiment was conducted at the University of Missouri Southwest Research Center in Mount Vernon, MO. This location contains a GrowSafe feeding system (Model 4000E, GrowSafe Systems Ltd., Airdrie, AB, Canada). The GrowSafe feeding system consists of 8 pens (7.3 x 26 m) with a total of 16 GrowSafe feed bunks and 4 Mirafount livestock waterers (Figure 3.1). Sawdust shavings were used for bedding in the pens near the feeders underneath the barn roof. Sawdust shavings were removed and replenished when they became soiled.

Baleage fed in this experiment was harvested from BarOptima and Kentucky 31 pastures located at the Southwest Research Center in Mount Vernon, MO. Toxic and novel endophyte-infected tall fescue was harvested in late May each year. The baleage was sampled after at least 30 days of ensiling was completed. Baleage samples were analyzed for dry matter, crude protein, neutral detergent fiber, acid detergent fiber, total digestible nutrients, and ergovaline concentration (Table 3.1). Forage nutritive value analysis was conducted at Custom Laboratory in Golden City, MO. Ergovaline concentration was determined using the HPLC methods by the Veterinary Medicine Diagnostics Lab at the University of Missouri (Rottinghaus et al., 1991). Total ergot alkaloid concentrations were determined by using the procedure from Agrinostics Ltd. Co. (Watkinsville, GA). In the third year of research, ergovaline levels were lower than the previous two years and on the low end of the toxicity range. Interestingly, total ergot alkaloid levels were similar in the first and third year of the study.

Diets consisted of tall fescue baleage, supplement, and corn (Table 3.2). Each year diets were balanced for energy and protein to promote a growth rate of approximately 0.5 kg d^{-1} using the Beef Ration and Nutrition Decision Software (BRaNDS, Iowa Beef Center, Ames, IA).

Research began in the last week of June or first week of July each year. In order to minimize the residual effects of the previous diet, heifers were fed an acclimation diet of novel endophyte-infected tall fescue baleage for 14 days. Acclimation diets were restricted to 14 days due to the limited availability of novel endophyte-infected tall fescue baleage. Following the acclimation diet, heifers were offered a diet consisting of toxic or novel endophyte-infected tall fescue.

Heifers were provided ad libitum access to either a toxic or novel tall fescue baleage diet during the trial. Feedings occurred once or twice daily depending on consumption. During each feeding, remaining feed in the GrowSafe bunks was mixed with the fresh feed to ensure minimal spoilage. GrowSafe bunks were cleaned weekly.

Data collection and sample analysis

Animal intake was measured continuously by the GrowSafe feeding system for 70 days. Heifer weights were recorded on two consecutive days at the beginning of the study, once after 35 days at the mid-point, and two consecutive days at the end of the study. Heifers were not held off feed or water for weighing. Metabolic average daily gain was calculated by using the start and end body weight to the 0.75 power and dividing by the number of days on feed. Body condition score (1 to 9 scale; Wagner et al. (1988)) and hair coat score (1 to 5 scale; Tucker and Ringer (1982)) were recorded on each heifer at the beginning and end of the trial. Body temperatures were measured on each heifer at the conclusion of the trial. Temperatures were determined using a thermometer (Model V966, Vicks, Procter & Gamble Company, Cincinnati, OH) in the rectum. Blood samples were collected from every heifer at the conclusion of the study via coccygeal venipuncture. Samples were collected into evacuated blood collection vials and stored on ice for approximately 1 hour. Blood samples were then centrifuged at 2,000 x g, for 10 minutes to separate the serum that was then collected and frozen until the analysis for prolactin using the radioimmunoassay procedure as previously described by Bernard et al. (1993). Prolactin analysis was performed in triplicate and averaged for each sample for statistical analysis.

Statistical analysis

Data were analyzed using the PROC GLIMMIX procedure of SAS 9.4 (SAS Inst. Inc., Cary, NC, USA). The models for animal performance contained treatment, average daily gain, and the interaction of treatment and average daily gain. Treatment is the combination of tall fescue type and animal genotype. The average daily gain variable was determined by dividing the treatments into four groups from high to low average daily gain. Year was a random effect. For each variable, observations outside two standard deviations from the mean were removed from the analysis. Least squares means were estimated using the LSMEANS statement and when significant effects were detected in the model ($P \leq 0.10$) the LINES option was used for mean separation.

RESULTS AND DISCUSSION

Feed intake

Kentucky 31 resulted in heifers that had lower (*P*=0.03) dry matter intake compared to heifers consuming BarOptima. Tolerant and susceptible heifers consuming BarOptima had intake rates of 6.26 and 6.68 kg d^{-1}, respectively. Tolerant and susceptible heifers fed Kentucky 31 had intakes of 5.90 and 5.79 kg d^{-1}, respectively (Table 3.3). This suggests tall fescue toxicosis is affecting feed intake. When evaluating dry matter intake as a percent of body weight, the tolerant heifers consuming Kentucky 31 did not differ (*P*=0.23) from tolerant heifers consuming BarOptima. Aldrich et al. (1993) reported a 10% reduction in intake of cattle consuming toxic endophyte infected tall fescue. It is possible that differences in intake could be attributed to the physical qualities of the Kentucky 31 and BarOptima diets. BarOptima is a soft-leaf tall fescue cultivar and that could influence intake. Grazing cattle favored BarOptima over toxic tall fescue and other novel endophyte-infected tall fescues (Kenyon, 2017). Another study reported steers grazing toxic tall fescue had decreased intake and spent less time grazing compared to steers grazing endophyte-free tall fescue (Stuedemann et al., 1989).

Average daily gain

BarOptima had increased (*P*<0.01) heifer average daily gain compared to heifers fed Kentucky 31, but no differences (P=0.88 and 0.98, respectively) were seen between genotypes of the heifers on BarOptima or Kentucky 31. BarOptima consumption increased (*P*<0.01) metabolic average daily gain compared to Kentucky 31. Reduced average daily gain is a common effect of tall fescue toxicosis and has been shown in many studies to occur while grazing Kentucky 31 (Crawford et al., 1989; Hoveland et al., 1983; Nihsen et al., 2004).

Kentucky 31 resulted in an increased (P<0.01) feed:gain ratio compared to heifers consuming BarOptima. Lower feed:gain implies that heifers consumed less feed per unit of gain. Feed:gain ratio is a measure of the efficiency of the animal to convert feed into increased body weight. Tolerant and susceptible heifers fed Kentucky 31 had no difference (P=0.52) in feed:gain ratio. Tolerant heifers consuming BarOptima had lower (P<0.01) feed:gain ratio than susceptible heifers fed BarOptima. Tolerant heifers fed BarOptima had a feed:gain ratio of 8.82, which was the least of all treatments.

Body temperature, body condition score, and hair coat score

Kentucky 31 resulted in heifers having greater (P=0.02; Table 3.3) body temperature than heifers fed BarOptima, and this occurred for both tolerant and susceptible genotyped heifers. Similarly, Rhodes et al. (1991) reported steers receiving high endophyte-infected diets had elevated rectal temperatures compared to steers on low endophyte level diets. Increases in body temperature are due to the constrictive effects that the ergot alkaloids have on the vasculature of the animal, which reduces the blood flow to the periphery (Klotz et al., 2008). Reduced blood flow has also been shown to increase rectal temperatures and hair coat scores (Aiken et al., 2001).

BarOptima consumption decreased (P<0.01) hair coat scores compared to heifers consuming Kentucky 31. No differences (P=0.79) in hair coat score were discovered between genotyped heifers fed Kentucky 31. Campbell et al. (2014) evaluated hair coat scores while grazing toxic tall fescue pastures and reported susceptible steers had higher hair coat scores compared to the tolerant genotype. Cattle with lower hair coat scores are likely able to dissipate heat quicker, which may help reduce the heat stress on the animal during the summer months. Olson et al. (2003) reported that cattle with short, slick hair coats maintained a lower rectal temperature and improved thermotolerance.

BarOptima resulted in heifers with greater (*P*<0.01) body condition scores compared to heifers consuming Kentucky 31. No difference (P>0.10) were discovered between body condition scores of tolerant or susceptible heifers consuming either BarOptima or Kentucky 31. Susceptible heifers fed Kentucky 31 had the lowest reported body condition score (Table 3.3). It is likely that reduced intake and decreased daily gains also results in the lower body condition scores for the heifers. Body condition score has been reported to be negatively affected by toxic endophyte-infected tall fescue. Watson et al. (2004) showed that body condition scores of cows grazing non-ergot alkaloid producing tall fescue pastures were higher at the end of the grazing period compared to wild type endophyte-infected tall fescue.

Serum prolactin

Kentucky 31 resulted in heifers with lower (*P*<0.01; Table 3.3.) serum prolactin levels than heifers consuming BarOptima. Decreases in serum prolactin concentration in beef cattle are commonly identified as a side effect of tall fescue toxicosis (Fribourg et al., 1991; Hoveland et al., 1983; Hurley et al., 1981; Parish et al., 2003; Rice et al., 1997). Kentucky 31 consumption resulted in heifers with an average serum prolactin concentration of 2.85 ng ml^{-1} compared to an average of 16.3 ng ml^{-1} for heifers consuming BarOptima (Table 3.3). Prolactin is involved with the shedding of the winter hair coat in cattle (McClanahan et al., 2008), which explains the lower hair coat score for heifers consuming Kentucky 31. Serum prolactin concentration did not differ (*P*=0.32) between genotype when heifers consumed BarOptima, which is expected. The BarOptima ration had an average ergovaline level of 52 μg kg^{-1}. That ergovaline concentration is well below the threshold of 400 to 750 μg kg^{-1} that has been reported to produce clinical symptoms of tall fescue toxicosis in cattle by Aldrich-Markham et al. (2007). BarOptima ergovaline concentration was also below the threshold of 150 μg kg^{-1} reported by Stamm et al. (1994). Susceptible heifers did not differ (P=0.58) in serum prolactin concentration from the tolerant heifers on Kentucky 31. It is possible that the negative effects of high environmental temperature during the summer months of this study overwhelmed any positive effect of cattle genotype (Campbell et al., 2014). These researchers evaluated steers consuming toxic tall fescue and found that susceptible steers had decreased serum prolactin concentrations compared to tolerant steers in May but not in June. In a second study, susceptible steers had lower prolactin concentrations and an increase in hair coat scores compared to the tolerant steers when consuming toxic tall fescue (Campbell et al., 2014).

CONCLUSION

Heifer intake, gain, body temperature, hair coat, and serum prolactin did not differ between genotype. This could be due to the fact that only one gene was used to determine tolerance. DRD2 genotype by itself shows an inconsistent response. Depending on environmental factors DRD2 gene is often important but there are likely other complementary genes that regulate the responses to the alkaloids. The amount of time heifers were exposed to the different diets may have limited the amount of response as well. Future research and analysis needs to be conducted to determine the complementary genes effects on gain and other physiological characteristics.

Table 3.1. Diets fed to heifers in the GrowSafe feeding system.

Diet composition for BarOptima[1]			
Ingredients	2013	2014	2015
Fescue baleage, %	94.0	98.5	93.5
Supplement, % [2]	6.0	1.5	4.0
Corn, %	0.0	0.0	2.5
Diet composition for Kentucky 31[1]			
Ingredients	2013	2014	2015
Fescue baleage, %	94.0	95.0	93.5
Supplement, % [2]	6.0	5.0	6.5
Corn, %	0.0	0.0	0.0

[1] Decoquinate [Deccox 10X (Consumers Supply Distributing, LLC, North Sioux City, SD] was added at a rate of 0.5 mg kg^{-1} d^{-1} for the duration of the research. Deccox® is a registered trademark of Zoetis, Inc. (Parsippany, NJ).

[2] Supplement consisted of 62% distillers grain, 36% soybean hull pellets, 1% triple 12 mineral (Producers Cooperative Association, Girard, KS), and 1% magnesium oxide (Feed Products and Service Company, St. Louis, MO)

Table 3.2. Analysis of tall fescue baleage fed to heifers in the GrowSafe feeding system.

BarOptima			
Baleage analysis[1], DM basis	2013	2014	2015
Dry matter, g kg^{-1}	537	585	543
Crude protein, g kg^{-1}	94	152	139
Neutral detergent fiber, g kg^{-1}	697	576	631
Acid detergent fiber, g kg^{-1}	434	344	411
Total digestible nutrients, g kg^{-1}	558	596	540
Ergovaline, μg kg^{-1}	60	47	50
Total ergot alkaloids, μg kg^{-1}	287	NR[2]	222
Kentucky 31			
Baleage analysis[1], DM basis	2013	2014	2015
Dry matter, g kg^{-1}	499	604	494
Crude protein, g kg^{-1}	98	145	133
Neutral detergent fiber, g kg^{-1}	697	613	639
Acid detergent fiber, g kg^{-1}	435	371	412
Total digestible nutrients, g kg^{-1}	576	574	540
Ergovaline, μg kg^{-1}	320	373	155
Total ergot alkaloids, μg kg^{-1}	2196	NR[2]	2157

[1] Forage nutritive value samples were collected from baleage after completion of ensiling process.
[2] Results not reported.

Table 3.3. Performance of genotyped heifers fed BarOptima or Kentucky 31 tall fescue diets in the GrowSafe feeding system.

	Treatment[1]					
Item	BO-T	BO-S	KY-T	KY-S	SEM	*P*-value
Average daily gain, kg d^{-1}	0.72^{a}	0.71^{a}	0.55^{b}	0.55^{b}	0.01	<0.01
Metabolic ADG[2], kg d^{-1}	0.13^{a}	0.13^{a}	0.10^{b}	0.10^{b}	0.002	<0.01
DM intake, kg d^{-1}	6.26^{b}	6.68^{a}	5.90^{c}	5.79^{c}	0.27	0.025
DM intake, % BW	2.11^{ab}	2.21^{a}	2.04^{bc}	2.01^{c}	0.04	<0.01
Feed:gain	8.83^{a}	9.72^{b}	12.17^{c}	11.89^{c}	0.53	<0.01

[a-c] Least squares means within a row with different superscripts differ ($P<0.10$).

[1] BO-T = tolerant genotyped heifers fed BarOptima, BO-S = susceptible genotyped heifers fed BarOptima, KY-T = tolerant genotyped heifers fed Kentucky 31, KY-S = susceptible genotyped heifers fed Kentucky 31

[2] Metabolic ADG is the weight gain to the 0.75 power divided by the number of days fed.

Table 3.4. Physical characteristics at the conclusion of research of genotyped heifers fed BarOptima or Kentucky 31 tall fescue diets in the GrowSafe feeding system.

	Treatment[1]					
Item[2]	BO-T	BO-S	KY-T	KY-S	SEM	*P*-value
Body temperature, °C	39.4[a]	39.5[ab]	39.7[b]	39.6[b]	0.18	0.02
Hair coat score[2]	2.21[a]	2.27[a]	3.07[b]	3.11[b]	0.18	<0.01
Body condition score[3]	5.86[a]	5.86[a]	5.62[b]	5.49[b]	0.23	<0.01
Serum prolactin, ng ml^{-1}	17.11[a]	15.49[a]	3.30[b]	2.40[b]	1.2	<0.01

[a-c] Least squares means within a row with different superscripts differ ($P<0.10$).

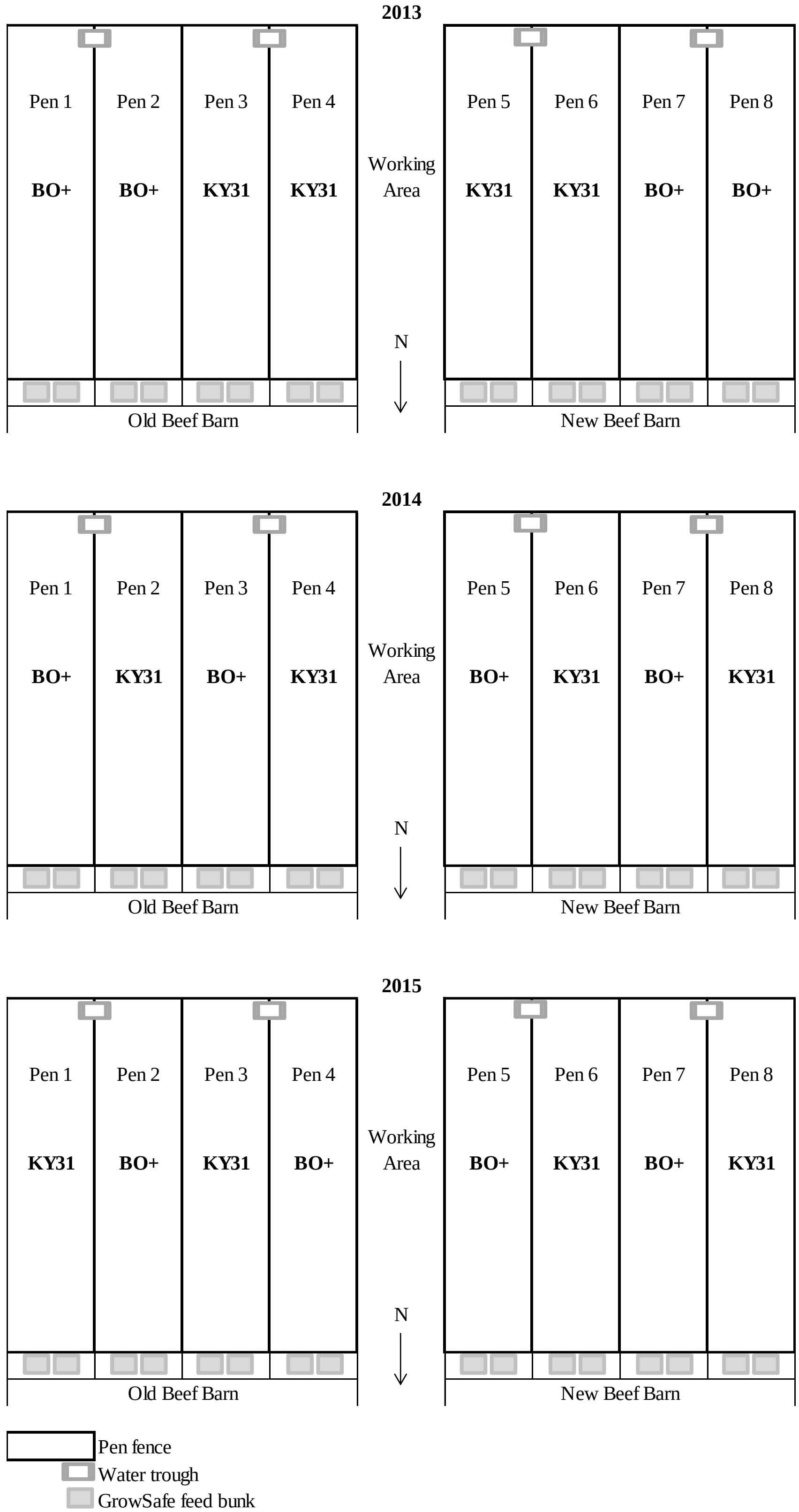
[1] BO-T = tolerant genotyped heifers fed BarOptima, BO-S = susceptible heifers fed grazing BarOptima, KY-T = tolerant genotyped heifers fed Kentucky 31, KY-S = susceptible heifers fed grazing Kentucky 31

[2] Hair coat scores were evaluated on a scale from 1 to 5 (1 - slick hair coat, 5 - rough hair coat).

[3] Body condition scores were evaluated on a scale from 1 to 9 (1 - emaciated, 9 - extremely fat).

[4] Measurements were taken at the conclusion of the research.

Figure 3.1. Diagram of the pen layout in the GrowSafe feeding system.



CHAPTER 4. IN VITRO FIBER DEGRADATION AS INFLUENCED BY ERGOT ALKALOID TOLERANT GENOTYPED BEEF CATTLE

ABSTRACT

Identifying the mechanism by which cattle tolerate exposure to ergot alkaloids will improve understanding of the pathogenesis of tall fescue toxicosis. A single nucleotide polymorphism on the dopamine receptor D2 (DRD2) gene has been identified as a marker for tolerance of ergot alkaloids produced by tall fescue (*Schedonorus arundinaceus* (Schreb.) Dumort.) infected with wild-type *Epichloë coenophiala* [(Morgan-Jones & W. Gams) C.W. Bacon & Schardl, comb. nov.]. This research was conducted to determine the effects that either novel or toxic endophyte-infected tall fescue, along with tolerant or susceptible genotyped cattle have on in vitro fermentation characteristics. Twenty-four single flow continuous culture fermenters were inoculated with rumen fluid obtained from tolerant or susceptible genotyped Angus-cross heifers consuming either novel or toxic tall fescue diets (n=6 replications per treatment combination per run). Fiber digestibility, pH, ammonia, and volatile fatty acid (VFA) concentrations were measured. Apparent digestibility of NDF, ADF, and CP did not differ ($P \geq 0.57$) between novel and toxic endophyte-infected tall fescue. Feeding novel tall fescue resulted in lower (P<0.01) true organic matter digestibility compared to toxic tall fescue. Novel tall fescue resulted in increased ($P \leq 0.01$) ammonia levels at 0 and 4 hours post feeding compared to toxic tall fescue. Novel tall fescue also showed decreased ($P \leq 0.01$) acetic acid at 0 and 4 hours post feeding, increased ($P \leq 0.07$) propionic acid at 0 and 4 hours post feeding, and increased ($P = 0.07$) butyric acid at 4 hours post feeding,

compared to toxic endophyte-infected tall fescue. No differences ($P \geq 0.34$) were found in total VFA concentration between BarOptima and Kentucky 31 diets. Under conditions of this experiment, rumen fluid from cattle expressing tolerant DRD2 genotype had little effect on rumen fermentation.

INTRODUCTION

Tall fescue [*Schedonorus arundinaceus* (Schreb.) Dumort.] is a common perennial cool season grass grown across the United States. The consumption of tall fescue infected with the wild-type endophyte, *Epichloë coenophiala* [(Morgan-Jones & W. Gams) C.W. Bacon & Schardl, comb. nov.], has negative effects on livestock performance (Bacon et al., 1977; Crawford et al., 1989; Nihsen et al., 2004; Stuedemann and Hoveland, 1988; Watson et al., 2004). The endophyte produces ergopeptine alkaloids, most notably ergovaline, the compound most commonly associated with tall fescue toxicosis (Bacon et al., 1986; Yates et al., 1985).

In response to the negative effects on livestock a novel endophyte-infected tall fescue, which produces less ergot alkaloids than the wild-type endophyte, was developed (Bouton et al., 2002). Research on novel endophyte has shown improvement in gains and reproduction in cattle compared to toxic endophyte-infected tall fescue (Nihsen et al., 2004).

A recently discovered animal genotype also shows potential as a way to identify animals that are tolerant or susceptible to ergot alkaloids from tall fescue. An association between a single nucleotide polymorphism on the dopamine receptor D2 (DRD2) gene and serum prolactin levels in beef cattle consuming toxic endophyte-infected tall fescue has been reported by Campbell et al. (2014).

Little is known about how or if the genetics, associated with tolerance to tall fescue toxicosis, affect the rumen microbial populations or rumen fermentation in cattle. Hill (2005) reported rumen microbial populations may affect the metabolism of ergovaline consumed by cattle. It is possible that ruminal microorganisms are capable of separating the alkaloids from the forage and transforming them into lysergic acid amides or ergopeptine alkaloids (Stuedemann et al., 1998). An in vitro study found that the response to toxic endophyte-infected tall fescue may be influenced by ruminal metabolism (Westendorf et al., 1993). Other possibilities are that ergovaline is not soluble in rumen fluid or the ergovaline remains in the solid matrix (Hill, 2005). Other research has been conducted evaluating the response of in vitro fermentation to diets of toxic or novel endophyte-infected tall fescue. Vibart et al. (2007) reported minimal effects of varying endophyte-containing fescues on in vitro fermentation products. Evaluating rumen characteristics based on the consumption of toxic or novel tall fescue and animal genotype as tolerant or susceptible to tall fescue toxicosis could help determine if animal genetics has an impact on rumen fermentation characteristics.

The objective of this research was to evaluate the effects that novel or toxic endophyte-infected tall fescue and tolerant or susceptible genotyped cattle have on fermentation characteristics in the continuous culture. The hypothesis was that rumen fluid from tolerant genotyped heifers fed Kentucky 31 would perform similar to rumen fluid from either genotype heifer fed BarOptima and better than rumen fluid from susceptible heifers fed Kentucky 31.

MATERIALS AND METHODS

The University of Missouri Animal Care and Use Committee approved the use of animals in this experiment (protocol #7820).

A total of twenty-four single flow effluent continuous culture fermenter polycarbonate vessels (Nalgene, Rochester, NY) were inoculated and maintained as described by Meng et al. (1999). Fermenters were continuously flushed with CO_2 gas, stirred with magnetic stir plates, and immersed in a water bath maintained at 39°C using thermostatically controlled heaters (Model 730, Fisher Scientific, Pittsburgh, PA). High buffer capacity solution modified by Slyter (1990) from McDougall's artificial saliva (McDougall, 1948), containing 470 g urea-N L^{-1} and 250 mg cysteine-HCL L^{-1}, was continuously infused into the fermenters using peristaltic pumps (Masterflex Model 7520-10, Cole Parmer Instrument Co., Chicago, IL). Fermenter dilution rates were calibrated prior to the research and held constant at 4% ± 0.5% h^{-1}. Effluent from the fermenters flowed into collection vessels that were immersed in an ice water bath.

Experimental design and treatments

Four treatments arranged in a two by two factorial were randomly distributed across six blocks of fermenters in two runs. Treatments were rumen fluid source (fluid from heifers genotyped as "fescue tolerant" or "fescue susceptible") and forage source (Kentucky 31 or BarOptima tall fescue).

Rumen fluid sample collection, storage and preparation

Rumen fluid was utilized from Angus-cross heifers provided ab libitum access to a forage diet in the GrowSafe feeding system at the University of Missouri Southwest Research Center in Mount Vernon, MO. Heifers were genotyped as tolerant or susceptible to tall fescue toxicosis, using the DRD2 genotype, prior to the start of feeding. Rumen fluid samples were collected from 24 heifers consuming either toxic Kentucky 31 or non-toxic BarOptima tall fescue in two different years. Rumen fluid samples were obtained using an esophageal tube with an oro-ruminal probe, vacuum pump and collection containers adapted from those outlined by Geishauser (1993). The rumen fluid collection apparatus can be seen in Figure 4.1. Individual samples were approximately 1 liter and immediately frozen after collection. Frozen samples were then transported from the Southwest Research Center to the University of Missouri in Columbia, MO.

Rumen fluid samples were stored at -20°C until the continuous culture research was conducted. Samples were thawed using a warm water bath at 40°C. Immediately after samples thawed they were strained through four layers of cheesecloth and diluted with McDougall's artificial saliva in a 1:2 dilution of rumen fluid to buffer. Individual heifer rumen fluid samples were used in each fermenter. Each fermenter was filled up to the effluent overflow port at approximately 1460 ml.

Fermenters were fed half the daily ration of 50 g d^{-1} at 12 h intervals. The experiment was conducted over a 7-day period, with 4 days adaptation and 3 days sampling. The two different collection years served as the two runs for the continuous culture.

Forage diet and analysis

Samples of the forage diet fed in the continuous culture were collected from a vertical mixer (LuckNow 2420, Lucknow, ON, Canada) immediately after mixing. Diets consisted of Kentucky 31 or BarOptima tall fescue and supplement. Diets (Table 4.1) were balanced for protein and energy to promote 0.5 kg d^{-1} gain. Heifers collected for rumen fluid samples consumed the same diets prior to rumen fluid sampling.

For diet analysis, the forage samples were freeze dried and ground through with a Wiley mill to pass through a 1-mm screen. Samples of the diet were analyzed for dry matter (DM) by drying at 105°C for 24 h, organic matter (OM) by incineration at 500°C, crude protein (CP) using the LECO, and neutral detergent fiber (NDF) and acid detergent fiber (ADF) using an ANKOM^{200} Fiber Analyzer (ANKOM Technology, Macedon, NY). Forage analysis results for toxic Kentucky 31 and non-toxic BarOptima tall fescue diets can be found in Table 4.2.

Fermenter sampling and analysis

Immediately before feeding (0 hours) and 4 hours after feeding (4 hours) during the final three days of each run, pH readings were taken and two 2 mL samples were collected directly from the fermenters and immediately frozen at -20˚C. The 0 and 4 hour samples were composited for each fermenter over the three day sampling period and analyzed for ammonia and volatile fatty acids (VFA).

At the conclusion of each run, fermenter contents were blended for 30 seconds to detach microbes from feed particles then centrifuged at 1000 x g for 5 min at 4°C to remove feed particles. Feed particles were air-dried in a 55˚C drying oven for 3 days to determine dry weight. Supernatant was re-centrifuged at 27000 x g for 30 min. The resultant pellet was washed once using 0.9% (wt. $vol.^{-1}$) saline solution then again using deionized distilled water. The final pellet, which contained bacteria, was transferred to plastic cups using deionized distilled water, lyophilized at 10°C (Genesis, Virtis, Gardiner, NY), and ground using a mortar and pestle. Subsamples of effluent (500 mL) were lyophilized at 10°C (Genesis, Virtis, Gardiner, NY), and ground using a mortar and pestle. Samples of effluent and fermenter pellets were then analyzed for purine content using the procedure of Zinn and Owens (1982). Ammonia concentration was determined colorimetrically (DU-65 spectrophotometer; Beckman, Palo Alto, CA) with the hypochlorite-phenol procedure adapted from Broderick and Kang (1980). Sample VFA concentration was determined using gas chromatography (Model 3400, Varian, Palo Alto, CA) following procedures outlined by Salanitro and Muirhead (1975).

Statistical analysis

Statistical analyses were performed using SAS (SAS version 9.4, SAS Inst. Inc., Cary, NC). Fermentation parameters were analyzed using the PROC MIXED procedure. The data were analyzed as a RCBD using four treatments with fermenter as experimental unit. The model for fermenter characteristics included forage, genotype, and forage x genotype as fixed effects, and with run and block serving as random effects. When the F-test was significant ($P \leq 0.10$), means separation was performed using Fisher's LSD.

RESULTS AND DISCUSSION

Digestion

Fermenters fed BarOptima had decreased (*P*<0.01; Table 4.3) true organic matter digestibility compared to fermenters fed Kentucky 31. Tolerant or susceptible genotype did not affect true organic matter digestion (P≥0.70) when fed BarOptima or Kentucky 31. Apparent digestion of NDF and ADF did not differ (P≥0.57) across forages and genotypes. De Lorme et al. (2007) and Stamm et al. (1994) reported apparent dry matter and ADF digestibility were not different between treatment groups of toxic and non-toxic tall fescue diets. It is important to note that these studies were conducted with tall fescue straw and were not conducted in a continuous culture. Burns and Fisher (2006) compared novel endophyte, wild-type endophyte, and endophyte-free tall fescue hays fed to cattle and found no difference in apparent NDF and ADF digestibility.

There was no difference (*P*=0.81) in apparent crude protein digestibility between BarOptima and Kentucky 31 diets. De Lorme et al. (2007), Stamm et al. (1994), and Burns and Fisher (2006) also found crude protein digestibility were not different when comparing toxic and non-toxic tall fescue. Matthews et al. (2005) evaluated the crude protein digestibility of toxic, novel, and endophyte-free tall fescue and reported that toxic tall fescue had lower crude protein digestibility compared to novel and endophyte-free tall fescue. It is possible that the difference in crude protein digestibility could be due to the use of a higher quality diet with hay providing the alkaloids.

Values for OM, NDF, and ADF digestibility are lower than expected (Table 4.3) when compared to the quality analysis of the forage (Table 4.2). It is possible these values were affected by the freezing of the rumen fluid samples or other variables in the process. Mansfield et al. (1995) reported a lack of continuity between in vitro continuous culture fermentation systems and in vivo digestion experiments They attributed this to reduced concentrations of viable bacteria and defaunation of the in vitro system.

Ammonia, pH, and volatile fatty acids

Ammonia at both 0 and 4 hours post feeding were greater (P<0.01) in the fermenters fed BarOptima compared to fermenters fed Kentucky 31. Increased ammonia suggests greater crude protein concentration in BarOptima, because the continuous culture system does not have a mechanism for immediate removal of nutrients. In the rumen, ammonia is absorbed across the rumen epithelium when concentrations increase (Abdoun et al., 2006).

BarOptima had higher pH levels at 0 hours (P=0.07) and 4 hours (P=0.02) post feeding compared to fermenters fed Kentucky 31. Reduced pH in the Kentucky 31 treatments could be indicative of increased fermentation, as OM digestion was greater in Kentucky 31-fed fermenters (Table 4.3). Stamm et al. (1994) reported ruminal pH was lower at high alkaloid concentrations and ammonia concentrations were not influenced by ergovaline concentration. De Lorme et al. (2007) evaluated endophyte-infected and endophyte-free tall fescue diets in lambs and found no difference in ruminal ammonia or ruminal pH levels.

Total volatile fatty acid concentration was not different between the treatments at 0 hours (P=0.71) but there was a forage by genotype interaction at 4 hours (P=0.01) post feeding. Fermenters dosed with rumen fluid from susceptible cattle had reduced total VFA when fed BarOptima but had increased total VFA when fed Kentucky 31 compared to the fermenters dosed with rumen fluid from tolerant cattle. The magnitude of difference across the treatment combinations is small and does not appear to have biological relevance, as other fermentation characteristics do not match this interaction. BarOptima fermenters had decreased acetate concentration at 0 hours and 4 hours (*P*≤0.01) post feeding compared to Kentucky 31. Fermenters fed novel endophyte-infected tall fescue had increased propionate concentrations at 0 hours and 4 hours (*P*≤0.07) compared to toxic endophyte infected tall fescue fermenters. An increase in propionate in ruminants has been identified as an indicator of more efficient rumen fermentation (Owens and Goetsch, 1988). Butyrate concentrations between the forage type were not different (*P*=0.23) at 0 hours but BarOptima had higher (*P*=0.07) butyrate concentrations at 4 hours post feeding compared to Kentucky 31. Interestingly, butyrate concentrations at 4 hours post feeding increased (P=0.03) in fermenters containing rumen fluid from tolerant cattle. Acetate:propionate was greater (*P*=0.06) at 0 hours for Kentucky 31 diets compared to BarOptima. Similarly, acetate:propionate ratio at 4 hours post feeding was higher (*P*<0.01) for Kentucky 31 compared to BarOptima diets. At 4 hours post feeding, fermenters containing rumen fluid from tolerant genotype cattle fed Kentucky 31 had a higher acetate:propionate ratio (*P*=0.04) compared to tolerant genotype fed BarOptima or susceptible genotype fed BarOptima. Acetate:propionate ratio for tolerant genotype fed BarOptima was lower (*P*=0.04) than tolerant genotype fed Kentucky 31 or susceptible genotype fed Kentucky 31. Vibart et al. (2007) reported no difference in total volatile fatty acid production in the continuous culture when feeding endophyte-infected, endophyte-free, and novel endophyte diets with varying amounts of grain supplementation, with the grain supplementation likely influencing results to a greater extent than in the current experiment, which mixed a supplement in with the forage at a modest rate (3.75 and 5.50% for BarOptima and Kentucky 31, respectively; Table 4.1). There is not a clear pattern to the differences in fermentation characteristics at feeding or 4 hours post feeding that would suggest forage type or rumen fluid from susceptible or tolerant cattle had any biologically significant influence under the conditions of this experiment.

CONCLUSION

This is the first continuous culture research project comparing rumen fluid collected from cattle genotyped as tolerant or susceptible to tall fescue toxicosis, and feeding the fermenters diets of novel or toxic endophyte-infected tall fescue. Under conditions of this experiment, there were minimal differences in ruminal fermentation associated with cattle of varying tolerance to tall fescue toxicosis. Nutrient profile of toxic endophyte-infected tall fescue differed from novel endophyte-infected tall fescue, which impacted fermentation characteristics.

Table 4.1. Diet ingredient composition for BarOptima and Kentucky 31 fed in continuous culture fermenters.

	Treatment	
Ingredients[1]	BarOptima	Kentucky 31
Tall Fescue	96.25	94.50
Supplement[2]	3.75	5.50

[1] Ingredients are estimates following mixing in vertical mixer.
[2] Supplement consisted of 62% distillers grain, 36% soybean hull pellets, 1% triple 12 mineral (Producers Cooperative Association, Girard, KS), and 1% magnesium oxide (Feed Products and Service Company, St. Louis, MO).

Table 4.2. Nutrient composition of diets fed to continuous culture fermenters.

	Treatment	
Item	BarOptima	Kentucky 31
Dry matter, g kg^{-1}	93.7	93.2
Organic matter, g kg^{-1}	91.3	91.9
Crude protein, g kg^{-1}	13.5	13.7
Neutral detergent fiber, g kg^{-1}	62.4	60.5
Acid detergent fiber, g kg^{-1}	35.9	35.1
Total ergot alkaloids, μg kg^{-1}	21.0	1497.0

Table 4.3. Fermenter digestibility parameters when BarOptima or Kentucky 31 tall fescue diets are fed to continuous culture fermenters.

	Treatment[1]					Forage	Genotype	Forage x Genotype
Parameter	BO-T	BO-S	KY-T	KY-S	SEM	*P*-value	*P*-value	*P*-value
True OM digestibility, %	39.94[b]	40.18[b]	44.03[a]	43.32[a]	1.74	<0.01	0.85	0.70
Apparent NDF digestibility, %	32.05	31.42	30.91	31.43	2.18	0.72	0.98	0.71
Apparent ADF digestibility, %	31.69	31.46	30.44	30.83	2.31	0.57	0.96	0.85
Apparent CP digestibility, %	59.17	60.13	60.62	57.61	3.15	0.81	0.65	0.38

[a-b] Least squares means within a row with different superscripts differ ($P<0.10$).

[1] BO-T = tolerant genotyped cattle fed BarOptima; BO-S = susceptible genotyped cattle fed BarOptima; KY-T = tolerant genotyped cattle fed Kentucky 31; KY-S = susceptible genotyped cattle fed Kentucky 31.

Table 4.4. Fermentation characteristics at 0 and 4 hours post feeding.

	Treatment[1]					Forage	Genotype	Forage x Genotype
Parameter	BO-T	BO-S	KY-T	KY-S	SEM	*P*-value	*P*-value	*P*-value
	0 h[*]							
Total VFA[2], mM	141.15	139.71	137.05	130.25	9.97	0.34	0.56	0.71
Acetate:Propionate	3.23^{b}	3.52^{b}	3.84^{a}	3.59^{a}	0.25	0.06	0.92	0.13
VFA, mol/100 mol								
Acetate	72.75^{b}	73.83^{b}	76.04^{a}	75.34^{a}	1.25	0.01	0.83	0.32
Propionate	23.69^{a}	22.48^{a}	21.28^{b}	21.63^{b}	1.24	0.07	0.63	0.38
Butyrate	1.78	1.89	1.70	1.80	0.10	0.23	0.16	0.95
pH	6.83^{a}	6.86^{a}	6.82^{b}	6.78^{b}	0.04	0.07	0.95	0.16
Ammonia, NH_3-N mg/dL	23.43^{a}	24.35^{a}	17.10^{b}	17.61^{b}	0.88	<0.01	0.26	0.75
	4 h[*]							
Total VFA[2], mM	158.32^{c}	142.50^{d}	146.33^{cd}	156.82^{c}	7.37	0.82	0.61	0.01
Acetate:Propionate	3.09^{be}	3.43^{bde}	3.90^{ac}	3.54^{acd}	0.23	<0.01	0.95	0.04
VFA, mol/100 mol								
Acetate	71.54^{b}	71.87^{b}	75.95^{a}	74.61^{a}	1.30	<0.01	0.58	0.37
Propionate	24.26^{a}	23.51^{a}	20.97^{b}	21.89^{b}	1.31	0.01	0.92	0.38
Butyrate	1.87^{a}	2.07^{a}	1.76^{b}	1.90^{b}	0.10	0.07	0.03	0.70
pH	6.76^{a}	6.80^{a}	6.70^{b}	6.70^{b}	0.05	0.02	0.51	0.50
Ammonia, NH_3-N mg/dL	17.56^{a}	18.16^{a}	12.29^{b}	12.70^{b}	0.67	<0.01	0.29	0.84

[a-b] Least squares means within a row with different superscripts differ in forage (*P*<0.10).
[c-e] Least squares means within a row with different superscripts differ in forage*genotype (*P*<0.10).
[*]Samples taken directly before feeding (0 h) and 4 hours after feeding (4 h).
[1] BO-T = tolerant genotyped cattle fed BarOptima; BO-S = susceptible genotyped cattle fed BarOptima; KY-T = tolerant genotyped cattle fed Kentucky 31; KY-S = susceptible genotyped cattle fed Kentucky 31.
[2] Total VFA = Acetate + Propionate + Isobutyrate + Butyrate + Isovalerate + Valerate.

Figure 4.1. Images of the collection containers and probe used for the extraction of rumen fluid to be used in the continuous culture.

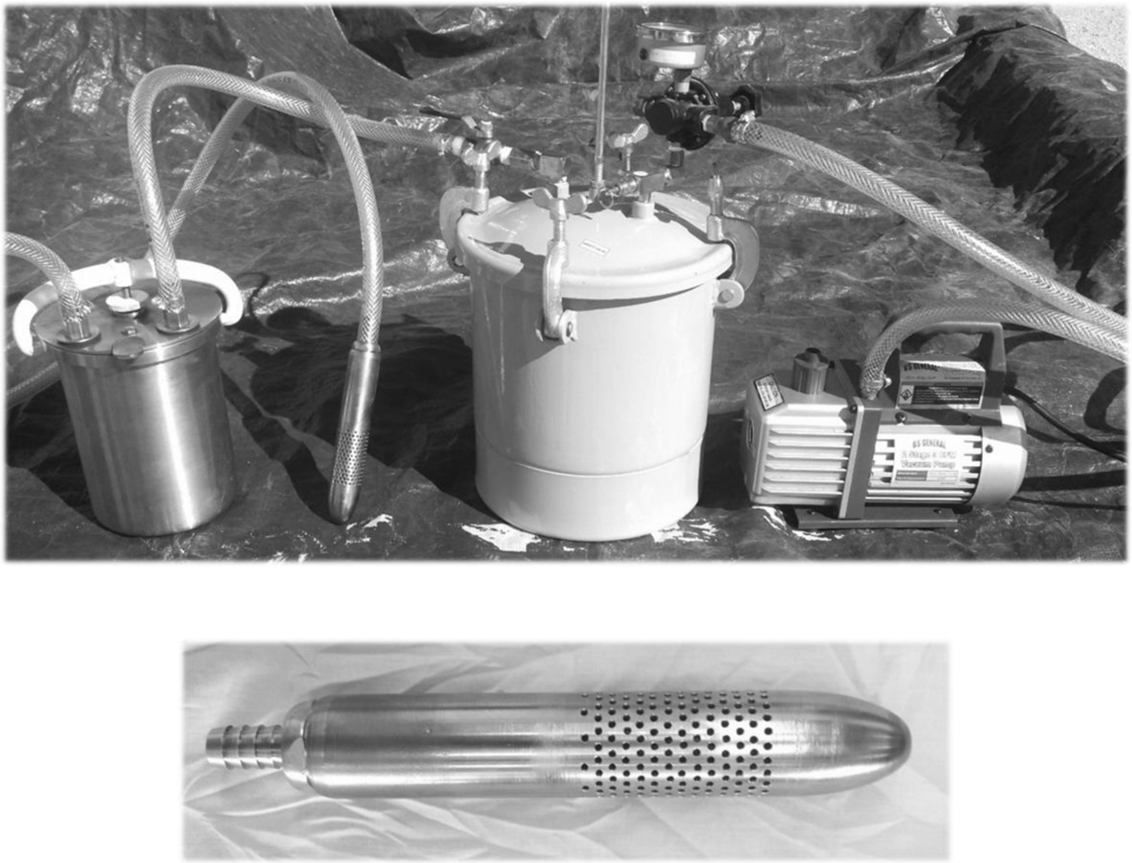


Figure 4.2. Images of the continuous culture fermenter system.

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FINAL REMARKS AND CONCLUSION

This work has provided insights into the ability of cattle to tolerate tall fescue toxicosis. It is apparent that more genetic variables are responsible for tolerance and these factors should be investigated in the future. With the release of new genetic tests, cattle producers will likely look to it for assistance in breeding animals that are tolerant to tall fescue toxicosis.

VITA

Brett T. Jones was born July of 1989 to Steve and Jeanne Jones. He grew up on a farm near Mount Vernon, MO. After graduating as Salutatorian from Mount Vernon High School in 2008. He then attended the University of Missouri-Columbia and graduated magna cum laude in 2012 with a bachelor's degree in Plant Sciences with minors in Agriculture Economics and Animal Sciences. He then began graduate school in August 2012 to complete a Doctorate in the Division of Plant Sciences under the mentorships of Dr. Robert Kallenbach and Dr. Craig Roberts. He is married to Courtnie Jones, who received a Master's degree in Education in 2019.