THE EFFECTS OF EXTENDED-RELEASE EPRINOMECTIN ON HEIFER PERFORMANCE AND FESCUE TOXICITY AND AN EVALUATION OF PARASITE RESISTANCE

BY

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THESIS

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ABSTRACT

Anthelmintics are commonly used by livestock producers. Parasite resistance has been reported in ruminants in multiple macrocyclic lactones, after their use over time. As new versions of anthelmintics are released to the market, evaluations for resistance need to be conducted. In addition, the use of anthelmintics has also been reported to be beneficial at reducing negative impacts of fescue toxicity. Previous work has shown improvements in growth performance of growing animals when treated with either ivermectin or extended-release eprinomectin. No work has determined the mechanism for this improvement though. The objective of this thesis was to determine if parasite resistance to extended-release eprinomectin was present on a cow-calf operation after three years of use and to explore CYP450 activity and liver function as potential mechanism for extended-release eprinomectin to mitigate fescue toxicity in fall-born beef heifers grazing endophyte-infected tall fescue.

Only one extended-release anthelmintic is currently on the market for ruminants and only limited data exist relative to parasite resistance. Therefore, the objective of this experiment was to determine if parasite resistance to extended-release eprinomectin (LongRange) was present in an operation with 3 yr of prior eprinomectin use and impacts on heifer growth performance. Fall-born Angus × Simmental heifers (224 ± 22 d of age) were randomly assigned 1 of 3 treatments: extended-release eprinomectin (**ERE**), extended-release eprinomectin and oxfendazole (**COMBO**), or saline control (**CON**). Control cattle had greater FEC from d 55 to 83 compared to ERE and COMBO heifers, although FEC was not different any other time point. Fecal egg count reduction (**FERT**) for ERE and COMBO were above the 90% threshold (91% and 98% reduction, respectively). Packed cell volume was similar between treatments throughout the experiment. Treated cattle had greater BW and BCS beginning on d 83 and 55, respectively.

Parasite resistance to extended-release eprinomectin was not present based on FERT, and treated heifers had greater growth performance compared to CON heifers.

The mechanism causing increased growth performance of growing cattle treated with extended-release eprinomectin has not been determined. Therefore, the objective of this experiment was to evaluate CYP450 and liver enzymes as potential mechanisms of extended-release eprinomectin mitigating fescue toxicosis in beef heifers grazing endophyte-infected tall fescue. Fall-born Angus × Simmental heifers (42 heifers; 170.2 ± 20.0 kg initial body weight (**BW**); 222 ± 21 d of age) were stratified by d -2 fecal egg count and BW and were assigned to one of six groups (7 heifers per group) using a stratified randomized design. Groups were then assigned to one of two treatments: extended-release eprinomectin (**ERE**; n = 3), or saline control (**CON**; n= 3). Body weight and BCS was improved in ERE heifers beginning on d 112 and 28 respectively. Respiration rates tended to be improved and HCS was improved in ERE heifers on d 55 and 83. Serum prolactin and total urinary ergot alkaloids were similar between treatments. In addition, ERE cattle tended to have greater total CYP3A4 activity. Liver enzymes including alkaline phosphatase and gamma-glutamyl transferase were greater in ERE heifers and total protein tended to be greater than control. Extended-release eprinomectin improved heifer growth performance, HCS, and tended to improve RR. In addition, extended-release eprinomectin tended to increase total CYP450 activity, and also had greater levels of several liver enzymes in heifer grazing endophyte-infected tall fescue.

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Dedication

To my parents Steve and Theresa Volk, and my grandparents, Leo and Annette Volk, who instilled a passion for agriculture and beef cattle in me at a young age and I will be forever grateful.

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CHAPTER 1

LITERATURE REVIEW

INTRODUCTION

Tall fescue (*Festuca arundinacea*) is a common forage that beef cattle in the United States graze. An issue caused by tall fescue is reduced performance and physiological conditions of beef cattle also known as fescue toxicosis. Many strategies have been evaluated to mitigate this condition while grazing endophyte-infected tall fescue, but none have completely solved the issue. Different variates of tall fescue including endophyte-free and novel endophyte tall fescue do not cause issues of fescue toxicosis. Although, these options are not feasible due to poor persistence and are expensive to reseed. Anthelmintics are commonly used in the beef industry to treat parasite infections and may be an option at reducing symptoms of fescue toxicosis, although the mechanism is not understood. However, with use of anthelmintics, parasite resistance becomes a concern. As new anthelmintics reach the market, research needs to be conducted to determine when resistance becomes an issue.

TALL FESCUE

Tall fescue (*Festuca arundinacea*) is a cool-season perennial grass that covers 14 million hectares in the United States and is persistent and productive in multiple environments (Thompson et al., 2001). The agronomic characteristics of tall fescue make it an ideal candidate for pastures as it can withstand drought, poor soil conditions, and intense grazing (Gunter and Beck, 2004; Kallenbach, 2015). In the United States, approximately 12 million beef cows graze tall fescue (Kallenbach, 2015). Tall fescue has these ideal attributes because of the endophyte infection in the plant (Gunter and Beck, 2004).

Endophyte and Ergot Alkaloids

The toxic endophyte *Neotyphodium coenophialum* was first reported by Bacon et al. (1977). The toxic endophyte is located in the intracellular spaces of sheath, stem, leaf, and seed of the plant but it does not invade the cells of the plant or become pathogenic (Thompson et al., 2001). The fungus and the plant have a symbiotic relationship in which both benefit from the relationship (Arachevaleta et al., 1989; Porter et al., 1994). The toxic fungus deters ruminants and insects from feeding on it and negatively affecting the animal's growth and reproduction (Siegel et al., 1990; Porter et al., 1994; Thompson et al., 2001). The toxic fungus also improves the drought tolerance of the plant (Siegel et al., 1990; Porter et al., 1994; Thompson et al., 2001). In exchange, the fungus is provided with protection, nutrients, reproduction and dissemination from the plant (Arachevaleta et al., 1989; Thompson et al., 2001).

Three classes of alkaloids are produced from the toxic endophyte: pyrrolizidine, peramine, and ergot alkaloids (Siegel et al., 1990). Of the three classes produced, ergot alkaloids are the only endophyte-derived alkaloid that deters ruminants from grazing as it is a mycotoxin to mammals (Strickland et al., 2011). Ergot alkaloids produce many different ergopeptines and the ergopeptine in highest concentration is ergovaline (Lyons et al., 1986; Strickland et al., 2011). The ergoline ring structure is similar to that of dopamine, norepinephrine, and epinephrine; which is likely the reason why ergot alkaloids can affect animals (Berde, 1980; Weber, 1980).

Fescue Toxicosis and Animal Performance

Fescue toxicosis is of great concern to producers with cattle grazing endophyte-infected tall fescue as cattle that graze tall fescue tend to have an unthrifty appearance and poor animal performance during the summer months (Schmidt and Osborn, 1993). Symptoms of fescue

toxicosis include decreased weight gain, decreased feed intake, increased vasoconstriction, rough hair coat, elevated body temperature, labored breathing, decreased serum prolactin levels and poor reproductive performance (Schmidt and Osborn, 1993; Paterson et al., 1995; Strickland et al., 2011; Aiken and Strickland, 2013).

Previous literature has strongly supported the association of fescue toxicosis with decreased weight gain and feed intake in cattle consuming ergot alkaloids. Schmidt and Osborn (1993) stated that a portion of the reduced weight gain in cattle grazing endophyte-infected (**E+**) tall fescue is an outcome from decreased feed intake; however, it could partially also be impacted by environmental temperature as well (Peters et al., 1992). Cattle grazing E+ tall fescue have been known to graze less time during the day, as they would spend their days in the shade or in bodies of water, and graze mostly at night (Bond et al., 1984). Most of the research focused on animal performance and E+ tall fescue was in beef steers grazing in the summer. Paterson et al. (1995) compiled data from multiple studies and concluded that steers grazing E+ tall fescue had decreased weight gain compared to steers grazing endophyte-free (**E-**) tall fescue despite multiple locations and management styles of the experiments. Additionally, for every 10% increase in endophyte infestation, average daily gain (**ADG**) was predicted to decrease by 0.045 kg per d (Paterson et al., 1995).

Less data is available for cows grazing E+ tall fescue compared to E- tall fescue; nonetheless, from the data available, it was concluded that cows grazing E+ tall fescue lost more weight compared to E- cows (Schmidt and Osborn, 1993; Paterson et al., 1995). Furthermore, these cows also had decreased pregnancy rates and milk production, as well as, weaning lighter weight calves. Schmidt et al. (1986) reported a large magnitude difference between pregnancy rates of heifers grazing E+ tall fescue to E- tall fescue (55% vs. 96%, respectively). When those

same animals were rebred as primiparous cows, only 33% of those grazing E+ were pregnant compared to the 93% of those grazing E- (Schmidt et al., 1986). Also, Boling (1985) and Gay et al. (1988) both reported reduced calving rates in cows grazing E+ compared to those grazing E- (67% vs 86% and 55% and 95%, respectively). Paterson et al. (1995) noted that the decreased conception rates could partially be related to the negative impact of the E+ fescue on cow weight loss and body condition compared to E- fescue. When comparing heifers and cows, Thompson et al. (2001) noted that beef heifers experienced a larger negative impact on reproduction than cows. Strickland et al. (2011) discussed the impacts of ergot alkaloids on heifer follicular development and concluded that there is a minimal direct effect; however, it could be escalated by an increased ambient temperature.

The similar ring structure of ergot alkaloids to biogenic amines, dopamine, norepinephrine and epinephrine allows ergot alkaloids to bind to biogenic amine receptors and elicit effects of decreased serum prolactin and vasoconstriction (Strickland et al., 2011). Unfortunately, prolactin is highly variable from animal to animal and can differ based on physiological factors of the animal in addition to environmental factors such as day length, season, and temperature (Hill et al., 2000). Although in previous research, serum prolactin has been most commonly used to determine if animals are exposed to fescue toxicity (Hill et al., 2000). It is important to note, that prolactin and animal weight gain are not highly correlated. However, circulating levels of prolactin indicate ergopeptide affects the dopamine receptors (Bacon and Hill, 2013).

Vasoconstriction is another symptom of fescue toxicosis and occurs as the vascular tone contracts and restricts blood flow to many tissues (Rhodes et al., 1991; Aiken et al., 2009; Strickland et al., 2011). Reduced blood flow affects the animal's ability to dissipate heat and

hinders the reproduction of the animal (Rhodes et al., 1991; Aiken et al., 2009; Strickland et al., 2011). With the inability to dissipate heat, cattle grazing endophyte-infected tall fescue will also have increased respiration as a mode to attempt to dissipate heat (Finch, 1986). In addition, hematocrit measures the animal's oxygen carrying capacity of the circulatory system and level of dehydration (Nordenson, 2006). Hematocrit can also be impacted after chronic ergot alkaloid exposure (Nordenson, 2006). However, previous research has shown inconclusive results on hematocrit values after exposure to ergot alkaloid exposure indicating more research is needed (Boling et al., 1989; Bacon and Hill, 2013; Poole et al., 2018). It is also important to note, one report indicated that hematocrit was impacted greater by temperature rather than E+ which indicates hematocrit may be a better indicator for heat stress than fescue toxicosis (Aldrich et al., 1993a). In addition to other symptoms of fescue toxicosis, cattle tend to have a rough hair coat when grazing tall fescue as a result of the combination of reduced serum prolactin concentration and vasoconstriction (Porter and Thompson Jr, 1992).

Research is lacking on the metabolism of ergot alkaloids in cattle; however, they are assumed to be biotransformed in the liver and may induce liver enzymes (Oliver, 1997; Strickland et al., 2011). Additionally, Oliver (1997) summarized previous fescue experiments and reported reduced levels of liver serum enzymes in cattle grazing E+ such as alkaline phosphatase, alanine transaminase, lipase, aspartate transaminase, and lactic acid dehydrogenase. No reports have determined the mechanism that causes the decrease in these serum enzymes (Oliver, 1997). Ergot alkaloids are alpha-2 adrenergic receptor agonists and in a review, Oliver (1997) hypothesized that this may be impacting some liver enzyme function differences. Alpha-2 receptor agonists in the liver inhibit adenyl cyclase levels and decreases overall liver function by reducing c-AMP regulated liver metabolism (Hems and Whitton, 1980; Jard et al., 1981; Stiles et

al., 1984). Additionally, alpha-2 adrenergic receptors agonists can cause reduced rumen contractions and feed intake in goats and sheep (Van Miert and Van Duin, 1991; Aldrich et al., 1993b). Alpha-2 adrenergic receptors are also present on blood vessels and can cause vasoconstriction when ergot alkaloids are present (Jie et al., 1987; Roquebert and Grenie, 1987; Badia et al., 1988). Although the exact mechanism is unknown, it is hypothesized that this is reducing animal performance when grazing E+ (Oliver, 1997).

Blood metabolites such as serum cholesterol, total protein, triglyceride, and blood urea nitrogen levels have also been reported to be decreased in cattle grazing E+ (Bond et al., 1984; Thompson and Stuedemann, 1993; Oliver, 1997). Suppressed levels of cholesterol and total protein are suggested to be related to reduced feed intake when the animals are experiencing heat stress (Martin and Edwards, 1986). In addition, reduced serum triglyceride levels is hypothesized to be associated with an increase in alpha-2 adrenergic receptor activity (Oliver, 1997). Reduced blood urea nitrogen levels was proposed to be due to mild dehydration of cattle grazing E+ (Oliver, 1997).

Ergot alkaloid levels can be measured in the urine after an animal has been exposed to the toxic endophyte as another measurement to determine the level of exposure. Stuedemann et al. (1998) reported that 96% of ergot alkaloids are excreted in the urine. Hill et al. (2000) compared serum prolactin concentrations and urinary alkaloid excretions from the same animals at the same time points to determine which is a better indicator of toxicity. In the experiment, Hill et al. (2000) noted that compared to serum prolactin concentrations, urinary alkaloid excretions were consistently at greater levels for cattle grazing E+ under different experimental conditions. Also, when switching from E+ to E- or E- to E+, urinary alkaloids was a more accurate assessment of fescue toxicity exposure than serum prolactin concentrations (Hill et al., 2000). Although urinary

alkaloids are not a commonly used indicator of fescue toxicosis. Additionally, Hill et al. (2000) described a relationship between animal performance and urinary alkaloid excretion; however, more research is needed to determine if this is applicable for multiple experiments.

Impact on the Beef Industry

Fescue toxicosis has a significant impact on the beef industry as over 12 million beef cows consume tall fescue (Kallenbach, 2015). Reduced reproductive efficiency and decreased weaning weights are the greatest economic impact on the beef industry related to fescue toxicosis (Porter and Thompson Jr, 1992) costing the industry close to $2 billion annually (Kallenbach, 2015). Reproduction issues such as failure to conceive or early embryonic loss due to fescue toxicosis is to blame for $1.5 billion annually (Hoveland, 1993; Kallenbach, 2015). Additionally, decreased weaning weights of calves grazing tall fescue with their dams is associated with the other $500 million in losses that the beef industry faces each year. Impacts on the stocker industry are difficult to quantify, as most stocker operations avoid grazing cattle on tall fescue because of the reduced performance (Hoveland, 1993).

Mitigation Strategies

Tall fescue is included in many grazing systems across the United States, and with the concern of fescue toxicosis, multiple strategies to mitigate symptoms have been researched. Diluting E+ tall fescue pastures with clover or another plant species is an option. However, Kallenbach (2015) acknowledged that although interseeding clover would add nutritional value to the pasture, it would not necessarily prevent fescue toxicosis. The increases in animal performance were similar between pastures with clover added to toxic and nontoxic tall fescue, indicating that the clover may only mask the toxicosis effects and not eliminate the problem (Roberts and Andrae, 2004; Aiken and Strickland, 2013). In the same manner, cattle grazing E+

tall fescue can be supplemented to dilute the intake of the endophyte; however, this has similar results to interseeding clover and will not eliminate the problem (Roberts and Andrae, 2004; Kallenbach, 2015).

The most toxic part of the tall fescue plant is the seed head, with ergot alkaloid concentrations being three to six times greater than the leaf blades or stems (Rottinghaus et al., 1991). Seed heads can be suppressed and pasture nutritive value and animal performance can be increased; but research shows that forage growth is diminished which impacts the practicality and stocking rates of the pastures (Aiken and Strickland, 2013; Kallenbach, 2015). In order to prevent fescue toxicosis, the cause of the toxicity needs to be removed: the endophyte. Endophyte-free tall fescue may decrease signs of fescue toxicosis, but decreased plant persistence limits this approach (Bouton et al., 2002; Aiken and Strickland, 2013; Kallenbach, 2015). Novel tall fescue (**NE**) contains a non-toxic endophyte that produces none to very small amounts of ergot alkaloids allowing the plant to still have persistence without negatively impacting the animal (Gunter and Beck, 2004). Aiken and Strickland (2013) noted that NE pastures are costly to establish and do require good grazing management to maintain the pastures. Strickland et al. (2011) noted that current management approaches are also looking into other mechanisms for mitigating symptoms of fescue toxicosis.

ANTHELMINTIC AND TALL FESCUE RESEARCH

Previous research has focused on the impact of management strategies that livestock producers often include on their farms already, such as the use of pharmaceuticals (Strickland et al., 2011). One area of pharmaceuticals that has been of interest to some researchers is the use of anthelmintic drugs. According to USDA (2015), anthelmintic drugs are those classified as

removing parasitic internal worms from the host. These anthelmintic drugs are categorized into groups based on their mode of action.

Classes of Anthelmintic Drugs

For cattle, there are currently three major classes of anthelmintics: imidazothiazoles, benzimidazoles, and macrocyclic lactones (Edmonds et al., 2010). Macrocyclic lactones are subdivided into two categories: first-generation avermectins and second-generation milbemycins. Each class of anthelmintics has multiple different drugs. An example of imidazothiazoles is Lavmisole (Edmonds et al., 2010; USDA, 2015). Benzimidazoles include albendazole, fenbendazole and oxfendazole (Edmonds et al., 2010; USDA, 2015). First-generation macrocyclic lactones include ivermectin, doramectin, eprinomectin, and abamectin, while an example of second-generation macrocyclic lactones is moxidectin (Edmonds et al., 2010; USDA, 2015).

Impacts of Internal Parasites on Beef Cattle

The most common internal parasite affecting the beef industry are gastrointestinal nematode (**GIN**) parasites (Williams and Loyacano, 2001). A survey conducted by the USDA National Health Monitoring System indicated that cow-calf producers consider GIN to be a major issue (Stromberg et al., 2015) with $2.5 billion being spent on anthelmintic drugs each year (Williams and Loyacano, 2001). Internal parasites can impact cattle of all ages, but younger cattle are more susceptible than cows (Miller, 1993). Mature cattle tend to have fewer issues with GIN as cattle typically develop an immunity to GIN during their second grazing season, which allows them to have minimal need for GIN control (McArthur and Reinemeyer, 2014). Previous research has shown that treated calves have improved weaning weights compared to untreated calves in spring-calving systems (DeRouen et al., 2009; Stromberg et al., 2015). Additionally, in

stocker cattle, improved ADG has been documented in treated vs untreated calves by up to 0.272 kg difference. Moreover, in cows, improved reproductive performance has been reported (Stromberg et al., 2015). Although, previous research has also demonstrated no differences in conception rates between heifers treated with ivermectin or untreated controls (Zajac et al., 1991). In addition to improvements from internal parasites, anthelmintics can impact external parasites on beef cattle as well.

Impacts of External Parasites on Beef Cattle

The horn fly, *Haematobia irritans (L.)*, is one of the most economically harmful pests to beef cattle throughout the United States (Byford et al., 1992). In a review conducted by Drummond et al. (1981), losses due to horn flies was approximately $730.3 million per year which was the largest loss among others including stable flies, ticks, lice, face flies, mosquitoes, and mites. Kunz et al. (1991) reported this number to rise to $876 million per year just 10 years later. Today, this number has increased to greater than $1 billion annually (Talley, 2018). Costly losses to beef cattle from horn flies are associated with reductions in weight gains, feed efficiency, and milk production (Campbell, 1976; Byford et al., 1992). Decreases in cattle performance are from blood loss along with the stress and annoyance of the flies (Campbell, 1976; Byford et al., 1992). A threshold of 100 horn flies per side of the animal was defined as being economically advantageous to intervene with fly control (Foil and Hogsette, 1994). Previous research has also shown that partial herd treatment for horn fly control can be adequate to avoid negative consequences of horn flies (Harvey and Brethour, 1981; Harvey and Brethour, 1983). Although internal and external parasite control was a valuable component of anthelmintics, additional benefits were investigated as well.

Ivermectin and Fescue Toxicosis

When ivermectin was first released on the market, many producers added it to their herd management plan because of the improved cattle performance (Gasbarre et al., 2009). Observational improvements were noted from anthelmintics beyond parasite control; however, Ellis et al. (1989) first documented improvements in steer weight gain of ivermectin steers compared to untreated controls when grazing E+ tall fescue. Unfortunately, parasite load was not monitored, so it is difficult to determine if parasite differences were impacting weight gain (Ellis et al., 1989).

Crawford Jr et al. (1990) focused on the impacts of injectable ivermectin, administered every 2 weeks, in steers without parasites and consumed either E+ hay or E- hay. Improvements in ADG were reported in steers fed E + hay when treated with ivermectin compared to steers not receiving ivermectin (Crawford Jr et al., 1990). However, no improvements in ADG were noted in steers fed E- hay and treated with ivermectin (Crawford Jr et al., 1990).

Another experiment focused on E+ tall fescue impact on cattle with or without corn gluten feed (**CGF**) supplementation and with or without ivermectin injections over 117 d (Crawford and Garner, 1991). All steers were treated with an anthelmintic, ivermectin, at trial initiation, but only half in each group received ivermectin injections every two weeks for the remainder of the experiment. Increasing supplementation levels were also used to determine if a specific threshold of supplementation had improved performance compared to others. Unfortunately, fecal egg counts (**FEC**) were not monitored throughout the experiment. However, it was expected that FEC would be low at least for the first 28 d since all cattle were treated with ivermectin initially. All steers that were treated with ivermectin every 2 weeks had improved ADG compared to steers not treated every 2 weeks with ivermectin, regardless of the level of

supplementation. Neither of the treatments alone were able to have weight gains similar to expected gains of cattle grazing E-, not experiencing fescue toxicosis; however, cattle supplemented with 8 lbs CGF plus treated every two weeks with ivermectin had similar gains to that of cattle on E- tall fescue (Crawford and Garner, 1991).

The first most notably known data published with FEC reported on cattle grazing E+ and treated with or without ivermectin was by Bransby et al. (1993). Bransby et al. (1993) reported a large improvement in cattle performance when using ivermectin and grazing E+ tall fescue compared to untreated cattle grazing E+ tall fescue. This experiment was conducted over two years with the first year having large FEC differences between treatments [120 eggs per gram (**EPG**) and 5 EPG for control and ivermectin treatments, respectively] but the second year had smaller differences between FEC (55 EPG and 14 EPG for control and ivermectin treatments, respectively).

In the second part of Bransby et al. (1993), another experiment was conducted comparing cattle grazing E + and E -, in addition to anthelmintic treatments of ivermectin, fenbendazole, and untreated controls administered on d 0 and 56 d later. In this experiment, parasite level was similar between all treatments ranging from 6 EPG to 39 EPG. Cattle treated with ivermectin had similar ADG between E+ and E-. Conversely, ivermectin cattle had improved ADG compared to untreated controls grazing both E+ and E- as well as cattle grazing E+ treated with fenbendazole. Although, cattle treated with fenbendazole grazing E- had the greatest ADG. The authors noted that visual symptoms of fescue toxicosis were decreased for ivermectin treated cattle; however, parameters utilized were not reported (Bransby et al., 1993). As these cattle had a low parasite load already, these results indicated that some of the improved performance could potentially be related to ivermectin mitigating fescue toxicosis, which led to more research in this area.

In a similar design, Ivy et al. (1993) compared ADG of cattle grazing E+ and E- tall fescue and three anthelmintic treatments of ivermectin, fenbendazole, or no treatment. Treatments were administered every 28 d of the experiment and FEC were noted to be below the economic threshold of 200 EPG (Ivy et al., 1993). No differences were reported between the three anthelmintic treatments on E- pastures. However, on E+ pastures ivermectin treated cattle had greater ADG compared to the other two anthelmintic treatments (Ivy et al., 1993). Unfortunately, no other parameters were reported from this experiment.

As research was lacking in this area, another experiment was conducted to determine if anthelmintic treatment and implanting cattle grazing E+ or E- had marked improvement (Bransby et al., 1995). Results from this experiment did not see marked improvement from ivermectin alone, but when the combination of the implant and ivermectin treated cattle was used on E+ pastures, cattle had improved performance (Bransby et al., 1995). Additionally, the combination of the implant and ivermectin and cattle grazing E+ was reported to be similar to all treatments grazing E-. Unfortunately, FEC were not reported so parasite differences are unknown. An indicator of fescue toxicosis, hair coat score (**HCS**), was reported to be improved in cattle grazing E- compared to cattle grazing E+ and improved in cattle treated with ivermectin, although data was not reported.

As previous research had varying levels of FEC differences, Rosenkrans et al. (2001) conducted an experiment of confined cattle consuming E+ hay to determine impacts of ivermectin and an immune challenge on cattle performance. Prior to experiment initiation, all cattle were administered a double dose of fenbendazole at two time points 10 d apart. This was done to ensure cattle did not have high parasite loads. The authors noted that steers were free of internal parasites, although FEC data were not reported (Rosenkrans et al., 2001). The day after

the last treatment of fenbendazole, cattle were assigned to treatment of a slow-release ivermectin bolus or no additional treatment. Over the 28 d period, there was no difference in ADG between treatments. Hematocrit (% packed cell volume) was increased in ivermectin treated steers at d 28. It is important to note that these cattle were not grazing pasture and the experiment was a short duration experiment, only 28 d. Most of the previous reports for improvement in ADG was reported over a longer grazing period. Overall, these studies indicated that cattle administered an anthelmintic, specifically ivermectin, could have fewer reduced symptoms of fescue toxicosis, and primarily focused on performance.

Little research has been conducted on another anthelmintic in the same class as ivermectin, eprinomectin, to determine if it would have similar results on cattle grazing E+. Gunter et al. (2006) conducted an experiment to determine the effects of a topical eprinomectin on fescue toxicosis compared to fenbendazole or untreated cattle when grazing E+ pastures. Results from this experiment only saw improved performance of treated cattle compared to untreated with no added improvement for the eprinomectin treatment (Gunter et al., 2006). Although, there were significant differences in FEC between treatments, which makes it difficult to compare growth differences between fescue toxicity and parasite burden. Moreover, indicators of fescue toxicosis were not measured. Additionally, this was only one experiment using eprinomectin. Therefore, more research is needed before the impacts of relief to fescue toxicosis anthelmintics in the macrocyclic lactone class could provide to the industry is determined.

EXTENDED-RELEASE EPRINOMECTIN

As previously mentioned, multiple anthelmintic drugs are available for livestock producers on the market today. In 2012, Merial released a new product on the market, LongRange, which is an extended-release injectable form of eprinomectin (Forbes, 2013). Unlike

other anthelmintic drugs on the market, LongRange is effective for up to 150 d, which is much more convenient for producers than applications every 28 d. This extended release of eprinomectin in this product is because of the Theraphase technology that slowly releases the active ingredient over a longer period of time. The plasma concentrations of eprinomectin peak a few days after administration and then decline until about d 25 where they remain constant until approximately d 70. The second peak is seen around d 90 and again declines until about d 160 when the product is then no longer in the animal's body.

As an injectable anthelmintic, administration of the product does not require specific tools to administer, as some topical and oral anthelmintic drugs require. Additionally, when the drug is administered as an injectable, it has a higher efficacy than being administered as a pour-on product, as weather or animal licking could impact efficacy of the pour-on products (Sutherland and Leathwick, 2011). Furthermore, the long-term release of the product makes it ideal to prevent parasite issues in herds throughout the grazing season without multiple administrations as it can stop the life cycle of the GIN and prevent resident adult worms from establishing in the host (Forbes, 2013).

Extended-Release Eprinomectin and Fescue Toxicosis

An experiment evaluating heifers treated with extended-release eprinomectin (**ERE**), a combination of moxidectin and oxfendazole (**MO**) and untreated controls (**CON**) was conducted to determine differences in heifer performance (Backes, 2016). Heifers in this experiment grazed endophyte-infected tall fescue, although, the impact on fescue toxicosis was not an objective. Only one parameter commonly associated with fescue toxicity was evaluated, hair coat score; although, no differences between treatments were reported. Fecal egg count were greater in CON heifers throughout the trial with the greatest difference being on d 84 with CON having 164 EPG

while treated cattle had 80 EPG and 6 EPG (MO and ERE, respectively). In addition, BW tended to be greater on d 84 for treated cattle compared to untreated cattle and the improvement continued from d 112 to 182. Moreover, ERE heifers tended to have greater BW on d 112 and had greater BW compared to MO heifers from d 140 to the end of the experiment (d 274). This improvement in BW also transitioned into an improvement in ADG and BCS at several time points throughout the experiment for treated heifers compared to CON heifers. It is important to note, that these cattle did have parasite differences, which could potentially impact the performance differences in this experiment. Furthermore, this experiment lacked replication of treatments as heifers grazed pastures separated by treatments with only one group per treatment.

Not only did Backes (2016) report differences in performance, but also noted greater heifer cyclicity, estrous detection, natural service and overall pregnancy rates in treated heifers compared to CON heifers. Additionally, ERE tended to have greater overall pregnancy rates compared to MO heifers (89 vs. 71%, respectively). Further research should be conducted in this area to confirm these results without the confounding issues.

In another experiment, Backes (2016) utilized spring-calving cows to determine differences in cows treated with extended-release eprinomectin (ERE), oral fenbendazole (**OXF**) and untreated controls (**CON**). Cattle grazed endophyte-infected tall fescue pastures, although determining impacts on fescue toxicity symptoms was not an objective. Hair coat score was the only parameter commonly associated with fescue toxicity that was evaluated. Hair coat score was greater for OXF cattle at d 91 compared to ERE and CON cattle, but it was not different at any other time point. Throughout the experiment, FEC were low (< 6 EPG), although, CON cattle had greater FEC at all time points compared to ERE and OXF cattle. There was a tendency for BW to be greater for CON cattle at d 14 compared to ERE and OXF cattle, although no

differences were reported at any other time. Cow BCS tended to be greater on d 14 and was greater on d 91 for OXF cattle compared to ERE cattle; however no differences were reported at any other time point. Pregnancy rate also tended to be greater for OXF cattle compared to ERE (81 vs. 61%, respectively). While this experiment had low FEC below the economic threshold, this experiment lacked replication of treatments as treatments grazed pastures in 2 groups per treatment and only 90 cattle in total were used in the experiment. Additional research in these areas should be conducted to determine differences without the confounding issues previously mentioned.

A case study was conducted on extended-release eprinomectin in fall-calving beef cows to determine differences in cow performance compared to injectable ivermectin (Andresen et al., 2018a). Treatments were administered in the fall, prior to the calving season, and both primiparous and multiparous cows were utilized. Based on the experimental design, ERE multiparous cows were heavier at experiment initiation than primiparous cows and ivermectin (**CONV**) multiparous cows. This greater BW continued throughout the experiment and ERE multiparous cows lost less BW over the duration of the experiment. However, there was no difference in pregnancy rates between treatments. Calves from ERE dams tended to have greater weaning weights compared to CONV calves and had greater adjusted weaning weights, as they were younger at weaning (Andresen et al., 2018a). It is important to note that this case study lacked replication of pastures, as well as, lacked animal numbers (n = 53 CONV and n = 66 ERE), and FEC were not evaluated so it is difficult to compare this data to other research. Additionally, forage type was not mentioned so it is difficult to determine if these cattle were exposed to fescue toxicosis.

In a similar second experiment, fall-born beef heifers were utilized to determine differences on heifer performance between fall treatments of injectable ivermectin (**CONV**) and ERE (Andresen et al., 2018a). Greater BW was reported at final pregnancy check for ERE heifers compared to CONV; however, a year after initial deworming, ERE only tended to have greater BW to CONV heifers. Additionally, ADG was greater for ERE heifers for the duration of the experiment and pregnancy rates were greater for ERE heifers after artificial insemination and the overall breeding season. However, this experiment is also lacking replication of pastures, as well as lacking animal numbers (n = 33 CONV and n = 41 ERE), and FEC were not evaluated so it is difficult to compare this data to other research. Again, forage type was not mentioned, so exposure to fescue toxicity is unknown.

In a larger study, 12 herds across seven states were utilized to determine impacts of ERE on production variables of beef cows and calves (Andresen et al., 2018b). Treatments included spring administration of ERE or injectable doramectin (**DOR**). Parasite infections were reported in this experiment, but remained low for both treatments (< 3 EPG) for the duration of the experiment. Cow BW and BCS was not different between treatments throughout the experiment. No differences were also reported for fly counts and pregnancy rates along with no differences in calf BW at birth, treatment, or weaning and ADG. However, fescue toxicosis was not an objective in this experiment so fescue toxicity parameters were not evaluated. Additionally, forage type was not mentioned so it is difficult to compare to other research.

Only one experiment has evaluated extended-release eprinomectin impacts on fescue toxicosis (Volk et al., 2019). Volk et al. (2019) utilized growing heifers grazing E+ tall fescue with relatively low FEC (< 14 EPG). Treatments included a spring injection of extended-release eprinomectin (**ERE**) or a saline control (**CON**). Typical fescue parameters such as hair coat

score, respiration rate and serum prolactin were not different between treatments, although there was an improvement in BW and BCS in ERE cattle. In addition, pregnancy rates were improved in ERE cattle as well. No mechanism was suggested and more research is required to determine the cause of increased growth and reproductive performance.

Research focusing on the effects of ERE on fescue toxicosis are lacking in beef cattle. Only one experiment has evaluated ERE on fescue toxicosis. Other research has been conducted utilizing ERE on beef heifers and cows; however, there have been issues in parasite infection differences as well as the lack of measuring fescue toxicosis parameters. In the future, the industry could potentially benefit from determining the effects of ERE on cattle grazing E+ tall fescue that are exhibiting symptoms of fescue toxicosis.

CYTOCHROME P450

Role in Ergot Alkaloid Metabolism

Ergot alkaloids have been shown to cause numerous effects on cattle performance (Strickland et al., 2011). Metabolism of ergot alkaloids in ruminants is a bit different than in other species as they have pregastric fermentation. An in vitro study conducted by Ayers et al. (2009) demonstrated that ergot alkaloid concentration increased over time in the rumen fluid which an in vivo study lead to increased absorption in the rumen. To alter the toxin in the body and eliminate them, ergot alkaloids go through a process called biotransformation which occurs through cytochrome P450 enzyme systems (Ball et al., 1992; Moubarak and Rosenkrans, 2000).

Cytochrome P450 enzymes (**CYP**) are located in the liver and are classified into families based on their function (Anzenbacher and Anzenbacherova, 2001). An example of a family would be denoted as CYP3 and then if the family needs to be divided into subfamilies, a letter is used to denote this classification (Anzenbacher and Anzenbacherova, 2001). For example, the

specific subfamily reported to biotransform ergot alkaloids is the CYP3A (Ball et al., 1992). Within this subfamily there are three genes: CYP3A4, CYP3A5 and CYP3A7. Two of these three (CYP3A4 and CYP3A5) are expressed in adults while CYP3A7 is expressed in fetal life (Strickland et al., 2011). Activity of CYP3A4 can vary greatly between individuals which is thought to be from DNA mutations and can vary up to 60-fold (Anzenbacher and Anzenbacherova, 2001; Strickland et al., 2011). This difference is also thought to potentially account for differences in individual's susceptibility to ergot alkaloid intoxication (Strickland et al., 2011). Ergot alkaloids can either activate or inhibit CYP activity which impacts the animal's ability to handle the ergot alkaloids (Settivari et al., 2006). Additionally, as an individual ages, there is a decrease in drug metabolism which can also cause variability in CYP enzyme activity (Anzenbacher and Anzenbacherova, 2001).

Drug-Drug Interactions

Not only are CYP enzymes known for metabolizing ergot alkaloids, but CYP enzymes are also known to be the main enzyme family that metabolizes therapeutic drugs (Cali et al., 2009). The most active CYP in metabolizing drugs is CYP3A4 and because of this, it plays an important role in adverse drug-drug interactions (Cali et al., 2009; Strickland et al., 2011). However, CYP3A5 is less active in xenobiotic (drug) metabolism (Strickland et al., 2011). A drug interaction occurs when one of two scenarios occurs. If a drug inhibits CYP3A4 enzyme activity, it will slow the clearance of a co-administered drug which can lead to drug toxicity (Cali et al., 2009). Additionally, if the drug induces the expression of CYP3A4, then it will increase the clearance of a co-administered drug which leads to reduced efficacy (Cali et al., 2009). Moreover, interactions are not only observed with drugs but components of the diet can also take part (Anzenbacher and Anzenbacherova, 2001). The first of these scenarios is likely what is

occurring when ergot alkaloids are consumed, as they can inhibit and are metabolized by CYP enzymes, which leads to fescue toxicosis. The second scenario is an option for a way to potentially mitigate fescue toxicosis if a drug that could induce CYP3A4 expression was identified as it could increase the clearance of the ergot alkaloids.

Characteristics of having the ability to alter a concurrently administered drug include having a relatively long elimination half-life, the ability to bind to cytochrome P450 and having a high lipid solubility (Anadon, 1982). Ivermectin has been reported to be a potent inducer of liver enzymes and it has all three of the previously mentioned characteristics (Bohlen et al., 1995). Bohlen et al. (1995) reported no drug-to-drug interactions when comparing the use of ivermectin along with either antipyrine or erythromycin. The authors mentioned that the lack of effect could mean ivermectin does not impact biotransformation of drugs, but the authors cautioned drawing conclusions on all drugs from this (Bohlen et al., 1995).

It is also important to note that the liver is the most important organ for xenobiotic metabolism and detoxification, which also makes it a main organ affected by fescue toxicosis (Settivari et al., 2006). Bacon and Hill (2013) summarized work that reported the liver is the first place ergopeptides are biotransformed and that drugs could impact this process. This work leads to the need for more research focusing on what drugs specifically could impact the process and potentially decrease impacts of the ergot alkaloids. Additionally, the impact on CYP in cattle that are experiencing fescue toxicosis is an area that needs more research. Analyzing CYP activity could be another method to identify cattle experiencing fescue toxicosis and could potentially be used to determine if mitigation strategies are effective.

Previous beef cattle work with CYP450

Previous work with CYP enzymes has mainly been conducted with rats and humans.

However, some more recent studies have been developed to focus on the impacts of beef cattle. Moubarak and Rosenkrans (2000) designed an experiment to determine the role of CYP3A on the metabolism of ergotamine in beef liver. They compared the metabolite profile of CYP3A in beef cattle liver that had grazed E+ tall fescue to rat liver both of which were incubated with ergotamine. Results from this experiment were the first to show that CYP3A is present in beef cattle liver and also indicates it has a role metabolizing ergot alkaloids in beef cattle (Moubarak and Rosenkrans, 2000). From this experiment, more work was conducted to determine the differences in susceptibility to fescue toxicosis.

Sales et al. (2012) focused on identifying single-nucleotide polymorphisms (**SNP**) for cattle CYP3A28 and also the relationship this SNP has on cattle grazing different forages at different body condition scores. Results from this experiment documented that cow productivity is negatively impacted by low body condition and E+ tall fescue along with the genetic alteration of a cytosine to guanine at base 994 which is known as C994G in CYP3A28 (Sales et al., 2012). A similar study was published the following year, which focused on Brahman-influenced cattle and stated that SNP CYP3A28 was associated with cattle productivity (Sales et al., 2013). Further research is needed in this area to determine the impacts genetic selection could have on the industry.

Moubarak et al. (2012) tested the P450-Glo CYP3A4 assay kit to confirm if this assay is appropriate for determining the toxic levels of E+ tall fescue. Commercially available ergot alkaloids were used as the control. From this experiment, one can conclude that the P450-Glo assay will be useful when studying specific effects of each component of the E+ tall fescue on the detoxification methods in the liver (Moubarak et al., 2012). Rosenkrans Jr. and Ezell (2015) continued working with this assay and confirmed that urine from cattle grazing E+ tall fescue

could be used to see differences in CYP activity. This experiment also found that urine inhibition tended to be correlated with ADG of the steers. Additionally, steer genotype impacted inhibition of CYP450 activity in the urine with the least inhibition in heterozygous steers (Rosenkrans Jr. and Ezell, 2015). With more research, the P450-Glo assay may be useful in the future for identifying cattle that will be susceptible to ergot alkaloids. Furthermore, this assay may also be useful in determining the level of exposure cattle have had to fescue toxicosis.

ANTHELMINTIC RESISTANCE IN CATTLE PARASITES

Resistance in the United States

The push for the industry to use anthelmintic drugs in cattle to improve animal performance appeared to be a step in the right direction at the time; however, good pasture management for parasites to prevent drug resistance was not as heavily focused on (Gasbarre et al., 2009; Sutherland and Leathwick, 2011). Sutherland and Leathwick (2011) defined anthelmintic resistance as having a greater number of GIN able to tolerate doses of the product than had previously been able to in a normal population of GIN. In small ruminants, GIN resistance is well documented in previous research to multiple classes of anthelmintic drugs (Waller, 1997); however, for cattle in the US, the first documented case has occurred in the past decade (Gasbarre et al., 2009). This resistance, specifically in macrocyclic lactones, has been seen and rapidly increasing in *Cooperia* spp. along with *Haemoncus* sp. (Gasbarre, 2014). According to the 2008 USDA National Animal Health Monitoring System's survey, in the cow-calf industry across the United States, *Cooperia* spp. is the most prevalent parasite (Stromberg et al., 2015). Stromberg et al. (2015) hypothesized that *Cooperia* spp. has greater resistance to macrocyclic lactones than in the past. Sutherland and Leathwick (2011) conducted a search on published literature to determine if resistance is increasing over time. Of the 145 documented

cases of GIN resistance in cattle parasites, majority of the cases occurred in the last five years prior to the search (Sutherland and Leathwick, 2011). An increase in resistance could be related to two factors: resistance is becoming a larger issue or that more producers and researchers are testing for resistance (Sutherland and Leathwick, 2011).

Resistance Detection Methods

Determining resistance in cattle can be more difficult than in other small ruminants as cattle tend to have lower FEC overall compared to small ruminants (McArthur and Reinemeyer, 2014). The most common method used to measure GIN resistance is the fecal egg reduction test (**FERT**; Coles et al., 1992; Gasbarre, 2014). Guidelines for the FERT were set by the World Association for the Advancement of Veterinary Parasitology in the early 1990s (Coles et al., 1992). This process involves sampling prior to anthelmintic treatment and again 14-28 days post-treatment depending upon the anthelmintic drug used. Groups of animals included in the study typically include a treated and an untreated group so that differences in GIN levels can be determined throughout the experiment (Taylor et al., 2002). The guidelines for sampling number in cattle are not clearly defined, but suggested to be around 10 animals (Coles et al., 1992) but for measuring resistance in a group, 20 animals is suggested to be the ideal sample size (Gasbarre, 2014). Resistance is present if the percentage of FERT is less than 95% (Sutherland and Leathwick, 2011) while others suggest 90% should be the threshold (Coles et al., 1992). Levecke et al. (2012) tested the detection limit of reduced efficacy of the tested anthelmintic for the FERT based on the method and number of FEC being excreted by the animal, sample size and sensitivity of the FEC method used. Overall results from this experiment concluded that with FEC methods having a sensitivity ≥ 5 EPG and sample sizes of < 15, unreliable results of the FERT would occur if the reduced-efficacy threshold was 95% (Levecke et al., 2012). However,

most combinations of sensitivity and sample size had reliable detection limits when the reduced efficacy-threshold was set at 90% (Levecke et al., 2012).

Another way to quantify anthelmintic resistance is to treat an infected host with the anthelmintic, and then kill the host to recover the GIN to be enumerated and classified by species (Gasbarre, 2014). This method allows for accurate quantification; however, it requires killing a large group of cattle for an experiment, which is not practical, so it is not commonly used (Gasbarre, 2014).

Previous Research with Anthelmintic Resistance

As more reports of resistance have been documented in cattle, the interest in researching the level of resistance between different anthelmintic drugs has increased. Edmonds et al. (2010) conducted an experiment in California with heifer calves from multiple sources that had previously been administered with macrocyclic lactones several months prior to the study initiation. Cattle were processed and an initial FEC was recorded which was then used to stratify the heifers to their treatments. Anthelmintic treatment groups consisted of injectable ivermectin, injectable moxidectin, oral fenbendazole, oral oxfendazole, and saline. Fourteen days post-treatment, cattle were necropsied for GIN infection to be determined. Reduced efficacy of all four anthelmintic drugs was detected as not all species of worms were reduced by >90%. Worm species that showed resistance included *Cooperia spp.* and *O.Ostertagi* (Edmonds et al., 2010).

Resistance was also measured in the southern United States by Walker et al. (2013) focusing on moxidectin or oxfendazole or a combination of the two given at separate times. Both heifers and steers were used in this FERT study. An addition of using coproculture procedure to classify the GIN remaining post-treatment was implemented so that no animals would need to be necropsied. Cattle given a combination of the two anthelmintic drugs at different times provided

greater parasite control and improved animal performance; however, it was noted that giving oxfendazole first provided better GIN control than if moxidectin was given fist. This study indicated signs of resistance of *Cooperia spp.* to moxidectin, but the authors noted that drought conditions were present during this experiment which could have impacted the results (Walker et al., 2013).

To determine the effectiveness of an extended-release eprinomectin (LongRange), Kunkle et al. (2013) conducted a study using 475 cattle across 7 locations in the United States. Control cattle were comingled with treated cattle throughout the experiment. Body weight, FEC, as well as a coproculture procedure was used to identify remaining GIN species post-treatment. All extended-release eprinomectin treated cattle had FEC reduced by ≥ 95% and the control cattle also had reduced FEC which could be related to the comingling of the cattle. Additionally, weight gain was significantly greater in eprinomectin treated cattle compared to control cattle (Kunkle et al., 2013). No resistance was seen in extended-release eprinomectin as an anthelmintic in this study; however, this experiment occurred shortly after extended-release eprinomectin was released to the market so more research on resistance should be conducted after extended-release eprinomectin has been used in the industry for a few years.

Reducing Resistance

Resistance to anthelmintic drugs can be significantly more of a problem when the dose is under or over administered to the animal (Smith et al., 1999). To delay resistance, these drugs can be used in combination with another drug that has lower resistance (Gasbarre, 2014). For example, an injectable macrocyclic lactone co-administered with either benzimidazole or levamisole could control parasites that are resistant to macrocyclic lactones but not the latter two. Injectable slow-release anthelmintic that are effective over longer periods of time are able to

maintain optimal productivity, but others suggest that this will shorten the effectiveness of the anthelmintic by allowing for even more selective pressures on the parasite genome (Gasbarre, 2014). However, mitigating the risk of resistance for long-acting products is essentially the same as shorter-acting products (Forbes, 2013).

The first case of anthelmintic resistance in cattle was documented in macrocyclic lactones the past decade (Gasbarre et al., 2009). It is important to note the heavy reliance on macrocyclic lactones in cattle production as they make up 88% of cattle anthelmintic sales (McArthur and Reinemeyer, 2014). One should also note, that implementing change in the use of anthelmintic drugs to reduce risk in the beef industry appears to be necessary, but, will require multiple discussions between producers, veterinarians, and researchers (McArthur and Reinemeyer, 2014). By implementing a change sooner rather than later, the industry may be able to prevent more issues of resistance from occurring that could be a larger detriment to the industry.

CONCLUSIONS

Tall fescue is productive in multiple environments and is still commonly grazed today by cattle across the United States. Fescue toxicosis is a concern to many cattle producers as it negatively impacts the industry greatly. Mitigation strategies to date have reduced symptoms of fescue toxicosis; however, they have not been cost effective or sustainable long-term. Some uses of anthelmintics in cattle grazing tall-fescue have shown improvements in cattle performance, with only previous experiment evaluating the impacts of extended-release eprinomectin.

In addition, macrocyclic lactone resistance has been reported in cattle parasites across the United States. Evaluations on parasite resistance to extended-release eprinomectin has not been widely reported at this time. Therefore, research in this thesis will evaluate parasite resistance to

extended-release eprinomectin on an operation after three years of use as well as discuss the effects of extended-release eprinomectin on heifer performance and fescue toxicity.

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CHAPTER 2

EVALUATION OF PARASITE RESISTANCE IN HEIFERS TREATED WITH EXTENDED-RELEASE EPRINOMECTIN

ABSTRACT

The objective of this experiment was to determine if parasite resistance to extended-release eprinomectin (LongRange) is present in a cow-calf operation after 3 years of eprinomectin use and effects on heifer growth performance. Fall-born Angus × Simmental heifers [224 ± 22 d of age; 171.4 ± 17.8 kg initial body weight (**BW**)] were stratified by d -2 fecal egg count (**FEC**) and BW and were assigned to one of nine groups (7 heifers per group) using a stratified randomized design. Groups were then assigned to one of three treatments: extended-release eprinomectin (**ERE**; n = 3, extended-release eprinomectin and oxfendazole (**COMBO**; n = 3), or saline control (**CON**; n= 3). At experiment initiation, all heifers were administered treatments according to label instructions. All heifers grazed endophyte-infected tall fescue in groups and were supplemented daily with a 50:50 mix of distillers grains and soybean hulls (1.8 kg per heifer per d). Body weight and body condition score (**BCS**) were recorded 7 times throughout the experiment, while FEC and packed cell volume (**PCV**) were recorded 6 times throughout the experiment. A fecal egg reduction test (**FERT**) occurred on d 28. There was a treatment × time interaction ($P < 0.01$) for FEC. Fecal egg count was not different ($P \geq 0.51$) at d -2, 28, 112, and 167, but CON heifers had greater FEC ($P < 0.01$) than ERE and COMBO heifers on d 55. Also, at d 55 ERE heifers had greater FEC ($P = 0.04$) than COMBO heifers. At d 83 CON heifers had greater FEC ($P < 0.01$) than ERE and COMBO heifers. However, FEC was not different on d -2, 28, 83, 112, or 167 ($P \geq 0.13$) between ERE and COMBO heifers. Fecal egg count reduction for ERE and COMBO were above the 90% threshold (91% and 98% reduction, respectively). There

was no treatment × time interaction or treatment effect ($P \geq 0.14$) for PCV. Additionally, there was a treatment × time interaction ($P < 0.01$) for BW. At d 83, ERE and COMBO tended ($P = 0.09$) to have greater BW than CON heifers and on d 112 through d 168 ERE and COMBO heifers had greater BW ($P < 0.01$) than CON heifers. Likewise, there was a treatment × time interaction ($P < 0.01$) for BCS with COMBO having greater BCS ($P < 0.01$) compared to ERE and CON at d 55. On d 83, 112, 147, and 167, COMBO heifers had greater BCS ($P < 0.01$) compared to CON heifers. In addition, ERE had greater BCS ($P < 0.01$) compared to CON on d 83, 112, and 167. Parasite resistance to extended-release eprinomectin was not present based on FERT, and ERE and COMBO heifers had greater growth performance compared to CON heifers.

Keywords: anthelmintic resistance, beef heifer, extended-release eprinomectin

INTRODUCTION

Anthelmintic drug resistance was first documented in small ruminants in 1964 (Waller, 1994), but more recently in 2003, resistance was reported in U.S. beef cattle (Gasbarre et al., 2009). This resistance specifically to macrocyclic lactones in cattle has been noted and rapidly increasing in *Cooperia spp.* along with *Haemonchus sp.* (Gasbarre, 2014). According to the 2008 National Animal Health Monitoring System's survey, *Cooperia* spp. is the most prevalent parasite in the United States cow-calf industry, and could be increasing resistance to macrocyclic lactones (Stromberg et al., 2015). Additionally, parasite treatment and prevention is costly to the beef industry with $2.5 billion being spent on anthelmintic drugs each year (Williams and Loyacano, 2001). Internal parasites can impact cattle of all ages, but younger cattle are more

susceptible than mature cows (Miller, 1993). One of the most notable and costly impacts of clinical parasitism is decreased growth performance of growing cattle (Hawkins, 1993).

Resistance to anthelmintic drugs can be significantly more problematic when the dose is under or over administered to the animal (Smith et al., 1999). To prevent resistance, a combination of anthelmintic classes can be used at the same time. For example, an injectable macrocyclic lactone co-administered with either benzimidazole or levamisole could control parasites that are resistant to macrocyclic lactones but not the latter two (Gasbarre, 2014). Injectable extended-release anthelmintics that are effective over longer periods of time are able to maintain optimal productivity, but some suggest that this will shorten the effectiveness of the anthelmintic by allowing for even more selective pressures on the parasite genome (Gasbarre, 2014). However, mitigating the risk of resistance for long-acting products is essentially the same as shorter-acting products (Forbes, 2013). An extended-release eprinomectin has been recently released to the market (Forbes, 2013), but research is limited on parasite resistance to this product. Edmonds et al. (2018), reported parasite resistance to extended-release eprinomectin; however, this was conducted in treatment groups with different initial FEC and was only evaluated at one location. Therefore, the objective of this experiment was to determine if parasite resistance [<90% fecal egg reduction test (**FERT**)] was present in an operation after three years of use of extended-release eprinomectin and to determine the corresponding impacts on heifer growth performance.

MATERIALS AND METHODS

Animals and Experimental Design

The Institutional Animal Care and Use Committee of the University of Illinois approved the procedures used in this experiment (protocol 18092) and followed the guidelines

recommended in the Guide for the Care and Use of Agricultural Animal in Agricultural Research and Teaching (FASS, 2010).

To determine if parasite resistance to extended-release eprinomectin (LongRange; Boehringer Ingleheim, Duluth, GA) is present in an operation after three years of use, 63 Angus × Simmental heifers [171.4 ± 17.8 kg initial body weight (**BW**); age = 224.2 ± 21.6 d; mean ± standard deviation] were utilized at the University of Illinois Dixon Springs Agricultural Center in Simpson, IL. Heifers were stratified by d -2 fecal egg count (**FEC)** and BW and were assigned to one of nine groups (7 heifers per group) using a stratified randomized design. Groups were then assigned to one of three treatments (3 groups per treatment): extended-release eprinomectin (**ERE**), extended-release eprinomectin and oxfendazole (Synanthic; Boehringer Ingleheim, Duluth, GA; **COMBO**), or saline control (**CON**). Extended-release eprinomectin and saline were subcutaneously injected at a rate of 1mL/50 kg of BW on d 0, while oxfendazole and water were given as an oral drench at a rate of 1mL/50kg of BW on d 0. Any cattle not receiving an extended-release eprinomectin injection received a saline injection, while cattle not receiving oxfendazole oral drench, received a water drench at the same dosage.

Cattle rotationally grazed endophyte-infected tall fescue ('Kentucky-31'; 84% infected; total ergot alkaloid concentration: June: 804 µg/L; July 199 µg/L; August: 350 µg/L; October: 1169 µg/L) pastures and had free-choice to a mineral supplement (Southern FS Services, Marion, IL; 12% Ca, 9.5% P, 17% salt, 5.9% Mg, 1.15% K, 24 mg/kg Co, 31 mg/kg I, 3,000 mg/kg Fe, 1,400 mg/kg Cu, 2,000 mg/kg Mn, 26.4 mg/kg Se, 4,000 mg/kg Zn, 550,000 IU/kg vitamin A, 3,300 IU/kg vitamin D, 220 IU/kg Vitamin E, and 6,600 mg/kg chlortetracycline). Pastures were grazed for three years prior by cattle treated with extended-release eprinomectin. Pasture size was 2.63 ± 0.11 ha, with an average of 0.38 heifers/ha. Groups were rotated every 14 d. As

groups were rotated, available forage was quantified in the pastures heifers were rotated out of and the pasture they went to by using a falling plate meter (Jenquip, Fielding, New Zealand) to collect 12 random measurements. A minimum of 99 kg DM/ha was available to all groups throughout the study, with 291 kg DM/ha of forage available on average (Fig. 2.2). Heifers were also supplemented daily with a 50:50 mix of soybean hulls and distillers grains [20.6% crude protein (**CP**), 51.9% neutral detergent fiber (**NDF**), 31.3% acid detergent fiber (**ADF**), and 5.1% crude fat] at a rate of 1.8 kg per heifer per d.

Sample Collection and Analytical Procedures

Body weights were collected seven times throughout the experiment with full 2-d BW measurements collected and averaged at the beginning (d -1 and 0) and end of the experiment (d 167 and 168). Throughout the experiment, 1-d full BW measurements were collected on d 28, 55, 83, 112, and 147. Body condition scores (**BCS**) were collected concurrent with BW and were evaluated using a 1-9 scale [emaciated =1; obese = 9; as described by (Wagner et al., 1988)].

Rectal fecal grab samples were collected on d -2, 28, 55, 83, 112, and 167 for determination of FEC per gram of feces using a modified version of the Modified Wisconsin flotation method described in Volk et al. (2019). Fecal egg reduction test (**FERT**) was performed on d 28 to determine percent reduction using the following formula: % reduction = [(untreated FEC - treated FEC)/ untreated FEC] × 100 (DeRouen et al., 2009). Coprocultures were performed on fecal samples collected on d -2, 28, and 112 at the University of Georgia. Fecal samples were composited by pasture and 3rd stage larvae were identified and enumerated (Agriculture, 1977). Up to one hundred infected larvae, if possible, were identified to genus for magnitude of population differences.

Blood samples were collected for plasma at six (d -1, 28, 55, 83, 112, and 167) time points during the experiment. Blood was collected via jugular venipuncture into one 10 mL plasma blood collection vacuum tube with K2 EDTA (Becton, Dickinson and Co., Franklin Lakes, NJ). Hematocrit [packed cell volume (**PCV**)] was determined using the micro-hematocrit procedure. Heparinized 75 mm micro-hematocrit capillary tubes (Fisher Scientific Co., Cat. No. 02-668-66) were filled 2/3 full with whole blood and sealed with Critoseal clay in duplicate. Samples were then centrifuged in an international micro-capillary centrifuge (Model MB; International Equipment Company, Boston, MA) for 10 minutes and read on a micro-hematocrit capillary tube reader (Oxford Labware, St. Louis, MO) to determine % PCV.

Forage samples were collected by randomly clipping approximately 5 cm from the ground, from at least 12 different locations, within each pasture. Feed and forage samples were collected every two weeks throughout the experiment. Forage and feed samples were dried at 55°C for a minimum of 3 d, ground through a 1 mm screen using a Wiley mill (Arthur H. Thomas, Philadelphia, PA). Forage samples were composited by group into 3 time periods and feed samples were composited for the entire experiment. Ground feed and forage were analyzed for NDF and ADF using an Ankom 200 Fiber Analyzer (Ankom Technology, Macedon, NY) as well as CP (Leco TruMac, LECO Corporation, St. Joseph, MI; Fig. 2.1). Feed samples were also analyzed for crude fat using an Ankom XT10 fat extractor (Ankom Technology, Macedon, NY). Total ergot alkaloid analysis of forages was conducted in a commercial laboratory (Agrinostics Limited, Co., Watkinsville, GA).

Statistical Analysis

A stratified randomized design was used and group served as the experimental unit. Body weight, BCS, PCV, FEC, and forage analysis were analyzed as repeated measures in the MIXED

procedure of SAS (SAS Inst. Inc., Cary, NC), with fixed effects of treatment, and time, and the interaction of treatment and time. Group nested within treatment was included as a random effect. The REPEATED statement was used to model the repeated measurements within animal for each variable and the heterogeneous autoregressive (1) covariance structure was used after considering the Log Likelihood. Fecal egg count residuals were not normally distributed, so FEC were transformed using the BoxCox procedure of SAS. Fecal egg counts were transformed using $(FEC +1)^{-1.25}$. Untransformed least square means are reported for FEC. The SLICE statement was used to separate least square means when the interaction of treatment and time was significant ($P \leq 0.05$). Significance was declared at $P \leq 0.05$, and tendencies were declared from $0.05 < P \leq 0.10$. Means reported in tables and figures are least squares means.

RESULTS

There was a treatment × time interaction ($P < 0.01$; Table 2.1) for FEC. Fecal egg count was not different ($P \geq 0.51$) on d -2, and d 28, but on d 55 CON heifers had greater FEC ($P < 0.01$) than ERE and COMBO heifers. Also, at d 55 ERE heifers had greater FEC ($P = 0.04$) than COMBO heifers. At d 83 CON heifers had greater FEC ($P < 0.01$) than ERE and COMBO heifers. However, FEC was not different on d -2, 28, or 83 ($P \geq 0.13$) between ERE and COMBO heifers. On d 112 and 167, FEC was not different ($P \geq 0.82$) between treatments. There was no treatment × time interaction or treatment effect ($P \geq 0.14$) for hematocrit. The FERT was above the threshold for both ERE and COMBO groups (>90%) at 28 d post-treatment (91% and 98%, respectively; Fig. 2.3).

On d -2, infective larvae were recovered by coproculture and recorded by treatment groups with 80, 101, and 76 larvae present for ERE, COMBO, and CON heifers, respectively. *Cooperia* was the primary species (63% ERE, 56% COMBO, and 59% CON; Fig. 2.4) and

Ostertagia was the second most prevalent (31% ERE, 37 % COMBO, and 34% CON). *Haemonchus sp.* represented a small portion of all infective larvae in all treatments (9% ERE, 7% COMBO, and 10% CON). Only COMBO heifers had *Oesophagostum* species; however, this was a low percent (1%).

Twenty-eight days post-treatment, after coproculture procedure, 25, 2, and 78 larvae were speciated for ERE, COMBO, and CON heifers, respectively. There were too few larvae from COMBO cattle to speciate. *Cooperia spp*. represented 44% of ERE larvae and 71% of CON larvae. *Ostertagia* represented 59% of the ERE larvae and 26% of the CON larvae. ERE cattle had no larvae from the *Haemonchus sp.* and *Oesophagosum sp. Haemonchus sp* represented 4% of CON larvae and *Oesophagostum* represented 2% of CON larvae.

After 112 d post-treatment, 36, 8 and 32 larvae were identified from ERE, COMBO, and CON cattle, respectively. Primarily *Cooperia spp.* was present (57% ERE, 81% COMBO, and 55% CON), with the remaining species including *Ostertagia* (21% ERE, 6% COMBO, and 30% CON), *Haemonochus sp.* (18% ERE, 13% COMBO, 11% CON), and *Oesophagostum* (7% ERE, 0% COMBO, 5% CON).

There was a treatment × time interaction ($P < 0.01$; Fig. 2.5) for BW. Heifer BW tended to be different ($P = 0.09$) at d 83 as COMBO and ERE heifers tended to have greater BW than CON heifers. At d 112, 147, and 167, ERE and COMBO heifers had greater BW ($P < 0.01$) than CON heifers. Although, BW was not different ($P \geq 0.41$) between ERE and COMBO heifers at any time point. Additionally, there was a treatment × time interaction ($P < 0.01$) for BCS. Heifer BCS was different ($P < 0.01$) at d 55 as COMBO heifers tended to have greater BCS ($P = 0.10$) compared to ERE, and ERE heifers tended to have greater BCS ($P = 0.10$) compared to CON. At d 83 and 112, ERE and COMBO heifers had greater BCS ($P < 0.01$) compared to CON heifers,

but BCS was not different ($P = 0.28$) between treated heifers. At d 147, COMBO heifers had greater BCS ($P < 0.01$) than ERE and CON heifers, but BCS was not different ($P = 0.43$) between ERE and CON. On d 167, ERE and COMBO heifers had greater BCS ($P < 0.01$) compared to CON heifers, but BCS was similar ($P = 0.57$) between ERE and COMBO.

DISCUSSION

In this experiment, a combination of extended-release eprinomectin and an oral oxfendazole was used to compare to treatment of only extended-release eprinomectin to determine if the combination provided greater efficacy against parasite infection. Prior to treatment, all cattle had a basal level of parasite infection. Twenty-eight days post-treatment, all cattle had decreased FEC. A reduction in FEC at 14, 32, and 61 d after the start of the experiment was noted in both untreated controls and treated cattle in Edmonds et al. (2018). The reduced FEC in treated cattle in this experiment is similar to previous literature for cattle treated with a macrocyclic lactone or benzimidazole (Gasbarre et al., 2009; Edmonds et al., 2010; Walker et al., 2013; Backes, 2016). Cattle in this experiment were maintained in treatment-specific pastures to prevent cross-over effects. Moreover, at the start of the experiment the cattle were moved to new pastures. Previous research conducted on other pastures at this station reported low FEC (< 14 EPG), so the pastures may not have been heavily contaminated with parasites. Additionally, the cattle in this experiment were kept in relatively low stocking densities (0.38 heifers/ha), which may have also impacted parasite infection on the pasture (Stromberg and Averbeck, 1999; Vercruysse and Claerebout, 2001). Furthermore, at this time, lush spring forage was available, and heifers had access to excess forage (minimum of 212.65 kg DM/ha available), so they did not have to graze close to the ground and fecal pats, which could have impacted parasite loads (Stromberg and Averbeck, 1999).

It is important to note that cattle tend to have lower FEC compared to small ruminants which can make determining resistance more difficult (McArthur and Reinemeyer, 2014). Despite the low FEC, control cattle had increased FEC at d 55 and 83 compared to ERE and COMBO cattle. This is similar to results in previous literature with untreated cattle having greater FEC compared to cattle treated with an anthelmintic (Kunkle et al., 2013; Walker et al., 2013; Backes, 2016; Edmonds et al., 2018).

Efficacy of anthelmintics are commonly reported after a FERT which compares pre- and post-treatment FEC to determine the reduction in fecal eggs (Coles et al., 1992; Gasbarre, 2014). In this experiment, a threshold of 90% or greater reduction was used to determine if resistance was present based on previous literature (Coles et al., 1992). Both anthelmintic treatments in this experiment had > 90% reduction in parasite infection at 28 d post-treatment. This is similar to results found in Kunkle et al. (2013) when evaluating the use of extended-release eprinomectin after it was first released to the market. In another experiment, Edmonds et al. (2018) reported a 71 % FERT for extended-release eprinomectin. The FERT equation was different than the one used in this experiment, in which they used [(control FEC at d 32 – extended-release eprinomectin FEC at d 32) / control FEC at d 32]. Prior to treatment, Edmonds et al. (2018) reported significantly lower FEC for the extended-release eprinomectin group compared to the saline control group and other treatment groups. To accurately compare these experiments, the reduction equation used in this current experiment was utilized on the data from Edmonds et al. (2018), and a 84% reduction was determined. Parasite resistance to extended-release eprinomectin was present at that location (Edmonds et al., 2018), but was not present in this current experiment. Other previous work has shown signs of resistance to macrocyclic lactones

such as ivermectin, eprinomectin and moxidectin (Gasbarre et al., 2009; Edmonds et al., 2010; Walker et al., 2013).

Using a combination of two different classes of anthelmintic treatments did result in greater reduction in this experiment. Similarly, previous literature has shown increased reductions when using a combination of a macrocyclic lactone and benzimidazole at the same time in cattle (Edmonds et al., 2018) and in sheep (Bartley et al., 2004). Moreover, increased reductions were also reported when using two different classes of anthelmintics administered 73 d apart (Walker et al., 2013).

Prior to treatment, all treatments had several species of parasites. At 28 d post-treatment, treated cattle had reduced parasite species of *Cooperia spp., Ostertagia,* and *Haemonchus sp.* Cattle treated with both extended-release eprinomectin and oxfendazole showed no parasite species present in the coproculture data, while ERE cattle had some level of *Cooperia spp.* and *Ostertagia* present. This indicates that extended-release eprinomectin may not be as effective against *Cooperia spp.* and *Ostertagia* as a combination of anthelmintics. *Cooperia spp.* and *Haemonchus sp.* have been reported to be increasing resistance to macrocyclic lactones in the United States and around the world (Gasbarre, 2014). *Ostertagia* has been reported to show resistance, but it is less wide spread (Gasbarre, 2014).

Walker et al. (2013) reported that cattle treated with moxidectin 73 d after treatment with oxfendazole had mostly *Cooperia spp*. and *Ostertagia* present. A small portion of the larvae was *Haemonchus sp.* 14 d after treatment with moxidectin (Walker et al., 2013). In cattle treated with moxidectin and then 73 d later treated with oxfendazole, no parasite species were present after coproculture (Walker et al., 2013). However, at 35 d post second treatment, all cattle had *Cooperia spp*. and *Ostertagia* present (Walker et al., 2013). It is important to note that both

anthelmintics utilized in Walker et al. (2013) were short-acting anthelmintics and were not expected to have long-term parasite control. Additionally, coproculture data was not reported prior to the first treatment in Walker et al. (2013), so initial parasite species are unknown.

Results from Gasbarre et al. (2009) reported presence of *Cooperia spp.* and *Haemonchus sp.* in cattle treated with pour-on eprinomectin. However, the data was collected from necropsy of a subset of the animals in the experiment, so it is unknown what the parasite species were prior to treatment (Gasbarre et al., 2009). Although this demonstrates that post-treatment, parasite resistance is present (Gasbarre et al., 2009).

Coproculture data was discussed in Edmonds et al. (2018) from anthelmintic treatments of doramectin, a combination of doramectin and albendazole, extended-release eprinomectin and an untreated control. Primary species reported in control animals throughout the experiment included *Cooperia spp*. and *Haemonchus sp.* Although not all treatments were mentioned in coproculture data at each time point, at d 32, extended-release eprinomectin cattle had decreased levels of *Ostertagia* and *Cooperia spp*.

Recovered larvae post-necropsy was reported in Edmonds et al. (2018) from anthelmintic treatments of doramectin, a combination of doramectin and albendazole, extended-release eprinomectin and an untreated control. Unfortunately, no samples were collected prior to treatment, although 118 d post-treatment, parasite levels were reported. *Ostertagia* was present in samples from extended-release eprinomectin calves, but at decreased levels compared to cattle treated with either doramectin, a combination of doramectin and albendazole, or a saline control. *Cooperia spp*. was present in similar level throughout all treatments, along with *Haemonchus placei* adults and late- L4. Although early L4 *Haemonchus placei* were reduced in extended-release eprinomectin calves compared to all other treatments.

Another indicator of parasite infection is anemia, which can be measured by hematocrit. Hematocrit evaluates the oxygen carrying capacity of the blood of the animal and can also be an indicator of dehydration level of the animal (Craig, 1988; Nordenson, 2006). In this experiment, there was no difference in hematocrit between treatments.

Although not monitored in this experiment, external parasites could have been different between treatments. Previous work with extended-release eprinomectin has noted improvements in fly control (Vesco et al., 2015; Trehal et al., 2017), but other studies have not reported differences (Andresen et al., 2018).

Growth performance of growing animals is decreased by parasite infection (Hawkins, 1993). Although, parasite level was low in this experiment (< 10 EPG) and well below the economic threshold of 200 EPG (Vercruysse and Claerebout, 2001), treated cattle had greater BW compared to CON cattle starting at d 83 and continued throughout the rest of the experiment. Body condition score was also improved in treated cattle compared to CON cattle. Similar results were reported in growing heifers treated with extended-release eprinomectin in Volk et al. (2019), although in that experiment, all cattle were treated with fenbendazole 14 d prior to the start of the experiment. Cattle in this current experiment were maintained in separate groups on pastures by treatment, thus forage quality and availability was evaluated to determine if there were differences between the treatment groups. Forage quality was not different between treatments at any time point for NDF, ADF, or CP content. Additionally, all groups had similar forage availability and likely did not impact the performance differences of these cattle. Previous literature has reported increased performance in treated cattle compared to untreated cattle; however, in those experiments, FEC levels were greater (Kunkle et al., 2013; Walker et al., 2013; Backes, 2016). Therefore, in those experiments, performance differences were likely

driven by parasite infection (Kunkle et al., 2013; Walker et al., 2013; Backes, 2016). In this current experiment, parasite load was low and the authors hypothesize that it is unlikely that parasite load explain the observed difference in performance.

Overall, based on the FERT (> 90%), parasite resistance was not present in an operation after three years of use of extended-release eprinomectin. Fecal egg count reduction demonstrated that using a combination of extended-release eprinomectin and oxfendazole reduced parasite species at a greater level than extended-release eprinomectin alone. Coproculture data indicated that *Cooperia spp.* and *Ostertagia* were the most prominent parasite species present. Fecal egg counts were low in all treatments throughout the experiment and treated cattle had greater growth performance compared to control cattle.

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Conflict of interest statement. None declared.

TABLE AND FIGURES

Table 2.1 Influence of anthelmintic treatments on heifer fecal egg count and hematocrit over time

	Treatment[1]				*P*-Value[2]		
Item	ERE	COMBO	CON	SEM	Trt	Time	Trt × Time
n	3	3	3				
Fecal egg count, eggs per gram[3]					0.01	<0.01	<0.01
d -2	10.0	9.9	9.9	-	0.97		
d 28	0.6	0.1	0.9	-	0.51		
d 55	1.4[b]	0.1[c]	10.6[a]	-	<0.01		
d 83	0.5[b]	0.1[b]	4.3[a]	-	<0.01		
d 112	0.1	0.0	2.9	-	0.82		
d 167	0.6	0.4	0.5	-	0.87		
Hematocrit, % packed cell volume					0.14	<0.01	0.95
d -1	37.4	36.5	36.2	0.54			
d 28	33.7	33.5	33.0	0.57			
d 55	36.7	36.2	36.1	0.51			
d 83	36.8	35.8	35.8	0.52			
d 112	36.6	35.8	35.4	0.58			
d 167	36.1	34.7	34.0	0.57			

[a,b,c] Treatment means with different superscript are different ($P < 0.01$).

[1]Treatments are defined as ERE, heifers that received injectable eprinomectin and oral dose of water; COMBO, heifers that received injectable eprinomectin and oral dose of oxfendazole; CON, heifers received a sterilized saline solution injection and oral dose of water at initiation of experiment. All treatments were administered at a rate of 1 mL per 50 kg of body weight.

[2]Abbreviations are defined as Treatment effect (Trt) and Treatment × time effect (Trt × Time).

[3] Fecal egg count least square means are from untransformed data while the p-values are representative of the transformed data.

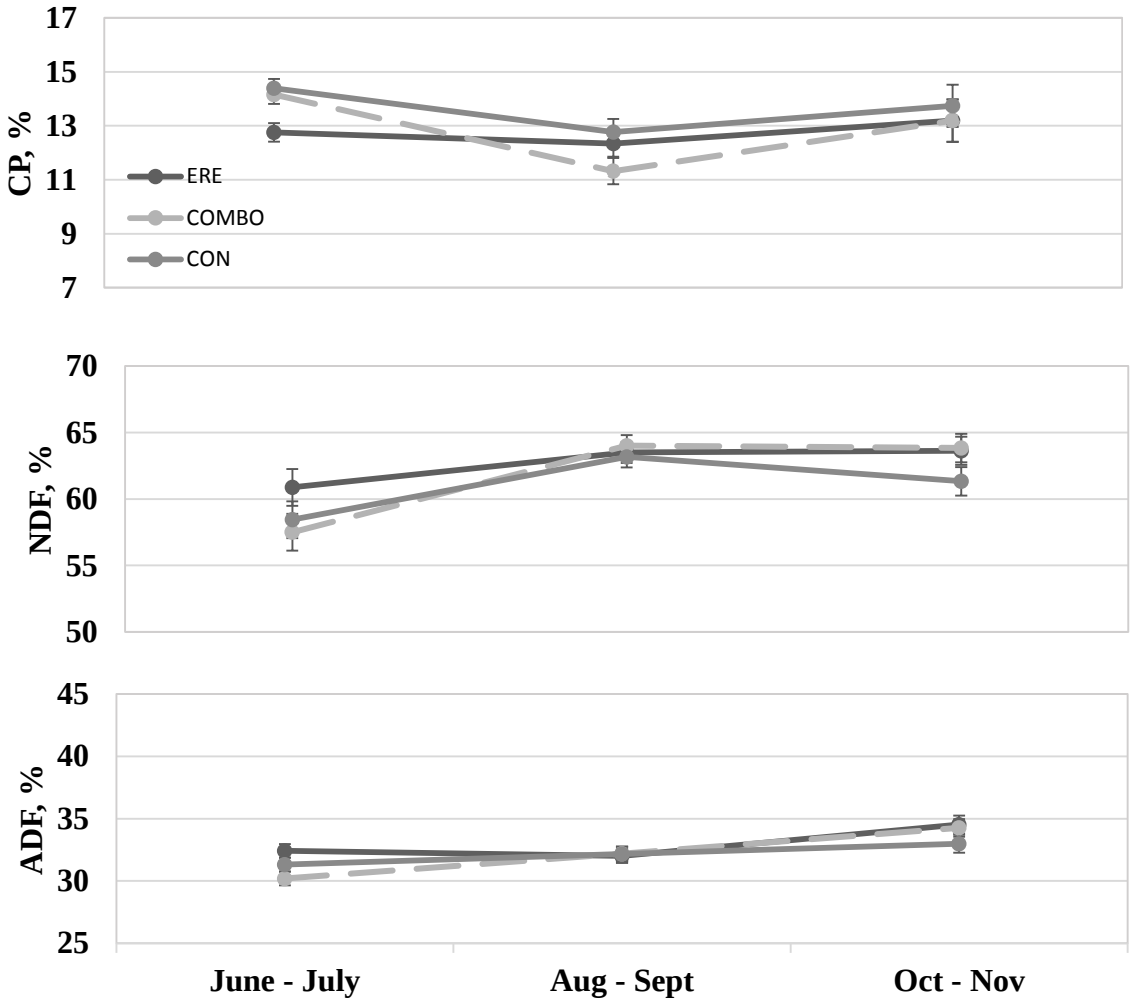

Figure 2.1 Forage quality [percentage crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber (ADF of endophyte-infected fescue (*Festuca arundinacea*) pastures from June 2018 to November 2018. Samples were collected as cattle rotated pastures and were composited every six weeks. There was no treatment × time interaction or treatment effect ($P \leq 0.12$) for NDF, ADF, or CP content. There was a time effect ($P < 0.01$) as NDF and ADF increased for all treatments over time while CP decreased at the midpoint and then increased at the end of the experiment.

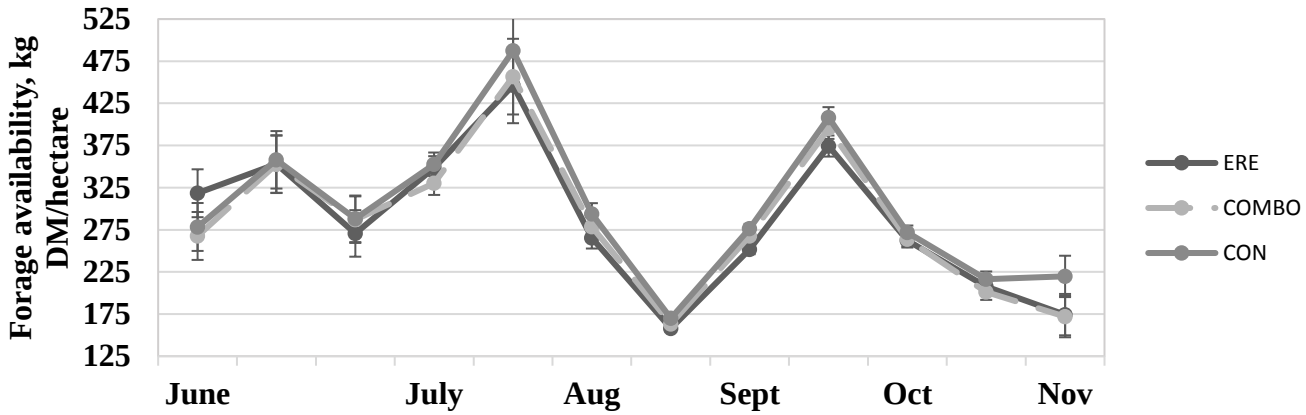

Figure 2.2 Forage availability of endophyte-infected fescue (*Festuca arundinacea*) pastures from June 2018 to November 2018. Samples were collected as cattle rotated every two weeks. There was no treatment × time interaction or treatment effect ($P \leq 0.11$) for forage availability. Forage availability fluctuated ($P < 0.01$) throughout the experiment.

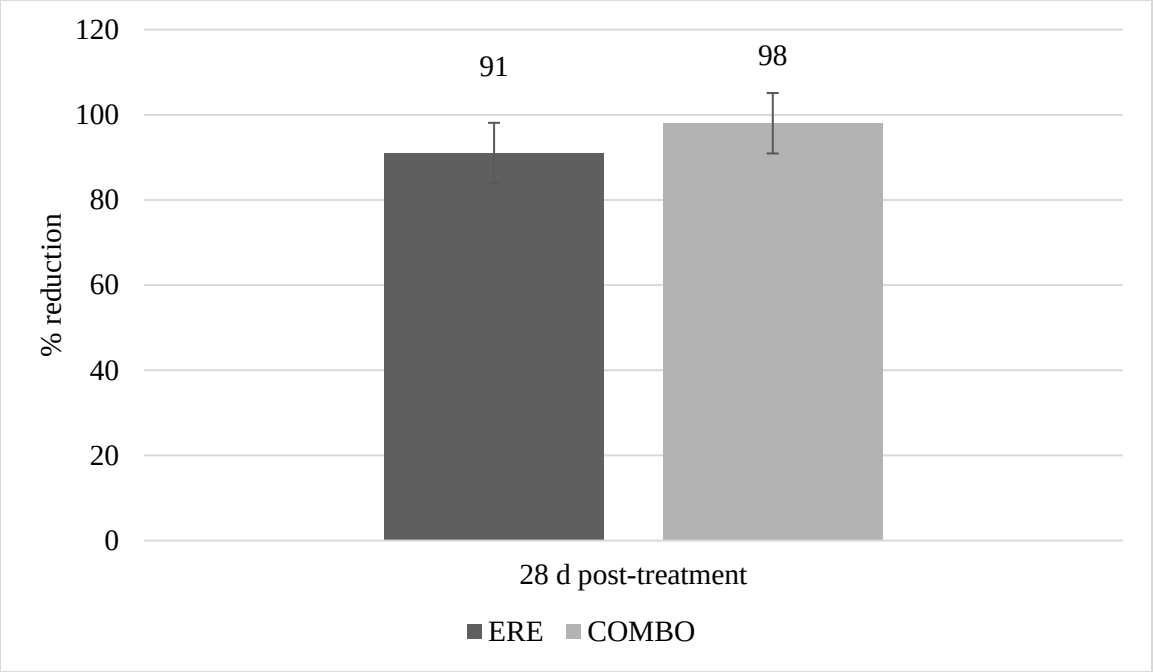

Figure 2.3 Fecal egg reduction test was determined on d 28 using the following formula: % reduction = [(untreated FEC - treated FEC)/ untreated FEC] × 100. Treatments are defined as ERE (n = 3), heifers that received injectable eprinomectin and oral dose of water and COMBO (n = 3), heifers that received injectable eprinomectin and oral dose of oxfendazole at initiation of experiment. All treatments were administered at a rate of 1 mL per 50 kg of body weight.

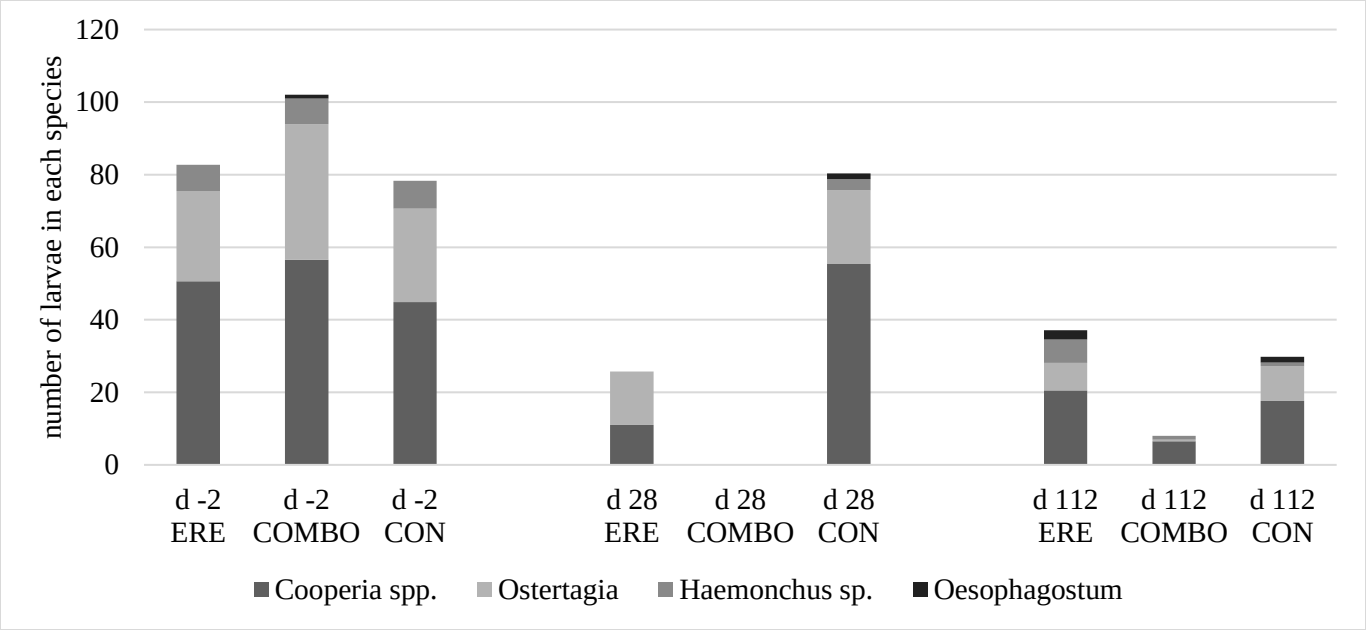

Figure 2.4 Influence of anthelmintics on heifer coproculture over time. Treatments are defined as ERE, heifers that received injectable eprinomectin and oral dose of water; COMBO, heifers that received injectable eprinomectin and oral dose of oxfendazole; CON, heifers received a sterilized saline solution injection and oral dose of water at initiation of experiment. All treatments were administered at a rate of 1 mL per 50 kg of body weight. Days are defined as d post treatment. Species are reported as the number larvae of the total count from that date. The following FEC correspond to the FEC reported after the coproculture procedure: d -2: 80, 101, and 76 larvae from ERE, COMBO, and CON respectively; d 28: 25, 2, and 78 larvae from ERE, COMBO, and CON respectively; d 112: 36, 8, and 32 larvae from ERE, COMBO, and CON respectively.

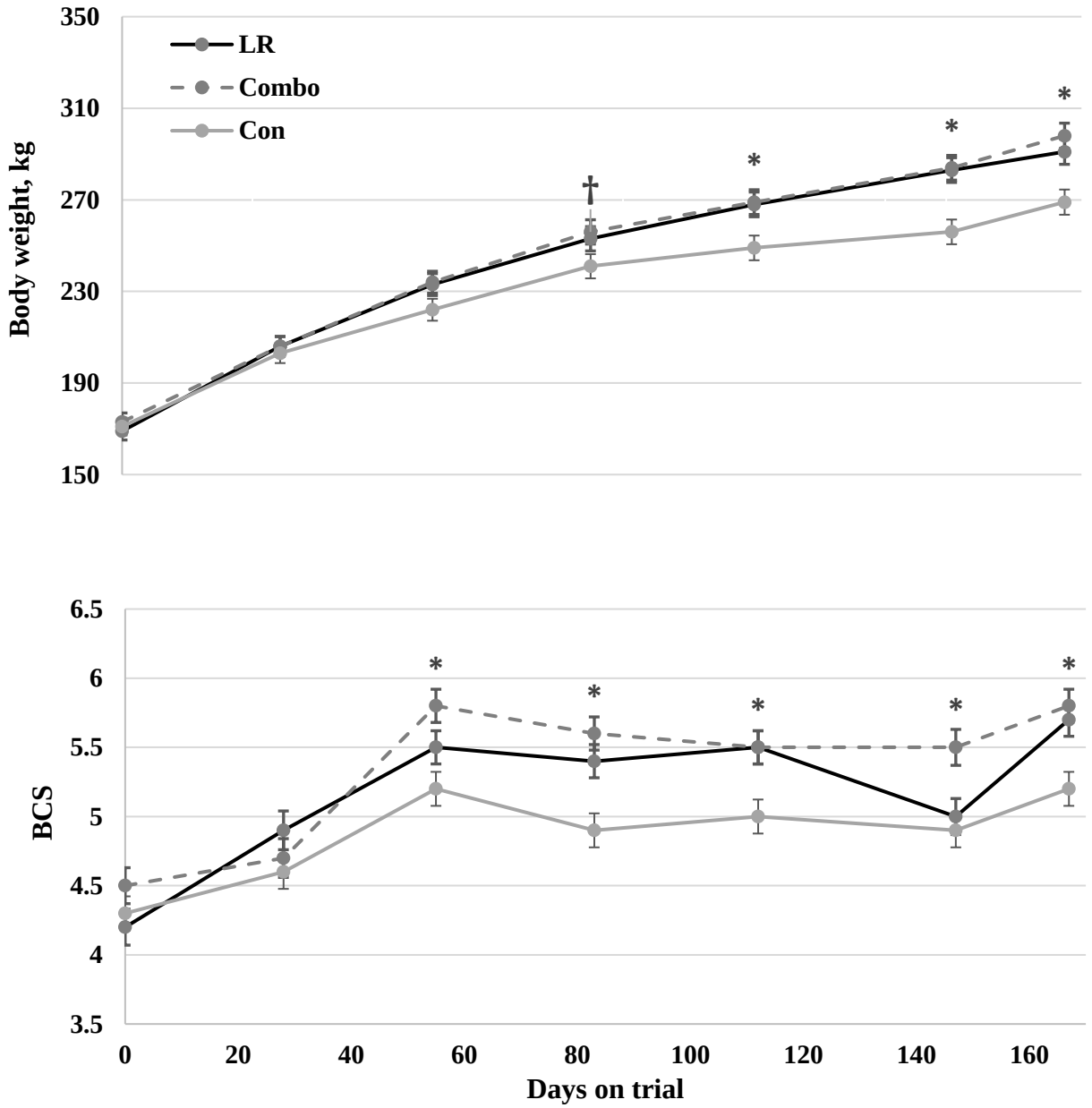

Figure 2.5 Effect of anthelmintics on heifer body weight (BW) and body condition score (BCS). Control cattle (n = 3) received a sterilized saline solution, ERE cattle (n = 3) received injectable eprinomectin, and COMBO cattle (n = 3) received injectable eprinomectin and oral oxfendazole at initiation of experiment. All treatments were administered at a rate of 1 mL per 50 kg of body weight. Significance of slice p-values are represented as: $P \leq 0.05$ defined by *, and tendencies from $0.05 < P \leq 0.10$ are defined as †. There was a treatment × time interaction ($P < 0.01$) for BW. On d 83 COMBO and ERE heifers tended ($P = 0.09$) to have greater BW than CON heifers and did have greater BW ($P < 0.01$) than CON heifers at d 112, 147 and 167. Additionally, there was a treatment × time interaction ($P < 0.01$) for BCS. On d 55, COMBO had greater BCS ($P < 0.01$) than CON and tended ($P = 0.10$) to have greater BCS than ERE. Throughout the rest of the experiment, COMBO had greater BCS ($P < 0.01$) than CON heifers at d 83, 112, 147, and 167, but only had greater BCS ($P < 0.01$) to ERE on d 147.

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CHAPTER 3

EVALUATION OF CYP450 ACTIVITY AND LIVER ENZYMES AS POTENTIAL MECHANISMS OF EXTENDED-RELEASE EPRINOMECTIN MITIGATING FESCUE TOXICITY IN BEEF HEIFERS GRAZING ENDOPHYTE-INFECTED TALL FESCUE

ABSTRACT

The objective of this experiment was to evaluate CYP450 activity and liver enzymes as potential mechanisms of extended-release eprinomectin mitigating fescue toxicosis in beef heifers grazing endophyte-infected tall fescue. Fall-born Angus × Simmental heifers [42 heifers; 170.2 ± 20.0 kg initial body weight (**BW**); 222 ± 21 d of age] were stratified by d -2 fecal egg count (**FEC**) and BW and were assigned to one of six groups (7 heifers per group) using a stratified randomized design. Groups were then assigned to one of two treatments: extended-release eprinomectin (**ERE**; n = 3), or saline control (**CON**; n= 3). At experiment initiation, all heifers were administered treatments subcutaneously at a rate of 1mL per 50 kg of BW. All heifers grazed endophyte-infected tall fescue in groups and were supplemented daily with a 50:50 mix of distillers grains and soybean hulls (1.8 kg per heifer per d). Body weight (**BW**), body condition score (**BCS**), and hair coat score (**HCS**) were recorded 7 times throughout the experiment, while respiration rates (**RR**) and serum prolactin and liver profile analysis was conducted at 5 times. Urine was collected at d 55 for total ergot alkaloid analysis and percentage total CYP activity. There was a treatment × time interaction ($P < 0.01$) for BW as ERE heifers had greater BW ($P \leq 0.03$) compared to CON heifers beginning on d 112. Additionally, there was a treatment × time interaction ($P = 0.02$) for BCS as BCS tended to be greater ($P \leq 0.09$) from d 28 to 55 and was greater ($P < 0.01$) from d 83 on for ERE heifers. There was a tendency for a treatment × time interaction ($P = 0.07$) for respiration rate. Furthermore, there was a treatment × time interaction for HCS, with CON having greater ($P = 0.05$) HCS at d 55 and tended to have greater ($P = 0.09$)

HCS at d 83. There was no treatment × time interaction or treatment effect ($P \geq 0.58$) on serum prolactin concentration. Total urinary ergot alkaloids were similar ($P = 0.62$) between treatments, although ERE cattle tended ($P = 0.06$) to have greater total CYP3A4 activity. There was a treatment × time interaction ($P = 0.04$) for alkaline phosphatase (**ALP**) as ERE cattle tended ($P = 0.06$) to have greater ALP on d 55; however, ALP was not different ($P \geq 0.15$) at any other time point. Additionally, there was a treatment × time interaction ($P = 0.02$) for gamma-glutamyl transferase (**GGT**). ERE cattle had greater ($P < 0.03$) GGT on d 28 and 112, and tended to have greater ($P = 0.07$) GGT at d 83. There was no treatment by time interaction ($P = 0.86$) for total protein, although ERE tended to have greater ($P = 0.07$) total protein than control. Extended-release eprinomectin improved heifer growth performance, HCS, and tended to improve RR. In addition, extended-release eprinomectin tended to increase total CYP450 activity, and increased several liver enzymes in heifer grazing endophyte-infected tall fescue.

Keywords: beef heifers, cytochrome P450, extended-release eprinomectin, fescue toxicosis

INTRODUCTION

Fescue toxicosis, caused by an ergot alkaloid producing endophyte in the tall fescue plant, negatively impacts beef cattle productivity and costs the industry an estimated $2 billion annually (Kallenbach, 2015; Klotz and Smith, 2015). Symptoms of fescue toxicosis include decreased weight gain, vasoconstriction which leads to increased respiration rate, rough hair coat, and decreased serum prolactin concentrations, along with poor animal growth and reproductive performance (Roberts and Andrae, 2004; Strickland et al., 2011).

Previous literature has reported increased weight gain of growing cattle grazing endophyte-infected tall fescue when treated with ivermectin, an anthelmintic (Ellis et al., 1989;

Bransby et al., 1993; Bransby et al., 1995). Ivermectin is only biologically active in the animal for 28 d, which makes it difficult to be considered as a fescue toxicity mitigation strategy. A new extended-release eprinomectin (**ERE**), also known as LongRange, has been released to the market and is biologically active for up to 150 d (Forbes, 2013). Since the release, ERE has been shown to improve heifer growth and reproductive performance; however these experiments did not evaluate the effects on fescue toxicosis (Kunkle et al., 2013; Backes et al., 2015; DeDonder et al., 2015; Backes, 2016). Additional work conducted by Volk et al. (2019), evaluated heifer performance when grazing endophyte-infected tall fescue and administered either ERE or a saline control. All cattle had relatively low fecal egg count (**FEC**; <14 eggs per gram), however, cattle treated with ERE had greater average daily gain (**ADG**), body weight (**BW**), and body condition score (**BCS**). In addition, heifers treated with ERE had greater pregnancy rate, but no differences were reported in typical fescue toxicosis symptoms. Although, no clear mechanism was identified for the improved performance in ERE treated cattle.

Ergot alkaloids are known to be biotransformed to be eliminated from the body by cytochrome P450 (**CYP**) enzymes in the liver (Ball et al., 1992; Moubarak and Rosenkrans, 2000). Moreover, CYP enzymes are also involved in drug-drug interactions as well as drug-toxicant interactions (Cali et al., 2009). The influence of one drug or toxicant can either inhibit or activate the CYP enzymes allowing for the second drug or toxicant to either increase or decrease clearance (Cali et al., 2009). Previous literature has reported that CYP enzymes in cattle play a role in metabolizing ergot alkaloids (Moubarak and Rosenkrans, 2000) and that a genetic single-nucleotide polymorphism (**SNP**) can be related to the animal's CYP activity. Additionally, Rosenkrans Jr. and Ezell (2015) reported that urine from steers heterozygous for

the SNP had decreased inhibition on CYP activity and that this inhibition tended to be correlated with ADG of the steers.

Another factor impacted by fescue toxicosis, is cattle liver function. Previous reviews have noted reduced liver enzyme levels in cattle experiencing toxicity (Thompson and Stuedemann, 1993; Oliver, 1997). This reduced level of liver enzymes is suggested to possibly be causing the poor weight gain of these cattle (Thompson and Stuedemann, 1993; Oliver, 1997). Although, further mechanisms have not been addressed. Previous literature has not determined if the CYP mechanism and liver enzyme activity is affecting animal performance when extended-release eprinomectin is administered. Therefore, the objective of this experiment was to evaluate CYP450 activity and liver enzymes as potential mechanisms of extended-release eprinomectin mitigating fescue toxicity in beef heifers grazing endophyte-infected tall fescue.

MATERIALS AND METHODS

Animals and Experimental Design

The Institutional Animal Care and Use Committee of the University of Illinois approved the procedures used in this experiment (protocol 18092) and followed the guidelines recommended in the Guide for the Care and Use of Agricultural Animal in Agricultural Research and Teaching (FASS, 2010).

To evaluate CYP450 activity and liver enzymes as potential mechanism of extended-release eprinomectin (LongRange; Boehringer Ingleheim, Duluth, GA) on fall-born beef heifers experiencing fescue toxicity, 42 Angus × Simmental heifers [170.2 ± 20.0 kg initial body weight (**BW**); age = 222.2 ± 21.4 d; mean ± standard deviation] were utilized at the University of Illinois Dixon Springs Agricultural Center in Simpson, IL. Heifers were stratified by d -2 fecal

egg count (**FEC)** and BW and were assigned one of six groups (7 heifers per group) using a stratified randomized design. Groups were then assigned to one of two treatments (n = 3): extended-release eprinomectin (**ERE**), or saline control (**CON**). All treatments were administered subcutaneously on d 0 at a rate of 1 mL/50 kg of BW. Any cattle not receiving an extended-release eprinomectin injection received a saline injection at the same dosage. Cattle rotationally grazed endophyte-infected tall fescue ('Kentucky-31'; 84% infected; total ergot alkaloid concentrations June: 459 ppb ERE, 340 ppb CON; July 143 ppb ERE, 364 ppb CON; August: 608 ppb ERE, 145ppb CON; Oct: 1156 ppb ERE, 1242 ppb CON) pastures and had free-choice to a mineral supplement (Southern FS Services, Marion, IL; 12% Ca, 9.5% P, 17% salt, 5.9% Mg, 1.15% K, 24 mg/kg Co, 31 mg/kg I, 3,000 mg/kg Fe, 1,400 mg/kg Cu, 2,000 mg/kg Mn, 26.4 mg/kg Se, 4,000 mg/kg Zn, 550,000 IU/kg vitamin A, 3,300 IU/kg vitamin D, 220 IU/kg Vitamin E, and 6,600 mg/kg chlortetracycline). Pasture size was 2.64 ± 0.13 ha, with an average of 0.38 heifers/ha. Groups were rotated every 14 d. As groups were rotated, available forage was quantified in the pastures heifers were rotated out of and the pasture they went to by using a falling plate meter (Jenquip, Fielding, New Zealand) to collect 12 random measurements. A minimum of 132 kg DM/ha was available to all groups throughout the study, with 294 ± 89 kg DM/ha of forage available on average (Fig. 3.2). Heifers were also supplemented daily with a 50:50 mix of soybean hulls and distillers grains [(20.6% crude protein (**CP**), 51.9% neutral detergent fiber (**NDF**), 31.3% acid detergent fiber (**ADF**), and 5.1% crude fat] at a rate of 1.8 kg per heifer per d.

Sample Collection and Analytical Procedures

Body weights were collected seven times throughout the experiment with full 2-d BW measurements collected and averaged at the beginning (d -1 and 0) and end of the experiment (d

167 and 168). Throughout the experiment, 1-d full BW measurements were collected on d 28, 55, 83, 112, and 147. Body condition scores (**BCS**) were collected concurrent with BW and were evaluated using a 1-9 scale [emaciated =1; obese = 9; as described by (Wagner et al., 1988)]. Hair coat scores (**HCS**; 1 to 5, in which 1= slick, short coat and 5 = unshed, full winter coat) were evaluated and recorded on d -1, 28, 55, 83, 112, 147, and 167. Additionally, respiration rates (**RR**) were evaluated on d 1, 29, 57, 85, and 113 in the afternoon. To determine respiration rates, two individuals simultaneously counted the number of breaths per heifer in 15 seconds. The two observations were then averaged and multiplied by four to determine breaths per minute.

Blood samples were collected for serum at five (d -1, 28, 55, 83, and 112) time points and plasma at one time point (d 167), during the experiment. Blood was collected via jugular venipuncture into one 10 mL serum blood collection vacuum tube (Becton, Dickinson and Co., Franklin Lakes, NJ) and one 10 mL plasma blood collection vacuum tube with K2 EDTA (Becton, Dickinson and Co., Franklin Lakes, NJ). Blood in the serum tube was allowed to clot at room temperature before being centrifuged at 1,300 × g for 20 min at 5° C. Serum was stored at -20° C for subsequent prolactin and liver profile analysis. Serum was pooled by group and was analyzed for prolactin concentration via a radioimmunoassay (Bernard et al., 1993). The intra assay CV was 5.9% and the inter assay CV was 7.6%. Serum liver profile was pooled by group and analyzed in a Beckman Coulter AU680 Chemistry Analyzer (Beckman Coulter, Brea, CA) at the University of Illinois Veterinary Clinical Pathology Lab. Parameters included in the liver profile were alkaline phosphatase (**ALP**), aspartate aminotransferase (**AST**), gamma-glutamyl transferase (**GGT**), cholesterol, glucose, creatine kinase (**CK**), blood urea nitrogen (**BUN**), total protein, and triglycerides. The blood in the plasma tube was kept on ice and were processed

within 24 h. Whole blood was used to isolate DNA in a procedure similar to Sales et al. (2012) to genotype all cattle at CYP3A28 single nucleotide polymorphism (**SNP**). Genotypes at SNP site C994G were homozygous cytosine (**CC**), homozygous guanine (**GG**), and heterozygous (**CG**). As not all genotypes were equally represented in both treatments (ERE: CG = 13 animals, GG = 8 animals; CON: CG = 8 animals, GG = 10 animals, CC = 3 animals), only cattle that were GG or CG were included for luminescence data.

On d 55, urine samples were collected from ERE and CON heifers. Approximately 50 mL of fresh urine was collected mid-stream when the animals were in the alley and chute. Urine samples were stored at -20° C for subsequent ergot alkaloid analysis. Total ergot alkaloids were determined in a commercial laboratory (Agrinostics Limited, Co., Watkinsville, GA).

Enzyme activity for CYP450 was evaluated on urine samples using the Promega P450-Glo Assay (product # V9920; Cali et al., 2009) following instructions in the kit. CYP3A4, specifically, was evaluated in this kit as it is known to biotransform ergot alkaloids and is a family in the CYP450 enzyme system (Ball et al., 1992). First, 12.5 µL of sample was added in triplicate to a 96-well plate (Corning Inc., Kennebunk, ME) along with 12.5 µL of CYP3A4 solution, and incubated at room temperature for 10 min. The reaction was then initiated by adding 25 µL of NADPH regeneration system and incubated for 10 min at room temperature. Next, 50µL of luciferin detection reagent was then add to each well and incubated at room temperature for 20 min. CYP3A4 luminescence was then recorded with a luminometer (Synergy HT; BioTek Instruments Inc., Winooski, VT) using no filters and an integration time of 1 s/well. CYP3A4 luminescence was recorded in relative light units (**RLU**) and is reported as percentage of total activity. This was calculated using the following equation for each plate: {[(sample RLU – average negative control RLU)/ (average positive control RLU)] * 100}.

Forage samples were collected by randomly clipping approximately 5 cm from the ground, from at least 12 different locations, within the pasture. Feed and forage samples were collected every two weeks throughout the experiment. Forage samples were dried at 55°C for a minimum of 3 d, ground through a 1 mm screen using a Wiley mill (Arthur H. Thomas, Philadelphia, PA), and were composited for all six groups. Forage samples were composited by group into 3 time periods and feed samples were composited for the entire experiment. Ground feed and forage were analyzed for NDF and ADF using an Ankom 200 Fiber Analyzer (Ankom Technology, Macedon, NY) as well as CP (Leco TruMac, LECO Corporation, St. Joseph, MI) (Fig. 3.1). Feed samples were also analyzed for crude fat using an Ankom XT10 fat extractor (Ankom Technology, Macedon, NY). Total ergot alkaloid analysis of forages was conducted in a commercial laboratory (Agrinostics Limited, Co., Watkinsville, GA).

Rectal fecal grab samples were collected on d -2, 28, 55, 83, 112, and 167 for determination of FEC per gram of feces using a modified version of the Modified Wisconsin flotation method. After collection, fecal samples were stored at 20°C until processed. Three grams of feces were mixed with 15 mL of a sodium nitrate solution (Feca-Med; VetOne, MWI Animal Health, Boise, ID) and strained into a 15 mL polypropylene conical tube (Corning; Corning, NY). Samples were then centrifuged for 7 minutes at 725 × g. After centrifugation, tubes were filled with the sodium nitrate solution to form a meniscus, and a cover glass slip (VWR VistaVision 18 × 18 mm; VWR International, Randor, PA) was placed onto the meniscus for 4 min. Cover slips were then removed and placed directly onto a microscope slide (VWR VistaVision 75 × 25 × 1 mm; VWR International, Randor, PA), which was scanned under a microscope to count the total number of eggs on the entire cover slip. Total number of eggs per 3 grams of feces was recorded and then converted to eggs per gram (**EPG**) of feces for statistical

analysis. All cattle had low FEC throughout the duration of the experiment [< 11 eggs per gram (**EPG**)].

Statistical Analysis

A stratified randomized design was used and group served as the experimental unit. Body weight, BCS, HCS, RR, average daily gain (**ADG**), serum prolactin concentration, forage analysis, and liver profile parameters were analyzed as repeated measures in the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC), with fixed effects of treatment and time, and the interaction of treatment and time. Group nested within treatment was included as a random effect. The REPEATED statement was used to model the repeated measurements within animal for each variable and the heterogeneous autoregressive (1) covariance structure was used for BW, BCS, HCS, RR, ADG, serum prolactin concentration, and forage analysis after considering the Log Likelihood. The compound symmetry covariance structure was used for all liver profile parameters after considering the Log Likelihood. Day -1 values were used as a covariate for alkaline phosphatase, cholesterol, and GGT analysis. Glucose, AST, and CK residuals were not normally distributed, so these parameters was transformed using the BoxCox procedure of SAS. Glucose was transformed using $(\text{Glucose})^{-1.25}$, AST was transformed using $(\text{AST})^{-2.75}$, and CK was transformed using $(\text{CK})^{-1.75}$. Untransformed least square means are reported for glucose, AST, and CK. The SLICE statement was used to separate least square means when the interaction of treatment and date was significant ($P \leq 0.05$). The MIXED procedure of SAS was also used to analyze urinary ergot alkaloids with fixed effect of treatment. The MIXED procedure of SAS was also used to analyze CYP3A4 luminescence with fixed effects of treatment, genotype, and the interaction of treatment and genotype. Group nested within treatment was included as a random effect for both urinary ergot alkaloids and CPY3A4

luminescence. Significance was declared at $P \leq 0.05$, and tendencies were declared from $0.05 < P \leq 0.10$. Means reported in tables and figures are least squares means.

RESULTS

There was no treatment × time interaction or treatment effect ($P \geq 0.14$; Fig 3.1 and 3.2) for NDF or CP content of the forage along with forage availability. There tended to be a treatment × time interaction ($P = 0.08$) for ADF; however, when sliced by time, there was no differences ($P \geq 0.21$). Additionally, there was no treatment effect ($P = 0.11$) for NDF, ADF, CP, or forage availability. Over time, NDF and ADF increased ($P \leq 0.03$) in both treatments while forage availability fluctuated over time ($P < 0.01$) with the least forage available in August. However, there was no time effect ($P = 0.18$) for CP content.

There was a treatment × time interaction ($P < 0.01$; Fig 3.3) for BW. Heifer BW was greater ($P \leq 0.03$) for ERE at d 112, 146, and 168 than CON. Additionally, there was a treatment × time interaction ($P = 0.02$) for BCS. On d 28 and 55, ERE heifers tended ($P \leq 0.09$) to have greater BCS compared to CON heifers. At d 83 and 112, ERE heifers had greater BCS ($P < 0.01$) compared to CON heifers. At d 147, BCS was not different ($P = 0.43$) between treatments, but at d 167 ERE heifers had greater BCS ($P < 0.01$) compared to CON heifers.

Likewise, there was a treatment × time interaction ($P = 0.05$; Table 3.1) for ADG. From d 0 - 55, and d 55 - 112 ERE heifers had greater ($P \leq 0.01$) ADG compared to CON heifers. Although, from d 112-168, ADG was not different ($P = 0.22$) between treatments. There was a tendency for a treatment × time interaction ($P = 0.07$) for respiration rate; however, there was no treatment effect ($P = 0.49$). Furthermore, there was a treatment × time interaction for hair coat score. On d 55 CON heifers had greater ($P = 0.05$) HCS and on d 83 tended to have greater ($P =$

0.09) HCS compared to ERE heifers. However, there was no difference ($P \geq 0.12$) in HCS at any other time point.

There was no treatment × time interaction or treatment effect ($P \geq 0.58$) on serum prolactin concentration. Over time, serum prolactin concentration decreased ($P < 0.01$) in both treatments. Total urinary ergot alkaloids on d 55 was not different between treatments ($P = 0.62$; Table 3.2). Interestingly, CYP3A4 luminescence on a percentage of total activity tended to be greater ($P = 0.06$) for ERE compared to CON.

There was a treatment × time interaction ($P = 0.04$; Table 3.3) for ALP. On d 55, ALP tended ($P = 0.06$) to be greater in ERE compared to CON cattle; however, ALP was not different ($P \geq 0.15$) between treatments at any other time point. Additionally, there was a treatment × time interaction ($P = 0.02$) for GGT. On d 28 and 112, GGT was greater ($P < 0.03$) for ERE cattle compared to CON, and tended to be greater at d 83 ($P = 0.07$) for ERE cattle. There was no treatment × time interaction or treatment effect ($P \geq 0.18$) on AST, cholesterol, and glucose. Over time, AST concentration decreased ($P < 0.01$) in both treatments, while cholesterol increased ($P < 0.01$) until d 83 and then decreased on d 112.

There was no treatment × time interaction effect ($P \geq 0.59$; Table 3.4) on CK, BUN, total protein, or serum triglycerides and no treatment effect ($P \geq 0.39$) on CK, BUN and serum triglycerides. There was a time effect for CK with values decreasing over time, with the only exception being an increase on d 55. There was a tendency for a treatment effect ($P = 0.07$) on total protein as ERE had greater total protein.

DISCUSSION

The objective of this experiment was to evaluate CYP450 and liver enzymes as a potential mechanism of extended-release eprinomectin mitigating fescue toxicity of fall-born beef heifers grazing endophyte-infected tall fescue. Reduced growth performance is an impact of fescue toxicosis (Roberts and Andrae, 2004; Strickland et al., 2011). In this experiment, heifer BW was similar until d 112, in which ERE cattle had greater BW compared to CON. Improved BW continued until the end of the experiment (d 168). Additionally, BCS tended to be improved on d 28 and 55 and was greater for ERE on d 83, 112, and 167 compared to CON. Furthermore, ADG was improved from d 0 – 55 and d 55 – 112 for ERE cattle compared to CON, but ADG was similar from d 112 – 167. These results are similar to Volk et al. (2019) where heifers treated with extended-release eprinomectin had improved BW compared to control heifers beginning on d 55 and maintained this difference throughout the remainder of the experiment (d 291). Similar patterns in improved BCS and ADG were noted as well (Volk et al., 2019). Other previous literature has reported that cattle grazing endophyte-infected tall fescue pastures and treated with ivermectin have improved BW gain compared to untreated controls (Ellis et al., 1989; Bransby et al., 1993; Ivy et al., 1993; Bransby et al., 1995).

Parasite loads have been known to have a negative impact on animal growth (Hawkins, 1993). Fecal egg counts were monitored throughout this experiment and were low throughout the experiment (< 11 EPG). Parasite infection was reported well below the economic threshold (< 200 EPG) in Bransby et al. (1993) and Ivy et al. (1993) but was not reported in other work (Ellis et al., 1989; Bransby et al., 1995). Moreover, FEC were reported to be low (< 14 EPG) in Volk et al. (2019) and likely were not impacting animal performance.

Cattle in this experiment were maintained in separate groups grazing separate pastures by treatment to prevent cross-over effects from parasite burdens. Forage quality between treatments was compared to ensure forage quality was not impacting cattle performance between treatments. In this experiment, forage quality and availability was not different between treatments.

Increased respiration rate is another symptom of fescue toxicosis as vasoconstriction occurs from the endophyte-infected tall fescue (Finch, 1986). Respiration rate in this experiment tended to have a treatment by time interaction; however, there was no treatment effect. In addition, other indicators of fescue toxicosis, such as HCS and serum prolactin were analyzed. Hair coat score was improved in ERE heifers on d 55 and 83, but no differences in serum prolactin were noted. Volk et al. (2019) reported no differences in RR, HCS, or serum prolactin in heifers, treated with ERE or saline control. In a fescue toxicosis review, HCS was reported to increase when cattle were experiencing fescue toxicosis, while serum prolactin concentrations were decreased (Strickland et al., 2011).

Ergot alkaloids are toxins in the body and more than 90% of them are excreted in the urine (Stuedemann et al., 1998; Hill et al., 2000). Urinary ergot alkaloids can be measured to determine toxicity level on an animal basis to provide more accurate results than just analyzing pasture level (Hill et al., 2000). Additionally, urinary ergot alkaloids have been reported to possibly have a cause and effect relationship with animal performance (Hill et al., 2000). In this current experiment, urinary ergot alkaloid concentration was similar to other work with cattle grazing endophyte-infected tall fescue (Hill et al., 2000); however, no differences were reported on d 55. Urinary ergot alkaloids were not analyzed in previous work focusing on both

anthelmintics and fescue toxicosis (Ellis et al., 1989; Bransby et al., 1993; Ivy et al., 1993; Bransby et al., 1995; Volk et al., 2019).

CYP450 enzymes are found in the liver and can be classified by their function into families (Anzenbacher and Anzenbacherova, 2001). Ergot alkaloids are reported to be biotransformed by CYP3A enzymes with the most prevalent being CYP3A4 (Ball et al., 1992; Anzenbacher and Anzenbacherova, 2001; Strickland et al., 2011). Therefore, CYP3A4 activity was measured in this experiment, and tended to be greater for ERE cattle at d 55. Previous literature has not evaluated the effects of macrocyclic lactones on CYP3A4 activity in the urine of animals (Ellis et al., 1989; Bransby et al., 1993; Ivy et al., 1993; Bransby et al., 1995; Volk et al., 2019). Rosenkrans Jr. and Ezell (2015) analyzed urine from steers grazing endophyte-infected tall fescue, and when luminescence was reported by genotype of the steers, steers that were heterozygous (CG) had greater activity. Considering CYP450 is involved in drug-toxicant metabolism, and can be observed interacting with components of the diet (Anzenbacher and Anzenbacherova, 2001), the authors propose that extended-release eprinomectin may be activating CYP450 and increasing clearance of ergot alkaloids. In this experiment, ergot alkaloid clearance was similar, but the trend for increased CYP450 activity, may explain the improved performance of cattle treated with extended-release eprinomectin.

Another indicator of fescue toxicosis is related to reduced liver enzyme function and was speculated in a review paper to impact the poor growth performance (Oliver, 1997). The authors acknowledge that all cattle in this experiment were grazing endophyte-infected tall fescue and experiencing toxicity. However, the authors speculate that increased liver enzyme function could potentially be a mode of action for extended-release eprinomectin to elicit the increased growth performance noted in previous experiments.

All liver enzyme function values in the current experiment were reported in the normal range according to the University of Illinois Veterinary Diagnostic Lab for the following parameters: ALP: 8 – 179 U/L; GGT: 4 U/L - 28 U/L; AST: 17-137 U/L; cholesterol: 44 – 150 mg/dL; glucose: 48-73 mg/dL; BUN: 5-19 mg/dL; total protein: 5.9-8.3 g/dL. Serum triglyceride levels were outside of the normal range (6-23 mg/dL) from d 55 – 112 and creatine kinase was outside of the normal range at d -1 (50-320 U/L). Cattle treated with extended-release eprinomectin had greater ALP, GGT, and total protein compared to CON heifers. In addition, AST, cholesterol, and creatine kinase was numerically increased in ERE heifers compared to CON. There were no differences in glucose, BUN and serum triglycerides between treatments.

Reduced levels of ALP, cholesterol, and total protein have been noted in cattle grazing varieties of endophyte-infected tall fescue that elicited varying levels of performance; however no differences were reported in AST, glucose, BUN, serum triglycerides, and creatine kinase (Bond et al., 1984). In another experiment, ALP, GGT, AST, creatine kinase were reduced in cattle consuming endophyte-infected tall fescue seed compared to cattle not consuming the endophyte; although no difference was reported in BUN between treatments (Dougherty et al., 1991).

Thompson and Stuedemann (1993) noted in a review that increased circulating ALP activity is an indication of induced activity in the liver, bone or other tissues suggesting that ERE cattle may have had increased liver activity. In the review, Thompson and Stuedemann (1993) also noted reduced levels of cholesterol but did not comment on serum glucose levels. In another review paper, GGT, AST, cholesterol, BUN, total protein, serum triglycerides, and creatine kinase was reported to be reduced and glucose levels were increased when cattle grazed endophyte-infected tall fescue, but the reviewer referenced unpublished results (Oliver, 1997).

The reduced BUN was hypothesized to be related to mild dehydration (Oliver, 1997). Moreover, another experiment reported reduced levels of ALP, AST, and creatine kinase after consuming endophyte-infected tall fescue (Piper et al., 1991).

Overall, heifer BW, BCS, ADG, and HCS were improved in cattle treated with ERE when grazing endophyte-infected tall fescue. Some parameters from the liver profile suggested improved liver enzyme function of ERE cattle compared to CON, while other parameters showed no differences. In addition, CYP3A4 activity tended to be increased in ERE cattle compared to CON, although other symptoms of fescue toxicosis were not different between treatments. Extended-release eprinomectin tended to increase CYP450 activity and improved some liver enzymes in heifers when grazing endophyte-infected tall fescue.

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Conflict of interest statement. None declared.

TABLES AND FIGURES

Table 3.1 Influence of extended-release injectable eprinomectin on heifer average daily gain, respiration rate, hair coat score, and serum prolactin concentration over time

	Treatment[1]			*P*-Value[2]		
Item	ERE	CON	SEM	Trt	Time	Trt × Time
n	3	3				
Average daily gain, kg/day				0.01	<0.01	0.05
d 0 - 55	1.16	0.94	0.030	<0.01		
d 55 - 112	0.61	0.47	0.036	0.01		
d 112 - 167	0.42	0.36	0.035	0.22		
Respiration rate, breaths per minute				0.49	<0.01	0.07
d 1	65.3	71.0	2.09			
d 29	53.4	55.3	1.56			
d 57	55.8	53.0	1.79			
d 85	31.5	33.4	1.56			
d 113	46.2	46.2	1.38			
Hair coat score[3]				0.23	<0.01	0.01
d -1	3.0	3.5	0.24	0.16		
d 28	2.5	3.0	0.22	0.12		
d 55	2.0	2.6	0.20	0.05		
d 83	2.1	2.6	0.20	0.09		
d 112	2.4	2.2	0.14	0.34		
d 147	2.9	3.0	0.15	0.65		
d 167	3.1	3.1	0.16	1.00		
Serum prolactin, ng/mL				0.83	<0.01	0.58
d -1	30.74	40.40	10.180			
d 28	31.68	30.05	6.986			
d 55	24.26	19.93	4.274			
d 83	10.86	13.09	2.883			
d 112	1.09	1.29	0.396			

[1]Treatments are defined as ERE, heifers that received injectable extended-release eprinomectin; CON, heifers received a sterilized saline solution injection at initiation of experiment. All treatments were administered subcutaneously at a rate of 1 mL per 50 kg of body weight.
[2]Abbreviations are defined as Treatment effect (Trt) and Treatment × time effect (Trt × Time).
[3]Hair coat score was evaluated on a 1 to 5 scale, in which 1= slick, short coat and 5 = unshed, full winter coat.

Table 3.2 Influence of extended-release injectable eprinomectin on heifer total urinary ergot alkaloids and CYP3A4 luminescence

	Treatment[1]			*P*-Value
Item	ERE	CON	SEM	Treatment
n	3	3		
Total urinary ergot alkaloids, ng/mg of creatinine				
d 55	70.7	63.5	9.44	0.62
CYP3A4 luminescence, % total activity				
d 55	18.58	12.56	2.31	0.06

[1]Treatments are defined as ERE, heifers that received injectable extended-release eprinomectin; CON, heifers received a sterilized saline solution injection at initiation of experiment. All treatments were administered subcutaneously at a rate of 1 mL per 50 kg of body weight. *P*-values for CYP3A4 luminescence are: Genotype: $P = 0.66$, Treatment × Genotype: $P = 0.43$.

Table 3.3 Influence of extended-release injectable eprinomectin on heifer alkaline phosphatase, aspartate aminotransferase, gamma-glutamyl transferase, cholesterol, and glucose over time

	Treatment[1]			*P*-Value[2]		
Item	ERE	CON	SEM	Trt	Time	Trt × Time
n	3	3				
Alkaline phosphatase[3], U/L				0.32	< 0.01	0.04
d -1	78.7	75.3	8.92	-		
d 28	121.4	99.9	8.92	0.17		
d 55	128.4	97.6	8.92	0.06		
d 83	113.1	90.6	8.92	0.15		
d 112	72.7	69.3	8.92	0.82		
Aspartate aminotransferase[4], U/L				0.18	< 0.01	0.89
d -1	71.3	62.7	-			
d 28	61.0	56.0	-			
d 55	64.0	61.0	-			
d 83	58.3	56.3	-			
d 112	56.7	54.3	-			
Gamma-glutamyl transferase[4], U/L				0.14	< 0.01	0.02
d -1	9.8	11.2	0.57	-		
d 28	13.8	11.8	0.57	0.03		
d 55	11.8	10.5	0.57	0.14		
d 83	12.8	11.2	0.57	0.07		
d 112	13.8	11.8	0.57	0.03		
Cholesterol[3], mg/dL				0.22	< 0.01	0.39
d -1	66.1	62.9	5.08			
d 28	76.5	69.9	5.08			
d 55	82.8	67.5	5.08			
d 83	83.1	70.2	5.08			
d 112	69.5	55.2	5.08			
Glucose, mg/dL[4]				0.66	0.23	0.97
d -1	58.0	55.3	-			
d 28	63.3	57.0	-			
d 55	67.3	71.0	-			
d 83	63.7	82.0	-			
d 112	54.7	51.3	-			

[1]Treatments are defined as ERE, heifers that received injectable extended-release eprinomectin; CON, heifers received a sterilized saline solution injection at initiation of experiment. All treatments were administered subcutaneously at a rate of 1 mL per 50 kg of body weight.
[2]Abbreviations are defined as Treatment effect (Trt) and Treatment × time effect (Trt × Time).
[3]d -1 was included as a covariate for all analysis (Aspartate aminotransferase: $P = 0.04$; Cholesterol: $P = 0.06$; Gamma-glutamyl transferase: $P = 0.10$).
[4]Untransformed LS means are reported with transformed p-values.

Table 3.4 Influence of extended-release injectable eprinomectin on heifer creatine kinase, blood urea nitrogen, total protein, and serum triglycerides over time

	Treatment[1]			*P*-Value[2]		
Item	ERE	CON	SEM	Trt	Time	Trt × Time
n	3	3				
Creatine kinase[3], U/L				0.39	< 0.01	0.59
d -1	332.3	323.0	-			
d 28	211.7	183.7	-			
d 55	236.7	241.7	-			
d 83	207.3	158.7	-			
d 112	141.0	133.0	-			
Blood urea nitrogen, mg/dL				0.72	< 0.01	0.72
d -1	16.7	16.7	1.06			
d 28	15.3	15.3	1.06			
d 55	15.7	17.3	1.06			
d 83	13.7	14.0	1.06			
d 112	14.7	15.0	1.06			
Total protein, g/dL				0.07	0.49	0.86
d -1	6.50	6.37	0.171			
d 28	6.70	6.47	0.171			
d 55	6.63	6.40	0.171			
d 83	6.57	6.27	0.171			
d 112	6.53	6.03	0.171			
Serum triglycerides, mg/dL				0.50	< 0.01	0.90
d -1	24.3	22.3	1.59			
d 28	22.0	22.0	2.08			
d 55	27.3	25.3	2.50			
d 83	34.7	32.0	3.74			
d 112	30.0	27.7	2.21			

[1]Treatments are defined as ERE, heifers that received injectable extended-release eprinomectin; CON, heifers received a sterilized saline solution injection at initiation of experiment. All treatments were administered subcutaneously at a rate of 1 mL per 50 kg of body weight.
[2]Abbreviations are defined as Treatment effect (Trt) and Treatment × time effect (Trt × Time).
[3]Untransformed LS means are reported with transformed p-values.

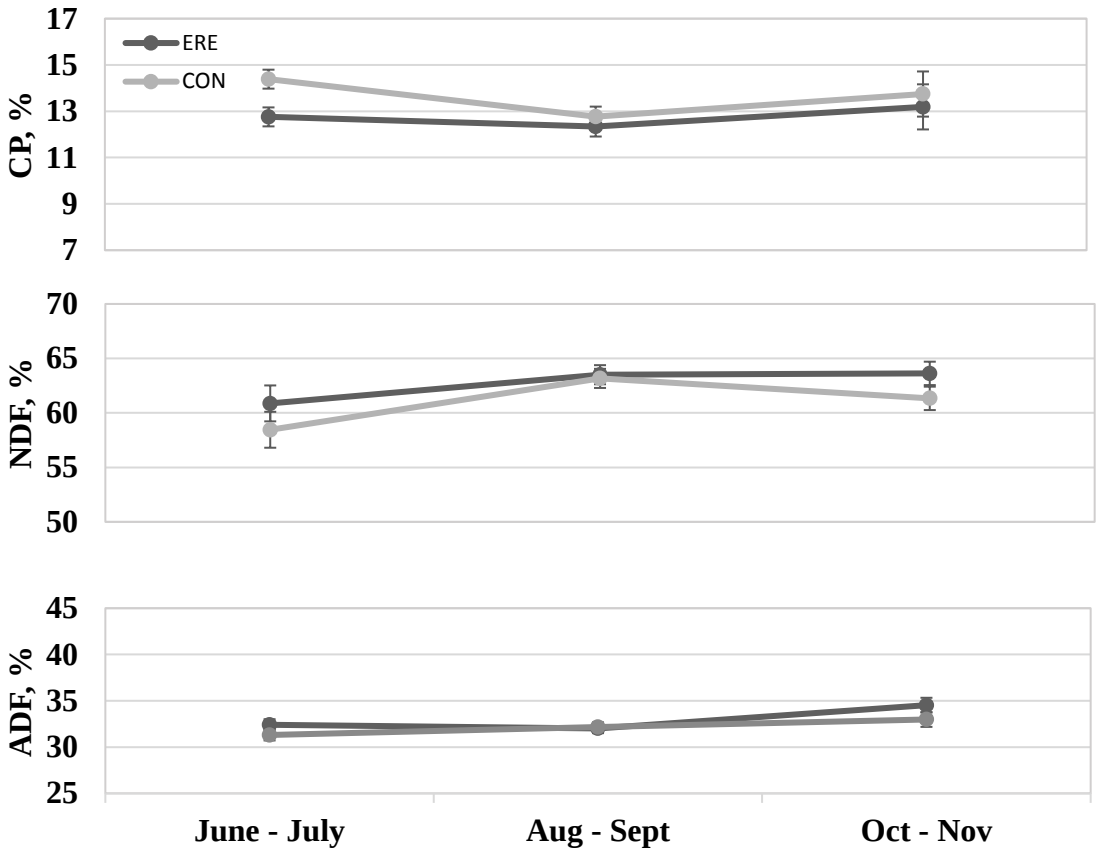

Figure 3.1 Forage quality [percentage crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber (ADF)] of endophyte-infected fescue (*Festuca arundinacea*) pastures from June 2018 to November 2018. Samples were collected as cattle rotated pastures and were composited every six weeks. There was no treatment × time interaction or treatment effect ($P \geq 0.14$) on NDF or CP content of the forage. There tended to be a treatment × time interaction ($P = 0.08$) for ADF; however, when sliced by time, there was no differences ($P \geq 0.21$). Additionally, there was no treatment effect ($P = 0.32$) on ADF. There was a time effect ($P \leq 0.03$) as NDF and ADF increased over time; however, there was no time effect ($P = 0.18$) for CP content.

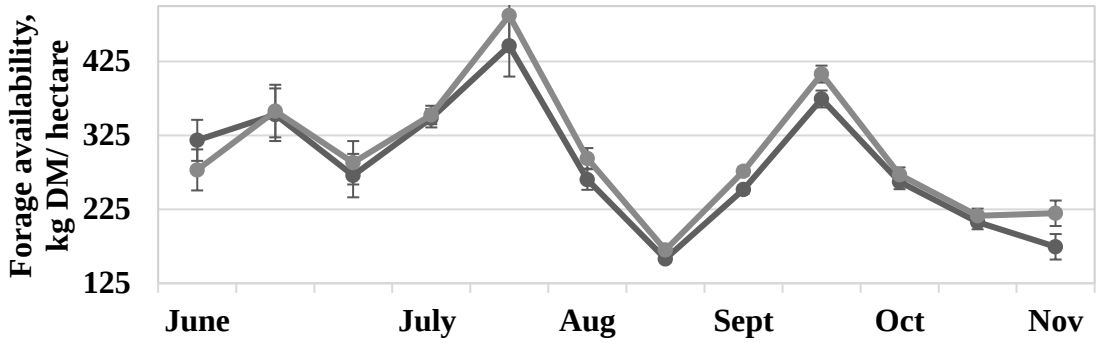

Figure 3.2 Forage availability of endophyte-infected fescue (*Festuca arundinacea*) pastures from June 2018 to November 2018. Samples were collected as cattle rotated pastures every two weeks. There was no treatment × time interaction or treatment effect ($P \geq 0.11$) on forage availability. There was a time effect ($P \leq 0.03$) as forage availability fluctuated ($P < 0.01$).

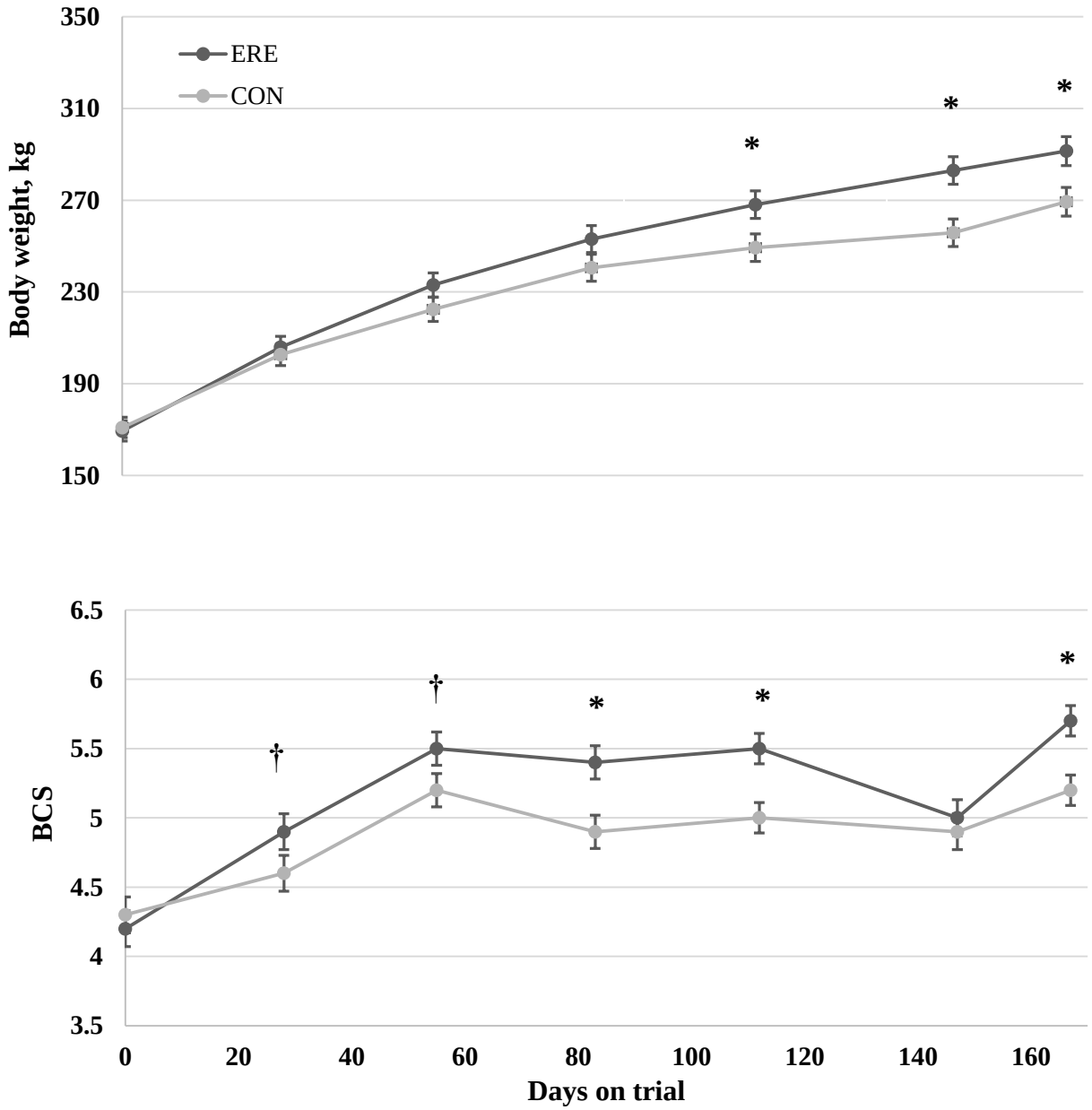

Figure 3.3 Effect of an extended-release injectable eprinomectin (LongRange) on heifer body weight (BW) and body condition score (BCS). Control cattle (n = 3) received a sterilized saline solution and ERE cattle (n = 3) received injectable eprinomectin at initiation of experiment. All treatments were administered subcutaneously at a rate of 1 mL per 50 kg of body weight. Significance of slice p-values are represented as: $P \leq 0.05$ defined by *, and tendencies from $0.05 < P \leq 0.10$ are defined as †. There was a treatment × time interaction ($P < 0.01$) for BW. Heifer BW was different ($P \leq 0.03$) at d 112, 146, and 168 as ERE heifers had greater BW compared to CON heifers. Additionally, there was a treatment × time interaction ($P = 0.02$) for BCS. Heifer BCS tended to be greater on d 28 and 55 ($P \leq 0.09$) for ERE heifers compared to CON heifers. At d 83, 112, and 167,ERE heifers had greater BCS ($P < 0.01$) compared to CON heifers, but BCS was not different ($P = 0.43$) at d 147.

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CHAPTER 4

CONCLUSIONS

The use of anthelmintics in ruminants has led to parasite resistance in cattle. Two classes of anthelmintics can be used in combination to limit the occurrence of resistance; however this strategy is not widely implemented in the beef industry. An extended-release eprinomectin was recently released to the market and limited data has evaluated parasite resistance at this time. The current data determined that parasite resistance to extended-release eprinomectin was not present on an operation that has used it for three years. Treated heifers had improved growth performance compared to untreated heifers even when parasite levels were low.

In addition, fescue toxicosis has negatively impacted the beef industry for many years. Multiple mitigation strategies have been attempted, although no one strategy has been adopted as an overall solution to this problem. Previous work has indicated that ivermectin, a macrocyclic lactone, may be effective at alleviating some negative impacts of toxicosis on growing cattle. Similar improvements in growth performance have been reported due to extended-release eprinomectin. In an attempt to understand the mechanism behind the growth performance differences, additional liver and fescue toxicity parameters were evaluated. In this current data, some fescue toxicity parameters were reduced and some liver enzyme functions were improved in cattle treated with extended-release eprinomectin. Furthermore, there was a trend for heifers treated with extended-release eprinomectin to have improved CYP3A4 total activity. These results suggest that extended-release eprinomectin may be working through the liver to improve cattle performance when grazing endophyte-infected tall fescue.