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Methods to Evaluate Ruminant Animal Production Responses

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Methods to Evaluate Ruminant Animal Production Responses

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy in Animal Science

by

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ABSTRACT

In experiment 1, 80 steers (197.0 kg initial body weight; BW for fall, 116.9 kg for spring), were stocked at 2.45 and 4.1 calves/ha in fall and spring, respectively in 16 tall fescue pastures [fall ergovaline (EV) = 1,475 ppb and spring EV = 1,173 ppb] under 2 treatments, mineral (MIN) (n = 8) and cumulative management (CM) (n = 8). Forage allowance did not differ ($P = 0.76$) between CM and MIN during fall but differed during spring ($P \leq 0.05$, 2.55 vs. 3.22 kg DM/kg BW, for MIN and CM, respectively). For fall, average daily gain (ADG) resulted in 0.41 × EV for MIN and 1.05 × EV for CM. For spring, ADG resulted in 0.80 × EV for MIN and 0.94 EV for CM resulting in an increase of ADG for CM as the level of EV increased. In experiment 2, steers (n = 3) were fitted with a device (Icetag; IceRobotics) strapped to left metatarsus that measured motion activity while on varying levels of EV toxicity. Initial lying bouts for CM were 18.4 but decreased by 0.9 bouts for every 1,000 ppb EV increase. Period 2 resulted in standing time for MIN calves of 858.01 min/day (14.3 h/d) whereas CM calves spent 792.01 min/day (13.2 h/d) standing and CM calves took 20% more steps daily than MIN calves. For every 1,000 ppb increase in EV, steps decreased by 275. In experiment 3, calves (n = 4) grazed long sward regrowth (LSR) or short sward regrowth (SSR) tall fescue and alfalfa paddocks for forage quality, visual observations, rumen volatile fatty acids and diet selectivity measurements. No differences in these behavior measurements were observed for either forage ($P < 0.05$). Within fescue paddocks, ruminal ammonia, total volatile fatty acids (VFA), acetate, and the branch-chain VFA were greater from SSR vs. LSR ($P < 0.05$), but these differences were not observed ($P \geq 0.11$) on alfalfa paddocks. In summary, the effect of combined management strategies offers potential to cope with toxicity in tall fescue pastures. Grazing activities of cattle grazing tall fescue or alfalfa may influence intake, but further

research is needed to determine these behavioral modifications when differences in sward height are small.

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DEDICATION

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CHAPTER I

INTRODUCTION

North America (Unites States, Canada and Mexico) produces 26% of the beef supply worldwide (Galyean et al.,, 2011). In Northwest Arkansas, beef cattle are produced using different forages such as tall fescue, alfalfa, and bermudagrass. Tall fescue is a common forage for producers and farmers because it persists for longer periods, resisting drought and diseases, and because it offers a fair amount of forage mass during the winter season being a cool-season grass. However, this promising grass triggers a long-term or chronic disease called “fescue toxicosis” caused by an endophyte fungus founded in the plant that produces ergot alkaloids. These alkaloids in turn cause animal reproduction and production reductions and alter physiological responses.

Management strategies are needed to minimize toxicity and enhance animal productivity. Development of novel fescue cultivars to replace fescue with high levels of toxicity, and legume incorporation are strategies implemented at the land and soil level. Strategic supplementation, growth promoting implants and ionophore are potential ways to overcome these difficulties and are strategies implemented at the animal level but need to be researched in more detail. Animal growth and behavioral studies were conducted in northcentral Arkansas to investigate how cattle growth, motion and grazing is affected by fescue grasses and combination management strategies.

Overall, improved forage utilization by ruminants needs to be accompanied by human interventions by planning strategically the proper forage utilization without having ecological impacts, but within a sustainable way to provide food for the animal and for humans. How forage quality is enhanced and how ruminants make the most of these forages is called the plant-animal-soil relationship. This relationship is to be enhanced with research and science put into practice.

CHAPTER II

REVIEW OF LITERATURE

Overall summary

Forages are “edible plants” offered as feed or grazed by herbivore animals (Wilkins, 2000). These forages consist of grasses, legumes, forbs and browse (Phelan et al., 2015). Forages are important to herbivores because herbivores possess bacteria, protozoa and fungi in their digestive system to breakdown forage cell wall components such as hemicellulose and cellulose (Minson, 1990) to produce energy (Wilkins, 2000). When ruminants graze forages, they take advantage of inexpensive herbage food resources that lower input or costs and increases sustainability of animal production (Soder et al., 2009). Ruminant livestock production is mainly in grasslands which comprise 26% of the world land area and 70% of the world agricultural area (FAO, 2019).

The availability of forages, or forage mass, is important in animal production. Forages need to have good nutritive value along with adequate forage production. Forage quality comprises components such as palatability, intake, digestibility and nutrient content that influence animal performance. Factors that affect forage quality include plant species and cultivar, maturity, environmental conditions, and diurnal effects. The most influential factor on forage quality, or how well forages translate into animal production, is maturity (Buxton, 1996). As forage maturity advances, nutrient content and fermentation and digestibility of plant components like sclerenchyma and xylem are restricted (Akin, 1989) which reduces their conversion into energy.

Some plants have components or chemical compounds that function as a protection against overgrazing or overutilization but also affect herbivores when grazing because they cause toxicity. For example, tall fescue pastures may cause toxicity in ruminants. Management strategies such as strategic protein supplementation, the use of implants, knowing the plant toxicity level and alkaloid concentration, and grazing pressure are tools to minimize production losses in animals grazing toxic plants (Pfister et al., 2001). Therefore, a number of factors must be considered in order to optimize utilization of forages by ruminants.

Ruminant animals are efficient in selecting their diet depending on the quantity of forage offered (Wilkins, 2000). However, that capability can be constrained or changed if they are grazing on homogeneous pastures. Because both, forage quality and animal condition affect animal performance, strategic management in animal production such as adequate grazing management, strategic supplementation (Delevatti et al., 2019) and forage demand calculations need to be developed by considering animal size, length of time grazing, and number of animals (Scott et al., 2018).

In summary, ruminants are important because they have microorganisms capable of breaking down forage components into energy to produce food for human consumption. However, forage production has constraints related to the plant itself and related to environmental conditions that limit quality and availability of forage. This results in low forage production and therefore forage quality declines. Therefore, it is necessary to review forages for their ability to maintain and improve animal performance.

Chemical composition

Forage chemical components

Concentrations of certain chemical components of forages are analyzed to assess forage quality (Sanderson et al., 1999). The most common chemical components that are analyzed to interpret forage quality are dry matter (DM), neutral detergent fiber (NDF), acid detergent fiber (ADF), crude protein (CP), acid detergent lignin (ADL; Wiersma et al., 1998), ether extract and mineral content. Lignin is probably the plant cell wall constituent that is most limiting for digestibility (Jung and Allen, 1995). These forage constituents can be used to calculate total digestible nutrients (Weiss et al., 1992) which is a measure of forage energy.

Some plants contain plant secondary metabolites that influence forage intake and digestibility. For example, condensed tannins decrease the breakdown of proteins in the rumen, thereby affecting the microbial population that is necessary to produce energy from carbohydrates (Barry and McNabb, 1999; Min et al., 2003). It therefore may be necessary to analyze an expanded number of forage chemical components in order to be more fully aware of how a particular forage might affect animal performance.

Management to alter chemical composition

Harvest management

Harvest management of forages includes cut frequency (the less frequent, the less stress plants suffer), timing, appropriate plant growth stage and maturity. These harvest management considerations affect yield, quality, and persistence of the plant (Sanderson et al., 1999; Wiersma et al., 1998). Harvest management needs to consider forage quality, nutrient yield, and plant longevity to achieve specific production goals (Brink and Marten, 1988). Furthermore, there are post-harvest factors that affect forage quality such as plant respiration (moisture, temperature, nutrient changes,), mechanical damage, and rain damage. Proper harvest management affects digestibility because it may decrease cell wall constituents such as hemicellulose and cellulose and affect CP that is necessary for microbial function. These can also affect forage intake, which then translates into less energy for the animal.

Silage is a method of harvesting and preservation of forages that uses fermentation for forage conservation (Heinritz et al., 2012). Whole plant corn silage is widely used as ruminant feed nationwide (Givens and Rulquin, 2004). Oat silage (Coblentz et al., 2016a) or ensiled legumes such as alfalfa are very common as well (Coblentz and Muck, 2012). Common ensiled grasses are tall fescue, meadow fescue and orchardgrass. Management of silage includes the proper maturity to cut the forage, weather conditions, wilting, and moisture; all indicators of ensilability (Coblentz and Muck, 2012) that affect silage chemical composition. However, silage quality can be enhanced by using additives that are arranged categorically as bacterial inoculants, enzymes, substrate sources, and inhibitors (Jennings, 2017).

Intake and digestion

Intake and digestibility are forage quality components, that if altered, interfere with animal production. Intake is the principal factor that determines animal performance. Intake is influenced by many factors including forage physical and chemical characteristics (Coleman and Moore, 2003; Fisher, 2002), animal physiological status, environmental factors (Zereu, 2016), and short-term physical-chemical mechanisms (Dulphy and Denarquilly, 1994). Forage related factors such as forage specie, stage of growth, and soil fertility, influence intake as well (Minson, 1990). Intake is controlled by other mechanisms and external factors such as gut capacity, ruminal distension, animal requirements, forage chemical composition, forage morphological characteristics, climate, feed resources and post-ingestive feedback (Decruyenaere et al., 2009). The post-ingestive feedback affects not only intake but also behavior of ruminants because it is based on a positive or negative experience (Fisher, 2002) that influences the animal to re-graze or to reject the forage. In addition, intake is influenced by animal physiological state and body size (Demment and Van Soest, 1985) because if the animal is larger in size it enhances gastrointestinal retention and capacity (Van Soest, 1996) and digestion rate and reticulo-rumen fill, (Allison, 1985) improving digestibility and animal production (Buxton et al., 1995).

Digestibility influences gut fill and distention, thereby influencing passage of ruminal contents through the rumen and reticulum thereby affecting intake (Allen, 1996). Digestibility is used to compare different forages and how they are consumed. For instance, Keyserlingk et al., (1996) simulated rumen in situ techniques of alfalfa silage, hay and corn to demonstrate dry matter and crude protein intestinal disappearance and digestibility. Ruminal digestion is different in small ruminants (Short et al., 1974) than from cattle because of gut capacity. Howevr, sheep and cattle comparisons are used as models to predict digestibility when fed either concentrate-based or forage-based diets (Chishti et al., 2019).

Toxicity

Effects on animals

When forages contain toxins or highly digestible nutrients, these may cause aversion by ruminants (Provenza, 1996). Anti-quality factors such as secondary plant compounds (glucosinolates), nitrates (Dillard et al., 2018), terpenoids, flavonoids, phenols, and alkaloids are components that occur in forages and potentially reduce average daily gains (Burns, 1978; Hemken, et al., 1984; Laca et al., 2001). Animal behavior and adaptation studies have evaluated how these anti-quality constituents have led to aversion to plants containing those substances (Allen and Segarra, 2001).

Certain alkaloids are anti-quality factors that interact with environmental temperature (Hemken et al., 1981). These alkaloids should be considered when choosing a forage and assigning the stocking rate in a determined area due to toxicity exposure. The existence of *Acremonium coenophialum* (Morgan-Jones and Gams) in tall fescue [*Lolium arundinaceum* (*Schreb.*) *Darbysh*.] pastures affects cattle and sheep productive performance (Allen, 1993) and can also cause ergotism in ruminants (Thompson et al., 2001). Klotz and Smith (2015) reviewed literature on ergot alkaloids about challenges in the past, present, and future. These authors concluded that past and present effects of ergot alkaloids in livestock present future challenges in agriculture.

Toxic plants have varied effects on physiological attributes in herbivores (Osborn et al., 1992). Eisemann et al., (2014) described widely how physiological responses such as respiration rate, heart rate (HR), surface temperature (ST), rectal temperature (RT), blood pressure (BP), certain serum hormones, and plasma metabolites are influenced in cattle by environmental temperature when offered tall fescue pastures containing high levels of ergovaline. These high levels of ergovaline in tall fescue decreased prolactin concentrations, increased temperature (Aldrich et al., 1993a), and increased incidence of fescue foot caused by vasoconstriction in peripheral body tissues (Klotz et al., 2016; Yates, 1962).

For many decades, the fungal endophytes contained in grasses have been of concern because of the cost-benefit relationship integrated into grazing management (Hoveland, 1993). In North America, tall fescue pasture is grazed probably more than any other cool-season grass (Kallenbach, 2015). This good-quality, persistent, but harmful grass has a mutualistic relationship with the endophyte (Aiken and Strickland 2013; Kallenbach, 2015; Latch, 1993). Thus, it is resistant to extreme environmental conditions which increases forage production (Hiatt and Hill, 1997; Johnson et al., 1985; Powell and Petroski, 1992) but it has toxic compounds that are harmful to ruminants.

The tall fescue endophytic fungus produces toxins (Browning and Leite-Browning, 1997; Klotz, 2015) that have a tendency to prevail over time (Clay, 1993). Young et al. (2013) compiled a vast literature of tall fescue produced and distributed in the United States, New Zealand, and Australia and the symbiotic relationships with fungi. They conclude that as these grass-endophyte symbioses have been sustainable in pasture systems to date, and they also need to be sustainable for the new grass-endophyte associations in the future.

Intake

Level of toxicity in plants has an effect on intake by ruminants. For instance, in tall fescue pastures, the use of nonergot alkaloid-producing endophytes have resulted in an increase in bite rate and DM intake by cattle , in contrast to endophyte - infected tall fescue pastures (Parish et al., 2003). Animals grazing E+ pastures had lower DMI resulting in lower ADG than animals grazing E- pastures (Parish et al., 2003). Early-life experiences with tannins influenced intake later in life (Catanese et al., 2012; Villalba et al., 2012). These authors concluded that lambs offered a balanced diet (beet pulp, oat grain, and a mix of milo:grape pomace, soybean meal, alfalfa, corn gluten meal) had greater ADG than lambs offered the same diet plus plant toxins with oxalic acid and quebracho tannins due to the fact that toxins decreased feed intake. Intake can also be affected by increased body and skin temperature (Carr and Jacobson, 1969), long, rough hair coats (Aiken et al., 2011) and foot rot (Prescott et al., 1994) leading to a decrease in daily grazing time and forage intake when ambient temperatures increase in toxic fescue fields.

Digestibility

Tall fescue infected with the endophyte fungus (E+) contains ergovaline and products of lysergic acid that affect digestion in ruminants (Humphry et al., 2002; De Lorme et al., 2007). If rumen normal function is altered, fiber digestion and OM intake can be reduced (Hannah et al., 1990).

Studies have evaluated including forages such as alfalfa (*Medicago sativa*) and birdsfoot trefoil (*Lotus Corniculatus*) into endophyte- infected tall fescue pastures to evaluate how this plant configuration may affect nitrogen fixation and plant secondary metabolites such as tannins and saponins. These compounds potentially bind ergovaline produced by the fungal endophyte (Clemensen et al., 2016). These authors concluded that E+ concentrations of ergovaline were greater in monocultures than in E+ mixtures with alfalfa. Therefore, animals may benefit from supplementation with alfalfa or birdsfoot trefoil (Owens et al., 2011) because of improved intake and digestibility (Clemensen et al., 2016).

Animal performance

As mentioned previously, toxicity acquisition from tall fescue is associated with an endophytic fungus that limits performance when grazing toxic plants (Schmidt et al., 1982). Understanding the effects and associations of these alkaloids leads to understand animal performance and economic losses (Poore and Washburn, 2013). Stuedeman and Hoveland (1998) stated that ADG increased from 30 to 100% in cattle grazing fescue with low levels of endophyte fungus infection compared with high levels of fungus infection. Average daily gain

(ADG) and prolactin concentrations were improved when cattle were fed soybean hulls and implanted with steroids while grazing toxic tall fescue (Carter et al., 2010). However, this increase of ADG may not alleviate fescue toxicosis per se (Aiken et al., 2008). Reductions in ADG are more related to intake reduction of 0.045 kg for each 10% accretion in E+ levels (Schmidt and Osborn, 1993).

Mitigating factors

Reviews of published studies about relieving the toxicity of tall fescue by applying the proper management practices have been described by Bacon et al. (1986). Dilution of tall fescue with other forages has been used to reduce the impacts of tall fescue toxicosis (Roberts and Andrae, 2004). Other management strategies to reduce fescue toxicosis and improve the forage production and quality are chemical treatment with herbicides to introduce alternative forages (Williamson, 2015). Aiken et al. (2012) utilized chaparral herbicide to suppress seedhead emergence in toxic tall fescue and evaluated animal performance. They concluded that ADG in cattle increased after applying chaparral herbicide to suppress reproductive development in tall fescue endophyte infected pastures.

Some studies with metoclopramide resulted in decreasing the skin temperature in cattle (Jones et al., 1994) and in sheep (Aldrich et al., 1993b) consuming toxic tall fescue. Samford-Grigsby et al. (1997) injected dopamine antagonist Ro 24-0409 into cattle to alleviate fescue toxicosis caused by the endophyte-infected fescue because the ergot alkaloids easily bind to dopamine receptors (D2) (Larson et al., 1999; Paterson et al., 1995).

Other production strategies to relieve fescue toxicity that affects weight gain are the use of steroidal implants. For instance, implants and protein supplementation to alleviate toxicosis in cattle and to enhance weight gain has been studied widely (Aiken et al., 2001). These authors found that protein supplementation and implants did not alleviated toxicity in fescue. Controlling the stocking rate and applying steroidal implants resulting in an increase in ADG if low stocking rates were applied but decreased ADG if the grazing intensity increases (Aiken et al., 2006).

Efforts to reduce the severity of tall fescue toxicosis include new varieties of tall fescue that include novel or non-toxic endophytes (NE+; Gunter and Beck, 2004; Nihsen et al., 2004; Aiken and Strickland, 2013). These NE+ associations alleviate fescue toxicosis and improve body weight (Aiken and Strickland, 2013). Comparisons of novel endophyte (NE+) and endophyte infected (E+) tall fescue cultivars resulted in enhanced growth performance in cattle (Hopkins and Alison, 2006; Parish et al., 2013). Beck et al. (2008) compared cattle performance from endophyte infected (E+) Kentucky-31 with that from Jesup AR542 infected with a non-toxic, novel endophyte (NE). Results showed greater performance for cattle grazing NE than E+. Caldwell et al. (2011) evaluated weaning and post-weaning performance of calves grazing infected tall fescue pastures, concluding that delaying weaning may benefit weight and immune function but careful attention is still needed later in the feedlot period.

Behavior

When animals respond to their environment is called behavior; how they respond to their natural habitat is called ethology (Arave and Albright, 1981). Behavioral strategies of grazers such as avoidance or tolerance help animals avoid plant secondary metabolites that affect their performance (Iason and Villalba, 2006). These foraging decisions influence grazing episodes that affect when and where animals graze and how they distribute their grazing day (Gregorini et al., 2006). These foraging decisions influence ingestive behavior, which is affected by paradigms such as regulation, learning, reward and neural control (Kissileff, 1991).

Behavior has been studied in ruminants fed different grasses such as tall fescue, alfalfa and switchgrass. For instance, in tall fescue, forage mass and canopy characteristics were studied for their effects on steer's ingestive behavior and performance revealing that selective consumption was not altered by forage mass and digestive behavior and that pastures with E+ need to be adequately managed to succeed in animal production responses (Burns et al., 2011; Burns and Fisher, 2013).

Grazing behavior in steers grazing endophyte-infected tall fescue (K-31) and offered two types of supplements to evaluate ADG and to mitigate fescue toxicosis resulted in no differences in ADG between control and supplement as self-fed liquid (Shockey et al., 2006). Galli et al. (2011) studied ingestive behavior by monitoring chewing and biting sounds to determine dry matter intake. The chewing-biting relationship helps to measure grazing behavior and herbage intake (Chelotti et al., 2016). Bite rate is an important foraging process, because animals need to acquire their required nutrients daily (Carvalho et al., 2015).

The grazing animal and the plant characteristics are related by a term called "plant-animal interface" that encompasses the interaction between plant morphological, physiological and chemical features with animal grazing activities (Forbes and Rouquette, 2011). This plant-animal interface influences behavioral patterns because the plant structure affects grazing. Sward structure such as leaf surface height influences bite size (Forbes, 1988) which is based on jaw movements and forage prehension (Ungar and Rutter, 2006).

Diet selectivity

Diet selection is based on a theoretical term called “optimal foraging theory” and relies on the assumption that animals select naturally to be fed efficiently. In other words, they prefer an “optimal diet” (Hanley, 1982) coming from a “nutritional wisdom” (Cassini, 1994). The foraging theory states that the animal should graze better if grazing forages are abundant and high in quality (Murden and Risenhoover, 1993). Diet selection in ruminants is accomplished by grazing patterns that these animals develop (Lu, 1998; Gregorini et al., 2006). Ruminants select a wide variety of forages that may or may not be of high quality (Provenza and Balph, 1987). Animals need to adapt behaviorally to have a better diet quality through enhanced diet selection. Gregorini et al. (2011) studied short-term herbage depletion on diet quality by steers and they concluded that the steers adapted behaviorally to support diet quality by increasing the number of steps per minute and reduced herbage intake per eating step.

Impacts of palatability

Palatability is a “complex phenomenon” that involves the animal-plant-environment relationship in a feed-offered and plant-preference level (Marten, 1978). The sense of taste influences behavior when ruminants have different flavors in the feed (Villalba et al., 2011). Alkaloids present in some plants may interfere with palatability because of a feedback called post-ingestive experiences (Provenza et al., 1992). Taste-feedback interactions are involved with food preference and post-ingestive feedback because they have an effect on palatability that may cause aversion (Provenza, 1996).

Forage mass/sward height

Structure and herbage shortage influence bite rate, dietary choices (Baumont et al., 2000) and animal performance (Carvalho et al., 2015; Fonseca et al., 2013; Mezzalira et al., 2014). Swards with greater leaf availability provide for an optimum and efficient herbage intake with less time grazing in a determined feeding station in cattle (Gregorini et al., 2009) and in sheep (Roguet et al., 1998a). Sward characteristics influence ingestive behaviors because low-quality diets make it difficult to select higher quality portions of the forage (Demment and Greenwood, 1988). Ruminants make foraging decisions on where and how to graze based on sward height and spatial patterns (Chapman et al., 2007). Furthermore, patterns of distribution of grazing ruminants are explained by the spatial distribution of forage quality and forage mass (Fryxell, 1991; Senft et al., 1985).

Motion

Motion in ruminants while grazing either heterogeneous or homogeneous swards is part of their foraging behavior on a feeding station scale because they have to adjust their time spent grazing, number of bites, and time spent moving because the forage is shorten or not abundant to maximize the energy intake (Roguet et al., 1998b). Activity and behavioral patterns have been studied using storage telemetry in small ruminants (Scheibe et al., 1998). The use of ear tags has also been used to track moving behavior (Trenel et al., 2009). Furthermore, the use of global positioning systems (GPS) have been used widely to track behavioral grazing activities such as location and movement (Richeson et al., 2018) and distance traveled (Schlecht et al., 2006) in ruminants. These authors concluded that cattle traveled 25km, goats 20km and sheep 21km daily on rangelands.

Animal performance

Forage effects

Livestock can take advantage of natural resources when grazing different pastures within the grazing system. Ruminants develop strategies to graze forages that enhance their performance. Affordable forage production and forage mass are also factors that influence animal production (Aiken, 2016). However, grazing management strategies such as the inclusion of an adequate stocking rate and rotational grazing systems (Bailey and Brown, 2011) may or may not enhance forage availability and improve economics (Beck et al., 2016). If forage consumption is altered due to low available forage mass, then animal maintenance will decrease due to lower energy input (Burns, 1978).

Forages have two fractions from a nutritional standpoint. One fraction contains cellular components such as proteins, carbohydrates and lipids. Another fraction is the plant cell wall that contains hemicellulose, cellulose and lignin (Van Soest, 1967) which provide the main energy source for ruminants (Wilson, 1994). This second fraction also restricts digestibility and intake influencing energy input to ruminants (Jung and Allen, 1995; Leng, 1990).

Additional impacts

Supplementation

Strategic supplementation is needed mostly in ruminants that are grazing grasses. Strategic supplementation is an effective program to ensure food intake and consumption of nutrients (Bowman and Sowell, 1997; Bowman et al., 1999). However, careful attention needs to be considered in terms of ADG because sometimes supplementation does not reach the target (Moore et al., 1999). Moore et al. (1999) reviewed a vast number of studies on the effect of supplementation on animal performance and concluded that even though supplementation with energy and protein were intended to augment growth rates, the results depended on the amount and type of supplement.

Another supplementation management was researched by Bodine et al. (2001) who offered protein supplement to cattle grazing low quality hay to improve digestibility resulting in an increase in ADG (Moore et al., 1999). Some supplementation with concentrates may increase growth rate in sheep when grazing forages with low herbage allowance (Prache et al., 1990). However, sheep fed diets with different protein supplements, soybean meal and meat meal, had no effect on animal performance (Manso et al., 1998). Supplementation can also modify the animal grazing behavior if it is a low protein source (Krysl and Hess, 1993). Other studies have reported improved animal performance by supplementing olive oil (Gomez - Cortez et al., 2008), sunflower oil (Gomez - Cortez et al., 2011), safflower seeds (Bottger et al., 2002), crude glycerin (Mach et al., 2009), and corn grain (Wright et al., 2015). These these supplements may improve ADG substantially because of the increased volatile fatty acids concentrations in the rumen.

Implants

Implants are used to improve animal performance. The use of growth promoting implants had no influence in BW, ADG and immunity in stressed cattle in one study (Richeson et al., 2015), but they may reduce costs (Barham et al., 2003; Reinhart, 2007) and increase feed efficiency (Duckett and Pratt. 2014). In ruminants, implanting with anabolic implants resulted in accretion of 18% in ADG, 6% in feed intake, 8% in feed efficiency, and 5% in carcass weight (Duckett and Pratt, 2014). In addition, implants may not influence carcass characteristics (Torrentera et al., 2017), or meat tenderness (Barham et al., 2003; Scaglia et al., 2004). Hutcheson et al. (1993) reviewed several studies on the effects of implants and re-implants containing androgen and estrogen on cattle performance and carcass characteristics, concluding that the implant strategies had an effect on ADG response. In addition, recently, Lean et al. (2018) compiled a meta-analysis of 31 experiments on how hormonal growth promotants influenced beef quality. They found out that multiple implants improve tenderness compared to a single implant.

Feed additives

The use of feed additives is intended to enhance rumen fermentation efficiency and reduce methane production. Some feed additives are plant extracts, dietary lipids, plant saponins, garlic oil, nitrates, microalgae, prebiotics, alkalizers and buffers. Combinations of feed additives such as lauric acid, myristic acid, linseed oil, and calcium fumarate may decrease protozoa and increase volatile fatty acids (VFA; Zijderveld et al., 2011). Feed enzyme additives improve digestibility of fiber components and feed utilization in ruminants (Beauchemin et al., 2003).

Summary

Forages comprise properties and physical characteristics that affect animal performance. Certain forages have chemical compounds that on one side prevent them from over utilization but on other side may cause toxicity to herbivores, thereby affecting intake rate and digestibility. Management strategies need to be developed to cope with the toxicity in ruminants fed forages that compromises their performance and physiological status as well as their behavior. Changes in behavior will be noticed because the animals need to adjust their grazing or feeding behavior in such a way to compensate for intake rate and energy input to the animal’s body in order to acquire those daily nutrients necessary for optimal animal performance. This behavior is influenced by diet selection, palatability, and forage mass and animal motion. Behavior of animals can be evaluated to account for diet selectivity and intake. Furthermore, diet selectivity and intake may be influenced by forage quality, type of forage preserved and fertilization type. Strategies to improve animal performance are the use of feed additives, the use of growth implants and the development of a strategic supplementation that enhances palatability and diet selectivity. For this reason, the objective of this research is to evaluate different common northwest Arkansas grasses fed to ruminants to evaluate forage quality, toxicity, grazing behavior, digestibility and intake depending on the type of forage offered, on the type of forage preserved and on the type of forage fertilized.

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CHAPTER III

PERFORMANCE-ENHANCING TECHNOLOGIES FOR STEERS GRAZING TALL FESCUE PASTURES WITH VARYING LEVELS OF TOXICITY

Abstract

The objective of this study was to evaluate a combination of best management practices strategy for steer calves grazing tall fescue pastures with a range of toxicity. The experiment was conducted over 2 grazing seasons (fall 2015 for 91 d and spring 2016 for 84 d). Steers (n = 80 within season, body weight [BW] = 197.0 ± 15.43 kg [fall] and 116.9 ± 4.88 [spring]) were stocked at 2.45 and 4.1 calves/ha in fall and spring, respectively, to 16 pastures with varying levels of toxicity based on interim ergovaline (EV) concentration within season. Pastures were assigned to either mineral (MIN, n = 8) only management (MGMT) or a cumulative MGMT (CM, n = 8). The CM treatment included an implant containing 40-mg trenbolone acetate, 8-mg estradiol, and 29-mg tylosin tartrate (Component TE-G with Tylan, Elanco Animal Health, Greenfield, IN), 150 mg/calf daily monensin (Elanco Animal Health), and 1% BW of a 50:50 corn gluten feed:soybean hull supplement (as-is basis). Data were analyzed within season using pasture as the experimental unit. For fall and spring, the EV concentration was 1,476 ± 883.2 and 1,173 ± 620.6 ppb, respectively, and ranged from 90 to 2,180 ppb. During the fall, forage allowance did not differ ($P = 0.76$) between CM and MIN. In the spring, however, forage allowance only differed for the month of June ($P \leq 0.05$, 2.55 vs. 3.22 ± 0.177 kg DM/kg BW, for MIN and CM, respectively). In the fall, average daily gain (ADG) responded to the simple effects of EV ($P = 0.01$) and MGMT ($P < 0.001$), and ADG for MIN steers was explained by $ADG = 0.41 - 0.000064 \times EV$, whereas ADG for CM was explained by $ADG = 1.05 - 0.000064 \times EV$. In the spring, there was an EV × MGMT interaction ($P = 0.03$) for ADG. For MIN, ADG

= 0.80 − 0.000278 × EV, whereas for CM, ADG = 0.94 + 0.000001835 × EV. In spring, the ADG response to CM relative to MIN increased as EV increased. The CM strategy resulted in lower blood urea nitrogen than MIN in fall and spring ($P < 0.01$), but prolactin and serum Cu were not affected by MGMT in either season. In conclusion, performance was improved within the fescue belt by implementing feeding strategies using implants, ionophores, and supplementation, but a detailed economic analysis is warranted. Further research is needed to evaluate CM programs under varied stocking rates and in combination with dilution of endophyte-infected fescue pastures with nontoxic grasses or legumes.

Introduction

Fescue toxicosis is a term used to qualify the clinical disease associated with reduced dry matter (DM) intake, reduced average daily gain (ADG), and elevated body temperature when cattle consume tall fescue [*Lolium arundinaceum* (Schreb.) Darbysh] forage containing mycotoxins produced by the *Epichloë coenophiala* [(Morgan-Jones & W. Gams) C.W. Bacon & Schardl] fungus (Aiken and Strickland, 2013). Several approaches to alleviate fescue toxicosis have been studied including those made at the plant level by incorporating complementary legumes or fescue replacement and those made at the animal level including treating cattle with various pharmacological compounds or pro- viding supplemental dietary nutrients (Roberts and Andrae, 2010; Gadberry et al., 2015). A meta-analysis of research results (Gadberry et al., 2015) indicated that cattle grazing toxic tall fescue pastures respond to growth promoting implants, medicated feed additives, and feed supplementation. Gadberry et al. (2015) also demonstrated supplemental feeding rate and form affected ADG with highly digestible, fiber-based supplements providing greater weight gain than starch based supplements. Most studies have focused on singular intervention and little has been published on cumulative strategies with treatments that have independently demonstrated effectiveness. Roberts and Andre (2010) suggested that additive benefits from applying multiple management strategies simultaneously may allow cattle grazing toxic fescue pastures to accomplish the same level of productivity as cattle on nontoxic pastures, although there would also be benefits to these strategies in the nontoxic or minimally toxic environments as well. It is hypothesized that weight gain would be greater for cattle grazing low-toxicity pastures and weight gain response to growth promotion management would be improved to a greater extent in cattle grazing highly toxic tall fescue. Therefore, the objective of this study was to evaluate the cumulative response of providing a growth promoting implant, ionophore, and supplementation with a digestible fiber–based feed provided to growing cattle grazing tall fescue pastures with a range of toxicity based on ergovaline (EV) concentration.

Materials and methods

All animal care and management procedures were approved by University of Arkansas Institutional Animal Care and Use Committee (protocol 16023).

Research Site and Pastures

The study was conducted at the University of Arkansas, Division of Agriculture Livestock and Forestry Branch Station (Batesville, AR; 35°50′ N, 91°48′ W). Pastures consisted of a gravelly silt loam soil type with 8% to 20% slope at an elevation ranging from 65 to 99 m. The pastures (n = 16, 3.24 ha/ pasture) were predominantly tall fescue [L. arundinaceum (Schreb.) Darbysh]: 8 pastures were endemic endophyte-infected [E. coenophiala (Morgan-Jones & W. Gams) C.W. Bacon & Schardl] Kentucky-31 and 8 pastures had been converted to a nontoxic endophyte-infected variety (cv Estancia, Mountain View Seeds, Salem, OR) 1 yr prior to the fall study. Pastures were fertilized with 60 kg/ha N prior to fall grazing and again in the spring, prior to grazing.

Within each of the 8 endemic and 8 converted pastures, 4 pastures were assigned to either mineral (MIN) only management (MGMT) or a cumulative MGMT (CM) resulting in 8 MIN pasture replicates and 8 CM pasture replicates. The original experimental design was a 2 × 2 factorial of MGMT by pasture type; however, EV test (described later) results on day 42 of the first study (fall, 2015) revealed that all pastures were contaminated with endemic toxic endophyte-infected tall fescue. The minimum and maximum EV concentrations were 427 and 3,060 ppb, respectively. On day 42 of a second study (spring, 2016), the minimum and maximum EV concentrations were <100 and 2,180 ppb, respectively. As a result, the idea of analyzing the study as a factorial design was abandoned. EV was therefore compared between MGMT to determine whether it could be incorporated into the statistical model as a predictor variable, and the following statistics are presented to confirm statistical analysis described later. Assessment of EV between MIN and CM pastures indicated that there was not a statistically significant difference between the 2 MGMT treatments in the fall study ($P = 0.44$), despite the MIN pastures (mean ± sem; 1,655 ± 316 ppb EV) having a numerically greater EV concentration than the CM pastures (1,296 ± 316 ppb EV). Assessment of EV on day 42 in a second study with identical pasture assignment to the same MGMT treatments (spring, 2016) revealed that EV did not differ ($P = 0.76$) between the 2 MGMT treatments with MIN averaging 1,221 ± 226 ppb EV and CM averaging 1,124 ± 226 ppb EV. Furthermore, fall and spring pastures were compared for EV rank using the Kruskal–Wallis rank sum test which indicated that pasture rank for EV did not differ ($P = 0.39$), meaning pastures that were more toxic in the fall study were also the more toxic pastures in the spring study. Given the range in EV among pastures but non-significant difference in EV between the MGMT treatments, an analysis using mixed continuous and discrete fixed effects was used and is described in the section titled Statistical Analysis.

Precipitation and temperature were recorded by the National Oceanic and Atmospheric Administration’s (NOAA) U.S. Climate Reference Network weather stations located at the Livestock and Forestry Research Station near the study site. The mean monthly temperatures and precipitation from fall through spring study months as well as the deviations from the historical reference period are reported in Table 1.

Animals and treatment allocation

Crossbred steers were used for this experiment in 2 separate grazing seasons. In the fall of 2015, spring born steers (n = 80, 197.0 ± 15.43 kg) were stocked at 2.5 steers/ha. In the spring of 2016, fall born steers (n = 80, 116.9 ± 4.88 kg) were stocked to pastures at 4.1 steers/ha. Steers were assigned randomly at 5 steers/pasture to each of the 16 pastures. Stocking rates were established by modifying the size of each of the original pastures using single-strand polywire electrified fencing (Gallagher USA, Riverside, MO). Pasture layout was created using QGIS software (http://www.qgis.org/en/site/, last accessed August 22, 2017) and fence perimeter waypoints transferred to a GPS (GPSMAP 64s, Garmin, Olathe, KS).

Pastures were allocated, as described previously, to either the MIN treatment where steers were allowed access to only pasture and free choice nonmedicated MIN (Vigortone 3V6 S; Provimi North America, Inc, Brookville, OH, Table 2); or were allocated the CM strategy. The CM strategy included the following: application of a growth promoting implant, on day 0, containing 40-mg trenbolone acetate, 8-mg estradiol, and 29-mg Tylosin tartrate (Component TE-G, Elanco Animal Health, Greenfield, IN); 150-mg monensin/d (Rumensin, Elanco Animal Health); 115 g/d of the MIN premix (Vigortone 3V6 SR; Provimi North America Inc., Table 2);

and 1% BW (as-fed basis) 50:50 corn gluten feed:soybean hull pellet supplement (Table 3). Prior to placement on pastures, steers were treated for internal parasites (Cydectin Pour-On; Boehringer Ingelheim Vetmedica, Inc, Duluth, GA for the fall and Dectomax injectable solution Zoetis, Inc, Kalamazoo, MI for the spring). Calves were weighed initially and at 28-d intervals following a 16-h removal from pasture and water. While grazing, all steers were allowed ad libitum access to water sourced from a well.

Feeding strategies

Cattle in MIN treatment were allowed free choice access to the MIN provided in a covered ground feeder (Sioux Steel Company, Lennox, SD). Calves were given a weekly allotment of MIN (114 g/calf, daily equivalent). MIN was weighed weekly, and quantity replenished was based on any uneaten portion. For CM, the monensin and MIN was incorporated into the supplemental feed at each morning feeding. For CM, feeding was pro-rated for a Monday through Friday delivery (7-d feed quantity fed over a 5-d period) and it was adjusted every 28 d based on shrunk BW. Supplement feed samples were collected weekly and composited for wet chemistry nutrient composition determination (Dairy One, Inc., Ithaca, NY).

Physiological measurements

Physiological measurements were taken on initial (day 0), interim (day 42), and final dates (day 91 or 84) of the fall and spring study following 16-h removal from pasture, without access to water. Rectal temperature was measured as a proxy for core body temperature using a GLA M700 Digital Thermometer (Agricultural Electronics, San Luis Obispo, CA). Skin temperature was measured near the tailhead with an infrared digital thermometer with scan averaging capabilities (IR1000, Klein Tools, Lincolnshire, IL). The tail skin temperature was taken, as a proxy for vasoconstriction effects on temperature exchange, approximately 10 to 12 cm below the drop of the tail-head region proximate the anus. Prior to scanning, a small area was clipped using an Oster (Jarden Consumer Solutions, Boca Raton, Florida) Cryogen-X size 10 blade leaving approximately 1.6-mm hair length. The mean temperature captured for each animal scan was recorded for analysis. Hair shedding score was evaluated during the spring study. Shedding was visually scored on a 1 to 5 scale using the shedding score system reported by Gray et al. (2011).

Blood samples were collected into 10-mL BD (Becton, Dickinson and Company, Franklin Lakes, NJ) vacutainer red top and 7-mL BD vacutainer blue top trace element tubes with clot activator by jugular venipuncture and centrifuged at 1,200 × g for 20 min in a Heraeus Megafuge 16R (Thermo Fisher Scientific, Inc., Waltham, MA) followed by freezing until analysis. Blood urea nitrogen (BUN) was analyzed within the University of Arkansas, Animal Science Nutrition Laboratory using the Urea Nitrogen Colorimetric detection kit (TECO diagnostics, Anaheim, CA), following the colorimetric method instructions. The interassay coefficient of variation (CV) was 13.69 mg/dL and intraassay was 3.11 mg/dL (reference values: 7 to 23 mg/ dL). For Cu analysis, blood samples were diluted 1:9 (vol/vol) with 1 N nitric acid and water to separate proteins from serum (protein precipitation), vortexed vigorously followed by centrifugation at 1,200 × g for 20 min at 20 °C (Beckman CS-6R, Palo Alto, CA). Copper (Cu) determination was completed at the University of Arkansas, Division of Agriculture, Altheimer Diagnostic Laboratory using a Spectro (Spectro Analytical Instruments, Kleve, Germany) Arcos inductively coupled plasma spectrophotometer with a detection limit of 0.03 mg/liter. Nonesterified fatty acid (NEFA) concentrations were analyzed at the University of Tennessee using commercially available kits (Wako Chemicals USA, Inc., Richmond, VA) according to the procedures described by Stratman et al. (2016). The NEFA intraassay and interassay CV was 6.52% and 8.64%, respectively. Serum prolactin was also analyzed at the University of Tennessee according to the procedures outlined by Bernard et al. (1993). The prolactin intraassay and interassay CV was 6.65% and 8.28%, respectively. Due to centrifuge malfunction, fall 2015 initial samples for Cu and BUN were lost.

Pasture sampling and analysis

At the time of animal data collection, pastures were sampled for forage mass and forage nutritive quality. Forage mass in each pasture was determined using a calibrated rising-plate (RP) meter (Michell and Large, 1983). Twenty height measurements were recorded within pasture on each sampling date; an additional set of samples were measured for calibration by clipping the forage within a 50 × 50 cm, fall, or 43.2 × 43.2 cm, spring, quadrant leaving a residual plant height of 2.5 cm. RP samples were dried to a constant weight at 50 °C in a forced-air oven. Forage mass predictions were developed separately for fall and spring, resulting in the following formulas: fall forage mass, kg/ha = 39.201 × RP and spring forage mass, kg/ ha = 189.379 × RP. The linear regression solution for predicting forage mass from RP height had a R2 ≥ 0.88 ($P \leq 0.01$).

Additional forage samples were collected on each sampling date by hand plucking to mimic forage consumed. Prior to sampling, areas were visually scanned for grazing patches and bite depths of plants apparently grazed. Samples were dried to a constant weight at 50 °C prior to storage for nutrient determination. Prior to nutrient analysis, the samples were ground to pass a 2-mm screen in a Wiley Laboratory Mill (Model 4, Thomas Scientific, Swedesboro, NJ) at the Southwest Research and Extension Center, Hope, AR. Plant composition of crude protein (CP), acid detergent fiber (ADF), and neutral detergent fiber (NDF) was determined by near-infrared reflectance spectroscopy (Feed & Forage Analyzer model 6500, FOSS North America, Eden

Prarie, MN). The CP calibration equation had a standard error of calibration (SEC) of 0.92, a standard error of cross validation (SECV) of 0.93, and R2 of 0.96. The NDF calibration equation had a SEC of 2.63, a SECV of 2.73, and an R2 of 0.95. The ADF calibration equation had a SEC of 1.66, a SECV of 1.70, and an R2 of 0.93.

Plant stand counts were conducted at the interim point of each study. Stand count determination was accomplished by traversing a zig-zag pattern across each pasture in a utility vehicle while dragging a rod with a metal pointed tip. At random stops, either the plant species in contact with the metal point or a record of bare ground was recorded. The objective was to capture a minimum of 50 and maximum of 100 random points per pasture.

Fescue tiller and leaf samples were collected on 12 November 2015 and 6 May 2016 for EV determination. Each pasture was sampled at 20 sites, traveling in a zig-zag pattern with a utility vehicle. At each stop, plants were cut by knife at the base of the crown and material was placed in a plastic sealable bag. Sample bags were kept on ice throughout the sampling process and then frozen. Frozen samples were shipped overnight in an ice chest with dry ice to the University of Kentucky Veterinary Diagnostic Laboratory (Lexington, KY) for EV plus ergovalinine concentration according to the procedures of Lea et al. (2014) modified for ultra-high performance liquid chromatography (UHPLC) with fluorescence detection. Briefly, fresh forage samples were flash frozen with liquid nitrogen (Scott Gross, Lexington, KY) and milled to a fine powder (Stein M-2 Mill, Steinlite Corp., Atchison, Kansas). Duplicate 1.25-g subsamples (0.25 g for freeze-dried samples) were extracted for 1 h with 5-mL extraction solution (50% aqueous 2-propanol/1% lactic acid containing 0.1- µM ergotamine) on a rotating mixer (Multi-Mixer & Rotator, United Products & Instruments, Inc., Dayton, NJ). The extraction solution supernatant was then syringe-filtered (PVDF 0.22 µm, Restek, Bellefonte, PA) into autosampler vials until UHPLC fluorescence analysis. A separate subsample was dried at 95 ± 5 °C overnight to determine moisture content (typically 75% to 80% for fresh forages and 4% to 8% for freeze-dried material).

The UHPLC system (Dionex Ultimate 3000 UHPLC, Thermo Fisher Scientific, Waltham, MA) utilized a Zorbax Eclipse Plus C18 RRHD analytical column (2.1 × 50 mm 1.8 µm, Agilent Technologies, Santa Clara, CA) with the fluorescence detector set to maximum sensitivity at 310 nm (excitation) and 410 n (emission). Mobile phases consisted of A (1:3) and B (3:1) mixtures of acetonitrile: 0.1 M aqueous ammonium acetate. Sample extracts and standard solutions (2 µL) were injected into the initial gradient conditions of 95% mobile phase A/5% mobile phase B with a 0.600 mL/ min flow rate. Immediately following injection, the mobile phase B was increased at a linear rate to 20% over the next 3.5 min, then further increased to 50% over the next 2.6 min, and finally increased to 90% over the next 2.7 min. The gradient profil was then held at 90% mobile phase B for 1 min. At 10.0 min after injection, the initial gradient conditions were resumed (11.0 min total run time).

EV calibrant solutions were prepared in methanol, ranging from 0.02 to 0.50 µM, in addition to an ergotamine internal standard concentration of 0.1 µM. Total EV concentration in each forage sample was interpolated from a calibration curve produced by plotting the peak area ratio of total EV to total ergotamine vs. total EV concentration. Final results were corrected for moisture content and reported in parts per billion (equivalent to ng/g) on a DM basis. Results were reported to a minimum resolution of 100 ppb. Assay coefficients for sample variation were not reported with the EV results.

Statistical analysis

Eight of the 16 pastures were anticipated to be low-toxicity or nontoxic; however, animal performance and EV testing revealed contamination with Kentucky 31 tall fescue among the pastures that had been renovated with the nontoxic fescue. Given the amount of variation among EV pasture concentration, it was decided to model study responses using an analysis of covariance approach (Littell et al., 2006). This approach combined discrete and continuous fixed effects for model intercept and slope parameter estimation, respectively. Responses to MGMT, EV, and MGMT × EV were modeled using the MIXED procedure of SAS (SAS Ins., Inc., Cary, NC). The model statement solution option was used to output fixed-effect intercept and slope parameter estimates. Plant stand counts were fitted to the same model using the GLIMMIX procedure for a binomial response distribution. In addition, hair score was also modeled using the GLIMMIX procedure fitted to a logit model for ordered response data and included an over-dispersion parameter. Pasture was the experimental unit for all responses. Pasture EV was modeled as a continuous covariate and model solutions were used to evaluate the EV slope effect on responses and whether the slope differed for MIN vs. CM (MGMT × EV). When appropriate, the model also included a repeated measures effect for month (pasture measurements) or period (temperature and blood chemistry measurements). Repeated measures were modeled with an autoregressive correlation structure and the denominator degrees of freedom estimation method was set to Kenward–Rogers. The initial, full model included all 2-way and 3-way interactions. Nonsignificant interactions were removed from the model. When an EV by period effect was detected for a response variable, simple correlations were used to describe relationships between the response variable and EV for each period. For table presentation, when at least one of the 2-

way interactions was significant for a response type, nonsignificant 2-way interaction P-values were also shown for table layout consistency.

Results and Discussion

Environmental conditions and forage characteristics

During the first half of the fall study, harsh growing conditions were encountered with above-average temperatures and below-average rainfall (Table 1). However, growing conditions improved during the second half of the study as rainfall amounts increased and temperatures remained well above normal. Fall 2015 EV levels were in a range between 427 and 3,060 ppb, averaging 1,475 ± 883.1. Research with EV as low as 260 and 520 ppb during heat stress was sufficient to suppress prolactin in lambs (Gadberry et al., 2003). Belesky et al. (1988) reported EV represented more than 80% of ergopeptines measured in tall fescue. Parish et al. (2003) measured 836 and 1,208 ppb total alkaloids at 2 study locations during fall in Georgia. At these levels of total alkaloids, reductions in prolactin and weight gain occurred by comparison to fescues containing endophytes that produced minimal toxin loads (≤28 ppb total alkaloids). In general, it appears the minimal fall EV in our study was at a level that would likely cause physiological and weight change.

Temperatures during the spring study, similar to the fall study, were above normal. There was a surplus of rain in March and May but a deficit in April and June compared with the normal reported at this location (Table 1). During the spring, EV levels ranged from <100 (90 ppb was used as a proxy for <100 ppb for the statistical analysis) to 2,180 and averaged 1,172 ± 620.6 ppb. As noted earlier, 100 ppb was the EV assay detection limit and EV was reported as <100 ppb. Given the previous fall level of EV within these same fields, we would anticipate EV to be closer to 100 than 0. Belesky et al. (1988) reported that ergopeptine alkaloid concentrations were greater in tall fescue with a high level of infection, and samples taken over a 228-d growing season showed greatest levels of ergopeptine alkaloids in the fall, similar to the EV difference in our study between November 2015 and May 2016. In addition to observing a lower EV in spring compared with the previous fall, the variation was also less among the spring samples.

The fall plant counts revealed 70.5 ± 2.32% tall fescue for MIN and 68.9 ± 2.43% tall fescue for CM which did not differ with MGMT ($P = 0.6$) or EV × MGMT ($P = 0.9$), but overall percent fescue increased as EV increased ($P < 0.01$). In the spring, the percentage fescue as influenced by level of EV tended to differ among pastures assigned to the MIN treatment compared with the CM treatment (EV × MGMT, $P = 0.06$). It is however unlikely that this interaction was attributed to MGMT creating a short-term effect on plant population diversity at different levels of pasture toxicity, especially after considering available forage.

Reducing the model to simple effects indicated no difference ($P = 0.96$) in fescue percentage between MIN (76.2 ± 1.73%) and CM (76.0 ± 1.70%). Similar to fall, EV levels were positively associated with fescue percentage ($P = 0.01$).

Forage mass (Table 4) during the fall and winter (October through January) was not affected ($P \geq 0.65$) by MGMT, EV, or an MGMT × EV interaction. Forage mass was greatest at the start of the fall grazing season and declined each month until the end of the grazing season ($P < 0.001$). Similar to forage mass, forage allowance (kg forage DM/kg steer BW) did not differ ($P \geq 0.13$) due to MGMT, EV, or an MGMT × EV interaction. Forage allowance was at its greatest level in October and declined as the season progressed, due to both declining forage mass and increasing steer BW. Forage allowance during the fall and winter was above the point identified by Beck et al. (2013) and NRC (2016) that would be limiting to performance of steers grazing pasture in the fall at full DM intake. It could be assumed that performance of steers grazing cool-season perennial pastures in the fall and winter would be affected by a similar break-point in forage allowance; thus, forage allowance would not be considered limiting in the present experiment. It should also be noted that exceptionally high-forage allowance which should provide ample opportunity for selective grazing did not prevent the effects of fescue toxicosis.

During the spring grazing season (March through June), forage mass (Table 4) was not affected by MGMT, EV, or an MGMT × EV interaction ($P \geq 0.18$). Forage mass was lowest at the beginning of the spring grazing season and increased as the season progressed ($P < 0.01$). Forage allowance, however, was affected ($P \leq 0.04$) by month, EV, and an MGMT × month interaction. As forage growth increased during the spring, forage allowance also increased, from 1.7 kg/ kg steer BW in March to over 3 kg/kg steer BW in April, May, and June. Forage allowance also increased ($P = 0.04$) 0.37 ± 0.165 kg/kg BW for every 1,000 ppb increase in EV. The increase in forage allowance with increasing toxicity is likely related to a combination of reduced forage DM intake (Beck et al., 2009; Aiken and Strickland, 2013) and reduced steer weight gain with increasing EV concentration. The MGMT × month interaction stems from lack of differences ($P \geq 0.10$) in forage allowance due to MGMT treatment in March, April, and May; yet, CM had lower ($P = 0.03$) forage allowance than MIN in June. The reduction in forage allowance for CM in June is related to increased steer weight and numerical reduction in forage mass at that time. Across all months and treatments, the forage allowance provided for steers in this grazing experiment were in excess of the 1 kg/kg steer BW indicated to maximize steer performance for spring growth cool-season annual pastures by Rouquette et al. (2012). Forage nutritive quality constituents were not affected by any of the 2- or 3-way interactions evaluated

($P \geq 0.07$), but was affected by month within the grazing season; thus, the forage chemical analysis is presented by MGMT treatment and month in Table 5. During the fall and winter grazing season, forage CP increased ($P \leq 0.05$) as the season progressed until January. The concentration of ADF and NDF generally decreased ($P \leq 0.05$) with the progressing grazing season, leading to an increasing estimate of total digestible nutrient (TDN) content as the season progressed with better moisture for growth during late season. In January, CP of MIN pastures was less ($P = 0.04$) than CP of CM pastures and NDF of MIN was greater ($P = 0.05$) than CM. Since the phenological growth stage at this time of year would not be different, this indicates that leaf age during January was likely less for CM pastures than MIN, leading to reduced NDF and increased CP. During the spring grazing season, CP and TDN decreased ($P \leq 0.05$), whereas ADF and NDF increased ($P \leq 0.05$) as the grazing season progressed, which is related to advancing forage maturity associated with stem elongation and seed head development which normally occurs at this time of year (Beck et al., 2013).

Animal growth performance

Fall grazing season

Initial BW of steers was 194 ± 5.7 kg for MIN and 199 ± 5.7 kg for CM steers ($P = 0.53$). Final BW for MIN steers was 28% less ($P < 0.001$) than CM (223 ± 5.6 vs. 286 ± 5.6 kg for MIN and CM, respectively). Cumulative management and MIN differed in overall ADG response ($P < 0.001$), ADG decreased with increasing EV ($P = 0.01$), and the magnitude of difference between MIN and CM did not differ across all levels of EV (MGMT × EV, $P = 0.19$). The weight gain of steers grazing tall fescue in the fall (Figure 1) was explained by $ADG = 1.05 - 0.000064 \times EV$ for CM and $ADG = 0.41 - 0.000064 \times EV$ for MIN. The expected performance of steers grazing tall fescue at the lowest level of toxicity (500 ppb EV) was 1.02 kg/d for CM and 0.38 kg/d for MIN, a 0.64 kg/d advantage for CM. At 2,500 ppb EV, expected performance of steers was 0.89 kg/d for CM and 0.25 kg/d for MIN. Performance of MIN steers during the fall was less than expected based on observations made by Beck et al. (2008, 2009) who reported gains in the fall and winter with steers grazing toxic tall fescue to be from 0.5 to 0.7 kg/d and gains of steers grazing nontoxic fescue to be from 0.8 to 0.9 kg/d. The reduced performance of steers grazing tall fescue during the fall in the current experiment is likely due to the growing conditions (Table 2) possibly resulting in different levels of toxicity between the fall and spring studies.

Spring grazing season

Initial BW of steers grazing in spring 2016 averaged 167 ± 1.6 kg for MIN and CM (P = 0.88). Final BW in the spring was 17% greater (P = 0.01) for CM than MIN (206 ± 4.9 vs 245 ± 4.9 kg for MIN and CM, respectively). ADGs (Figure 2) were affected by an MGMT × EV interaction (P = 0.03). The ADG of steers during the spring was explained for MIN by ADG = 0.80 − 0.000276 × EV, whereas for CM, ADG = 0.94 + 0.000001835 × EV indicating that EV had much greater impact on ADG of MIN steers compared with CM. Thus, at the lowest EV concentration, MIN gained 0.77 kg/d compared with 0.94 kg/d for CM and a 0.17 kg/d advantage for CM, whereas at the greatest EV concentration, CM gained 0.7 kg/d more than MIN (0.94 vs. 0.24 kg/d for CM and MIN, respectively). The performance of steers in the MIN treatment grazing tall fescue with a range in EV concentration in the current experiment is in line with observations made by Beck et al. (2008, 2009) with steers grazing nontoxic and toxic tall fescue in the spring. Contrarily, the gains of steers in the CM treatment were similar to gains by steers grazing nontoxic tall fescue reported by Beck et al. (2008, 2009) regardless of EV concentration, indicating that the combination of supplementation and growth-promoting technologies counterbalanced the negative weight gain response to increasing EV.

Hoveland (1986) indicated that steer performance declines precipitously as levels of toxic endophyte infection increase in a stand, indicating that steers grazing endophyte-free tall fescue had gains in excess of 0.9 kg/d, whereas steers grazing 90% toxic endophyte tall fescue had gains of only 0.6 kg/d. The reduced impact of EV on performance of steers with CM confirms the findings of the meta-analysis of growth promoting technology use for steers grazing toxic tall fescue reported by Gadberry et al. (2015) who found an average response of 0.1 kg/d with implants and 0.06 kg/d with feed additives. The additive effect of these growth promoting technologies is in agreement with observations of additive response to implants and feed additives by steers grazing wheat pasture (Beck et al., 2014). Based on the growth response reported by Gadberry et al. (2015), at the lowest level of spring EV, there appears to be a response level that would align with the expected response from implants and feed additives but not supplementation. At greater levels of toxicity, providing supplemental feed appeared to offset possible reductions in DM intake often associated with tall fescue toxicosis (Aiken and Strickland, 2013) along with the additive weight gain effects of implants and feed additives.

Results showed an improvement in performance that substantiates the additive approach idea suggested by Roberts and Andre (2010). Gadberry et al. (2015) indicated that digestible fiber–based supplemental feed response was ADG = 0.06 × % BW0.75 + 0.13. In the present study, the average supplementation rate in spring and fall was 4% shrunk metabolic weight. Based on the work of Gadberry et al. (2015), the theoretical additive response in this study was 0.53 kg/d (0.37 kg from supplementation, 0.1 kg from implant, and 0.06 kg from ionophore). Applying the current study fall and spring mean EV and ADG prediction equations, the average benefit to enhanced management (CM – MIN) in fall and spring was 0.64 and 0.47 kg/d, respectively, which falls above and below the theoretical additive response, supporting the concept of an additive approach. Furthermore, Carter et al. (2010) observed a 0.51 kg/d improvement in ADG with steroidal hormone–implanted steers fed 2.3 kg/d soybean hulls. Their study did not include an ionophore but complements the observed weight gain in this study.

Predicted weight gain response was also modeled using formulas published in the NRC (2016) and supporting software. Mean shrunk weight, ADG, supplement nutrient composition, supplemental feeding rate, and pasture nutrient composition were available for basic empirical model assessment. Standard model adjustment for growth implant and ionophore was used in the CM evaluation. Forage intake was unknown but also was not restricted in fall or spring. The NRC predicted intake, adjusted to mimic the observed overall mean ADG for MIN during fall (0.3 kg/d) and spring (0.46 kg/d), was predicted at 1.8% shrunk BW, despite differences in mean shrunk weight and forage TDN between the 2 seasons. Using the predicted forage intake for MIN and the known overall mean supplemental feed rate in fall (2.0 kg/d DM), fall metabolizable energy allowable gain for CM exceeded the observed average (1.14 vs. 0.96 kg/d) and input DM intake exceeded predicted DM intake by 9%. Similarly, using the overall mean supplemental feed rate for spring (1.78 kg/d DM), spring metabolizable energy allowable gain for CM exceeded the observed average (1.2 kg vs. 0.94 kg/d) and input DM intake exceeded predicted DM by 11%. Modeled responses suggest that DM intake on fescue pasture without supplementation was likely restricted and therefore resulting in the observed ADG being less than expected for the quality (protein and fiber) of the forage. Introducing supplemental feed at 1% BW increased weight gain; however, the model suggested that forage substitution may be occurring at this level of supplemental feeding or fescue toxins were limiting weight gain response to the feed supplement.

Blood chemistry

Copper status

There were no effects ($P \geq 0.12$) of MGMT, EV, Period within the grazing season, or MGMT × EV on serum Cu (Table 6) during the fall study. Although there were no effects ($P \geq 0.09$) of MGMT or EV on serum Cu status during the spring study, serum Cu concentrations decreased as the grazing season progressed (Period, $P < 0.01$).

The byproduct feed supplement was low in Cu (6 ppm); therefore, for both MIN and CM, the main contribution to supplemental Cu was the MIN supplement (1,000-ppm Cu). The CM mineral was incorporated into the daily supplemental feed, whereas MIN was free choice. During the fall study, mineral disappearance for MIN was 91 ± 8.1 g/d (91-mg Cu), whereas during spring, mineral disappearance for MIN was 127 ± 3.7 g/d (127-mg Cu). For a 211- kg calf with an estimated dietary requirement of 10 ppm (NRC, 2016), the MIN group was consuming approximately 216% and 301% of their requirement in fall and spring study, respectively, at a 2% BW intake. The serum Cu observed in the current experiments, however, would be considered deficient (Lopez-Alonso et al., 2006).

It is thought that exposure to tall fescue toxins leads to reduced Cu status (Coffey et al., 1992; Saker et al., 1998; Stewart et al., 2010). Exposure of spring calving cows to toxic endophyte tall fescue pastures resulted in decreased blood Cu compared with cows grazing only nontoxic endophyte-infected tall fescue (Caldwell et al., 2013). Cattle that graze toxic endophyte tall fescue pastures may be Cu deficient in plasma and liver tissues (Coffey et al., 1992) with below normal serum Cu concentrations (Lopez-Alonso et al., 2006) leading to a decrease in immune responses in stress-challenged animals (Saker et al., 1998).

Results from Saker et al. (1998) demonstrated that steers grazing toxic endophyte-infected tall fescue pastures had lower Cu status than steers grazing endophyte-free tall fescue from July through September, ranging from normal (0.7 to 1.1 mg/ kg) to deficient (0.2 to 0.4 mg/kg). Contrary to the observations in the current experiment, Oliver et al. (2000) found Cu levels at about 0.7 mg/kg for steers grazing endophyte-free tall fescue and 0.6 mg/kg for steers grazing toxic endophyte-infected tall fescue. Likewise, Stewart et al. (2010) found that beef steers grazing endophyte-free tall fescue had greater liver Cu concentrations than steer grazing toxic endophyte-infected tall fescue pastures. Copper status in the current experiment was generally not associated with pasture level of toxicity ($P = 0.98$ and 0.09 for fall and spring study, respectively).

Blood urea nitrogen

During the fall, there were no effects ($P \geq 0.07$) of EV, Period, or MGMT × EV on BUN (Table 6), but MIN steers had greater ($P < 0.01$) BUN concentrations than CM steers, despite the CM steers receiving supplemental feed that contained a greater percentage protein content (19.4% DM) in comparison to the protein content of available forage (12.3% to 17.3% DM). During the spring, the same reduction ($P < 0.01$) in BUN was observed for CM compared with MIN. But, BUN concentrations were found to increase ($P < 0.01$) as the spring grazing season progressed, even though forage CP concentrations decreased during this time (Table 5). Unlike fall, the forage CP ranged from 15.4% to 25% while the supplemental feed averaged 16.9% CP.

BUN concentration is a metabolic indicator of protein-energy status, which can indicate dietary problems (Kohn et al., 2005) and ammonia production in the rumen (Torell et al., 1974).

Levels of BUN greater than 19 or 20 mg/dL indicate high dietary protein intake and BUN concentrations lower than 7 mg/dL indicate protein deficiency (Hammond, 1992). Blood urea N concentrations were within the adequate range for dietary N (Hammond, 1992) at all times during the current experiment (Table 6). In the current study, EV did not affect BUN; likewise, Oliver et al. (2000) observed similar BUN levels among steers on endophyte-free and endophyte-infected pastures.

Nonesterified fatty acid

The concentration of NEFA (Table 6) was less ($P < 0.01$) in CM steers compared with MIN steers during the fall study. During the spring study, however, the magnitude of the mean NEFA concentration was half of the difference observed in fall and therefore did not differ ($P = 0.32$) between treatments. Level of EV did not influence NEFA in the fall study ($P = 0.57$) or spring study ($P = 0.23$). NEFA did change ($P < 0.01$) throughout both the fall and spring seasons. In the fall, NEFA increased as the season progressed, whereas in the spring, NEFA decreased as season progressed. Diet, DM intake, and other nutritional and non-nutritional factors affect NEFA (Bowden, 1971). Overall, it appears that changes in pasture conditions greatly influenced NEFA. Generally, forage protein concentration increased and fiber concentration decreased as the fall study progressed, whereas forage protein concentration decreased and fiber concentration increased as the spring study progressed. Pasture quality (based on protein and fiber concentrations) in the fall study was not as good as the levels observed during the spring study and it was during the fall study that CM resulted in lower NEFA levels and thus improved metabolic status. The samples collected for blood chemistry were collected following a 16-h fasting period; therefore, the reduced NEFA concentrations in CM compared with MIN may have been due to greater adipolysis by MIN (Grummer and Carroll, 1991).

Prolactin

Serum prolactin concentrations (Table 6) during the fall study were not affected ($P \geq 0.19$) by MGMT, EV, or the MGMT × EV interaction. However, serum prolactin in the fall differed throughout the season ($P < 0.01$) with interim study prolactin being less ($P < 0.05$) than initial and final values. Final winter prolactin values had returned to initial levels ($P = 0.5$). Further data are needed to determine whether EV had diminished in these late season pastures. Kallenbach et al. (2003) observed a reduction in stockpiled fescue EV from early to late winter.

During the spring, serum prolactin was not affected ($P \geq 0.79$) by MGMT or the MGMT × EV interaction. There was, however, an EV × Period interaction (data not shown) for prolactin in the spring ($P = 0.01$).

Reduced serum prolactin concentration is the most recognized physiological change associated with fescue toxicosis (Hurley et al., 1980; Hoveland et al., 1983) and these results are consistent with previous studies involving comparisons of toxic endophyte, nontoxic endophyte-infected, and endophyte- free tall fescue (Parish et al., 2003; Nihsen et al., 2004) and sheep fed endophyte-infected and endophyte-free tall fescue seed (Gadberry et al., 2003). In the fall study, there was not a statistically significant relationship between EV and prolactin which suggests that the low end of the EV range (427 ppb) was just as detrimental to prolactin production as the fescue pastures at the greatest end of the EV range. In the spring, EV was generally associated with prolactin ($P = 0.03$), whereby, as EV increased, prolactin decreased. This may have occurred in the spring, unlike the fall, because EV was as low as 100 ppb in certain spring pastures, and numerically, the overall mean EV at spring sampling was less than the overall mean EV at fall sampling. As previously mentioned, the EV effect on prolactin was not consistent across the 3 measurement periods in spring. Initial prolactin would not be expected to correlate with EV because the study had not started. The interim prolactin mean was less than initial prolactin, evident to the effect of grazing toxic fescue. The interim period exhibited the greatest correlation (data not illustrated) between EV and prolactin ($r = -0.66$, $P = 0.006$). Prolactin at the end of the spring study was at its lowest and did not correlate (data not illustrated) with EV ($r = -0.33$, $P = 0.22$). In the spring, end of study, mean prolactin was numerically more similar to the fall study mean prolactin levels and there was no EV effect on prolactin in the fall study. The reduced correlation at the end of the spring study was likely due to fields becoming more toxic as the season progressed.

A MGMT effect on prolactin was not detected in either the fall or spring study. Carter et al. (2010) reported soybean hulls fed at 2.3 kg, daily (as-fed basis), increased serum prolactin but steroidal implants did not affect prolactin in steers. The supplemental feeding rate in that study was approximately 0.7% of average study body weight. Shappell et al. (2015) found increased estrogenic activity within serum of steers fed soybean hulls and the level of estrogenic activity was greatest for steers fed soybean hulls in combination with a steroidal implant. Research with rats (Gudelsky et al., 1981) demonstrated that estrogen had some dopamine sparing effect on prolactin and ergot alkaloids are considered dopaminergic (Larson et al., 1995). Current research indicates that estrogen and estrogen- like compounds may help ameliorate fescue toxicosis; however, prolactin did not respond to CM in the current study. This may be due to lesser soybean hull consumption and implant estradiol level in the present study.

Rectal and tail skin temperatures and hair score

Temperatures

Rectal and tail skin temperatures (Table 7) were not affected ($P \geq 0.16$) by EV in the fall study. Rectal temperatures were affected ($P \leq 0.04$) by MGMT, Period, and the MGMT × Period interaction. Initial and final rectal temperatures for CM and MIN did not differ, but interim rectal temperatures during the fall study were less ($P \leq 0.05$) for MIN compared with CM. Rectal temperatures in the spring did not differ ($P \geq 0.46$) due to MGMT or EV, but were lowest ($P < 0.01$) at the intermediate sampling period. These results parallel studies reported by Aiken et al. (2008) who found no difference between rectal temperatures in steers fed soybean hulls or nonsupplemented steers on toxic tall fescue pastures. Aldrich et al. (1993) found that cattle grazing endophyte-free tall fescue had lower rectal temperatures than cattle grazing or toxic endophyte-infected tall fescue; however, rectal temperatures were elevated in response to environmental temperature change as was observed during the spring in the current experiment.

A similar MGMT × period interaction ($P = 0.01$) was found for tail skin temperatures (Table 7) in the fall and winter grazing season. Initial and intermediate tail skin temperatures did not differ between MIN and CM in the fall, but tail skin temperatures at the end of the grazing season were less ($P \leq 0.05$) for MIN compared with CM. Tail skin temperatures in the spring were less ($P < 0.01$) for MIN than for CM, and increased as the grazing season progressed ($P < 0.01$) which is likely due to the increased ambient temperatures observed in the late spring (Table 1) and observations of increased grazing activity of CM steers (Diaz et al., 2017). Aldrich et al. (1993) confirmed that environmental temperature influenced skin temperature at a fixed level of toxicity, and Gadberry et al (2003) demonstrated that toxicity level influenced skin temperature at a fixed level of heat stress. There were no sufficient slope differences over the spring season to result in an MGMT × Period interaction; however, as season progressed, the difference in tail skin temperature between CM and MIN increased, contributing to the observed MGMT effect. The CM management strategy appears to have interceded in steers to allow them to cope with higher ambient temperatures by vasodilation and physical relief to dissipate body heat through increasing peripheral blood flow (Al-Haidary et al., 2001) and thus skin temperature. It is unclear if there was beneficial effects from increased estrogenic activity (Shappell et al., 2015) of the treatments in the current study. Aiken et al. (2016) demonstrated that forage isolflavones improved vascular blood flow in goats (Aiken et al., 2016).

Hair score

Hair score was numerically high and hair shedding was not evident among calves until the end of the spring study in mid-June. Hair shedding score in June was affected by MGMT (P = 0.03) but not EV (P = 0.39), and the mean final hair coat score was 4.5 ± 0.24 and 3.7 ± 0.21 for MIN and CM, respectively. Nihsen et al. (2004) reported improved hair coat scores with nontoxic endophyte-infected fescue compared to toxic, Kentucky 31. Carter et al. (2010) also observed an improvement in hair coat due to supplementation with soybean hulls or with steroidal implants but not an additional benefit for combining these practices. Others have speculated that prolactin may influence hair growth and retention (McClanahan et al., 2008; Gray et al., 2011); unlike hair score, prolactin did not differ according to MGMT in the spring study.

In conclusion, it is unlikely that the majority of toxic tall fescue pastures throughout the fescue region of the southeastern United States will be converted to nontoxic fescue in the near future. Based on experience in the current experiment, some efforts to convert fields from toxic to nontoxic endophyte-infected tall fescue will not be successful. Cumulative management strategies including growth promoting implant, ionophore, and supplemental feeding offer a best management practice solution to improve the welfare and weight gain of growing cattle grazing toxic tall fescue. Further research is needed to evaluate this program at varied stocking rates and in combination with dilution of endophyte-infected fescue pastures with other nontoxic grasses or legumes. In addition, carry-over effects of cumulative management strategies on calf performance throughout the feedlot finishing phase of production and carcass composition need assessment to establish protocols for cattle producers that stocker cattle on fescue pastures and market after the stocker phase of production or retain ownership through feedlot finishing.

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Table 1. Record of climatological observations at the University of Arkansas, Division of Agriculture, Livestock and Forestry Research Station Batesville, AR.

	Temperature, ° C	Departure from long-term average, ° C	Precipitation, mm	Departure from long-term average, mm
September, 2015	21.94	+ 1.1	30.73	- 85.9
October[1]	15.83	+ 1.0	54.61	- 71.1
November[1]	10.56	+ 2.1	253.49	+ 133.1
December[1]	8.33	+ 5.1	231.39	+ 144.3
January	2.50	+ 0.8	23.37	- 63.8
February	6.39	+ 2.1	42.16	- 72.7
March[2]	11.67	+ 2.8	178.31	+ 37.3
April[2]	15.00	+ 1.3	104.39	- 13.1
May[2]	18.33	- 0.7	108.71	+ 14.1
June[2]	25.28	+ 1.6	46.74	- 53.8
July	27.22	+ 1.4	95.00	+ 19.8

[1] Fall study began October 6, 2015 and ended January 5, 2016.

[2] Spring study began March 22, 2016 and ended June 14, 2016.

Table 2. Guaranteed analysis of the self-fed mineral supplement offered to MIN steers and mineral premix included in supplement fed to CM steers.

	MIN[1]	CM[2]
Monensin (as Monensin Sodium) g/kg	-	1.32
Calcium (Ca), % minimum	18.60	18.60
Calcium (Ca), % maximum	22.30	22.30
Phosphorus (P), % minimum	3.00	3.00
Salt (NaCl), % minimum	18.20	18.20
Salt (NaCl), % maximum	21.80	21.80
Magnesium (Mg), % minimum	1.00	1.00
Copper (Cu), minimum, mg/kg	1,000	1,000
Selenium (Se), minimum, mg/kg	26.40	26.40
Zinc (Zn), minimum, mg/kg	3,750	3,750
Vitamin A, minimum, IU/kg	300,000	300,000
Vitamin D3, minimum, IU/ kg	44,092	44,092
Vitamin E,minimum, IU/ kg	220.46	220.46

[1] Vigortone 3V6 S, Provimi North America, Inc., Brookville, OH

[2] Vigortone 3V6 SR w/monensin active drug ingredient, Provimi North America, Inc., Brookville, OH.

Table 3. Ingredient composition of the 50:50 soybean hull pellet:corn gluten feed supplement fed to steers grazing tall fescue during the fall of 2015 and spring of 2016.

Component (dry matter basis)	Fall	Spring
Crude protein,%	20.1	16.9
Acid detergent insoluble crude protein, %	0.7	1.5
Acid detergent fiber, %	27.5	31.4
Neutral detergent fiber (ash-free), %	45.9	50.3
Crude fat, %	3.4	3.5
Total digestible nutrients, %	74	69
Net energy for maintenance, Mcal/kg	1.70	1.54
Net energy for gain, Mcal/kg	1.08	0.95
Calcium, %	0.32	0.33
Phosphorus, %	0.60	0.62
Copper, ppm	6	6

Table 4. Forage mass (kg DM/ha) and forage allowance (kg forage DM/kg steer BW) for tall fescue pastures with varying ergovaline concentrations (EV) grazed by growing steers fed mineral only (MIN) or cumulative growth promoting management (CM) including supplementation, ionophore, and hormonal implant.

				P-value			
Item	MIN	CM	SEM	MGMT[1]	EV[2]	Month	Month × MGMT
Forage mass, kg DM/ha							
Fall study				0.73	0.65	< 0.001	0.91
October[a]	2,794	2,769	116.6				
November[b]	2,471	2,472	116.6				
December[c]	2,234	2,300	116.6				
January[d]	2,031	2,169	116.6				
Spring study				0.69	0.13	< 0.001	0.18
March[a]	1,156	1,190	130.2				
April[b]	2,348	2,416	130.2				
May[c]	2,524	2,771	130.2				
June[c]	2,686	2,564	130.2				
Forage allowance, kg DM/kg BW							
Fall study				0.13	0.67	< 0.001	0.78
October[a]	5.84	5.65	0.253				

(Table 4 (Cont.)...) Forage mass (kg DM/ha) and forage allowance (kg forage DM/kg steer BW) for tall fescue pastures with varying ergovaline concentrations (EV) grazed by growing steers fed mineral only (MIN) or cumulative growth promoting management (CM) including supplementation, ionophore, and hormonal implant.

				P-value			
Item	MIN	CM	SEM	MGMT[1]	EV[2]	Month	Month × MGMT
November[b]	4.99	4.61	0.253				
December[c]	4.33	3.76	0.253				
January[d]	3.70	3.04	0.253				
Spring study				0.36	0.04	< 0.001	0.03
March[a]	1.68	1.73	0.177				
April[b]	3.02	2.94	0.177				
May[b]	3.01	2.94	0.177				
June[c]	3.22[e]	2.55[f]	0.177				

[1] MGMT – effect of cumulative growth promotion management CM vs MIN.

[2] Effect of ergovaline concentration.

[abcd] Least squares means for month within grazing season with differing superscripts differed at $P \leq 0.05$.

[ef] Least squares means within rows differed at $P \leq 0.05$.

Table 5. Forage nutritive quality for tall fescue pastures with varying ergovaline concentrations (EV) grazed by growing steers fed mineral only (MIN) or cumulative growth promoting management (CM) including supplementation, ionophore, and hormonal implant.

			P-value[1]	
Item	MIN	CM	MGMT[2]	EV
		Fall grazing season		
Crude protein, % of DM				
October[a]	12.3 ± 0.64	13.7 ± 0.65	0.75	0.64
November[b]	14.4 ± 0.35	15.8 ± 0.35	0.73	0.47
December[c]	15.8 ± 0.39	17.3 ± 0.39	0.29	0.43
January[b]	12.3 ± 0.64[e]	15.1 ± 0.34[f]	0.04	0.80
Acid detergent fiber, % of DM				
October[c]	38.9 ± 0.93	37.0 ± 0.95	0.79	0.91
November[c]	39.3 ± 0.62	37.0 ± 0.63	0.56	0.78
December[b]	35.5 ± 0.88	31.5 ± 0.89	0.08	0.97
January[a]	32.1 ± 1.06	28.9 ± 1.07	0.07	0.95
Neutral detergent fiber, % of DM				
October[d]	66.6 ± 1.19	63.9 ± 1.20	0.93	0.85
November[c]	64.9 ± 0.61	62.7 ± 0.62	0.97	0.84
December[b]	60.6 ± 0.94	56.9 ± 0.95	0.13	0.74
January[a]	56.5 ± 1.25[f]	52.8 ± 1.27[e]	0.05	0.88
Total digestible nutrients, % of DM				
October[a]	59.0 ± 1.04	61.1 ± 1.05	0.79	0.91
November[a]	58.5 ± 0.69	61.1 ± 0.70	0.56	0.78
December[b]	62.8 ± 0.98	67.2 ± 0.99	0.08	0.97

(Table 5 (Cont.)…) Forage nutritive quality for tall fescue pastures with varying ergovaline concentrations (EV) grazed by growing steers fed mineral only (MIN) or cumulative growth promoting management (CM) including supplementation, ionophore, and hormonal implant.

Item	MIN	CM	P-value[1] MGMT[2]	EV
January[c]	66.6 ± 1.18	70.1 ± 1.20	0.07	0.95
		Spring grazing season		
Crude protein, % of DM				
March[d]	24.9 ± 0.28	25.0 ± 0.29	0.21	0.10
April[c]	20.2 ± 0.56	20.9 ± 0.56	0.68	0.51
May[a]	15.4 ± 0.52	15.6 ± 0.52	0.86	0.08
June[b]	18.6 ± 0.26	19.2 ± 0.27	0.26	0.23
Acid detergent fiber, % of DM				
March[a]	16.0 ± 0.51	15.2 ± 0.52	0.53	0.93
April[b]	29.3 ± 0.64	28.8 ± 0.65	0.89	0.73
May[d]	33.9 ± 0.65	34.4 ± 0.66	0.83	0.10
June[c]	32.6 ± 0.35	32.1 ± 0.36	0.87	0.16
Neutral detergent fiber, % of DM				
March[a]	35.2 ± 0.60	33.9 ± 0.61	0.28	0.33
April[b]	52.9 ± 0.92	51.5 ± 0.94	0.92	0.34
May[d]	60.0 ± 0.80	60.1 ± 0.81	0.86	0.06
June[c]	57.3 ± 0.58	56.0 ± 0.58	0.79	0.02
Total digestible nutrients, % of DM				
March[d]	84.5 ± 0.57	85.4 ± 0.58	0.53	0.93
April[c]	69.7 ± 0.72	70.3 ± 0.73	0.89	0.73
May[a]	64.5 ± 0.73	64.0 ± 0.73	0.83	0.10

(Table 5 (Cont.)…) Forage nutritive quality for tall fescue pastures with varying ergovaline concentrations (EV) grazed by growing steers fed mineral only (MIN) or cumulative growth promoting management (CM) including supplementation, ionophore, and hormonal implant.

			P-value[1]	
Item	MIN	CM	MGMT[2]	EV
May[a]	64.5 ± 0.73	64.0 ± 0.73	0.83	0.10
June[b]	65.9 ± 0.39	66.5 ± 0.40	0.87	0.16

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[1] There were no significant 2- or 3-way interactions ($P \geq 0.07$) so all were removed from the final model. There was a month effect ($P < 0.01$) for all forage nutritive quality components.

[2] MGMT – effect of cumulative growth promotion management CM vs MIN.

[abcd] Months with least-squares means for forage nutritive quality components within grazing season with differing superscripts, differ ($P \leq 0.05$).

Table 6. Cu, blood urea N, and prolactin analyses of steers fed mineral only (MIN) or cumulative growth promoting management (CM) including supplementation, ionophore, and hormonal implant while grazing tall fescue pastures with varying ergovaline concentrations (EV).

	MGMT[1]			Season				P-value		
	MIN	CM	SEM[2]	Initial	Interim	Final	SEM[3]	MGMT	EV	Period
Serum Cu, mg/L										
Fall	0.10	0.10	0.003	-	0.09	0.10	0.003	0.49	0.98	0.12
Spring	0.09	0.10	0.003	0.12^{c}	0.10^{b}	0.08^{a}	0.003	0.11	0.09	< 0.01
Blood urea nitrogen, mg/dL										
Fall	12.5	9.5	0.42	-	11.6	10.4	0.41	< 0.01	0.65	0.07
Spring	11.0	8.7	0.42	8.6^{a}	9.6^{b}	11.3^{c}	0.52	< 0.01	0.23	0.01
Non-esterified fatty acid, ug/dL										
Fall	432	343	21.1	292^{a}	377^{b}	493^{c}	25.5	0.01	0.57	< 0.01
Spring	518	477	27.9	520^{b}	561^{b}	411^{a}	34.1	0.32	0.23	0.01
Prolactin, ng/mL										
Fall	10.0	16.5	3.29	20.6^{b}	2.3^{a}	16.8^{b}	3.98	0.19	0.29	< 0.01
Spring	50.6	48.3	5.87	83.8^{b}	39.4^{a}	25.2^{a}	7.18	0.79	0.03	0.01

[1] MGMT = effect of cumulative growth promotion management CM vs. MIN.

[2] Standard error of the mean for MGMT.

[3] Standard error of the mean for Period.

[a–c] Least squares means within row for season effect differ ($P \leq 0.05$).

Table 7. Rectal and tail skin temperatures of steers fed mineral only (MIN) or cumulative growth promoting management (CM) including supplementation, ionophore, and hormonal implant while grazing tall fescue pastures with varying ergovaline (EV) concentrations.

	MIN			CM				*P*-value			
	Initial	Interim	Final	Initial	Interim	Final	SEM[2]	MGMT	EV	Period	MGMT × Period
Rectal temp. °C											
Fall	39.2^{c}	38.4^{a}	39.2^{c}	39.2^{c}	38.9^{b}	39.4^{c}	0.09	<0.01	0.16	<0.01	0.04
Spring	39.3^{a}	38.6^{b}	39.7^{c}	39.2^{a}	38.6^{b}	40.0^{c}	0.13	0.46	0.47	<0.01	0.27
Tail skin temp. °C											
Fall	26.6^{d}	20.8^{a}	23.4^{b}	26.3^{cd}	21.0^{a}	25.4^{c}	0.39	0.08	0.56	<0.01	0.01
Spring	24.6^{a}	27.7^{b}	31.5^{c}	25.1^{d}	29.3^{e}	33.4^{c}	0.51	<0.01	0.48	<0.01	0.37

[1] MGMT = effect of cumulative growth promotion management CM vs. MIN.
[2] Standard error of the mean for MGMT × Period. $^{a\text{–}e}$Least squares means within row differ ($P \leq 0.05$).

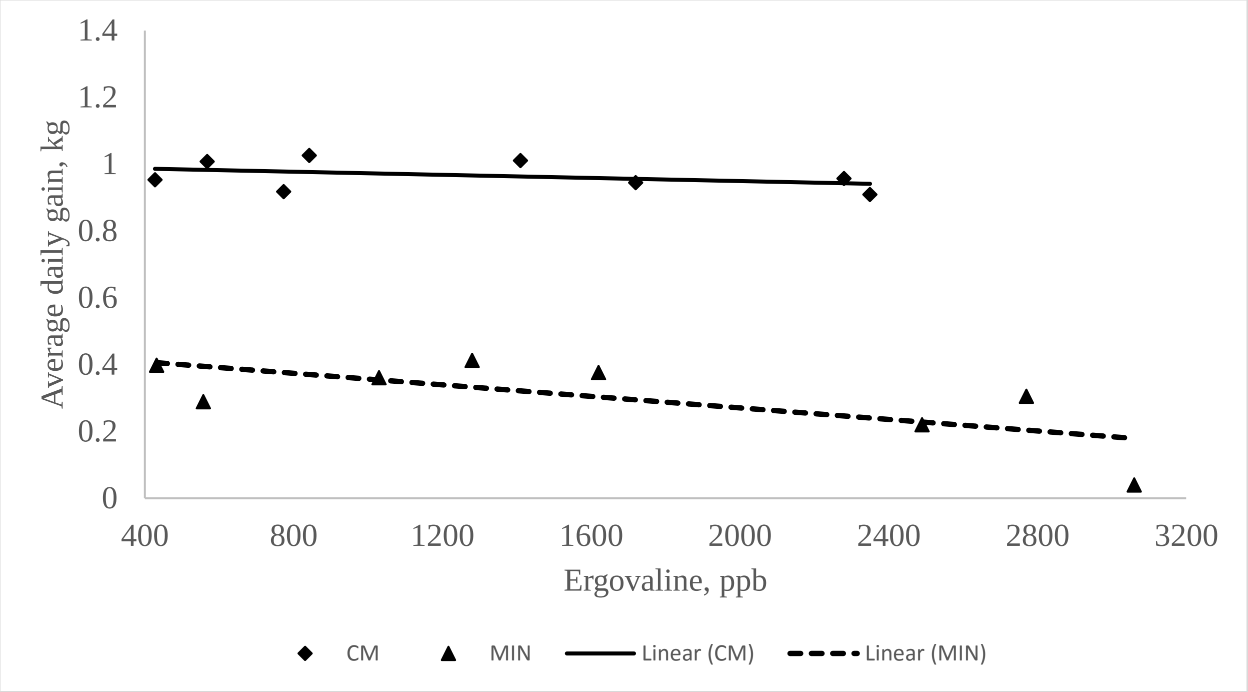

Figure 1. Effect of growth promoting management (CM = cumulative growth promoting management including hormonal implant, ionophore, and supplementation versus mineral (MIN) (free choice access to a non-medicated mineral only) and ergovaline (EV) concentration on performance of steers grazing tall fescue in the fall.

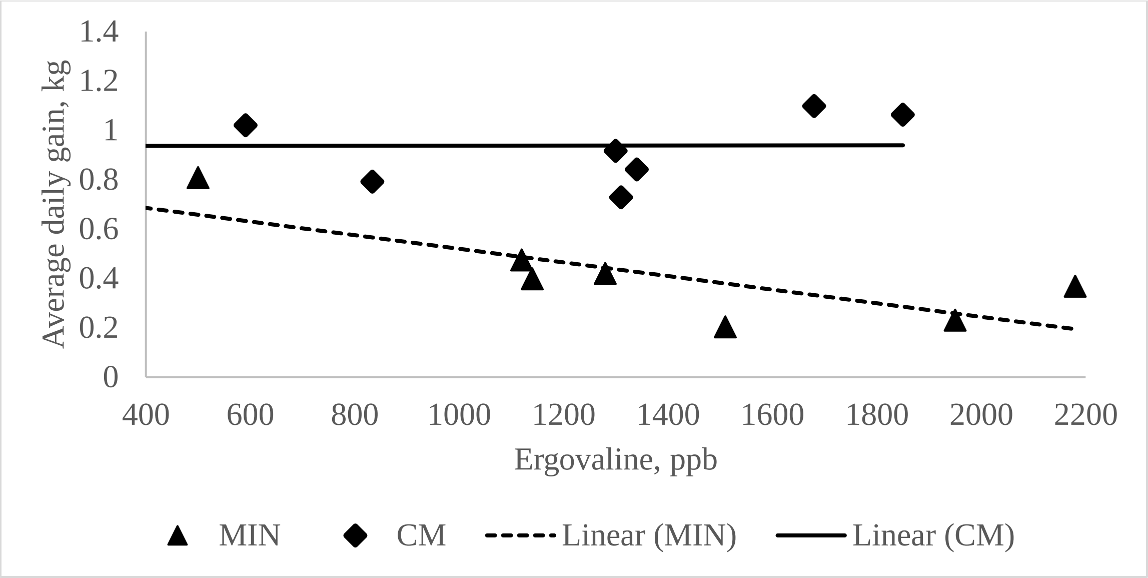

Figure 2. Effect of management (MGMT) as cumulative management (CM, including hormonal implant, ionophore, and supplementation) or mineral (MIN) (free choice access to a non-medicated mineral only) and ergovaline (EV) concentration on ADG during the spring.

CHAPTER IV

MANAGEMENT PROGRAM FOR STEERS GRAZING TOXIC FESCUE ALTERS ACTIVITY

Abstract

Grazing toxic, endophyte-infected tall fescue influences grazing activity. The objective was to assess activity of steers grazing tall fescue pastures varying in toxicity and managed with and without a combination of management practices including supplemental feed, ionophore, and steroidal implant. Activity of steers was monitored using IceQube (IceRobotics, Ltd., Edinburg, UK) accelerometers. Steers (n = 45, 116.9 ± 4.88 kg initial BW) grazed 1 of 15 pastures differing in ergovaline concentration (**EV**) in spring, 2016. For 7 pastures, steers were offered mineral (**MIN**) only management (**MGMT**). In the remaining pastures, steers received a cumulative (**CM**) MGMT strategy including 1% BW of a 1:1 corn gluten feed:soybean hulls mixture, 150-mg/d monensin and a steroidal implant containing 40-mg trenbolone acetate, 8-mg estradiol, and 29-mg Tylosin tartrate. Physical activities of lying bouts, steps and standing time were reported in two periods of 27 and 26 d. In period 1, lying bouts were not different for MGMT ($P = 0.11$) or EV ($P = 0.26$). Period 2 lying bouts exhibited a MGMT × EV interaction ($P = 0.02$). Time steers spent standing was not different between CM and MIN during period 1 ($P = 0.79$) but were lower for CM during period 2 ($P < 0.01$). Behavioral changes due to EV and MGMT appeared more prevelant during period 2 when warmer weather and changing forage quality would be expected to worsen the effects of fescue toxins. The CM may elicit greater satiety or reduced heat stress. Strategies that improve productivity of cattle on toxic fescue may also improve welfare as expressed through physical activity changes.

Key words: Activity, Fescue, Management, Steers

Introduction

Cattle consuming tall fescue [*Lolium arundinaceum* (Schreb.) Darbysh] forage containing mycotoxins produced by the *Epichloë coenophiala* [(Morgan-Jones and Gams) C.W. Bacon and Schardl] fungus (Aiken and Strickland, 2013) exhibit reduced dry matter intake, average daily gain, and increased body temperature. Parish et al. (2003) observed cattle grazing non-toxic fescue, either endophyte-free or nonergot-alkaloid producing endophyte-infected, spent less time standing during spring months compared to those grazing toxic fescue. Howard et al. (1992) also noted greater standing activity by cattle grazing toxic fescue. Standing is a behavior to cope with heat stress in cattle (Allen et al., 2013).

Gadberry et al. (2015) showed through meta-analysis that cattle consuming toxic fescue respond to growth promoting implants, medicated feed additives, and supplemental feed. There may be additive benefits to stacking management strategies (Roberts and Andre, 2010). Diaz et al. (2018) reported that a cumulative management strategy increased performance and in some instances sustained performance as fescue toxins increased. Although efforts to alleviate fescue toxicosis often document growth rate and physiological responses, most have not reported changes in behavior. Today, assessment of behavior is important in understanding impacts of various treatments on animal welfare.

Data logging accelerometers are used to remotely monitor behavior and modifications in behavior associated with disease, oestrus, and management (Richeson et al., 2018). The IceQube (IceRobotics, Ltd., Edinburg, UK) is a triaxial accelerometer that functions mainly as a pedometer attached to the leg and has been validated in both cows and calves (McGowan et al., 2007; Trénel et al., 2009). This device is capable of logging motion data including standing and lying time, lying bouts, and steps. This allows capturing data during times that might not otherwise be observed or collecting more data than practical when human observation is limited.

Scalgia et al. (2009) used this technology to study the effect of supplement feed and feed timing on behavior. They observed similar lying time and steps among calves grazing ryegrass and either not supplemented or supplemented at 0.5% BW either morning, noon, or evening.

Our hypothesis is management that improves the metabolic status of steers grazing toxic fescue would result in a change in physical activity. Our objective was to determine activity differences for steers grazing toxic fescue and provided a combination growth promotion management strategy consisting of supplemental feed, ionophore, and steroidal implant to steers grazing toxic fescue without intervention.

Materials and Methods

The study was conducted at the University of Arkansas, Division of Agriculture Livestock and Forestry Research Station (Batesville, AR; 35°50’ N, 91°48’ W), and all procedures involving animal care and management were conducted within the guidelines of the University of Arkansas Institutional Animal Care and Use Committee (protocol 16023). Diaz et al. (2018) described the experimental design in great detail. In brief, the study compared the effects of a cumulative (**CM**) management (**MGMT**) strategy to a supplemental mineral (**MIN**) only MGMT program for steers grazing fescue pastures with varying levels of toxicity. Pasture toxicity was based on ergovaline (**EV**) concentration on d 42 (study mid-point). The minimum and maximum EV concentrations were <100 and 2,180 ppb, respectively, and did not differ between CM and MIN pastures ($P = 0.76$). The CM strategy included 1% BW, as-fed, supplemental feed (1:1 corn gluten feed:soybean hulls), 150 mg monensin/d (Rumensin, Elanco

Animal Health), 115 g/d of the MIN premix (Vigortone 3V6 SR; Provimi North America Inc.), and a growth promoting implant, on d0, containing 40 mg trenbolone acetate, 8 mg estradiol, and 29 mg Tylosin tartrate (Component TE-G, Elanco Animal Health, Greenfield, IN). The MIN steers had access, through a self-feeder, to a non-medicated version of the mineral formulation offered to CM steers. The CM steers were offered their supplement for a Monday through Friday delivery (7-d feed quantity prorated over a 5-d period) in a feed trough adjacent to the perimeter fence and near the pasture gate entrance based on 1% of BW/d. Steers had access to water from a trough and little to no shade was available in pastures.

Steer activity was monitored during the spring study. The study began March 21 and continued for 84 d; however, activity monitoring did not begin until d 28. Activity was recorded using an IceQube (IceRobotics, Ltd., Edinburg, UK) affixed to the left metatarsus. The IceQube continuously records standing and lying activity, step count, and a motion index for up to 60 d. Within each pasture, 3 of 5 steers were randomly selected to wear an IceQube device. There were 15 pasture replicates; 8 CM pastures and 7 MIN pastures. An 8th MIN pasture was excluded from activity monitoring due to not being able to restrict steers from a small pond adjacent to the water trough. By d 84, 7 of 8 CM pastures had complete data for 3 of 3 steers; 1 pasture had complete data for 2 of 3 steers. Four of 7 MIN pastures had complete data for 3 of 3 steers; 3 MIN pastures had complete data for 2 of 3 steers. Data losses were attributed to unit failure, unit movement on the leg, or complete unit loss. The IceQube strap design was large for the size of steers used in this study (116.9 ± 4.88 kg initial BW).

Activity was partitioned into 2 periods based on the idea that forage quality would be greater, temperature milder, and effects of fescue toxins less severe for early compared to late in the season. The accelerometers were affixed on April 20 (d 29). Period 1 activity began April

21 and ended May 17 (d 30). Period 2 began May 18 (d 57) and continued through June 21 (d 82). June 12 was the last day for complete 24 h records because the accelerometers were activated before shipment from West Texas A&M University (Amarillo, TX). Period 1 and 2 consisted of 27 d and 26 d, respectively.

Pasture was the experimental unit. Responses were aggregated within pasture prior to statistical analysis. Responses analyzed included number of daily lying bouts, daily standing time (minutes), and number of daily steps. Responses were modeled by period. Study mid-point ergovaline (EV) was modeled as a continuous covariate. The full model included MGMT, EV and MGMT×EV to test for different slopes for each MGMT treatment. Removal of non-significant interactions were based on an acceptance criteria of $P \leq 0.10$. Linear models were fit with the lm function within the R (www.r-project.org) stats package. Analysis of variance type III sum of squares and F-tests were computed using the car package. Management effects on activity were more prevalent during period 2 than period 1 and to a greater extent than the effect of EV. Therefore, period 2 standing data was subset and averaged within treatment, pasture, and hour to study the repeated measures response of standing as affected by MGMT, hour, and MGMT × hour. The model included a first-order autoregressive correlation structure for the repeated measure of hour. The experimental subject was pasture. The model was fit using the lme function of the nlme package for R.

Results and Discussion

Daily lying bouts

Lying bouts are the number of unique lying events that occur daily. During period 1, the lying bouts averaged 21.2 ± 0.3, daily and were not affected by MGMT ($P = 0.11$) or EV ($P = 0.26$) nor the MGMT × EV interaction ($P > 0.05$) (Figure 1a). During period 2, lying bouts were influenced by MGMT × EV (*P* = 0.02; Figure 1b). The base lying bouts for CM steers was 18.4 and decreased by 0.9 bouts for every 1000 ppb increase in EV. The base lying bouts for MIN steers was 11.1 and increased by 3.5 for every 1000 ppb increase in EV. Interestingly, the lying frequency was greater with CM across most of the EV concentrations, but the effects of EV appeared greater with MIN. Lying bouts appeared more similar at high levels of pasture toxicity during period 2.

Daily steps

The number of daily steps per steer was also determined within each period. During period 1, there was a tendency for MGMT × EV (*P* = 0.08) indicating the effect of pasture toxicity differentially affected the number of daily steps taken for MIN and CM (Figure 2a). Step counts decreased rapidly in MIN pastures as pasture toxicity increased in period 1. During period 2, steps taken were influenced by MGMT (*P* = 0.01) and tended to be affected by level of EV (*P* = 0.09) but not MGMT × EV (*P* = 0.31) (Figure 2b). During period 2, CM steers exhibited 20% more steps daily than MIN steers, and for every 1000 ppb increase in EV, steps decreased by 275.

Daily standing time

The total minutes per day spent standing was determined for each steer each period. During period 1, steers stood for 792.6 ± 9.37 minutes throughout the day. Amount of time spent standing during period 1 was not affected by MGMT (*P* = 0.68), EV (*P* = 0.55), or MGMT × EV (*P* = 0.79). During period 2, MGMT (*P* < 0.01) but not EV (*P* = 0.87) affected standing time and the interaction MGMT × EV was not significant. Steers fed MIN spent 858.01 ± 10.4 min/d standing while CM steers spent 792.01 ± 9.73 min/d standing. Therefore, MIN spent 60% of their day standing, while CM spent 55% of their day standing. Period 2 standing time for CM resembled period 1 standing time. Similar to our CM response, steers on toxic fescue had a greater percentage standing time compared to steers grazing non-toxic fescue in spring (Parish et al., 2003). The greater standing time with MIN may be associated with coping with heat stress.

Hourly standing time (Period 2)

Recognizing MIN steers spent 8% more time standing during period 2, we further examined the hourly difference in standing behavior to determine if this additional standing time was clustered around particular hours of the day. A MGMT × hour (*P* = 0.005) interaction occurred during period 2 (Figure 3). Hourly MGMT comparisons indicated MIN exhibited greater standing time at 000 (*P* = 0.07), 0400 (*P* = 0.06), 0500 (*P* = <0.01), and 2100 h (*P* = 0.06). It appears MIN steers were standing more frequently near the start and end of civil twilight but not throughout the entire day length nor entire nighttime.

While Scaglia et al. (2009) did not see a significant change in activity associated with calves grazing ryegrass and supplemented at 0.5% BW, our CM strategy that included 1% BW supplementation for steers grazing toxic fescue did alter activity. Overall, the CM strategy resulted in activity differences that included greater step counts, more time at rest during the latter part of the study, and more lying bouts during period 2. The lying activity may demonstrate greater satiety under CM management. Tail temperature but not rectal temperature differences were noted (Diaz et al., 2018) which may suggest thermoregulatory influence as well. Allen et al. (2013) reported heat stress results in greater standing time. We believe the greater step counts was partly attributed to either the addition of monensin to the feed, feed palatability, or a combination thereof which needs objective assessment. Steers would come to the bunk at morning feeding but not completely eat the entire feed portion immediately. We observed steers would leave feed in the trough and return to eat later. Troughs were typically clean by the next morning. Monensin can affect intake, eating and ruminating behavior (Baile et al., 1979; Deswysen et al., 1987).

The CM calves were supplemented 5 d of the week. While our primary objective and hypothesis was focused on CM compared to MIN; we subset the CM data from the dataset and aggregated responses to the level of pasture and a feed-day category [fed (Monday through Friday average) or not fed (Saturday and Sunday average)]. There were 8 weekends (16 of 53 d) CM calves did not have feed placed in the trough. Our goal was not to present this as a valid experimental design to study the effect of skipped meals on behavior but to determine if and by how much not feeding on the weekend influenced our CM responses. Overall, there was no difference in standing time ($P = 0.21$) or steps ($P = 0.27$) between the days CM calves received supplement or did not receive supplement. There was a tendency ($P = 0.07$) for more lying bouts on days fed; however, the mean and standard error of the difference was 0.67 ± 0.345. These comparisons suggest not feeding on the weekend minimally influenced our assessment of the effect of CM on behavior. Morais et al. (2014) studied the effect of supplementation frequency (daily, Monday through Friday, or Monday-Wednesday-Friday) on performance and foraging behavior on a warm-season grass in a tropical environment. Overall, supplementation frequency did not affect average daily gain, mean forage intake or mean supplement intake. Morais et al. (2014) also reported Monday through Friday grazing time increased 0.96 h on the day not supplemented, but the additional grazing time did not differ from the grazing time of daily supplemented calves. The grazing time of daily supplemented calves increased 0.48 h on the day the alternative supplementation frequency treatments were not supplemented. It is possible that fence-line contact among treatments may have influenced the grazing time of supplemented calves on the day supplementation was not provided to the remaining treatments.

It is plausible to think the non-significant difference in standing and steps between supplemented days and non-supplemented days with CM treatment in our study may be due to foraging activity replacing activity visiting the feed trough. Morias et al. (2014) observed more grazing activity early during observation hours on the day supplement was not offered. Further research is needed to establish how daily versus alternative supplementation frequencies alters grazing behavior, in addition to motion activities such as steps, standing, and lying bouts, with cattle grazing toxic fescue.

Implications

The objective of this study was to assess the physical activity changes associated with a CM strategy to improve metabolic status of steers grazing toxic fescue. The CM strategy increased lying bouts and reduced standing time during the second half of the study when fescue nutritive value would be lesser and environmental temperatures greater, making fescue toxins more detrimental to production. The physical activity change is probably the result of greater satiety and/or reduced heat stress. Management that improves performance may concomitantly benefit the overall welfare of steers grazing toxic, endophyte-infected fescue.

ACKNOWLEDGEMENTS

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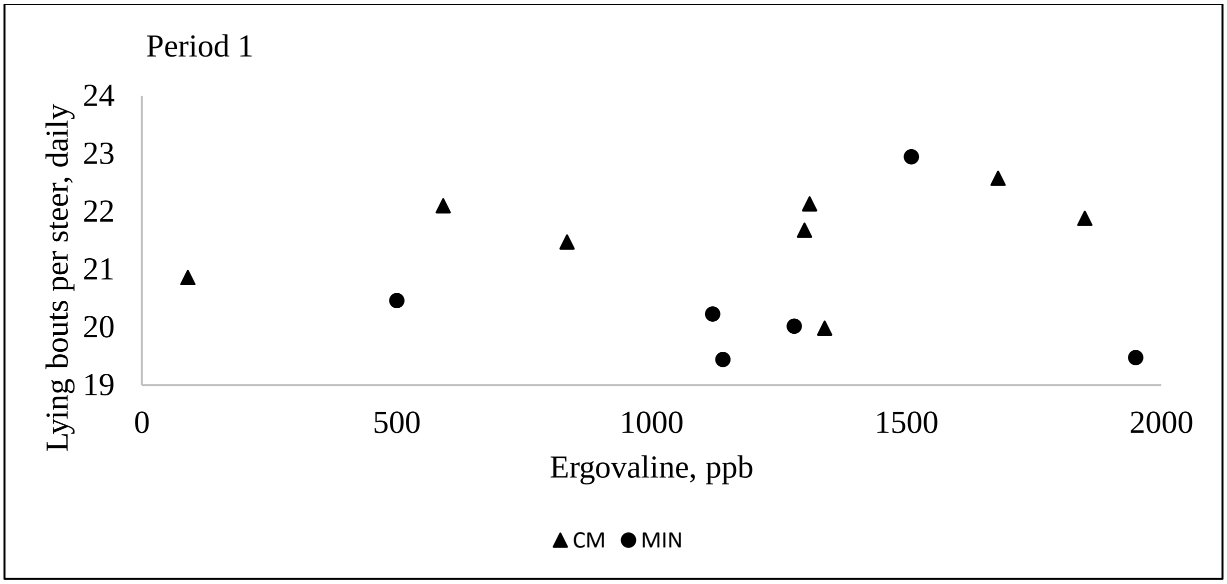

Figure 1a. Effect of management (MGMT) as either mineral only (MIN) or cumulative management (CM) including a growth promoting implant, ionophore, and 1% body weight supplementation with a 1:1 blend of corn gluten feed and soybean hulls on lying bouts with pastures of varying levels of ergovaline (EV). Period 1 lying bouts did not differ for MGMT (*P* = 0.11) or pasture (*P* = 0.26).

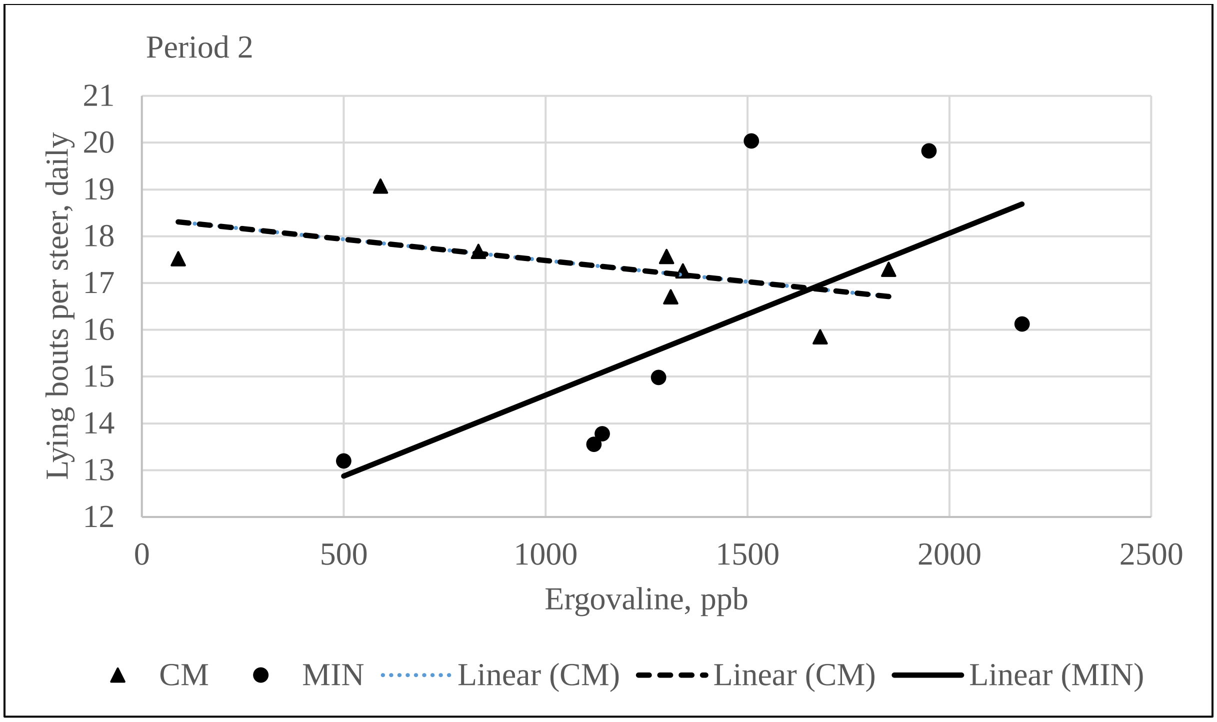

Figure 1b. Effect of management (MGMT) as either mineral only (MIN) or cumulative management (CM) including a growth promoting implant, ionophore, and 1% body weight supplementation with a 1:1 blend of corn gluten feed and soybean hulls on lying bouts with pastures of varying levels of ergovaline (EV). In period 2, there were an interaction between MGMT × EV ($P = < 0.05$).

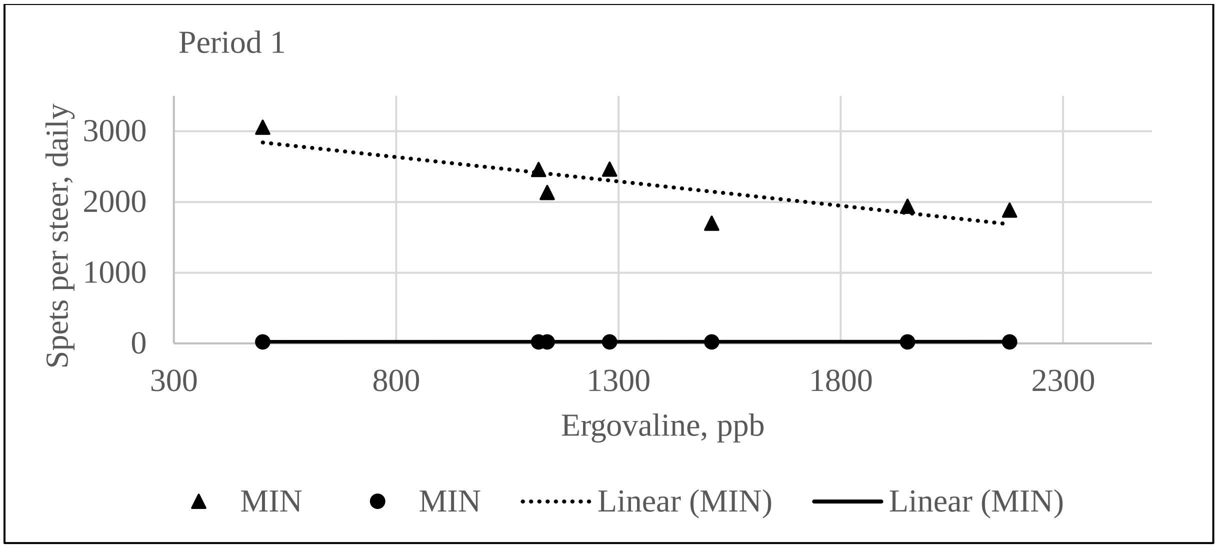

Figure 2a. Effect of management (MGMT) as either mineral only (MIN) or cumulative management (CM) including a growth promoting implant, ionophore, and 1% body weight supplementation with a 1:1 blend of corn gluten feed and soybean hulls on step activity with pastures of varying levels of ergovaline (EV). In period 1 there were significant interaction MGMT × EV ($P < 0.05$).

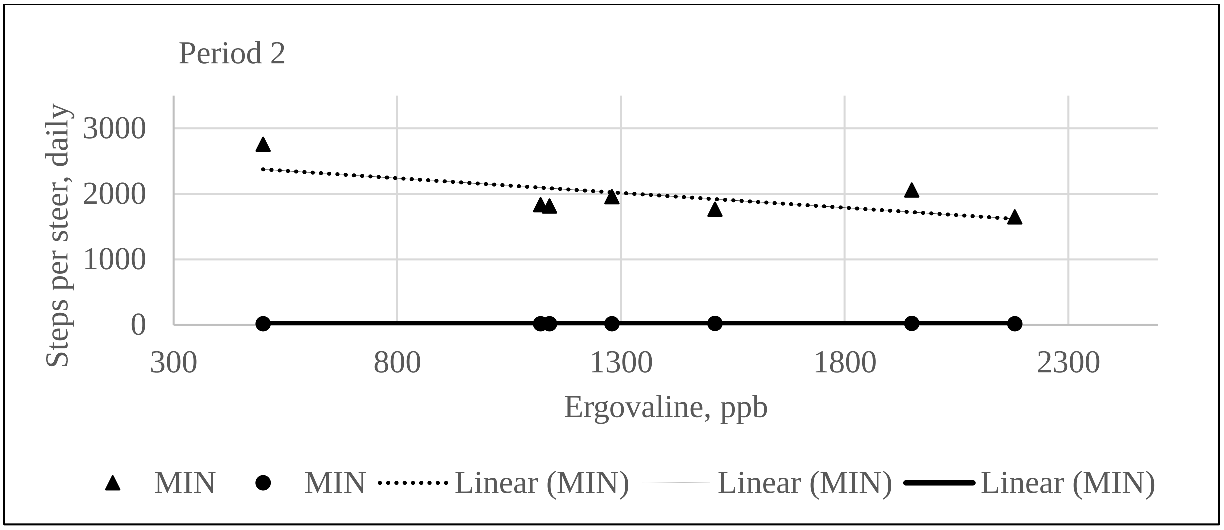

Figure 2b. Effect of management (MGMT) as either mineral only (MIN) or cumulative management (CM) including a growth promoting implant, ionophore, and 1% body weight supplementation with a 1:1 blend of corn gluten feed and soybean hulls on step activity with pastures of varying levels of ergovaline (EV). Period 2, MGMT × EV had no significant differences ($P > 0.05$) but EV response had a tendency ($P = 0.007$).

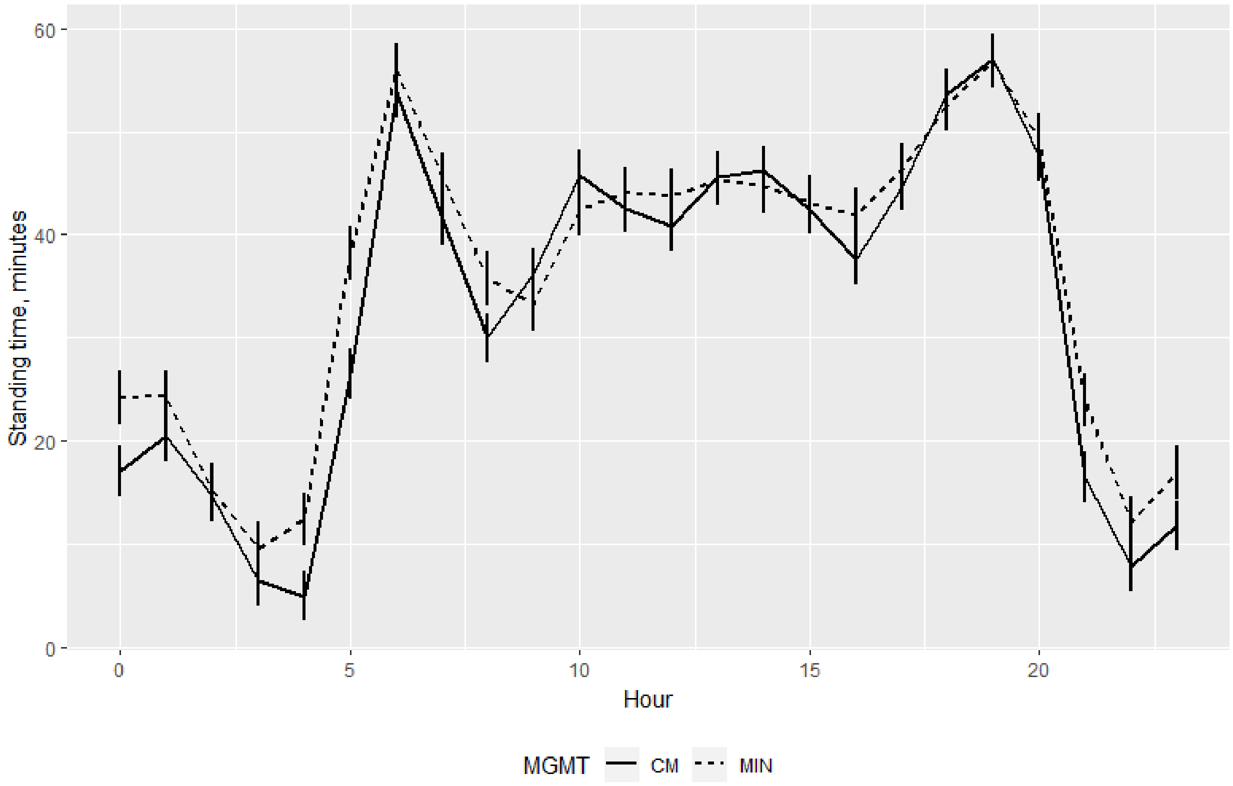

Figure 3. Effect of management (MGMT) as either mineral only (MIN) or cumulative management (CM) including a growth promoting implant, ionophore, and 1% body weight supplementation with a 1:1 blend of corn gluten feed and soybean hulls on hourly (0 hour = 12am) standing time during period 2.

CHAPTER V

EFFECT OF ALFALFA AND TALL FESCUE SWARD HEIGHT ON GRAZING BEHAVIOR AND DIET PREFERENCES BY STEERS

Abstract

Sward heights influence ingestive behavior in cattle. The objective of this experiment was to evaluate the effects of two different sward heights, long sward regrowth (LSR) and short sward regrowth (SSR) with target heights of 12 and 10 cm respectively, on diet composition, rumen fermentation, and grazing behavior of 3 heifers and 1 steer (402 ± 30.1 kg BW) grazing novel endophyte fescue and (493 ± 26.57 kg BW) grazing alfalfa. For both experiments, forage samples were taken to evaluate forage nutritive value prior to stocking animals. Visual observations, grazing time per minute, and bites per min were recorded for two days every hour from 0700 to 1900 h in two periods (d 1 and 2 of each period). Rumen fluid was collected every 2 hrs on d 3 to measure pH, ammonia and volatile fatty acids. A rumen evacuation followed by a 20-min grazing time was conducted on d 4. Diet selectivity from grazing was measured by analyzing neutral detergen fiber (aNDF), acid detergent fiber (ADF), and N content. For tall fescue, forage nutritive value and diet selection did not differ between treatments ($P \geq 0.07$) for NDF (55.0 ± 1.43), ADF (26.8 ± 0.53), CP (22.6 ± 6.65) and OM (91.9 ± 0.001). No differences ($P \geq 0.19$) in grazing behavior between treatments were observed. However, ruminal ammonia, total VFA, concentrations of acetic, isobutyric, butyric, isovaleric and valeric acids were greater ($P < 0.05$) from SSR vs LSR. Alfalfa forage nutritive value and diet selectivity were not different ($P \geq 0.24$) between LSR and SSR. No differences were observed ($P \geq 0.65$) with regard to grazing behavior; time spent grazing averaged 6.33 min/10 min and bite rates averaged 13 bites/min. No treatment differences ($P \geq 0.11$) were observed for ammonia and for total VFA between LSR and SSR but ruminal pH was greater from LSR than SSR (*P* = 0.03). Variability of grazing behavior is high among animals and further research is needed to detect differences based on forage sward heights and to increase sample size in order to get a power test.

Key words: Behavioral activities, diet composition, rumen fermentation, sward height

Introduction

Sward height influences diet choice and voluntary intake (Galyean and Gunter, 2016) in grazing animals through their influence on activities such as bite weight and bite depth dimensions (Ungar et al., 1991; Ungar et al., 2002), movements or motion indices, and steps per minute (Gregorini et al., 2011). The behaviors are called ingestive behaviors and measure the rate of bites, chewing, and motion, the animal uses to get the highest energy intake per unit of time (Demment and Greenwood, 1988). These foraging dynamics vary by swards height (Gregorini et al., 2009) and by previous experiences of the animals (Villalba et al., 2015). Diet selection by cattle is, thus, influenced by forage structure and foraging strategies in forages such as tall fescue and alfalfa. Sward height and regrowth length influence forage quality measurements such as N content and fiber content resulting in differences in diet selectivity in ruminants because the way the amount of forage acquired daily. Lower plant N should result in lower rumen ammonia concentrations, bacterial growth and fermentation (Erdman et al., 1986). If ruminal ammonia is low, digestion may be suppressed, which in turn reduces forage intake (Krysl and Hess, 1993; Scaglia et al., 2009; Swanson et al., 2017). Legumes such as alfalfa have higher crude protein but they may cause digestive problems if intake greatly exceeds animal needs (Netto et al., 2014). Tall fescue and alfalfa consumed at two canopy heights, 12 cm and 25 cm, influenced the number of bites in cattle (Galli et al., 2017). In other research, sward height influenced bite dimensions and grazing movements in sheep and cattle when grazing homogeneous ryegrass (Rook et al., 2004).

Materials and Methods

Both studies were conducted at the University of Arkansas, Agricultural Research and Extension Center, Fayetteville, (36º18'N, 94º16'W) and all procedures involving animal care and management were conducted within the guidelines of the University of Arkansas Institutional Animal Care and Use Committee, approval number 18005. The tall fescue study was conducted in fall of 2017, and alfalfa study was conducted in spring 2018.

Pasture management

Existing stands of fescue and alfalfa used for the experiments were planted in 2014 and 2011 respectively. To conduct each experiment, respective fields were split into eight paddocks measuring 30.48 m wide and 45.72 m length representing 2 sward treatments replicated with 2 animals per period for 2 periods. To achieve similar grazing times with different canopy heights, paddocks were mowed at 12 cm (LSR) and 10 cm (SSR) 7 days apart for long sward regrowth (LSR) and 14 days apart for short sward regrowth (SSR) representing 28 days and 21 days of regrowth, respectively.

Forage sampling

Prior to stocking animals, initial sward heights were measured with a ruler; fescue measured 33 cm for LSR and 28 cm for SSR, and alfalfa measured 55 cm and 45 cm for LSR and SSR, respectively. Forage grab samples were obtained immediately prior to the start of grazing 1 d before the behavior and fermentation measurements began. Samples (n = 5) were hand-clipped using a 0.5 m × 0.5 m square and in harvested randomly throughout the field to provide a representative sample, placed in paper bags and transported to an oven and dried to a constant weight at 50° C prior to subsequent analysis. Content of OM was obtained by ashing in a muffle furnace at 500 °C for 6 h (procedure no. 942.05; AOAC, 2000).

Animal handling

Four ruminally-cannulated calves, 1 steer and 3 heifers, with an initial body weight (BW) of 402 ± 30.1 kg for the fescue study and 494 ± 26.6 kg initial BW for alfalfa study were randomly assigned and stocked on experimental paddocks on October 6, 2017 to initiate the tall fescue study and May 4, 2018 to initiate the alfalfa study. Prior to the 4-d experimental period, animals were kept in an adjacent pasture of a similar forage type for a 10-d adaptation period. During the alfalfa study in period 1 on the rumen fermentation collection day, one calf was removed because of bloat issues.

Visual observations

After a 10-d adaptation period, calves were individually allocated randomly to 4 of the 8 individual paddocks the evening prior to 4 d of data collection. Visual observations were recorded for two consecutive days from 0700 h through 1900 h by two experienced observers. At the beginning of each hour, grazing activity was recorded for 10 minutes as either grazing (GR), ruminating (RM), lying (LY) or idling (ID). If animals were grazing, the total number of bites was recorded, then divided by the total grazing time during the 10-minute period to determine bite rate. At the end of the first period, calves were co-mingled on a common fescue or alfalfa adjacent pasture for 10 days. Calves were then reallocated to the remaining 4 paddocks for a second period but to a different sward height than they grazed in period 1, and procedures were repeated.

Rumen fluid collection and chemical analysis

For both studies, rumen fluid was collected every 2 h from 0700 to 1900 h on d 3 of each period and analyzed for ammonia, VFA, and pH. Rumen samples were taken from four different areas in the rumen, placed in a rubber bucket, mixed thoroughly, squeezed through 4 layers of cheesecloth, and drained gently into a cup. The pH was measured immediately using a portable pH meter (Toledo EL2 model, Greifensee , Switzerland). Samples were then placed on ice in an insulated container for transportation to the lab for later analyses. Calves were moved to a corral to collect the rumen fluid (10 meters distance from the padock), then were placed back onto their respective paddocks to continue grazing immediately following collection of ruminal fluid.

Rumen fluid and a metaphosphoric acid solution (125 mL/L) containing 2-ethylbutyric acid as an internal standard were combined (5:1 ratio) for subsequent VFA analysis. A second aliquot of rumen fluid was combined (2:1 ratio) with 0.1 *N* HCl for subsequent ammonia N analysis. Ammonia N concentrations were colorimetrically determined (Broderick and Kang, 1980). Volatile fatty acids were analyzed by gas−liquid chromatography using the method and equipment described by Akins et al. (2009).

Diet selectivity and chemical analysis

Following the last rumen sampling at 1900 h, calves were individually placed into cattle working pens to facilitate rumen evacuation the following morning (d 4). Immediately after emptying the rumen, calves were placed onto their respective paddocks to graze for 20 to 30 minutes and then transported to a working facility to remove a representative sample of the consumed forage. This forage masticate was then placed into zip-lock bags and transported to the laboratory. Samples were lyophilized for subsequent laboratory analyses. All values were corrected to a DM basis based on drying the samples overnight at 100° C. All samples were ashed in a furnace at 500 ºC to determine OM. Samples were also used to determine aNDF, ADF and N content. After forage samples were ground 1mm, a representative sample was weighed and placed in an Ankom 200 fiber analyzer for fiber analysis following Van Soest method (Van Soest, 1967. For N analysis, a representative sample (~0.15 g) was placed in a Leco FP-528 N analyzer.

Statistical analysis

Data was analyzed using the PROC MIXED procedure of SAS version 9.3 (SAS Institute Inc., Cary, NC). Forage samples, rumen fluid and diet selectivity data were analyzed as a cross-over design. Visual observations were analyzed using repeated measures using calf within treatment as a sub-variable. Each experimental paddock was considered as the experimental unit and calf considered as observational unit.

Results

There were no differences in forage CP, aNDF or ADF for either fescue or alfalfa for LSR or SSR before animals were stocked on the experimental units ($P \geq 0.05$; Table 1). Alfalfa OM ($P = 0.04$) was greater for LSR vs. SSR prior to grazing, and that of fescue tended ($P = 0.07$) to be greater from LSR vs. SSR, but this difference was small (≤ 0.6 percentage units). Foraging behavior data from visual observations are summarized in Table 2. Grazing time spent (per 10 min) by calves was not different between sward height treatments ($P \geq 0.35$) in either fescue or alfalfa. Bite rate (bites/min) in calves grazing tall fescue did not differ ($P = 0.38$) between treatments, showing the generally large variability in behavior between animals. Bite rates in alfalfa (14 bites/min) were numerically different from those in tall fescue (32 bites/min) although they were not compared statistically because the measurements were gathered in different seasons. Similar to tall fescue, bite rates did not differ between those grazing LSR and SSR ($P = 0.67$).

In fescue paddocks, the total time spent ruminating per 10 minutes was not different between LSR and SSR ($P = 0.19$). Lying periods were likewise not different between LSR and SSR ($P = 0.78$) and calves idling under LSR and SSR treatments did not differ ($P = 0.45$) between treatments. In alfalfa paddocks, no differences were observed in ruminating, lying and idling grazing activities ($P \geq 0.75$).

Rumen fermentation

Ruminal ammonia concentrations from calves grazing fescue were greater ($P < 0.05$) from calves grazing SSR compared with those grazing LSR (Table 3). Total VFA were also greater ($P < 0.05$) from calves grazing SSR vs. LSR. Concentrations of acetic, propionic, isobutyric, butyric, isovaleric and valeric acids from calves grazing fescue were greater from SSR than LSR ($P \leq 0.05$). Conversely, concentrations of total VFA, ammonia, acetic, propionic, isobutyric, butyric, isovaleric and valeric acids from calves grazing alfalfa were not different between SSR and LSR ($P \geq 0.11$). Ruminal pH tended to be greater from LSR than SSR ($P = 0.06$) when calves grazed tall fescue, but was greater ($P = 0.03$) from SSR when calves grazed alfalfa.

Diet selectivity

Masticate samples collected on tall fescue paddocks following rumen evacuation resulted in no differences ($P = 0.11$) in aNDF and averaged 52.8 % across LSR and SSR, respectively. There were no differences ($P = 0.83$) in ADF averaging 24.5 % across LSR and SSR respectively. Crude protein was not different in LSR from SSR ($P = 0.52$), averaging 25.9 %

between LSR and SSR respectively. Organic matter did not differ from LSR and SSR (*P* = 0.99) averaging 89.7 % across LSR and SSR respectively.

Selectivity of animals grazing alfalfa did not influence aNDF between treatments (*P* = 0.47) averaging 33.9 % for LSR and SSR masticate samples. Similar to NDF, ADF, CP, and OM were not different (*P* ≥ 0.16) between treatments on alfalfa paddocks.

Discussion

Despite the fact that the two studies were conducted separately, CP, aNDF and ADF content were not affected by the sward heights imposed in this study in either forage. This could be attributed to the small (5 cm difference between the two sward heights for fescue and 10 cm difference for alfalfa) or the seasonal influence. Belesky et al. (1998) studied the nutritive value of swards on clipping frequency and concluded that sward growth declined between 3 and 6 week intervals affecting forage composition and nutrient inputs. The masticate aNDF from tall fescue samples is only slightly less than the aNDF of hand-clipped samples gathered before grazing. This could be a result of animals grazing more desirable plant parts during the first 3 d of grazing the individual paddocks. Alfalfa aNDF in masticate was greater than in hand-clipped samples, probably due to rapid fermentation of masticates in rumen and selectivity during the first days leaving lower quality forage. The lack of significant differences in fiber content between LSR and SSR in tall fescue and alfalfa could be attributed to the short time frame in days of regrowth between treatments leading them to have similar fiber contents. Stem NDF is impacted by the environment (Lamb et al., 2014). Forage characteristics influence diet selectivity and grazing behavior affecting daily nutrients acquisition (Baumont et al., 2000). The CP concentrations from tall fescue masticate were slightly more than from the hand-clipped forage samples taken before the study, and can be attributed to short time difference between forage sampling and diet selectivity data collection. The CP concentration of the alfalfa masticate was slightly greater than from the hand-clipped probably due to the difference in days between sample collection and masticate collection. The difference in VFA's in the present study coincide with the ones from Morvay et al. (2010) that VFA may vary largely among diets, especially acetate.

Conclusions

The objective of this study was to evaluate the effects of two different sward heights on diet composition, rumen fermentation, and behavior of calves grazing novel endophyte fescue and alfalfa in separate experiments. Ammonia concentration and total VFA were the only observed differences in tall fescue between forage heights. Differences in VFA with no difference in chemical composition could be attributed to low metabolism and digestion in rumen and reticulum. The expectations were that sward height would influence forage composition and therefore influence the ingestive behavior and diet selectivity by calves. The results of the present study did not reflect any differences between the two sward heights and further research is needed.

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Table 1. Forage nutritive value of two different sward heights (LSR and SSR) of tall fescue and alfalfa stands before animals were stocked[1].

	Tall fescue				Alfalfa			
	Treatment				Treatment			
Item[2]	LSR	SSR	SEM[3]	*P*- value	LSR	SSR	SEM	*P*- value
	% of DM				% of DM			
aNDF	54.3	55.8	1.43	0.52	27.7	24.7	1.44	0.24
ADF	26.2	27.4	0.53	0.21	23.0	21.1	1.82	0.52
CP	22.5	22.7	6.65	0.84	31.3	32.8	0.75	0.25
OM	91.7	92.0	0.001	0.07	89.6	89.0	0.001	0.04

[1] Initial forage heights were 33 cm and 28 cm for tall fescue LSR and SSR, respectively, and 55 cm and 45 cm for LSR and SSR for alfalfa, respectively.

[2] aNDF = neutral detergent fiber inclusive of residual ash; ADF = acid detergent fiber; CP = crude protein; OM = organic matter percentages.

[3] SEM = standard error of the mean.

Table 2. Visual observations of cannulated calves grazing long sward regrowth (LSR) or short sward regrowth (SSR) in tall fescue and alfalfa pastures[1].

	Tall fescue				Alfalfa			
	Treatment				Treatment			
Item[2]	LSR	SSR	SEM[3]	*P*- value	LSR	SSR	SEM	*P*- value
Grazing/10 min	3.8	4.3	0.34	0.35	2.8	3.0	0.37	0.65
Bites/10 min	335	308	0.37	0.38	144	130	33.04	0.67
Ruminating/10 min	2.8	2.1	0.34	0.19	0.5	0.5	0.21	0.96
Lying/10 min	1.5	1.6	0.28	0.78	3.1	3.0	0.49	0.97
Idling/10 min	1.9	1.9	0.47	0.45	3.7	3.5	0.59	0.75

[1] Initial forage heights were 33 cm and 28 cm for tall fescue LSR and SSR, respectively, and 55 cm and 45 cm for LSR and SSR for alfalfa, respectively.

[2] Grazing time activities refer to the number of minutes within a 10-min window around each full hour. Data was collected at each full hour for 10 min per animal. Minute values do not add to full 10 min, as below values are averages across all sampling times between 0700 h and 1900 h.

[3] SEM = standard error of the mean.

Table 3. Rumen fluid concentration from cannulated calves grazing long sward regrowth (LSR) or short sward regrowth (SSR) in tall fescue and alfalfa pastures[1]

	Tall fescue				Alfalfa			
	Treatment				Treatment			
Item[2]	LSR	SSR	SEM[3]	*P*-value	LSR	SSR	SEM	*P*-value
Ammonia/mM	28.1	31.8	1.36	0.03	49.7	43.7	6.68	0.11
VFA/mM	71.8	80.5	5.22	0.01	125	118	9.54	0.63
Acetic acid/mM	47.6	52.4	3.68	0.01	74.1	71.2	5.65	0.72
Propionic acid/ mM	12.1	13.8	0.89	0.05	25.7	24.1	1.68	0.52
Isobutyric acid/ mM	1.1	1.2	0.04	<0.01	2.2	1.1	0.17	0.62
Butyric acid/ mM	8.6	10.1	0.56	0.01	17.6	16.1	1.56	0.52
Isovaleric acid/ mM	1.6	1.8	0.08	<0.01	3.1	3.0	0.30	0.75
Valeric acid/ mM	1.0	1.1	0.06	0.04	2.3	2.2	0.22	0.55
pH	6.8	6.6	0.09	0.06	5.7	5.9	0.10	0.03

[1] Initial forage heights were 33 cm and 28 cm for tall fescue LSR and SSR, respectively, and 55 cm and 45 cm for LSR and SSR alfalfa, respectively.

[2] Rumen fermentation values are averages across all sampling times between 0700 h and 1900 h (every 2 hours).

[3] SEM = standard error of the mean.

Table 4. Diet selectivity by cannulated steers grazing long sward regrowth or short sward regrowth in tall fescue and alfalfa pastures[1].

	Tall fescue				Alfalfa			
	Treatment				Treatment			
Item[2]	LSR	SSR	SEM[3]	*P*-value	LSR	SSR	SEM	*P*-value
	% of DM			% of DM				% of DM
aNDF	51.2	54.3	1.14	0.11	30.9	36.1	3.94	0.47
ADF	24.1	24.8	0.61	0.83	25.1	31.1	5.13	0.51
CP	25.6	26.2	0.60	0.52	24.7	23.7	2.47	0.81
OM	88.9	88.9	0.003	0.99	84.1	78.2	0.02	0.16

[1] Initial forage heights were 33 cm and 28 cm for tall fescue LSR and SSR, respectively, and 55 cm and 45 cm for LSR and SSR alfalfa, respectively.

[2] aNDF = neutral detergent fiber inclusive of residual ash; ADF = acid detergent fiber; CP = crude protein; OM = organic matter percentages.

[3] SEM = standard error of the mean.

CHAPTER VI

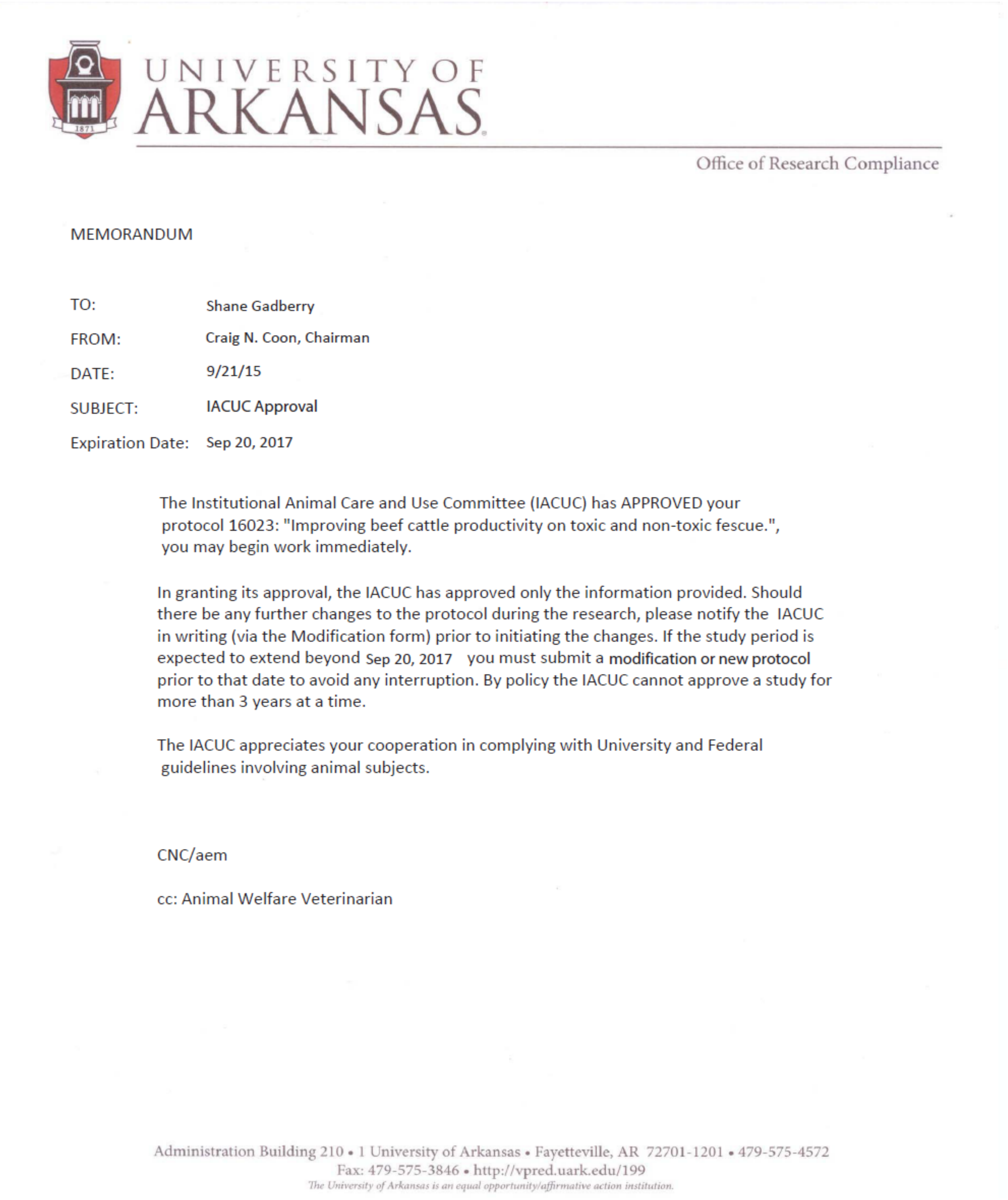
CONCLUSION

In steers grazing tall fescue pastures that contain the toxic endophyte (E+) fungus, management strategies may alleviate toxicosis caused by different levels of the ergot alkaloid ergovaline. Average daily gain increased when a cumulative management that included an implant, ionophore and supplemental feed were offered. Conversely, in when only mineral supplementation was offered, steer weight gains responded negatively to increasing levels of ergovaline in the pasture. Motion activity by steers while on pastures with varying levels of toxicity, may or may not be reduced depending on the extra management strategies imposed. Displacement and number of steps daily increased by about 20% in steers under a combined management strategy. Lying bouts decreased when toxicity levels increased.

Forage sward heights influence diet selectivity because animals need to graze enough to acquire their daily nutrient requirements. Thus, grazing strategies need to be developed strategically throughout the grazing period. In the present study, no differences were observed in diet selection, rumen fermentation and behavioral grazing activities by calves grazing different sward heights in alfalfa and in tall fescue pastures, but this is likely because differences in sward height were not great enough to elicit a response. It is also possible that animal to animal variability was too great to elicit response differences.

CHAPTER VIII

APPENDIX



UNIVERSITY OF
ARKANSAS

Office of Research Compliance

MEMORANDUM

TO: Shane Gadberry

FROM: Craig N. Coon, Chairman

DATE: 9/21/15

SUBJECT: IACUC Approval

Expiration Date: Sep 20, 2017

The Institutional Animal Care and Use Committee (IACUC) has APPROVED your protocol 16023: "Improving beef cattle productivity on toxic and non-toxic fescue.", you may begin work immediately.

In granting its approval, the IACUC has approved only the information provided. Should there be any further changes to the protocol during the research, please notify the IACUC in writing (via the Modification form) prior to initiating the changes. If the study period is expected to extend beyond Sep 20, 2017 you must submit a modification or new protocol prior to that date to avoid any interruption. By policy the IACUC cannot approve a study for more than 3 years at a time.

The IACUC appreciates your cooperation in complying with University and Federal guidelines involving animal subjects.

CNC/aem

cc: Animal Welfare Veterinarian

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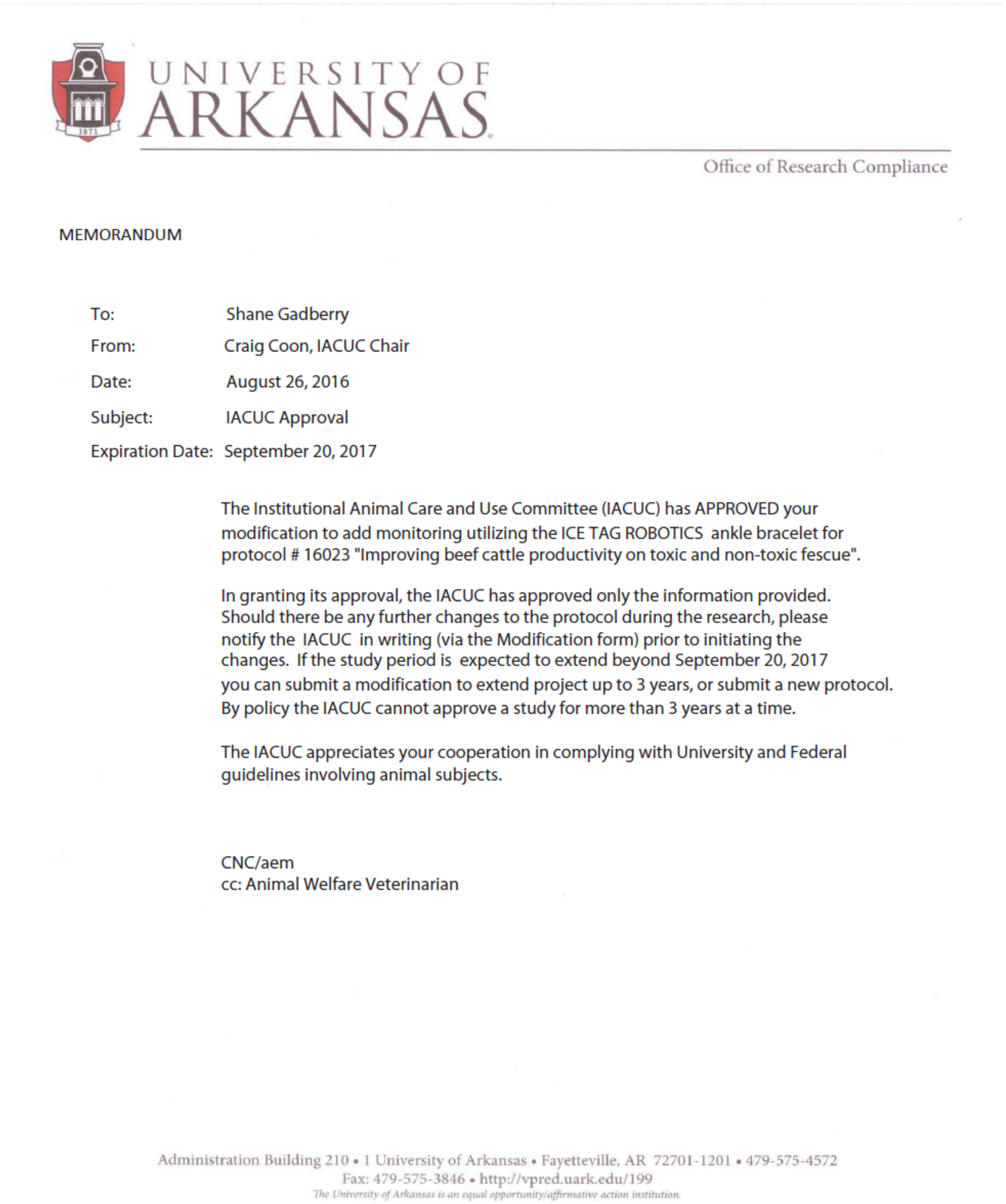
Fax: 479-575-3846 • http://vpred.uark.edu/199



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ARKANSAS

Office of Research Compliance

MEMORANDUM

To: Shane Gadberry
From: Craig Coon, IACUC Chair
Date: August 26, 2016
Subject: IACUC Approval
Expiration Date: September 20, 2017

The Institutional Animal Care and Use Committee (IACUC) has APPROVED your modification to add monitoring utilizing the ICE TAG ROBOTICS ankle bracelet for protocol # 16023 "Improving beef cattle productivity on toxic and non-toxic fescue".

In granting its approval, the IACUC has approved only the information provided. Should there be any further changes to the protocol during the research, please notify the IACUC in writing (via the Modification form) prior to initiating the changes. If the study period is expected to extend beyond September 20, 2017 you can submit a modification to extend project up to 3 years, or submit a new protocol. By policy the IACUC cannot approve a study for more than 3 years at a time.

The IACUC appreciates your cooperation in complying with University and Federal guidelines involving animal subjects.

CNC/aem
cc: Animal Welfare Veterinarian

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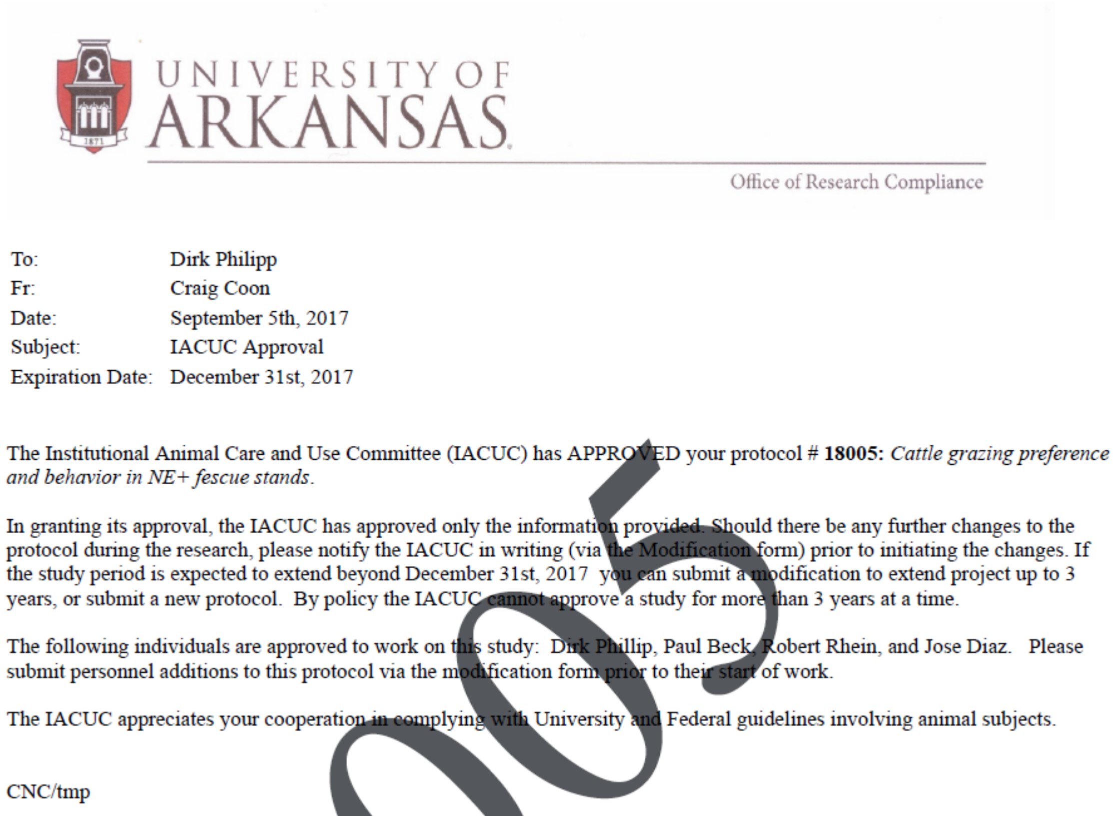


UNIVERSITY OF ARKANSAS
Office of Research Compliance

To: Dirk Philipp
Fr: Craig Coon
Date: September 5th, 2017
Subject: IACUC Approval
Expiration Date: December 31st, 2017

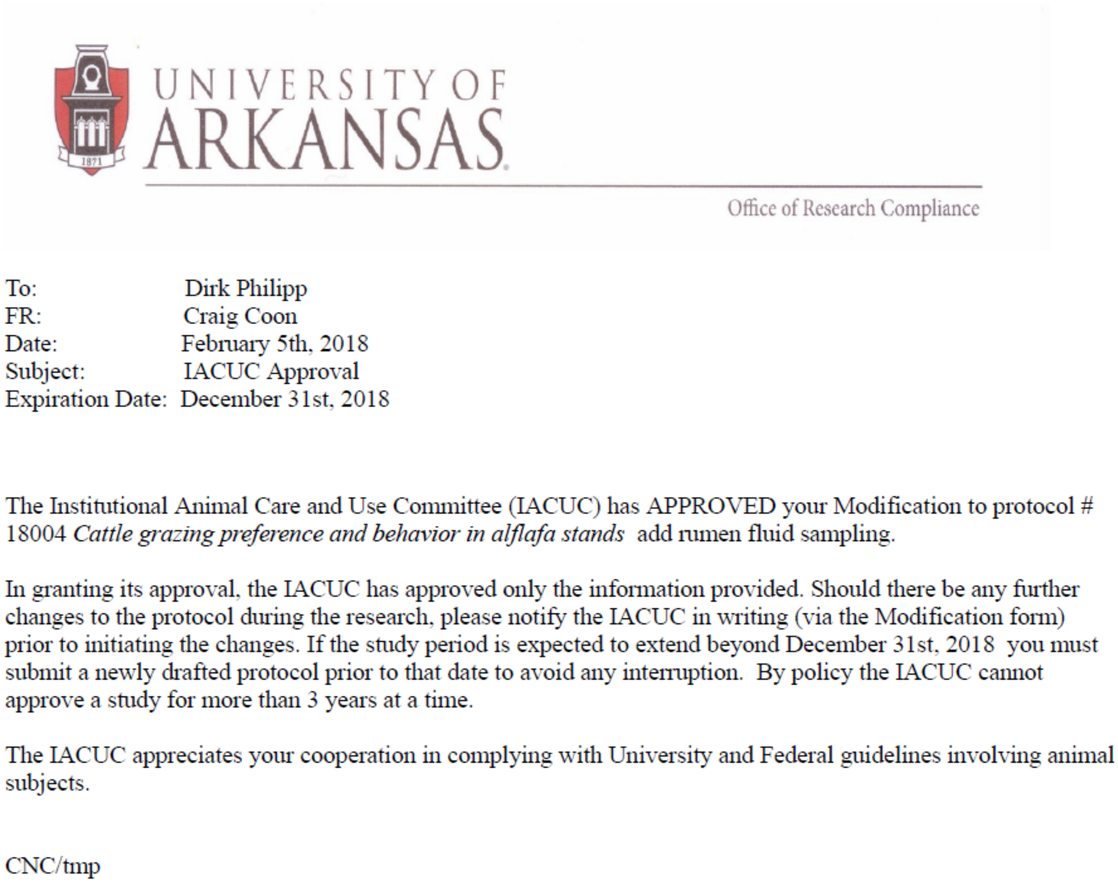
The Institutional Animal Care and Use Committee (IACUC) has APPROVED your protocol # **18005:** *Cattle grazing preference and behavior in NE+ fescue stands*.

In granting its approval, the IACUC has approved only the information provided. Should there be any further changes to the protocol during the research, please notify the IACUC in writing (via the Modification form) prior to initiating the changes. If the study period is expected to extend beyond December 31st, 2017 you can submit a modification to extend project up to 3 years, or submit a new protocol. By policy the IACUC cannot approve a study for more than 3 years at a time.

The following individuals are approved to work on this study: Dirk Phillip, Paul Beck, Robert Rhein, and Jose Diaz. Please submit personnel additions to this protocol via the modification form prior to their start of work.

The IACUC appreciates your cooperation in complying with University and Federal guidelines involving animal subjects.

CNC/tmp



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Office of Research Compliance

To: Dirk Philipp
FR: Craig Coon
Date: February 5th, 2018
Subject: IACUC Approval
Expiration Date: December 31st, 2018

The Institutional Animal Care and Use Committee (IACUC) has APPROVED your Modification to protocol # 18004 *Cattle grazing preference and behavior in alflafa stands* add rumen fluid sampling.

In granting its approval, the IACUC has approved only the information provided. Should there be any further changes to the protocol during the research, please notify the IACUC in writing (via the Modification form) prior to initiating the changes. If the study period is expected to extend beyond December 31st, 2018 you must submit a newly drafted protocol prior to that date to avoid any interruption. By policy the IACUC cannot approve a study for more than 3 years at a time.

The IACUC appreciates your cooperation in complying with University and Federal guidelines involving animal subjects.

CNC/tmp