INTERACTION OF METSULFURON AND NITROGEN FERTILIZER IN TALL FESCUE FORAGE SYSTEMS

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MIKAELA ADAMS

Dr. Eric Bailey, Thesis Supervisor

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The undersigned, appointed by the Associate Vice Chancellor of the Office of Research and Graduate Studies, have examined the thesis entitled.

INTERACTION OF METSULFURON AND NITROGEN FERTILIZER IN TALL FESCUE FORAGE SYSTEMS

Presented by Mikaela Adams,

a candidate for the degree of Master of Animal Sciences,

and hereby certify that, in their opinion, it is worthy of acceptance.

Dr. Eric Bailey
Assistant Professor
Division of Animal Sciences

Dr. Derek Brake
Assistant Professor
Division of Animal Sciences

Dr. Harley Naumann
Associate Professor
Division of Plant Sciences

Dr. Bryon Weigand
Division Director
Division of Animal Sciences

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ABSTRACT

Tall Fescue (*Schedonorus arundinaceus*) is an introduced cool-season bunch grass that is a vital component of forage systems that support beef cattle production in Missouri and much of the eastern United States. This cool-season grass is known for its high forage yield and resistance to environmental stressors such as drought, pest, and grazing. The resilience of tall fescue to stress is attributed to a symbiotic relationship with the fungal endophyte *Epichloë coenophiala*, which produces ergot-like alkaloids that can negatively impact livestock performance. Replacement of alkaloid producing tall fescue cultivars, such as K31, has been suggested as a strategy to reduce ergot-like alkaloid consumption by livestock; however, this requires extensive resources and time. It has been reported to cost $250 to $400 per acre to replace tall fescue, with an additional 18 months of no grazing while new species emerge. Most current research efforts have focused on managing tall fescue to reduce toxicity risk by decreasing amounts of alkaloids consumed by livestock. Removing parts of the plant, such as the stems and seedheads, that contain greater concentrations of alkaloids by physical or chemical seedhead suppression has proven to be a successful strategy. A metsulfuron herbicide and nitrogen fertilizer were applied on pastures grazed by stocker cattle for three years to evaluate impacts on toxicity and productivity of the forage system. Metsulfuron herbicide reduced seedhead density by 80% and decreased ergovaline production; however, this management strategy also caused a decrease in forage yield. Nitrogen fertilizer recovered forage yield losses due to metsulfuron herbicide. Seedhead reduction in response to metsulfuron has potential for reducing alkaloid exposure when grazing endophyte-infected tall fescue.

CHAPTER 1. REVIEW OF LITERATURE

Introduction - Stocker Cattle Industry

There are three segments within the beef industry prior to harvest; cow-calf, background/stocker, and finishing. The cow-calf sector represents a system that is focused on the management of a breeding herd, with the goal of producing an annual calf crop. The cow/calf production system is often an extensive, grazing-dependent system. The stocker, or backgrounder, sector of the industry consists of growing weaned steer and heifer calves (or yearlings) prior to feedlot placement. The feedlot/finishing sector is an intensive production system aimed towards weight gain in preparation for processing. This phase feeds high-grain/low-forage diets.

The stocker, or backgrounder, segment seeks to optimize cost per unit of weight gain. These 300-to-900-pound calves or yearlings are grown in a variety of management systems after being weaned from the dam. Stocker cattle generally gain 100 to 400 pounds throughout the backgrounder/stocker stage of production. Weight gain is dependent on animal health and condition, forage quality and availability, supplemental ration provided, and feeding period. This segment of the beef industry aggregates cattle from numerous cow-calf operations, creating large groups of like characteristics for feedlots. Missouri is ranked 3rd in the United States for beef cow inventory (USDA Beef Cow Inventory); however, only approximately 30% of the terminal beef calf crop remains in Missouri after weaning (Horner et al., 2014). There are nearly 1.8-million beef calves born in the state of Missouri each year, with approximately 1.1 million sold out of state at weaning. Enhancing opportunities for the backgrounder/stocker industry creates new business models within the state, adding value to calves post-weaning. Utilizing abundant, high-quality forage resources in Missouri could provide a cost-effective feed source for growing stocker cattle.

The eastern United States is a major production region for grazing livestock, primarily beef cattle. Producers growing stocker cattle in this region are predominantly grazing tall fescue, a cool season perennial forage. Stocker cattle producers are interested in grazing a forage that is persistent and productive, meeting the nutritional requirements of young, growing animals without a need for additional supplementation. While tall fescue is persistent when heavily grazed by livestock, poor animal performance of cattle is often observed (Bush and Buckner, 1973). Reduction of average daily gain (ADG) in steers grazing tall fescue has been associated with a fungal endophyte (Hoveland et al., 1980). The adverse effects on the health and performance of livestock grazing tall fescue infected by toxic endophytes have been studied extensively.

Section 1 - Tall Fescue

Tall fescue is a cool-season perennial forage, thought to be introduced to the United States through contaminated grass seed from Europe (Hoveland, 1993). ‘Kentucky 31’ (K31) tall fescue was an ecotype discovered in Menifee, Kentucky in 1931. Kentucky 31 tall fescue was brought to the attention of local producers due to hardiness under poor growing conditions. In 1942, the Kentucky Agricultural Experiment Station released K31 tall fescue, and the cool-season forage cultivar was included in Kentucky’s seed certification program in 1945 (Fergus and Buckner, 1972). Tall fescue occupies just over 14 million hectares of pasture in the eastern temperate region of the United States; this region is often referred to as the Fescue Belt (Buckner et al., 1979).

Arachevaleta et al. (1989) reported the hardiness of K31 tall fescue to be a result of a symbiotic relationship between the grass and a fungal endophyte (endo = within; phyte = plant). Greater than 95% of fescue pastures containing K31 variety are infected by the endophyte fungus, *Epicholë coenophiala* (Shelby and Dalrymple, 1987). The plant supplies nutrients to the endophyte, and the fungus generates toxins that are distributed throughout the plant increasing its resistance to environmental stressors (Ferguson et al., 2021). An increase in productivity, specifically seedling vigor and persistence of the K31 tall fescue, is an additional benefit of this symbiotic relationship (Hill et al., 1990).

The fungal endophyte can limit the usefulness of the forage for grazing systems due to the endophyte production of toxins (Hill et al., 1990). After K31 tall fescue was released and producers reported reduced cattle performance and symptoms of an unknown toxicosis. Research determined the cause of reduced performance was the fungal endophyte *Epicholë coenophiala*, causing tall fescue toxicosis in grazing livestock (Bacon et al., 1977).

Many researchers have explored the hardiness of tall fescue and the symbiotic relationship between the forage and the fungal endophyte. There are three well-known cultivars of tall fescue; endophyte-infected (E+) tall fescue containing the fungal endophyte, non-endophyte-infected (E-) tall fescue that does not contain the fungal endophyte and "novel" endophyte-infected tall fescue containing a reduced alkaloid producing endophyte (Hopkins and Alison, 2006). When compared to non-endophyte-infected tall fescue, endophyte infected tall fescue produces greater forage mass under normal conditions after 10 to 14 weeks of growth (Clay, 1987). Endophyte infected tall fescue expresses greater tolerance to stressors such as drought, flooding, and varying N rates than non-endophyte-infected tall fescue (E-, Arachevaleta et al., 1989; West et al., 1988). Arachevaleta et al. (1989) found that 75% of non-endophyte-infected (E-) tall fescue plots died when exposed to extreme drought conditions, whereas no deaths were reported for the endophyte infected (E+) tall fescue plots. These drought conditions consisted of three different water levels imposed for 40 days at -0.50 (alongside two lower levels of -0.03, -0.05MPa) matric potential. Clay (1987) reported that the symbiosis among the plant and the fungal endophyte increased germination and more than doubled the percentage of filled seeds, furthering the competitive abilities over non-endophyte-infected (E-) tall fescue. Greater forage pest resistance resulted in an increase in productivity seen in the endophyte infected (E+) tall fescue. West et al. (1988) found greater quantities of soil-borne nematodes in the endophyte-free (E-) soil than the endophyte infected (E+) treatment groups at all irrigation levels. It was reported that root-knot nematode infestation of the root reduced root growth; the endophyte infection also inhibited the reproduction cycle of the nematode and mitigated the pest population (West et al., 1988). Johnson et al. (1985) reported that three aphid species (*Rhopalosiphum padi*, *Schizaphis graminum*, and *Rhopalosiphum maidis*) were unable to survive after 3 days when confined to solely endophyte-infected (E+) tall fescue; however, the population of aphids thrived when confined to solely non-infected (E-) tall fescue for 10 to 15 days.

Section 2 - Ergot-like Alkaloid Production

Ergot is a term used to generalize all species of the fungus *Claviceps*; ergotism refers to diseases associated with mammals consuming toxic levels of ergots. Alkaloids are produced by the fungus, *Claviceps purpurea,* causing toxic effects in mammals, referred to as ergotism. For livestock, the primary exposure is from ergot-infected feed such as barley, rye, wheat, and oats, as well as infected seeds in forages consumed in grazing systems, hay, or silage. Tall fescue can be infected by a separate fungus, *Epicholë coenophiala.* Ergot-like alkaloids associated with fescue toxicosis are produced by the endophyte fungus, *Epicholë coenophiala*. The signs of ergotism can be indistinguishable from those of fescue toxicosis in beef cattle; gangrene of the extremities, hyperthermia and production loss are reported in both diseases (Evans et al., 2012). However, ergotism is less dependent on ambient temperature than fescue toxicosis and can be fatal.

Throughout the K31 tall fescue growing season, ergot-like alkaloid concentrations fluctuate, peaking in late spring, decreasing through summer, and reaching a second spike in the fall (Belesky et al., 1988). Rottinghaus et al. (1991) observed a difference in alkaloid concentration among plant parts, reporting mature seedheads to have the greatest concentration of ergot-like alkaloids. Ergot-like alkaloids are found in the intracellular space of the plant, concentrated at growing points (nutrient sinks) within the plant. While the level of ergot-like alkaloids in the leaves, stem and seedheads differs significantly, the stage of maturity of the plant also has a major influence on the ergot-like alkaloid concentration consumed (Guerre, 2015). Rottinghaus et al. (1991) and Guerre (2015) found the greatest concentration of ergot-like alkaloids to be in June when the plant reached maturity.

Researchers made efforts to uncover compounds that are responsible for the poor ruminant performance associated with animals grazing endophyte infected tall fescue. The presence of the fungal endophyte (*Epicholë coenophiala*) in tall fescue results in the production of various ergot-like alkaloids, which have a broad spectrum of toxicity to grazing animals that can differ considerably (Guerre, 2015). Lyons et al. (1986)

discovered ergot-like alkaloids were produced in vitro by the *Epicholë coenophiala* fungus but were not present in the uninfected samples. It is logical to assume ergot-like alkaloids causing tall fescue toxicosis are synthesized by this fungus, not by the forage itself. Inadequate measuring techniques of ergot-like alkaloids within animal tissues and lacking an adequate understanding of the complex interactions among the animal, plant, endophyte, and environmental factors involved have slowed development of fescue toxicosis management strategies.

Section 3 - Fescue Toxicosis

Poor performance of cattle grazing infected tall fescue has been noted in the literature (Hoveland et al., 1980; Hoveland et al., 1983; Read and Camp, 1986). Reduced performance of livestock grazing K31 tall fescue is associated with a toxicity from consumption of ergot-like alkaloids (Hoveland et al., 1983). The fungal endophyte found in tall fescue produces ergot-like alkaloids that cause negative effects in animals grazing the forage (Guerre, 2015). Read and Camp (1986) reported weight gains to be inconsistent among cattle grazing endophyte-infected tall fescue, along with other health problems. Hoveland et al. (1983) found that steers grazing non-endophyte infected tall fescue pastures had a greater average daily gain (ADG) than those grazing infected tall fescue pastures. The multitude of negative changes to the animal's health while grazing tall fescue is referred to as "fescue toxicosis". Strickland et al. (2011) estimated over a $1 billion loss annually in the United States due to ergot-like alkaloid ingestion in livestock, posing a detrimental economic loss for producers.

Fescue toxicosis has various effects across livestock species; in cattle it is primarily reported as reduced performance due to the 'summer slump'. The summer slump refers to the warmest time of year when the mature forage is in the reproductive stage, concurrent to a peak in ergot-like alkaloid concentrations. During this time, the animal may experience greater heat stress due to elevated temperatures combined with a thicker hair coat and vasoconstriction due to fescue toxicosis. The combination of these negative effects causes reduced performance in cattle grazing K31 tall fescue pastures. Hoveland (1993) reported beef cows consuming endophyte-infected tall fescue had reduced pregnancy rates, poor average daily gain and decreased milk production which directly relates to decreased calf weaning weight.

Depressed weight gain reported in livestock grazing endophyte-infected tall fescue is an early indicator of toxicity (Hoveland et al., 1980; Hoveland et al., 1983; Read and Camp, 1986). Paterson et al. (1995) found one contributing factor of decreased ADG in beef cattle was reduced dry matter intake. Read and Camp (1986) and Hoveland et al. (1983) reported steers grazing low endophyte-infected tall fescue (less than 5% of tall fescue tillers tested were infected) had nearly double the ADG of steers grazing high infected tall fescue (averaging 94% of tall fescue tillers tested were infected). Stuedemann et al. (1985) noted inconsistencies in grazing behavior of steers grazing either high or low endophyte infected tall fescue. Steers on high endophyte-infected tall fescue pastures spent less time physically grazing, when compared to steers consuming the low endophyte infected tall fescue. The differences in grazing behavior were greatest from 12:00PM to 4:00PM (the hottest hours of the day). Hemken et al. (1981) reported a 50% reduction in DM intake for heat stressed calves; therefore, reductions in grazing time was likely due to animals experiencing increased heat stress when grazing endophyte infected tall fescue.

Poor performance of cattle grazing endophyte-infected tall fescue is correlated to environmental temperature. Hemken et al. (1981) fed one of two varieties of tall fescue that had previously altered animal performance during the summer period in temperature-controlled rooms. In both years of the experiment, calves consuming tall fescue with greater alkaloid concentrations had elevated rectal temperature and respiratory rates when compared to calves consuming low endophyte tall fescue, indicating heat stress. Hemken et al. (1981) also reported calves consuming highly toxic tall fescue, housed in temperature-controlled rooms kept at greater than 31 degrees Celsius, had less dry matter intake and weight gain than calves fed the less toxic tall fescue in the cooler environments (10-13 or 21-23 degrees Celsius). “Less toxic” and “highly toxic” were not clearly defined by the authors (Hemken et al., 1981). These findings led to the conclusion that poor performance of cattle grazing tall fescue is related to environmental temperature, even though toxicity is present throughout the forage growing season. A study by Crawford et al. (1989) fed Holstein heifers different concentrations of infected tall fescue through the summer and reported their growth over time. The ADG of the heifers decreased by 0.05 kg for every 10% increase in endophyte infection.

Increased heat stress in cattle grazing endophyte-infected tall fescue could be associated with the differences in hair coats that several studies have reported (Hemken et al., 1981; Hoveland et al., 1983; Read and Camp, 1986; Peters et al., 1992). A study by McClanahan et al. (2008) fed a group of calf’s endophyte-infected tall fescue and either left their coat unclipped or clipped the hair coat monthly to a length of 6mm. Calves with an unclipped hair coat had elevated rectal temperature compared to calves with a clipped haircoat. The rough hair coats of unclipped calves led to greater core body insulation and therefore increased heat stress. Aiken et al. (2011) bleached two patches of hair (bleached April 23) on steers grazing endophyte-infected tall fescue (grazing endophyte-infected tall fescue prior to start of experiment, initial grazing of experiment began April 23) and observed the hair growth and shedding patterns of each animal. The patches of bleached coat were clipped, one patch on June 5 and the other on July 7. When measurements were taken, if the clipped hairs were completely bleached it was considered un-shed winter hairs, whereas hairs with some bleach were summer growing hairs and non-bleached hairs were summer growing hairs following the shedding of winter hairs. Calves with summer growing hair that grew to excessive lengths and winter grown hair that failed to shed, resulted in elevated core body temperature to intensify hyperthermia triggered by fescue toxicosis. Prolactin was noted as a possible mechanism of this phenomenon, as it is involved in the hair growth cycle and the shedding of the winter coat (McClanahan et al., 2008).

Cattle consuming endophyte-infected tall fescue have decreased success in reproduction. Brown et al. (1992) found a 7.6% decrease in calving rates for cows grazing endophyte-infected tall fescue, when compared to cow herds grazing bermudagrass. Poole et al. (2019) reported a decrease in pregnancy rates for cows consuming a toxic tall fescue diet in seven states among the humid temperate United States. Danilson et al. (1986) exposed cows to diets with varying levels of fescue toxicity (low – 0 to 20%, medium – 25 to 60% or high – 80 to 99%) and reported decreased pregnancy rates of 95.8%, 81.8%, and 54.5%, respectively as the level of endophyte infection increased.

Reduced milk production is a contributing factor to economic losses of fescue toxicosis in beef and dairy cattle. Brown et al. (1996) reported both milk yield and percent milk fat decreased when Angus, Brahman, and crossbred cows grazing endophyte-infected tall fescue pastures. In another study, a 25% decrease in milk production was noted in cows grazing endophyte infected versus non endophyte infected tall fescue (Peters et al., 1992). The authors (Peters et al., 1992) also reported a decrease in offspring weight when the dam was grazing endophyte-infected tall fescue. The decrease in milk production in the dam was likely the cause of the decreased in ADG reported in the calves.

Symptoms of fescue toxicosis outwardly expressed by the animal (reduced weight gain, agalactia, and thick hair coats) make diagnoses easy for producers; however, there are physiological responses to the endophyte that aren't as obvious. Ergot-like alkaloids can interact with serotonin (Schöning et al., 2001; Klotz et al., 2013), dopamine (Larson et al., 1995, 1999), and androgen (Oliver et al., 1998) receptors causing various responses when consumed. Although there are several negative effects of fescue toxicosis in grazing ruminants, most note vasoconstriction, as it alters blood flow and hormone release throughout the body. Ergot-like alkaloid consumption has demonstrated a contractile response in multiple vascular models; Pesqueira et al. (2014) reported Holstein steers consuming tall fescue seed extract (containing ergopeptine alkaloids) showed a persistent contractile response that is thought to be a primary contributing factor to vasoconstriction. Additionally, increased rectal temperature and respiration rate is reported in multiple species consuming endophyte-infected tell fescue (Hemken et al., 1981; Osborn et al., 1992; Browning and Browning, 1997). The increase in rectal temperature indicates heat stress, which could help to explain the decrease in intake, poor growth, and poor reproductive performance seen in the summer slump.

Ergot-like alkaloids consumed and absorbed into the bloodstream effect levels of prolactin. These ergot-like alkaloids are identified as a dopamine agonist (Larson et al., 1995, 1999); the hormone dopamine inhibits prolactin secretion (Lamberts and Macleod, 1990). Foote et al. (2013) found prolactin concentrations decreased by 46% in steers consuming endophyte infected tall fescue, and only by 18% in steers consuming non-endophyte infected tall fescue. Decreased serum prolactin concentration in beef cattle consuming ergot-like alkaloids in the diet is a side effect of tall fescue toxicosis (Hoveland et al., 1983; Hurley et al., 1980; Parish et al., 2003; Rice et al., 1997). Hurley et al. (1980) reported Holstein calves consuming toxic endophyte infected tall fescue to have lower basal prolactin concentrations than those calves fed a less toxic endophyte infected tall fescue. This study also found increasing ambient temperature increased basal prolactin levels in calves fed less toxic endophyte infected tall fescue but not in the calves consuming more toxic endophyte infected tall fescue, concluding that some factors in toxic fescue inhibit prolactin secretion, especially at high temperatures associated with the "summer slump" (Hurley et al., 1980).

"Fescue foot" is another symptom reported with K31 tall fescue consumption in cattle. There are a multitude of symptoms associated with fescue foot, including necrosis of tail, feet and ears, rough coat, swelling of coronary bands and shedding of the hoof. The ergot-like alkaloids produced by the fungal endophyte cause peripheral vasoconstriction in the cow, resulting in these symptoms (Rhodes et al., 1991). Symptoms of fescue toxicosis occur during the summer months but issues with fescue foot occur in the colder months. Williams et al. (1975) injected calves with endophyte-infected tall fescue extracts and monitored them for physical changes in colder temperatures. Throughout this study, lameness was observed as well as swelling of coronary bands and discoloration of tails (Williams et al., 1975). In a study by Read and Camp (1986), 83 percent of animals grazing high-infected tall fescue pastures had symptoms of "fescue foot", while none of the animals grazing the non-endophyte-infected tall fescue exhibited any symptoms. Ergot-like alkaloid consumption can affect livestock productivity and health across much of the calendar year, limiting its potential as a forage base for production systems across the Fescue Belt without proper management.

Section 4 – Tall Fescue Management Strategies

Extensive research has been conducted to evaluate potential solutions or management strategies to decrease the negative effects of fescue toxicosis. Removal of the K31 tall fescue from pastures and reseeding with tall fescue cultivars that do not contain toxic endophyte is a strategy for eliminating fescue toxicosis among grazing animals. Livestock consuming endophyte-free forage had improved performance and no symptoms of fescue toxicosis (Hoveland, 1993); however, stand persistence was reduced compared to endophyte infected tall fescue (Read and Camp, 1986).

New tall fescue cultivars with a non-toxic endophyte that provides the plant with vigor, while avoiding the negative effects on grazing animal are now available. Phillips and Aiken (2009) evaluated animal performance and well-being between novel endophyte-infected and endophyte-free tall fescue. Novel endophyte infected tall fescue can handle repeated defoliation by livestock, a trait that is highly sought after in the endophyte-infected tall fescue (Phillips and Aiken, 2009). Lomas and Moyer (2018) evaluated three tall fescue cultivars; E+ is K31 tall fescue, E- is a non-endophyte-infected tall fescue (no endophyte), and MaxQ is a novel endophyte-infected tall fescue (reduced alkaloid producing endophyte). Steers grazing low-endophyte and novel endophyte pastures were 20% heavier at the end of the grazing period, had 39% greater grazing gain, 39% greater daily gain and produced 39% greater gain/acre than steers grazing high-endophyte K31 tall fescue (Lomas and Moyer, 2018). However, the average available forage (DM basis) of high-endophyte K31 tall fescue pastures was greater than that of low-endophyte and novel endophyte pastures. This finding is confounded with intake, as it is suspected that more forage was left behind due to the cattle grazing less endophyte infected K31 tall fescue than cattle grazing non-infected pastures. While the endophyte-free and novel endophyte tall fescue cultivars result in greater ADG (1.8 lbs/head/day or greater), the forage modification of endophyte-free tall fescue is associated with reduced drought and heat tolerance, increased sensitivity to insects and susceptibility to overgrazing leading to forage persistence problems.

Novel endophyte and endophyte free tall fescue are two alternative forage types proven to lessen reduction in performance for grazing animals; however, it is a financial investment of over $260 per acre (Grassland Renewal, 2021) to replace endophyte-infected tall fescue. There is a major opportunity cost associated with lost forage production in the first two years, when the pastures are out of commission while the new cultivar emerges.

Dilution of the endophyte in grazing diets by interseeding pastures or providing supplementation can reduce the negative effects on livestock. Establishing a legume in tall fescue pastures can reduce fescue toxicosis, while also reducing the need for nitrogen fertilizer to maximize forage yield. Hoveland et al. (1986) found legumes grown within tall fescue pastures can produce equivalent yield as tall fescue fertilized with 67 to 168 kg N/ha (60 to 150 lb/acre), depending on the legume interseeded. Thompson et al. (1993) reported the addition of legumes (approximately 15% and 10% clover in spring and summer stands, respectively) provided steers grazing tall fescue pastures with an additional gain of 0.07 kg d^{-1}. Hoveland et al. (1981) found greater ADG and body weight (BW) gain per hectare for white clover-tall fescue pastures (0.74 kg/d and 504 kg/d respectively) than for monoculture tall fescue pastures (0.37 kg/d and 293 kg/ha respectively) during spring grazing. Legumes, such as clover, increase protein and energy availability of the forage, leading to improved animal performance and reduced losses due to fescue toxicosis (Roberts et al., 2009). Lomas and Moyer (2013) reported grazing performance was similar ($P > 0.05$) among steers grazing endophyte infected tall fescue interseeded with either lespedeza, ladino clover or red clover for two consecutive years of an experiment. Grass-legume interseeding requires rotational grazing management to maintain the legume within the mixture. Under continuous grazing systems, the legume cannot withstand cattle preferentially grazing and will die out leaving a monoculture pasture (Undersander et al., 2022)

Seedhead suppression is a pasture management technique that can reduce effects of tall fescue toxicosis on grazing cattle. Rottinghaus et al. (1991) reported seedheads to contain five times greater concentration of ergovaline than the leaves or stems; therefore, reducing the seedhead production of tall fescue reduces the amount of toxin the animal may consume. Goff et al. (2012) found cattle to selectively graze endophyte-infected seedheads; indicating a greater concentration of alkaloids in cattle diets grazing tall fescue. Roberts and Andrae (2004) found clipping pastures to be an effective way to reduce the ergot alkaloids available to the animal, while also maintaining forage quality. Belesky and Hill (1997) sampled leaves and pseudo stems of uncut fescue plants and plants cut to 5- or 10-cm heights at 7-d intervals for a 6-wk period, reporting uncut plants yielded twice the ergot alkaloid concentration. Salminen and Grewal (2002) found greater ergovaline concentrations in tall fescue plants clipped biweekly than those clipped weekly. Moyer and Kelley (1995) reported metsulfuron, a broad leaf herbicide, reduced the presence of weed species while maintaining forage value of tall fescue. Recently, research was conducted to explore the ability of metsulfuron to suppress seedhead emergence in tall fescue. Sather et al. (2013) reported clipped or metsulfuron treated pastures reduced seedhead density by 36 to 55%, increased crude protein levels by 1.03 to 2.14% and reduced acid detergent fiber (ADF) levels by 1.60 to 2.76%. Treatments containing the broad leaf herbicide, reduced ergot alkaloid concentrations 26 to 34% (in the spring harvest, Sather et al., 2013). Aiken et al. (2012) showed treating K31 tall fescue pastures with the broadleaf herbicide reduced seedhead emergence. This study (Aiken et al., 2012) reported suppressing seedhead emergence boosted weight gain in steers and reduced severity of fescue toxicosis symptoms in animals. This broadleaf herbicide works by inhibiting production of branched chain amino acids; valine, leucine, and isoleucine, resulting in a stunting effect and delayed maturity (Brown, 1990).

Nitrogen fertilizer is applied to tall fescue pastures to increase forage production. Bélanger et al. (1992) applied nitrogen at increasing levels (160, 240, 200, and 180 kg N ha−1) to tall fescue pastures in the spring and summer, measuring shoot growth over the forage growing season and reported DM increased by an average of 168% for spring growth and 408% for summer growth. Lyons et al. (1986) and Rottinghaus et al. (1991) found nitrogen fertilizer to increase alkaloid concentration in endophyte-infected tall fescue. Additionally, ergovaline increased by 549, 639 and 992 µg/kg on a whole plant average concentration, from plots applied with either 0, 67.4, or 134.8 kg/ha of nitrogen (Rottinghaus et al., 1991). Application rates of N for grazing pastures should correlate with the associated grazing intensity of the pasture. The rate of N application should be limited to reduce waste of excess growth; current extension recommendations for spring N application are less than 67 kg/ha (Roberts et al., 2009). Limiting amount of Nitrogen fertilizer applied to tall fescue pastures matches animal requirements within the pastures, without creating excessive concentrations of alkaloids.

Section 5 – Stocker Cattle Management on Tall Fescue

Kentucky 31 tall fescue has one of the longest growing seasons among cool season grasses (Roberts et al., 2009). When adequate moisture is available, tall fescue can provide excellent grazing in the spring and good grazing in the fall for weaned calves entering the stocker segment of the beef industry. Due to reduction in ADG of stockers grazing endophyte infected tall fescue, a perception that calves stockered on tall fescue will not gain as well in the feedlot has developed. Duckett et al. (2001) reported feedlot performance of cattle grazing toxic, endophyte-free and novel endophyte tall fescue pastures had no differences in performance, feed efficiency or carcass quality. However, cattle grazing endophyte-free and novel endophyte tall fescue entered the feedlot heavier and reached the targeted feedlot weight sooner (Duckett et al., 2001). Beck et al. (2013) concluded endophyte-infected tall fescue provides a nutrient dense diet for grazing cattle;

any differences in animal performance are likely due to the anti-nutitonal factors associated with the fungal endophyte.

An approach to mitigating production losses from grazing endophyte infected tall fescue is specialized animal management. Addition of supplemental feed (concentrates) to the diet can reduce forage intake and dilute ergot alkaloid consumption, therefore reducing the negative effects of fescue toxicosis. Richards et al. (2006) reported soybean hulls (fed at 0.60% of BW, on organic matter (OM) basis) decreased OM intake of green chop toxic endophyte infected tall fescue by 14%; however, total OM intake and digestion increased. Stokes et al. (1988) reported dairy steers receiving ad libitum toxic endophyte tall fescue hay and corn at 0.65% of BW had greater ADG than steers fed endophyte-infected tall fescue hay without corn supplementation. Aiken et al. (2008) found an increase in ADG of steers grazing endophyte infected tall fescue and supplemented soybean hulls (2.3 kg/steer/day) but did not find a difference in serum prolactin or hair coat ratings among treatments.

Rapid growth of tall fescue in the spring and fall allows for increased stocking rates, focusing the grazing pressure on smaller areas during periods of rapid pasture growth. Cattle grazing the fescue tiller that has started to produce a seedhead remove the growing point, preventing the tiller from producing a seedhead. Therefore, pastures stocked heavy enough to maintain the forage in the vegetative growth stage can maintain a higher-quality forage, if managed properly. K31 tall fescue pastures should be stocked heavily early in the growing season, allowed time for rest and regrowth, and then grazed heavily again to maintain the vegetative stage of growth. Lightly stocked endophyte-infected tall fescue limits animal performance due to ergot alkaloid consumption, while heavily stocked fescue pastures limit animal performance due to forage mass. Rotational grazing improves forage utilization and has been shown to increase animal gains per acre on tall fescue pastures. Bransby et al. (1988) proposed an increase in pasture utilization; increased stocking rates decreased the emergence and selective grazing of the high-ergot-alkaloid-containing seedheads (Goff et al., 2012). Belesky and Hill (1997) reported tall fescue plants under frequent, intensive grazing will prioritize plant carbohydrate reservoirs to regrowth, limiting substrate available for alkaloid production by the endophyte. Detling and Painter (1983) found close, frequent grazing can increase leaf blade to stem ratios, which reduces the toxicity of the tall fescue tiller. Low ergot alkaloid concentrations in leaf blades relative to other plant parts led Rottinghaus et al., (1991) to suggest that ergot alkaloid intake can be reduced by utilizing grazing management strategies that maximize consumption of leaf blades. Forage utilization represents a critical cost factor in stocker production, achieving a proper stocking rate is key to stocker profitability, ensuring maximum profitability attributes to both plant and animal performance.

Estrogenic growth promoting implants are used within the beef industry for increasing weight gain of growing cattle. Ford et al. (1977) reported estrogen administration decreased vasoconstriction. It is hypothesized by evidence from Edwards (2005) that estrogen can induce vasodilation, and progesterone + estrogen can collectively induce vasodilation. Aiken et al. (2016) found implantation with steroid hormones (200 mg progesterone and 20 mg estradiol) increased ADG with lower stocking rates (3, 4 steers/ha), but the effect diminished with increased grazing intensity (5, 6 steers/ha). Heavy stocking rates likely reduced diet quality and/or animal DMI.

Performance diminished with higher stocking rates likely due to decreased nutrient availability per head.

Research utilizing multiple management strategies can further reduce negative effects of fescue toxicosis on the grazing animal. Carter et al. (2010) reported a 0.51 kg/d greater ADG in steers with a steroidal hormone-implanted (200 mg progesterone and 20 mg estradiol) and supplemented 2.3 kg/d soybean hulls while grazing endophyte infected tall fescue compared to control. Steers fed soybean hulls with hormone-implants had greater serum prolactin concentrations and a greater percentage of steers has slick hair coats at the end of the study (Carter et al., 2010). Diaz et al. (2018) conducted an experiment comparing a cumulative management strategy of a hormonal implant (40-mg trenbolone acetate, 8-mg estradiol, and 29-mg tylosin tartrate), 150 mg/calf daily monensin, and 1% BW of a 50:50 corn gluten feed:soybean hull supplement to a treatment group receiving only free choice mineral. Pastures within the experiment expressed a variety of ergovaline concentrations throughout; therefore, the authors regressed average daily gain against pasture ergovaline concentration. Diaz et al. (2018) reported the y-intercept of the two treatments was similar; however, each treatment expressed different slopes as the ergovaline concentration increased. For the mineral only treatment group, there was a linear decrease in ADG as ergovaline concentrations increased. For the cumulative management treatment group, the slope was not different from zero, indicating increases in pasture ergovaline concentration did not effect ADG of steers subjected to the cumulative management strategy. Further research is needed to evaluate cumulative management strategies incorporating varied stocking rates and dilution of endophyte-infected tall fescue pastures.

Endophyte-infected tall fescue houses the fungal endophyte, *Epicholë coenophiala*, that produces ergot-like alkaloids that may disrupt dopamine-signaling pathways resulting in the symptoms commonly termed fescue toxicosis (Strickland et al., 1994). Avermectin anthelmintic demonstrated potential remediation of tall fescue toxicosis in grazing beef cattle but did not demonstrate a significant improvement in body weight gain (Bransby, 1997). A common anthelmintic, domperidone, is a dopamine antagonist and is thought to alleviate poor performance due to fescue toxicosis. The most abundant of the ergot-like alkaloids produced is ergovaline, a known dopamine agonist. Jones at al. (2003) reported body weight gains of non-pregnant, cycling heifers receiving a once daily injection of domperidone (at a concentration of 0.44 mg/kg BW) did not differ from animals consuming endophyte-free fescue, and both treatment group body weight gains were greater than animals consuming endophyte-infected tall fescue with no additional treatment. Further research should be conducted in growing steers, as the current research on anthelmintics alleviating the symptoms of fescue toxicosis focuses primarily on reproductive success in replacement heifers.

Conclusion

Herbicide application, such as metsulfuron, act as growth regulators on K31 tall fescue by delaying maturity and extending peak quality of forage beyond the typical growing season. This chemical technique reduces seedhead development, which is thought to reduce livestock exposure to toxins, due to blades and sheaths contains 5 times less ergovaline concentration than reproductive seedheads (Rottinghaus et al., 1991). The broadleaf herbicide improved the quality and effective use of K31 tall fescue into August, compared to untreated forage biomass in June (Turner et al., 1990), provides an opportunity for extended stocker cattle growth in a grazing management system. Nitrogen application is known to increase tall fescue yield; however, ergot alkaloid concentration has been reported to increase with increasing levels of N in both field and controlled-environmental experiments (Rottinghaus et al., 1991). Applying N to tall fescue pastures, as a forage yield booster, alongside Metsulfuron application, to suppress seedhead emergence, has not been investigated as a possible management strategy of fescue toxicosis. The overall objective of this research was to evaluate effects of metsulfuron and different N rates on stocker cattle performance, forage nutritive value, species composition, seedhead density and ergovaline concentration. We hypothesized metsulfuron application would partially depress ergot alkaloid increases commonly seen in response to nitrogen fertilization resulting in a more productive grazing system for growing stocker cattle.

CHAPTER 2. INTERACTION OF METSULFURON AND NITROGEN FERTILIZER IN TALL FESCUE FORAGE SYSTEMS

ABSTRACT

Chemical seed head suppression of endophyte infected tall fescue (*Schedonorus arundinaceus*) purportedly improves stocker cattle performance but may decrease forage yield. Spring nitrogen application increases tall fescue growth with a concomitant increase in ergot-like alkaloids, produced by the symbiotic endophyte *Epicholë coenophiala*. We hypothesized that greater amounts of nitrogen applied to tall fescue would increase forage yield and offset losses in forage production from chemical suppression of seed heads with Metsulfuron without effect on alkaloid concentration. Ninety-six steers (266.25 ± 19.25 kg) were randomly assigned to one of sixteen, 1.8 ha pastures in 2019, 2020 and 2021. The animals were turned out to pasture on April 17th ± 1 day and continuously grazed their respective pastures for 57 days each year. Pastures were randomly assigned to one of four treatments; no metsulfuron or nitrogen (NEGCON), metsulfuron (13 g/ha) with 0 kg/ha (MET0), 67 kg/ha (MET67), or 134 kg/ha (MET134) of nitrogen, applied as ammonium nitrate. Ammonium nitrate was applied on March 7th ± 3.6 days and metsulfuron was applied approximately 72 hours before cattle were turned out to graze. Steers grazing MET0 pastures were removed on May 30 ± 4.9 days all three years of the experiment due to insufficient forage mass. Steer weight, standing forage height, forage nutritive value and alkaloid concentrations in tall fescue tillers were measured each month. Tall fescue seedhead frequency and forage species frequency measurements were collected June 14 ± 3.1 days. We observed a

reduction (*P*<0.01) of tall fescue seedheads within pastures receiving the Metsulfuron application. Metsulfuron application did not affect (*P*=0.57) ergovaline concentration but ergovaline concentration increased (*P*<0.01) at each monthly sampling across treatments. Forage mass was less (P<0.01) for MET0 than NEGCON in May and June. Forage mass was greater (P<0.01) for MET67 and MET134 than MET0 in May and June. Steer ADG was not affected (*P*=0.29) by treatment. Under the conditions of this experiment, metsulfuron decreased seedhead concentration in tall fescue but did not affect ergovaline concentration. Nitrogen fertilizer improved forage mass that was lost to metsulfuron application in previous experiments but did not impact steer gain in a short duration, continuous grazing system.

INTRODUCTION

Tall fescue (*Schedonorus arundinaceus*) is a cool-season perennial grass covering over 14 million hectares in temperate humid regions of the United States (Casler and Kallenbach 2007), with the primary tall fescue cultivar in this region being Kentucky 31 (K31). There is a symbiotic relationship between K31 and the fungal endophyte *Epicholë coenophiala*, which produces ergot-like alkaloids. The endophyte is noted as contributing to the improved production and hardiness that the forage is known for (Clay, 1987; Arachevaleta et al., 1989). However, a toxicosis in beef cattle is found when fungal endophyte infected K31 forage is consumed (Lyons et al., 1986). Fescue toxicosis causes an estimated loss of over $2 billion annually in the United States due to poor weaning weights, pregnancy rates and stocker cattle performance (Strickland et al., 2011).

Ergot-like alkaloids are toxins produced by *Epicholë coenophiala,* the fungal endophyte that infects K31 tall fescue (Lyons et al., 1986). Alkaloids are concentrated in the seedheads within the reproductive tissue during the reproductive stage (Rottinghaus et al., 1991). Rottinghaus et al. (1991) reported that different parts of the tall fescue plant have varying alkaloid concentrations with the highest concentration in the seedhead, followed by stems and leaf blades.

The seedhead of the forage containing a greater alkaloid concentration has led to management strategies to control seedhead production in K31 pastures. Aiken et al. (2012) reported application of metsulfuron during the boot stage reduced tall fescue seedheads by over 90% and steer ADG increased by 39%. Application of herbicides for seedhead control may reduce forage production. A greater mean forage mass was seen in untreated pastures when compared to the pastures treated with metsulfuron (Aiken et al., 2012). Likewise, Israel et al. (2016) reported a 54% decrease in forage mass in pastures treated with metsulfuron.

Spring application of a nitrogen fertilizer increases tall fescue forage production (Bélanger et al., 1992). Nitrogen (N) fertilizer increases tall fescue growth rate; however, it also increases the alkaloid concentrations found in the plant tissues (Rottinghaus et al., 1991). Therefore, nitrogen fertilizer in the spring is recommended at moderate rates (<50kg/ha) in tall fescue pastures intended for grazing to avoid the increase in alkaloid production by the endophyte (Rottinghaus et al., 1991).

The combination of metsulfuron with increased nitrogen fertilization rates may regain lost yield found with the solo herbicide application. Most current metsulfuron studies focus on the effects of the herbicide on tall fescue under a modest spring nitrogen fertilizer recommendation. Israel et al. (2016) applied nitrogen at a rate of 67 kg/ha to all pastures within the experiment 2 weeks after the varying combinations of herbicide was applied. Nitrogen fertilizer was applied to all pastures in a study by Aiken et al. (2012) at a rate of 62 kg/ha approximately one month prior to the application of Metsulfuron. The interaction of metsulfuron and nitrogen, as well as the impact on alkaloid production, is currently unknown in K31 tall fescue. Our objectives were to evaluate the effects of metsulfuron combined with different nitrogen rates on stocker cattle performance, forage nutritive value, species composition, seedhead density and ergovaline concentration. We hypothesize Metsulfuron application will depress the ergot-like alkaloid increase reported in response to nitrogen fertilization in K31 tall fescue.

MATERIALS AND METHODS

All animal handling practices, and experimental procedures were reviewed and approved by the University of Missouri Animal Care and Use Committee (approval no. 9506).

Site Description

The experiment was conducted at the University of Missouri Southwest Research Center (37˚04'55" N, 93˚53'21" W, elevation 400 m) near Mount Vernon, Missouri. Soil was a mix of Keeno cherty silt loam (loamy-skeletal, siliceous, mesic Mollic Fragiudalf) and Gerald silt loam (fine, mixed, mesic Umbric Fragiaqualf). Precipitation totals recorded by the Lawrence County weather station and the historical average are shown in Table 2.1. Pastures (n=16; 1.8 ha per pasture) of established K31 tall fescue (*Schedonorus arundinaceus*) were determined to be 88% infected with the fungal endophyte (*Epichloë coenophiala*) based off 100 tested tillers using a Western blot (Hiatt et al., 1999; Agrinostics Ltd. Co., Watkinsville, GA). Tillers were randomly selected from the pastures and tested for endophyte infection in April 2019.

Experimental Design & Treatment Structure

The experiment was arranged as a randomized complete block design, with blocks consisting of four pastures, one assigned to each treatment within block. Six steers were randomly assigned per pasture (3.3 steers/ha) at a fixed stocking rate. Blocks were determined based on usage in the previous growing season before year one of the experiment. Each year, treatments were reassigned within blocks. Treatments consisted of a negative control with no metsulfuron or nitrogen fertilizer (NEGCON), metsulfuron with no nitrogen fertilizer (MET0), metsulfuron with 67kg/ha nitrogen provided from ammonium nitrate (MET67), and metsulfuron with 134kg/ha nitrogen provided from ammonium nitrate (MET134).

Pasture Management & Sampling

Ammonium nitrate was applied to MET67 and MET134 pastures on March 7th ± 3.6 days. Metsulfuron (Chaparral, Corteva Agriscience, Wilmington, DE) was applied to the MET pastures at a rate of 13 g/ha approximately 72 hours before cattle were turned out to graze each year of the experiment with a 0.25% volume per volume, high quality non-ionic surfactant (Perference 90%, WinField United, Arden Hills, MN). Steers were turned out to graze on April 17th ± 1 day for a total of 57 days. Animal measurement and forage collection occurred on day 0 at trial initiation, day 28 for an intermediate collection and day 57 at trial conclusion each year.

Tall fescue tillers were sampled from each pasture monthly by walking in a W pattern and clipping the tall fescue tiller nearest to the technician's boot every twenty paces, for a total of 30 samples per pasture. The tall fescue was clipped at the base and the whole plant was collected. Tiller samples were stored on ice until frozen at -20˚C, lyophilized, and later analyzed via HPLC using methods of Rottinghaus et al. (1991).

A rising plate meter (F400, Agricultural Supply Services, Whitminster, UK) was used to measure forage height monthly. Approximately 75 readings were taken while walking a W pattern through each pasture, measuring every five paces. Five calibration measurements were taken in each pasture across a range of forage heights. Plate meter height was recorded and the forage directly under the plate meter was clipped inside a 0.1 m^2 quadrat, air dried at 55˚C in a forced-air oven and weighed. A total of 720 calibration clippings were collected throughout all three years of the experiment and used to create a regression describing the relationship between forage height and forage mass. Data were analyzed using the REG procedure of SAS (SAS Institute, Inc., Carry, NC) with forage height, date of measurement and date squared in the model to predict forage mass. Studentized residuals greater than 3 were removed (n=16). The regression produced the following prediction equation: Forage mass(kg/ha) = -921.69 + 23.80(height) – 1308.11(year) + 333.32 $(\text{year})^2$ + 15.98(date) with an adjusted R-square of 0.66. Calibration clippings were composited within pasture and subsequently analyzed for CP, ADF, and NDF after weights for forage mass were recorded.

June 14 ± 3.1 days, 30 quadrats measuring 0.1 m^2 were randomly placed every 15 paces while walking an W pattern throughout each pasture. Tall fescue seedheads within the quadrat were counted and the frequency of tall fescue, Kentucky bluegrass, orchardgrass, clover, birdsfoot trefoil, and buckhorn plantain were recorded within each quadrat. Individual quadrat data were averaged within pasture to report seedhead density and species frequency.

Steer Management

Steer calves weighing 266.25 ± 19.25 kg were provided an anthelmintic (LongRange® Injectable, Boehringer Ingelheim Vetmedica, Inc, Duluth, GA and Safe-Guard drench, Merck Animal Health, Kenilworth, NJ) and a growth promoting implant containing 40 mg trenbolone acetate and 8 mg estradiol (RevalorG®, Merck Animal Health, Kenilworth, NJ) approximately 14 days before the start of the trial.

Steers were limit fed a 50:50 dried distiller's grains with soluble/soyhull pellet mix at 2% body weight in a drylot for three days before the start of the trial to equalize gut fill. Cattle were individually weighed on two consecutive days at the end of the limit fed period. Before measures of body weight were collected, the scale was validated to 454 kg in 45.4 kg increments. The scale was recalibrated if any weight was >2.5% greater or less than the stated calibration weight. Six steers were randomly assigned to each pasture based on a stratification of initial body weights. The pastures were 1.8 ha resulting in a stocking rate of 2.0 animal units/ha. Steers continuously grazed the single pasture assigned throughout the experiment. A single day weight was collected for the interim weight on day 28 of the experiment and a 4% pencil shrink was applied across treatments. Cattle were provided free choice access to white salt for the duration of the trial.

On May 30 ± 4.9 days forage mass was (forage height less than 50mm as measured by rising plate meter) to be inadequate to support the steers in MET0 pastures. The same day steers from the experiment for all three years. Remaining steers were removed from pastures on the morning of day 57 of the experiment, limit fed with the DDGS/ soyhull pellet mix at 2% body weight for three days and weights recorded on the last 2 consecutive days.

Forage Analysis

Plate meter calibration clippings (n=5 per pasture) were dried in a forced air 55˚C oven to obtain partial dry matter (DM). The calibration clippings from each pasture were then composited into a single sample per plot and ground to pass through a 1-mm screen using a Wiley mill (No. 4, Thomas Scientific, Swedesboro, NJ). Ground samples were analyzed for non-sequential ADF, NDF (Van Soest et al., 1991), lab DM and CP. Lab DM was determined by drying samples in a 105˚C oven overnight. Nitrogen content was determined by combustion (LECO FB-428, LECO Corporation, St. Joseph, MI) in year 1 of the experiment. Frozen tall fescue tillers were lyophilized and ground using a Cyclotec mill (1093 sample mill, Foss A/S, Hillerod, DK) to pass through a 1-mm screen. Ground tiller samples were analyzed using HPLC for ergopeptine alkaloids (ergovaline, ergosine, ergotamine, ergocornine, ergocryptine, ergocrostine) using procedures described by Rottinghaus et al. (1991).

Statistical Analysis

Data were analyzed as a randomized complete block design using SAS version 9.4 (SAS Institute, Inc., Cary, NC). Seedhead frequency, steer weight gain, and botanical composition data points were averaged within pastures and the means of each were analyzed using the MIXED procedure with seedheads, weight, rate of gain, and botanical composition as dependent variables and treatment and block as independent variables. Block was initially included the model as a fixed effect. Block was not significant ($P \geq$

0.16) across the response variables and was removed from the model. Year was included in the model as a random effect in order to avoid differences in growing conditions from year to year. The LSMEANS function was used to separate means and calculate SEM. The PDIFF function was used when the *F*-statistic was significant ($P \leq 0.05$).

The CP, NDF, ADF, alkaloid and standing forage crop data were analyzed with repeated measures using the MIXED procedure with the model containing treatment, month, and treatment × month. Kenward-Roger denominator degrees of freedom adjustment was used for the repeated measures. Year was included as a random term. The repeated term was month with pasture × month serving as the subject. Compound symmetry was the covariance structure. The LSMEANS function was used to separate means and calculate SEM. The PDIFF function was used to separate means when the *F*-statistic was significant ($P \leq 0.05$). Tendencies were declared at ($P \leq 0.15$).

RESULTS AND DISCUSSION

Seedhead and Botanical Composition

Metsulfuron application decreased ($P<0.01$; Table 2.2) tall fescue seedhead production in pastures; average seedhead reduction in treatment groups was 68% compared to the negative control. Aiken et al. (2012) found a 93% reduction in seedhead concentration on pastures treated with metsulfuron. Israel et al. (2016) reported Metsulfuron applied alone or in combination with a second herbicide reduced seedhead density by 36 to 55% when compared to the nontreated control. Sather et al. (2013) found Metsulfuron-containing herbicides to reduce seedhead density in K31 tall fescue pastures from 14 to 61% when applied during the vegetative stage. Metsulfuron applied in the boot stage of plant growth equates to a greater seedhead suppression (Sather et al., 2013).

Findings in the current experiment are within range of what is to be expected for seedhead suppression, but efficacy of treatment is dependent on the timing of application. The herbicide inhibits the production of the branched chain amino acids; valine, leucine, and isoleucine, resulting in a stunting effect and delayed maturity (Brown, 1990). The varying levels of nitrogen within the metsulfuron treatment did not affect (P<0.20) seedhead production. An increase in the number of seedheads within pastures treated with fertilizer was not something we expected; however, Fairey and Lefkovitch (1998) showed an increase in the total mass of seedheads from pastures treated with greater concentrations of Nitrogen.

Botanical composition of pastures was measured June 14 ± 3.1 days. Frequency of quadrats containing K31 tall fescue did not differ (P=0.50) across treatment groups. Greater than 98% of quadrats measured contained K31 tall fescue. Kentucky bluegrass was reported in over 76% of quadrats but did not differ (P=0.30) among treatment groups. There was a tendency for orchardgrass to be greater (P=0.07) in the NEGCON, than the pastures receiving varying levels of Metsulfuron (MET0, MET67, MET134). Goff et al. (2014) reported spring metsulfuron application to tall fescue pastures tended to increase the density of non-fescue cool season forage species (Kentucky bluegrass and orchardgrass) after one application. However, amongst other forage species recognized, botanical composition did not change (P>0.20) in response to treatment. Frequency of birdsfoot trefoil was not affected (P=0.53) by treatment; however, it was only detected in 1 to 4% of quadrats across treatments. Clover and buckhorn plantain were not detected in pastures treated with metsulfuron but were detected in the control group. The lack of quadrats containing these species in the control group inhibited statistical analysis. The frequency of other forbs was less (*P*=0.01) in pastures treated with metsulfuron. This was expected as metsulfuron mainly targets forbs; Sather et al. (2013) reported forage legume groundcover was nearly eliminated on the metsulfuron treated portions of the pastures.

Alkaloid Production

Metsulfuron application did not affect (*P*=0.57; Figure 2.1) ergovaline concentration in tall fescue tillers collected. Most metsulfuron literature tested the effects of the herbicide on tall fescue under a single concentration of nitrogen fertilizer across the experiment. Israel et al. (2016) applied Nitrogen at a rate of 67 kg/ha to experimental pastures 2 weeks after the varying combinations of herbicide was applied. Nitrogen fertilizer was applied to all pastures in a study by Aiken et al. (2012) at a rate of 62 kg/ha approximately one month prior to the application of Metsulfuron. For the current experiment, applying varying levels of nitrogen fertilizer alongside the herbicide allowed for further investigation of the relationship between Nitrogen and Metsulfuron application. Israel et al. (2016) reported herbicide treatments containing Metsulfuron reduced total ergot-like alkaloid concentrations 26 to 34% when compared to the nontreated control. The reduction in seedhead concentration in tall fescue treated with Metsulfuron agrees with this reported decrease in alkaloid production, due to the seedheads having the greatest concentration of ergot-like alkaloids when compared to the leaf and stem.

Nitrogen application did not affect (*P=0.*57) ergovaline concentration in the current experiment. Lyons et al. (1986) watered tall fescue tillers twice weekly with solution containing 0.5 millimolar or 1.0 millimolar of Nitrogen. The greater level of nitrogen fertilizer increased total ergot-like alkaloid concentrations. Rottinghaus et al.

(1991) reported ergovaline concentrations in all parts of the plant (stem, leaves and seedheads) increased with nitrogen fertilizer. Conversely, nitrogen is vital nutrient for fungal endophyte growth (Naffaa et al., 1998). Delivering additional nitrogen to the plant, and the endophyte, may boost the fungi growth and alkaloid producing potential. Rottinghaus el al. (1991) observed an ergovaline increase by 81% in plant tissues as a result of the 135 kg/ha of nitrogen applied (similar N level to the current experiment), with the greatest ergovaline concentration in the stems and seedheads. The decreased production of seedheads and stems seen with metsulfuron application is thought to help avoid the large increase of ergovaline concentration commonly observed in response to nitrogen fertilizer; however, a reduction ergovaline concentration was not observed in this experiment. When tiller samples were collected, plant material was cut at ground level and not segregated into plant parts for analysis.

Pasture ergovaline concentration increased ($P<0.01$; Figure 2.2) across the three months of the experiment with the largest increase between May and June. Alkaloid concentrations commonly peak in late spring with seedhead extension, followed by a decrease in the summer as tall fescue goes dormant. Belesky et al. (1988) detected an increase in alkaloid concentrations, as seedheads developed, through the end of May. Peters et al. (1992) reported estimated ergovaline consumption of cow-calf pairs grazing K31 tall fescue to be greater in June than August. Ergovaline concentration varied in different portions of the fescue plant from May to June, with an exponential increase in ergovaline in seedheads while concentration in leave blades and stems stayed flat. (Rottinghaus et al., 1991).

Forage Nutritive Content and Yield

For all three years of the experiment, MET67 and MET134 had greater (treatment x month, $P<0.01$; Figure 2.3) biomass than NEGCON and MET0 in April. The increase in forage mass in April is a result of the nitrogen fertilizer that was applied on March 7 ± 3.6 days each year. Watson and Watson (1982) applied nitrogen at rates of 0, 67 and 134 kg/ha and reported dry matter yield increased with increasing rates of nitrogen, with the maximum forage yield pairing with the greatest rate of nitrogen (134 kg/ha). Dry matter yield of cool-season grasses can be increased by applying nitrogen fertilizers; spring application of nitrogen results in greater forage production when compared to a fall or winter application (Kroth et al., 1977). Forage nitrogen conversions result in the plant-available forms of nitrogen available as a nutrient source; however, nitrogen is water-soluble and easily leached into the soil before it is utilized by the plant for energy. Applied nitrogen increases the availability of nutrients for plant growth, as it is a major component of chlorophyll. The forage uses sunlight energy to produce sugars from water and carbon dioxide via photosynthesis. The increase in chlorophyl from Nitrogen, as well as being a major component of amino acids as a protein source, provide the plant with resources for growth and reproduction. Metsulfuron was applied 3 days prior to forage yield measurement and would have little effect on April forage due to the herbicide stunting forage growth over time, rather than affecting the forage stand that is already in the field.

In May, there was less (treatment x month, $P<0.01$) forage biomass in MET0 relative to other treatments. The MET0 treatment had the least biomass of all treatments, while MET134 forage mass was numerically greater than NEGCON and MET67. In May, herbage mass increased less across treatments that had metsulfuron applied. While not measured, this reduction in forage mass may be explained by an inversion of forage growth rate and forage consumed by the steers. Forage mass was less (P<0.01) for MET0 than NEGCON. We hypothesize this was due to metsulfuron application because reduced forage mass was reported when metsulfuron was applied to tall fescue during the boot stage of growth (Sather et al., 2013). The reduction in forage mass in MET0 pastures is not likely due differences in forage consumption by cattle. Depressed gain and lower DMI is reported when cattle consume endophyte infected tall fescue (Paterson et al., 1995). In the current experiment, tall fescue samples collected from MET0 and NEGCON had >200 ppb Ergovaline in May, which is above the toxic threshold (150 ppb; Stamm et al., 1994), thus it is plausible that cattle were experiencing fescue toxicosis irrespective of treatment.

Forage mass increased irrespective of treatment from May to June. There was a large increase in forage mass observed for MET0 pastures in June as they were ungrazed from May 30 ± 4.9 days each year until forage collections on June 14 ± 3.1 days. Williamson (2015) reported continuously grazed tall fescue pastures treated with Metsulfuron allowed selective grazing of vegetative tall fescue. This overgrazing led to the accumulation and maturation of forage, causing the lower quality forage to be grazed out of necessity in the latter parts of the grazing season (Williamson, 2015). Aiken et al. (2012) reported visual observation of preferential consumption of vegetative tall fescue (treated with metsulfuron) in a continuous grazing system, likely causing the aboveground herbage mass of the fescue to be reduced. The yield loss in K31 tall fescue due to Metsulfuron treatment is thought to be irrelevant, the reduced forage mass is likely due to less stems and seedheads in pastures as the forage is maintained in a vegetative growth stage (Aiken et al., 2012).

There was no effect of treatment (*P* = 0.56; Figure 2.4) for neutral detergent fiber (NDF), but NDF increased by month (*P* <0.01). There was no effect of treatment (*P* = 0.51; Figure 2.5) for acid detergent fiber (ADF), but ADF increased by month (*P* <0.01). This is to be expected as tall fescue matures and develops seedheads in the late spring (Sinclair et al., 2006). Typically, there is a decline in forage quality when the plant transitions from a vegetative stage to a reproductive stage of growth (Glenn et al., 1980). Sather et al. (2013) reported boot-stage application of metsulfuron-containing herbicide resulted in NDF concentrations that were less than nontreated controls. There was a significant response to ADF content with boot-stage applications, reducing the ADF content when metsulfuron-containing herbicide was applied. This was not seen in the current experiment. However, Sather et al. (2013) differed from the current experiment in that it was a small-scale plot study, while the current was pasture-level research with grazing animals. Additionally, Sather et. al (2013) study reported hand removal of any weeds that emerged in the experimental areas so as not to interfere with forage yield or nutritional value data. In the current experiment, forage was collected by clipping all forage present above the soil inside a 0.1 m^2 quadrat, there was no weed removal strategies incorporated into the collection.

We did not observe a time by treatment interaction (*P*=0.84; Figure 2.6) for crude protein (CP), and there was no difference (*P* = 0.46) among treatments. However, there was a difference (*P* <0.01) in CP concentration across the three months of the experiment. CP concentration decreased as the fescue matured from April to May as expected and stayed constant through June. Wolf and Opitz von Boberfeld (2003) reported nitrogen application to tall fescue can allow for greater protein synthesis (and forage CP concentration) when nitrogen is the limiting factor for plant growth; however, this was not seen with the current experiment. Metsulfuron did not appear to impact CP concentrations as there were similar concentrations reported for MET0 and NEGCON. Sather et al. (2013) reported no effect of metsulfuron on forage CP concentration.

Steer Performance

Initial steer body weight did not differ ($P = 0.61$; Table 2.3) among treatments. Intermediate steer body weight (collected on day 28) and final steer body weight (collected on day 57) did not differ ($P > 0.21$). The final body weight included only NEGCON, MET67 and MET134 treatment groups. Steers grazing pastures assigned to MET0 were not included in the analysis of the second period as they were removed from pastures on May 30 ± 4.9 days each year due to insufficient forage mass.

For period one of the experiment, day 0 to day 28, there was a tendency (P=0.06) for the NEGCON ADG to be less than other treatments. While forage mass differed across treatments in April, all treatments averaged over 2,000 kg/ha. Allden and Whittaker (1970) established a grazing threshold of 2,000 kg/hectare of available forage will not limit intake potential for the animal. In these conditions, livestock can readily select a diet of their choice in an acceptable grazing time. Findings from this experiment were likely not due to a nitrogen fertilizer effect among treatment groups as the ADG of steers grazing MET0 pastures were not different from the ADG of steers grazing MET67 and MET134.

Ergovaline concentration in forage samples collected in May and June (Figure 2.1) was well above the toxic threshold (150 ppb; Stamm et al., 1994) for all three months of the experiment, across all treatments. Thus, it seems unlikely that alkaloids impacted steer gains differently across treatment groups during this study. Aiken et al. (2012) reported steers grazing metsulfuron treated tall fescue pastures had an increase in steer gain. Improved performance was discussed as improved forage quality and decreased alkaloid concentration in forage; however, application of metsulfuron did not decrease alkaloid concentration or affect forage quality in the current experiment. Metsulfuron application to tall fescue pastures reduces total ergot-like alkaloid reservoirs by reducing seedhead concentrations and improves forage quality. Aiken et al. (2012) credited those two factors to his findings of a 39% increase in ADG of growing steers in response to metsulfuron application. There was a month effect ($P < 0.01$; Figure 2.2) for ergovaline concentration (May to June). This effect may have caused the reduction in steer performance during the final 28 days of the experiment; however, untangling the confounded effects of declining forage quality and increasing alkaloid concentration and environmental stress on cattle ADG in a pasture setting is difficult. Diaz et al. (2018) found as ergovaline concentration in the diet increased, cattle ADG decreased, which matches what was reported in the current experiment. Reduced performance in period 2 could be attributed to declining forage quality. Increasing forage maturity is associated with increase in GI fill and reduction in turnover rates, suggesting that mature forage could be limiting particle reduction and turnover with digestion (Telford, 1980). Environmental conditions may also explain reduced performance in period 2. Steudemann et al. (1985) found inconsistencies in grazing behavior of steers grazing varying levels of endophyte infected tall fescue. Steers grazing endo-phyte infected tall fescue spent less time grazing than steers on E- tall fescue especially during the warmest period of the day. Hemken et al. (1981) observed a 50% reduction in DMI for heat stressed calves; the reduction in grazing time measured in this experiment may be due to heat stress. ADG was not different ($P = 0.86$; Table 2.3) for period two across treatment groups but was less than the ADG reported in period one.

Reduced nutritive value of forage in the last 28 days of the experiment may have influenced poor steer gain reported, irrespective of treatment, from the first to second 28 days (1.13 to 0.65 kg/d). There was no difference in ADF ($P = 0.51$; Figure 2.5), NDF ($P = 0.56$; Figure 2.4) or crude protein ($P = 0.46$; Figure 2.6) among treatment groups across the three-year experiment. However, there was a month effect ($P < 0.01$) for crude protein and NDF; crude protein was lowest and NDF was at its highest in the last 28 days.

Overall performance was not different ($P = 0.29$; Table 2.3) among treatment groups. Aiken et al. (2012) conducted a similar experiment, grazing steers on fescue pastures treated with Metsulfuron, however those animals were grazed for 83 days in year one and 92 days in year two at a slightly greater stocking rate (2.7 animal units per hectare). Steer grazing MET0 in the current experiment showed a lesser ADG than what was reported by Aiken et al. (2012); however, steers grazing NEGCON pastures showed a greater ADG. We attribute the differences between studies to length; steers in the current experiment grazed for 57 days each of the three years.

SUMMARY AND CONCLUSION

The application of metsulfuron to K31 tall fescue pastures reduced seedhead concentration while nitrogen fertilizer improved forage yield, largely overcoming yield drag reported with metsulfuron application to tall fescue pastures. There were no differences among treatments (NEGCON, MET0, MET67, MET134) for ergovaline concentration in forage samples. Reduced seedhead concentration and improved forage growth should result in improved cattle performance and the ability to increase stocking rate of pastures. However, there were no differences in ADG of the steers or forage quality due to treatment, which may be due to the continuous stocking rate applied to pastures throughout the experiment.

Table 2.1 University of Missouri Southwest Center precipitation data, centimeters per month

Month	2019[1]	2020[1]	2021[1]	Historical average[2]
March	8.36	20.12	15.72	9.42
April	12.34	12.52	10.24	10.67
May	34.67	26.82	18.42	12.73
June	15.47	4.45	13.64	13.31

[1]http://agebb.missouri.edu/weather/stations/lawrence/index.htm
[2]https://wrcc.dri.edu/ (Period of record: 1960 – 2013)

Table 2.2 Tall fescue seedhead concentration & species frequency in infected[1] K31 tall fescue pastures, treated with metsulfuron herbicide and nitrogen fertilizer application[2]

Item	NEGCON	METO	MET67	MET134	SEM[3]	*P*-value
Seedheads, # per 0.1 m^2	9.60[a]	2.41[b]	3.06[b]	3.65[b]	0.996	<0.01
Frequency, % of quadrats containing						
Tall fescue	99.44	98.61	98.89	98.89	0.553	0.50
Kentucky bluegrass	76.11	82.75	86.11	85.56	4.086	0.30
Orchardgrass	15.60	5.30	10.32	18.09	5.139	0.07
Other grasses	9.72	10.28	6.94	8.61	3.413	0.91
Clover	7.38	0.00	0.00	0.00	-	-
Birdsfoot trefoil	1.65	3.31	2.45	2.45	2.450	0.53
Buckhorn plantain	1.33	0.00	0.00	0.00	-	-
Other forbs	7.22[a]	4.44[ab]	1.39[b]	1.67[b]	1.37	0.01

[1]88% endophyte infection rate

[2]Nitrogen applied March 7th ± 3.6 days and metsulfuron applied 72 hours prior to the cattle being turned out to graze each of the three years

[3]SEM = standard error of means

[a b]Means within rows lacking common superscripts differ ($P<0.05$)

Table 2.3 Steer performance grazing infected[1] K31 tall fescue in spring, following metsulfuron herbicide and nitrogen fertilizer application[2]

	NEGCON	MET0	MET67	MET134	SEM[3]	*P*-value
BW, kg						
Initial (d0)	268	264	267	264	2.2	0.61
Intermediate (d28)	292	297	300	297	3.5	0.21
Final (d57)	304	-[4]	310	306	3.6	0.49
ADG, kg						
Period 1 (d0 to d28)	0.96	1.26	1.27	1.27	0.112	0.06
Period 2 (d29 to d57)	0.30	-[4]	0.23	0.23	0.121	0.86
Overall (d0 to d57)	0.65	-[4]	0.76	0.76	0.058	0.29

[1]88% endophyte infection rate
[2]Nitrogen applied mid-March and metsulfuron applied mid-April each of the three years
[3]SEM = standard error of means
[4]ADG for period 2 and overall, not included as MET0 steers were removed May 30 ± 4.9 days due to insufficient forage mass

Figure 2.1 Effect of metsulfuron herbicide and nitrogen application on ergovaline concentration in K31 tall fescue pastures. Treatment effect ($P = 0.57$); SEM = 71.463.

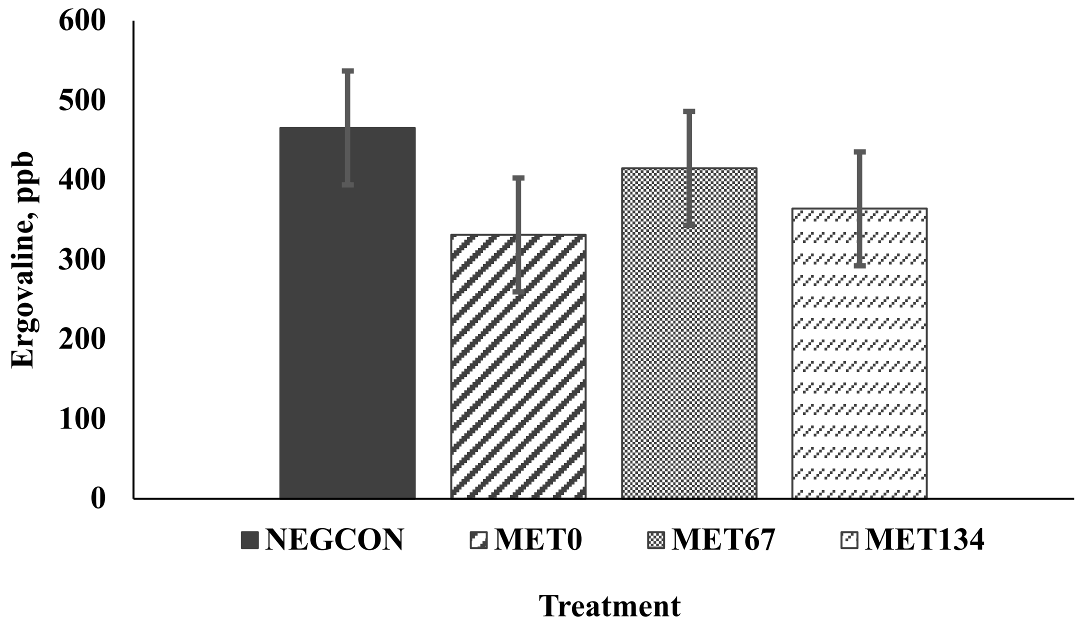
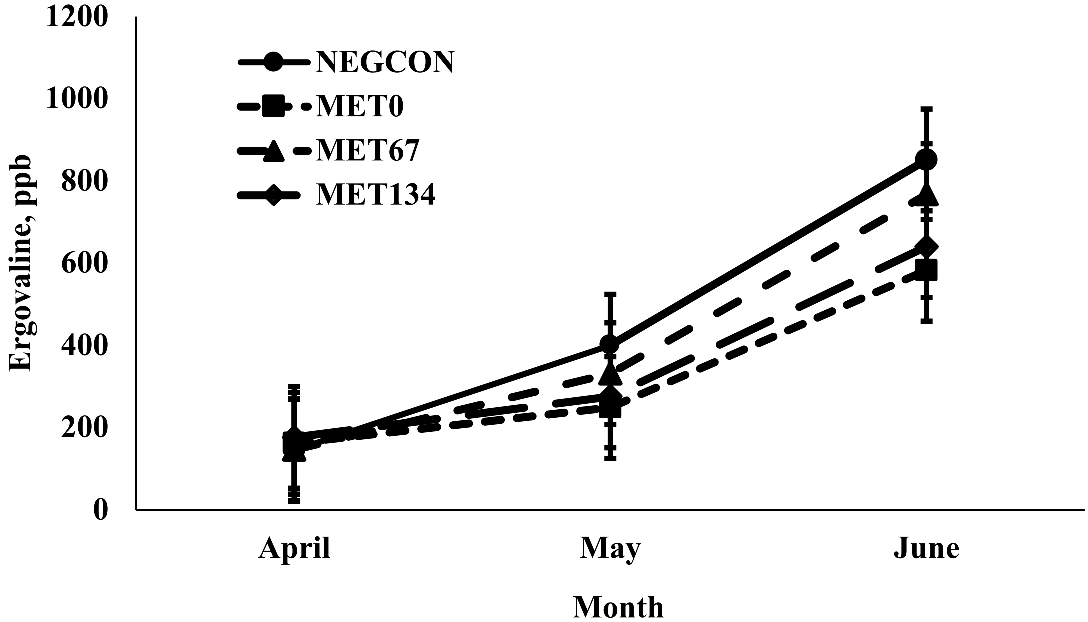
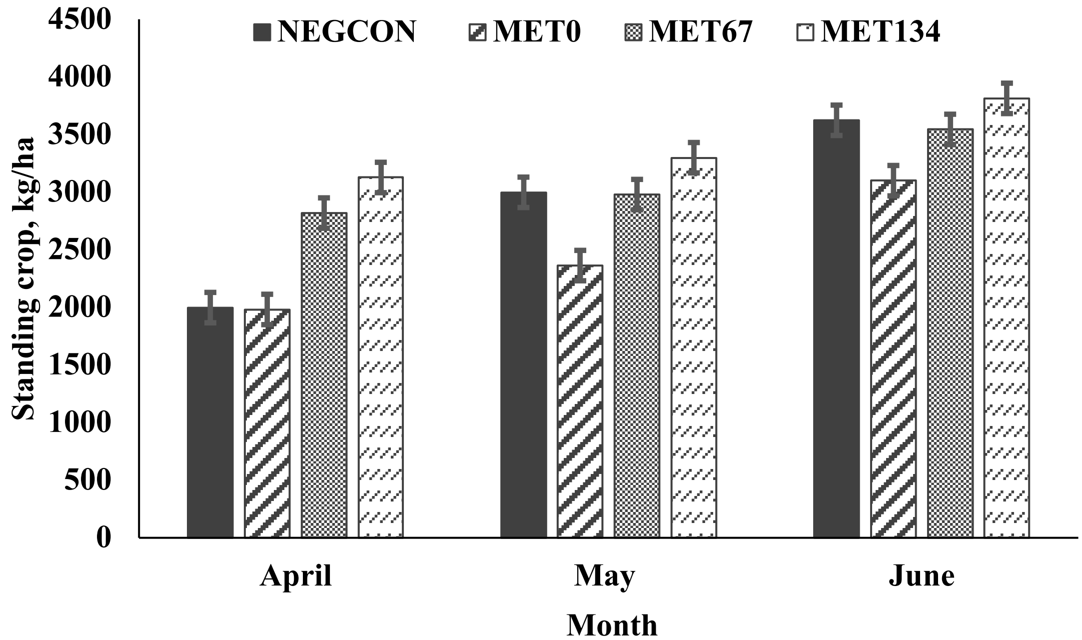


Figure 2.2 Effect of metsulfuron herbicide and nitrogen application on ergovaline concentration[1] in K31 tall fescue pastures. Treatment effect ($P = 0.57$); month effect ($P < 0.01$); month*treatment interaction ($P = 0.94$); SEM = 123.78.



[1]Data reported are from 2019 and 2021, 2020 samples were lost due to freeze dryer malfunction.

Figure 2.3 Effect of metsulfuron herbicide and nitrogen application on forage mass in K31 tall fescue pastures[1]. Treatment*month interaction ($P < 0.01$); SEM = 131.93.



[1]Pastures were continually stocked at 3.3 steers/ha throughout the experiment

Figure 2.4 Effect of metsulfuron herbicide and nitrogen application on monthly neutral detergent fiber concentrations in K31 tall fescue pastures. Treatment effect (*P* = 0.56); month effect (*P* < 0.01); month*treatment interaction (*P* = 0.99); SEM = 1.608.

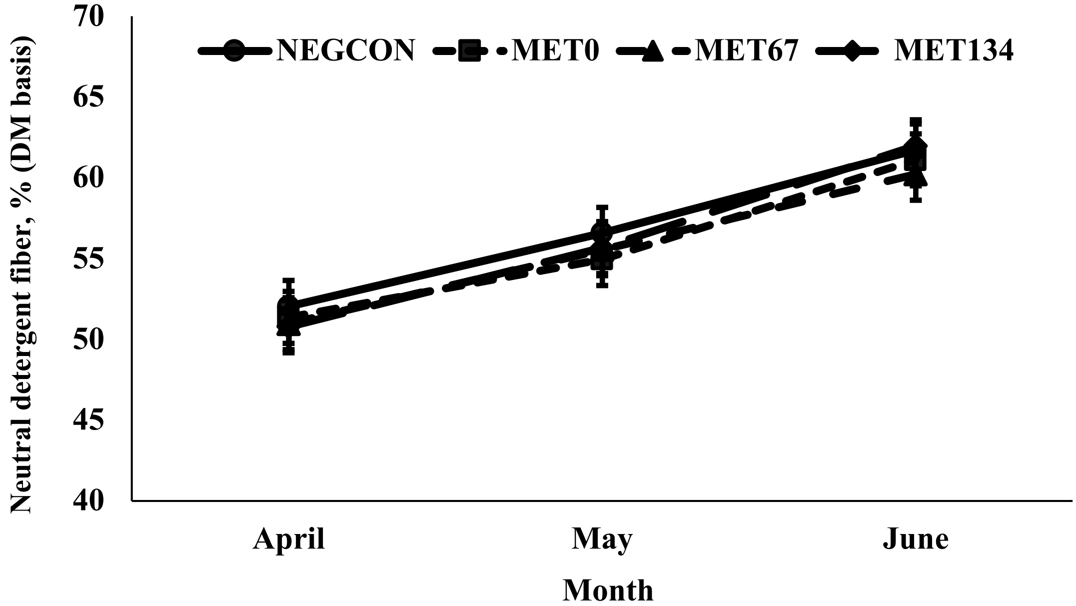


Figure 2.5 Effect of metsulfuron herbicide and nitrogen application on monthly acid detergent fiber concentrations in K31 tall fescue pastures. Treatment effect ($P = 0.51$); month effect ($P < 0.01$); month*treatment interaction ($P = 0.99$); SEM = 1.860.

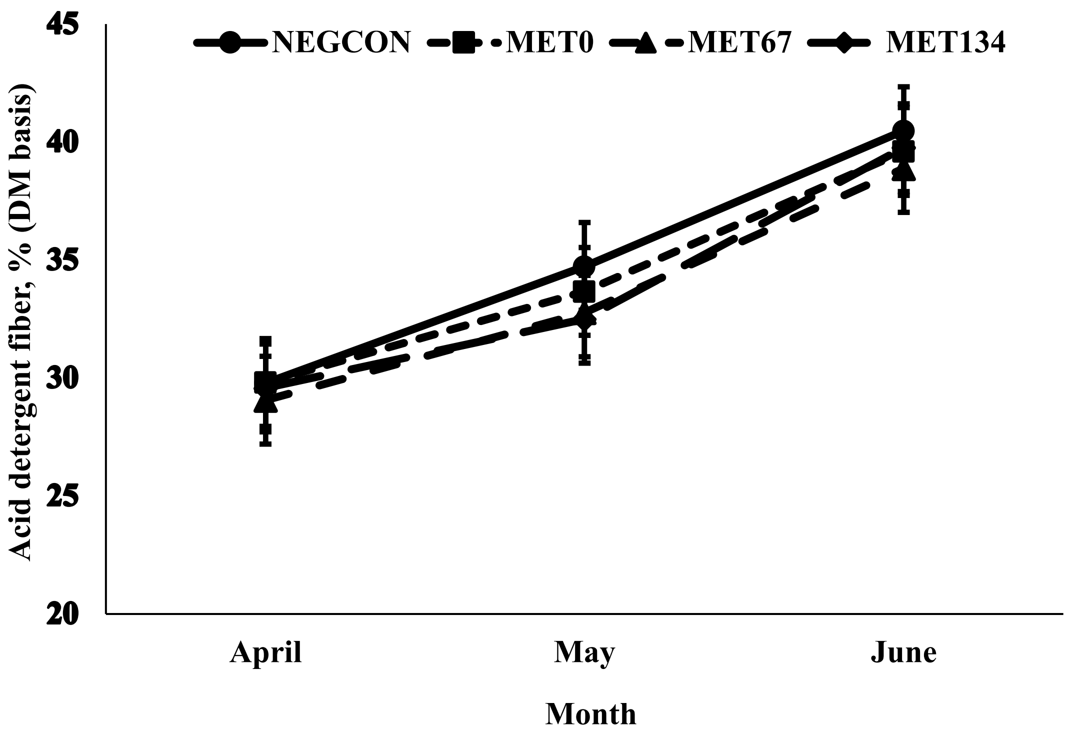
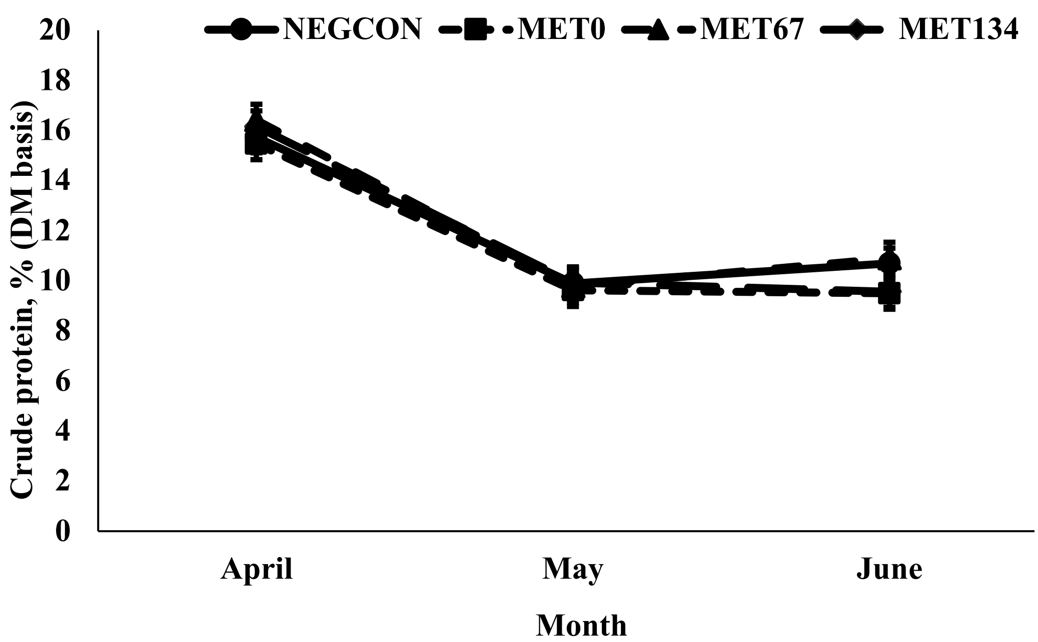


Figure 2.6. Effect of metsulfuron herbicide and nitrogen application on monthly crude protein concentrations in K31 tall fescue pastures. Treatment effect (P = 0.46); month effect (P < 0.01); month*treatment interaction (P = 0.84); SEM = 0.636.



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