NOVEL ALKALOID MANAGEMENT STRATEGIES FOR IMPROVED BEEF CATTLE PRODUCTION IN THE FESCUE BELT

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ABSTRACT

Tall fescue (*Schedonorus arundinaceus*) is an important forage crop for agriculture in Missouri and much of the eastern United States. Tall fescue is known for hardiness and herbage accumulation, much of which is attributed to a symbiotic relationship with the fungal endophyte *Epichloë coenophiala*. This endophyte produces alkaloids, which negatively affect livestock performance after consumption. Tall fescue is a species well adapted to Missouri forage systems and takes considerable resources and time to remove from pastures and replace with a new species. Due to these negative factors, most research efforts have focused on managing tall fescue to reduce toxicity risk. One strategy includes reducing alkaloid load by removing one of the most toxic part of the plant (seedheads). The results will be presented from research projects using prescribed fire on infected tall fescue plots and metsulfuron plus nitrogen on pastures grazed by stocker cattle as management strategies to reduce toxicity and improve productivity of tall fescue forage systems. Prescribed burning of tall fescue in April resulted in reduced seedheads by 49%, lower alkaloid concentrations and improved forage nutritive value in months following burning. Metsulfuron herbicide reduced seedhead density by 80% and decreased ergovaline production while nitrogen fertilizer recovered forage yield losses due to metsulfuron. Seedhead reduction in response to prescribed fire and metsulfuron has potential for reducing alkaloid exposure when grazing endophyte-infected tall fescue.

CHAPTER 1. REVIEW OF LITERATURE

Tall fescue

Tall fescue, a cool season C3 grass, was thought to have been introduced to the United States from Europe through contaminated grass seed (Hoveland, 1993). In 1931, an ecotype of tall fescue found in the hills of eastern Kentucky gained attention for its high productivity and hardiness and the Kentucky Agricultural Experiment Station began researching it soon after (Fergus and Buckner, 1972). Seed from this ecotype, now called Kentucky 31, was released in 1942 and over the following decades was rapidly adopted by farmers in the eastern United States (Fergus and Buckner, 1972). Today, tall fescue covers 14 million ha in the eastern USA with Kentucky 31 being the main variety (Buckner et al., 1979). It was discovered that Kentucky 31 along with other ecotypes possess a symbiotic relationship with an endophytic fungus (Bacon et al., 1977). The symbiotic relationship with tall fescue plants provides productivity and hardiness that popularized tall fescue.

Under normal conditions, endophyte-infected (E+) tall fescue produces more herbage and tillers than non-infected tall fescue (Clay, 1987), and expresses greater drought tolerance than non-infected (E-; Arachevaleta et al., 1989; West et al., 1988). Arachevaleta et al. (1989) showed that E+ tall fescue produces more dry herbage under low and moderate drought stress. However, three out of four E- plots died in high stress conditions compared to no deaths for E+. Endophyte infection increased germination and more than doubled percentage of filled seeds, furthering their competitive advantage over E- tall fescue (Clay, 1987).

Improved pest resistance is also a contributing factor to greater productivity. West et al. (1988) measured fewer nematodes per 100 ml of soil samples near E+ tall fescue. Johnson et al. (1985) observed significantly fewer *Rhopalosiphum padi*, *Schizaphis graminum*, and *Rhopalosiphum maidis* aphids preferring E+ plant tissue compared to E-. In addition, virtually no aphids confined with E+ plants were alive after three days compared to aphids confined with E- plants, where the population thrived and grew in 10- 15 days of confinement.

Fescue toxicosis

While endophyte-infected tall fescue has many positive agronomic attributes, poor performance of cattle grazing infected tall fescue is observed despite a nutrient profile supportive of greater animal performance than what is achieved (Hoveland et al., 1980; Hoveland et al., 1983; Read and Camp, 1986). The endophyte produces alkaloids that contribute to numerous production and health issues in grazing livestock (Bacon et al., 1977). The aggregated health issues associated with alkaloid ingestion are referred to as, "fescue toxicosis". This poses a significant financial issue for producers, as Strickland et al. (2011) estimated losses in the US of over $1 billion annually.

Fescue toxicosis is separated into two symptom groups: summer slump and fescue foot. Summer slump occurs at increased temperatures and is associated with poor performance of livestock. Consumption of endophyte-infected tall fescue has been linked to reduced pregnancy rates, poor cattle growth, and decreased milk production directly resulting in decreased weaning weights (Hoveland, 1993).

Depressed weight gain by grazing livestock was an early indicator of all fescue toxicity reported (Hoveland et al., 1980; Hoveland et al., 1983; Read and Camp, 1986). Read and Camp (1986) reported beef steers grazing low endophyte-infected tall fescue had double the average daily gain (ADG) of steers grazing high infected tall fescue. A causative agent of depressed gain is reduced dry matter intake (DMI; Paterson et al., 1995). Stuedemann et al. (1985) observed a change in grazing behavior of steers grazing either high or low endophyte infected tall fescue. Steers on E+ tall fescue spent less time grazing than steers on E- tall fescue especially from 12:00 PM to 4:00 PM, the warmest period of the day. This behavioral change was sustained even after removal from E+ tall fescue for up to 26 days. Heat stress likely caused reduced grazing time in this experiment. Hemken et al. (1981) reported a 50% reduction in DMI for heat stressed calves.

Ambient temperature plays a significant role in fescue toxicosis. Hemkin et al. (1981) placed Holstein calves in temperature-controlled rooms at three different temperatures (10-13 ºC, 21-23 ºC, and 34-35 ºC) and fed them either high endophyte or low endophyte tall fescue. They noted decreased growth and dry matter intake only in the high-temp treatment. Both rectal temperature and respiratory rate were elevated at 34-35 C˚ when compared to low endophyte tall fescue, indicating heat stress. Crawford et al. (1989) exposed Holstein heifers to varying levels of infected tall fescue over summer months and measured growth over time. Heifer ADG decreased by 0.05 kg for every 10% increase in endophyte infection.

Consumption of endophyte-infected tall fescue is associated with decreased reproduction in cattle. Brown et al. (1992) observed a decrease of 7.6% in calving rate for cows grazing E+ tall fescue verses bermudagrass. Danilson et al. (1986) recorded decreasing pregnancy rates (95.8%, 81.8%, and 54.5%, respectively) as infection increased from low (0-20%), to medium (25-60%) and high (80-99%).

Alkaloid consumption also reduces milk production in cattle. In one study, milk yield and percent milk fat decreased when Angus, Brahman, and reciprocal cross cows were grazing E+ tall fescue versus Bermudagrass (Brown et al., 1996). Peters et al. (1992) observed a 25% decrease in milk production determined by weigh-suckle-weigh in cows grazing E+ versus E- tall fescue, along with reduced offspring weight gain. This decrease in milk production likely caused decreases in calf gains.

The second condition of fescue toxicosis is known as, “fescue foot.” Symptoms include gangrene of tail, feet and ears, rough coat, swelling of coronary bands and sloughing of the hoof. Opposite to summer slump, fescue foot is prevalent in colder months. Williams et al. (1975) observed lameness, swelling of coronary bands, and discoloration of tails when calves were injected with tall fescue extracts. These symptoms are due to peripheral vasoconstriction caused by the tall fescue toxins (Rhodes et al., 1991).

Several studies have recorded differences in the hair coats of cattle grazing E+ versus E- tall fescue (Hemken et al., 1981; Hoveland et al., 1983; Read and Camp, 1986; Peters et al., 1992). McClanahan et al. (2008) assigned calves grazing E+ tall fescue to treatments of hair unclipped or clipped monthly to a length of 6mm. Rectal temperature for unclipped calves was elevated compared to clipped on measurement dates with ambient temperatures above 25˚C. It is likely that the rough hair coats of unclipped calves led to more insulation of core body temperature and possibly increased heat stress. Aiken et al. (2011) bleached two patches of hair on steers grazing E+ tall fescue in order to observe hair growth and shedding. One patch was clipped June 7 and the second on July 5. Clipped hairs completely bleached were considered un-shed winter hairs, hairs with some bleach were summer growing hairs and non-bleached hairs were summer growing hairs following the shedding of winter hairs. They observed summer growing hair that grew to excessive lengths and winter grown hair that did not shed. They cited prolactin as a possible mechanism of this phenomenon, as it is involved in the hair growth cycle. Serum prolactin declined in cattle consuming alkaloid-enriched diets.

Ergot-like alkaloids

When endophyte infected forages became associated with poor ruminant performance, researchers made efforts to learn what compounds were directly responsible. Fungi from the genera Epicholë, Neotyphodium, and Balansia found in tall fescue have been shown to produce ergot-like alkaloids (Lyons et al., 1986).

Ergot-like alkaloids are separated into classes of clavine alkaloids, lysergic acid and derivatives, and ergopeptine alkaloids (Strickland et al., 2011). Alkaloids contain an ergoline ring that is similar to several endogenous neurotransmitters (Strickland et al., 2011). Alkaloids 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 several reactions in the body. Vasoconstriction is regarded as the primary response to alkaloid consumption. These compounds alter blood flow and hormone release in a range of tissues.

The hormone prolactin is affected by alkaloids absorbed into the bloodstream. Serum prolactin concentrations are commonly decreased by over 50% in cattle consuming infected tall fescue (Aiken et al., 2009; Foote et al., 2013). Dopamine inhibits prolactin secretion (Lamberts and Macleod, 1990) and alkaloids are known to be dopamine agonists (Larson et al., 1995, 1999). Prolactin plays a role in the initiation and maintenance of lactation (Akers et al., 1981) but suppressed serum prolactin is commonly used as an expression of alkaloid activity in ruminants (Thompson and Stuedemann, 1993).

Peripheral tissue dysfunction is commonly associated with fescue toxicosis. Oliver et al. (1993) and Klotz et al. (2013) observed vasoconstriction in biopsied bovine lateral saphenous veins and dorsal metatarsal arteries in response to alkaloids. Sheep also displayed decreased blood flow in leg skin when fed a high endophyte versus a low endophyte diet (Rhodes et al., 1991). This reduced blood flow to the leg could play a role in lameness and sloughing of the hoof. Walls and Jacobson (1970) and Aiken et al. (2009) created a vasoconstrictive response in tails of heifers by feeding alkaloid-enriched diets, which could be a cause of gangrene symptoms observed in livestock grazing endophyte infected tall fescue.

Skin temperature in a range of locations is decreased in animals consuming E+ versus E- tall fescue (Walls and Jacobson, 1970; Rhodes et al., 1991; Osborn et al., 1992; Browning and Browning, 1997). This reduced skin temperature has been attributed to reduced blood flow to skin (Osborn et al., 1992). Additionally, increased rectal temperature and respiration rate is reported in response to alkaloid consumption in multiple species (Hemken et al., 1981; Osborn et al., 1992; Browning and Browning,

1997) indicating heat stress, which could play an important role in decreased intake, poor growth, and reproductive performance symptoms of the summer slump.

In addition to the peripheral, alkaloids also affect blood flow to internal organs. Dyer (1993) exposed bovine uterine and umbilical arteries to ergovaline in vitro and observed a potent vasoconstrictive response in both tissues. Reduced blood flow to uterus and fetus could be the cause of poor pregnancy rate observed in cattle grazing E+ tall fescue. Foote et al. (2013) dosed the rumen of cannulated steers with either E+ or E- tall fescue seed daily. After 8 days the rumen was vacuumed out, rinsed and a buffer solution was allowed to incubate for 30 minutes. Blood flow to the reticulorumen was reduced from 28.4 to 20 L/h for E+ steers and VFA flux from rumen was also decreased. This compromised absorptive capacity of the rumen would also be a factor in depressed performance by cattle consuming alkaloid-enriched diets.

Alkaloid production

Alkaloid concentration in E+ tall fescue fluctuates throughout the growing season. Ergopeptine alkaloids peak in late spring, decrease through summer followed by a second spike in the fall for both grazed (Belesky et al., 1988) and ungrazed (Rottinghaus et al., 1991) fescue. A difference of alkaloid concentration among plant parts also exists in addition to temporal variation in alkaloid concentration. Rottinghaus et al. (1991) reported mature seedheads as being the largest reservoir of ergovaline in June, but Kenyon et al. (2018) observed greatest ergovaline concentrations in 0- to 5-cm segment at the base of the plant in October. Goff et al. (2012) observed cattle selectively grazing infected tall fescue seedheads. Grazing behavior like this would result in a high concentration of alkaloids in cattle diets. When tall fescue was stockpiled over the winter, ergovaline concentrations steadily declined until early March (Kallenbach et al., 2003).

Nitrogen fertilizer is used to increase forage production in tall fescue swards. Bélanger et al. (1992) fertilized tall fescue with N at increasing levels in the spring and summer and measured resulting shoot growth over the following growing period. Shoot dry matter increased by an average of 168% for spring growth and 408% for summer growth. Nitrogen fertilization consistently increases alkaloid concentration in E+ tall fescue (Lyons et al., 1986; Rottinghaus et al., 1991). Rottinghaus et al. (1991) measured ergovaline concentration of leaf blades, stems/sheaths and seedheads from plots applied with either 0, 67.4, or 134.8 kg/ha of nitrogen. Ergovaline increased by 227, 509 and 593 μg/kg in leaf blades, stems/sheaths and seedheads, respectively. Form of nitrogen fertilizer may also influence alkaloid production (Arechavaleta et al., 1992; Rogers, 2010). Kulkarni and Nielsen (1986) cultured the fungal endophyte in different nitrogen sources and observed growth in ammonium chloride and ammonium nitrate cultures and little growth in potassium nitrate or urea cultures. Conversely, Naffa et al. (1998) reported growth of the endophyte in all four nitrogen sources. Rogers (2010) fertilized tall fescue with either ammonium nitrate or poultry litter and measured ergovaline concentrations in response. Poultry litter fertilization resulted in a 25% less ergovaline compared to ammonium nitrate. Rogers hypothesized this reduction was partially due to the lower bioavailability of poultry litter compared to ammonium nitrate.

Alkaloid metabolism and absorption in the rumen

The mechanism of how alkaloids are metabolized in ruminants remains unclear. Hill et al. (2001) showed that when consumed by ruminants, the alkaloids lysergic acid, lysergol, ergonovine, ergotamine, and ergocryptine are actively transported across rumen and reticulum wall. Ayers et al. (2009) suggests that the rumen, omasum and reticulum are the main site of absorption for alkaloids. However, Ayers et al. (2009) also demonstrated minimal ability of ergovaline to be absorbed in the rumen. This contradicts popular views that ergovaline is the main alkaloid driving fescue toxicosis, as it would have little effect if unable to be absorbed by the ruminant. Hill et al. (2001) alternatively showed other ergopeptine alkaloids (ergonovine, ergotamine, and ergocryptine) which ergovaline is of the same class, to possess some transport potential but still at a fraction of lysergic acid's potential for transport across the rumen epithelium.

Despite poor absorption, ergovaline may still play a significant role in fescue toxicosis. Ergovaline and other members of the ergopeptine family are potent agonists of neurotransmitters (Larson et al., 1995). Larson et al. (1999) revealed lysergic acid's ability to agonize neurotransmitters to be $1/100^{th}$ of ergovaline and ergotamine tartate. This would allow low concentrations of ergovaline absorbed to elicit a significant vasoconstrictive response.

However, ergovaline is likely metabolized in the rumen, which may create molecules that contribute to the etiology of fescue toxicosis. De Lorme et al. (2007) fed E+ tall fescue to lambs and measured the concentration of different alkaloids in feces and urine. Digestibility of ergovaline was 64.2% and -12.5%, respectively, for lysergic acid.

De Lorme et al. (2007) reported 35% of dietary ergovaline and 248% of lysergic acid was collected from bodily excretions, indicating that ergovaline was metabolized into lysergic acid. Metabolism likely took place in the rumen by ruminal microbes since ergovaline is poorly absorbed. Lysergic acid is more readily absorbed than ergovaline. Thus, it seems that ergovaline contributions to fescue toxicosis may be a function of both ergovaline directly absorbed from the diet and lysergic acid metabolized from dietary ergovaline by ruminal microorganisms.

Management solutions

A myriad of management practices have been developed to mitigate impacts of alkaloids on ruminant production. One option is the renovation of infected tall fescue to a non-toxin producing, or “novel” endophyte variety. Replacement if toxic tall fescue with non-toxic tall fescue involves spraying infected tall fescue with herbicide (e.g., glyphosate) in early spring before becoming reproductive planting a smother crop, haying this smother crop, spraying regrowth with herbicide, no-till drilling novel tall fescue seed into stubble and applying fertilizer (Gunter and Beck, 2004). Novel endophyte varieties have been shown to possess similar forage productivity and stand persistence as infected tall fescue (Gunter and Beck, 2004). Renovation of tall fescue costs over $600/ha and was only suggested if expected forage production potential is significant and endophyte infection rate exceeds 35% (Kallenbach, 2015).

Conversion of K31 to novel tall fescue varieties increased ADG of beef cattle by 47% from studies in five different states due to the absence of alkaloids (Gunter and Beck, 2004). While potential cattle production increase is high for this strategy, it is not an ideal solution for all. Producers that lease grassland will be less willing to make this investment in someone else's land. Additionally, not all pastures are suitable to be renovated.

Tall fescue stockpiled through winter has decreasing ergovaline levels starting in January (Kallenbach et al., 2003). Ergovaline concentration decreased in stockpiled tall fescue by 85% by March with minimal deterioration of forage nutritive value (Kallenbach et al., 2003). Differed grazing of fall growth would allow producers to reduce alkaloid consumption. Studies have also shown that haying tall fescue grass substantially reduces alkaloid concentrations (Roberts et al., 2002; Norman et al., 2007). Ergovaline level is further reduced by 30% when hay is subjected to ammoniation (Kallenbach et al., 2006).

Dilution of toxic tall fescue with other feedstuffs in cattle diets is an approach that can increase cattle performance (Kallenbach 2015). This is achieved through interseeding legumes or providing supplemental feed. Increased cattle performance from addition of legumes has been observed but not proven to be the result of alkaloid dilution (Aiken and Strickland, 2013). Increased cattle gains from legume interseeding may be the result of a greater quality diet rather than diluted alkaloid consumption (Aiken and Strickland, 2013). A true dilution effect has been observed with use of supplemental grains or byproducts as supplements provided additional increases in animal performance for supplementation on toxic tall fescue compared to novel tall fescue (Kallenbach, 2015).

Another strategy to mitigate effects of alkaloid is eliminating seedheads before consumption by livestock. Increased stocking density can be used to keep tall fescue in a vegetative state and discourage seedhead growth (Paterson et al., 1995). Bransby et al. (1988) reported ADG of cattle increased as stocking density increased on infected tall fescue compared to the typical response of ADG decreasing with increased grazing pressure observed in noninfected plots. A second method to remove seedheads is chemical suppression. Reynolds et al. (1993) used six different plant growth regulators (sethoxydim, fluazifop-*p*-butyl, glyphosate, haloxyfop methyl, mefluidide, and amidochlor) applied in April to tall fescue plots and measured forage and seedhead production. Only sethoxydim, haloxyfop methyl and mefluidide consistently reduced seedhead numbers. Forage production decreased across treatments by 53, 34 and 50% in year one and 65, 58 and 50% in year two for sethoxydim, haloxyfop methyl and mefluidide, respectively. Moyer and Kelley (1995) applied the plant growth regulator metsulfuron on tall fescue and recorded a 59% reduction in seedheads and 54% decrease in forage production.

Turner et al. (1990) applied mefluidide to tall fescue pasture in early spring and observed increased weight gain in stocker cattle. Cattle consuming treated E+ tall fescue recorded greater organic matter intake and weight gain during July and August. Turner hypothesized that increased cattle performance was partially due to greater quality forage available on treated plots. Aiken et al. (2012) grazed steers on metsulfuron treated or untreated tall fescue and reported a 39% increase in ADG. Serum prolactin was greater for steers grazing seedhead suppressed pastures and rectal temperature was decreased in year one and tended be decreased in year two indicating reduced impact of alkaloid intoxication in the cattle. Seedhead frequency was reduced by more than ten times for treated pastures. This study (Aiken et al., 2012) was not able to determine whether increased cattle gains were a result of decreased seedhead consumption or greater forage quality resulting from the treatment. Goff et al. (2015) conducted a similar experiment using endophyte free tall fescue and recorded an increase in ADG of 16% when compared to non-treated tall fescue.

Israel et al. (2016) applied metsulfuron at either 7 or 12 g/ha to tall fescue plots and measured seedhead suppression, herbage accumulation, quality and alkaloid concentrations. Seedheads were reduced by 36 to 55% and alkaloids were reduced by 26 to 34% compared to the untreated control. Differences in alkaloid concentrations were no longer present at the summer harvest. Herbage accumulation was reduced by 35 to 39% at the low rate and 46 to 51% at the high rate. Tall fescue stem yield was reduced by 42 to 67% while leaf yield was reduced by 34 to 50%. The CP, ADF and NDF content of spring harvest samples were all improved in response to metsulfuron, likely due to the change in the leaf to stem ratio of tall fescue.

Metsulfuron falls into the class of sulfonylurea herbicides, which act by inhibiting the production of branch-chain amino acids valine, leucine and isoleucine (Brown 1990). This results in decreased cell division, plant growth and bud death, therefore slowing the development of the plant. Sulfonylurea herbicides antagonize plant species at variable rates through differential uptake, active site sensitivity and metabolic break down of the herbicide. Dernoeden (1990) applied metsulfuron in the fall on mixed swards of Kentucky bluegrass and tall fescue in an attempt to remove tall fescue from the swards. The herbicide inflicted little injury to the Kentucky bluegrass while decreasing tall fescue frequency by greater than 90%. Goff et al. (2014) applied metsulfuron at variable rates in either October, March or April on tall fescue plots. Effective seedhead suppression was observed in the October and April treatments with April having the greatest suppression. The tall fescue tiller density was reduced at the high rate of metsulfuron with the greatest reductions observed in March (40%) and less in October and April (20%). There was also a tendency for increased crown density of other grass species in response to metsulfuron such as orchardgrass, Kentucky bluegrass and timothy.

The basic aboveground morphology of a perennial grass consists of one or more tillers extending from the plant base (Moore and Moser, 1995). These tillers are made up of one or more phytomers, which consist of a leaf blade, sheath, internode, node and axillary bud. The reproductive tissues are known as the inflorescence or spike. Moore et al. (1991) divided the growth stages of perennial grasses into 1) germination, 2) vegetative, 3) elongation, 4) reproductive and 5) seed ripening. Each of these stages is further divided into several substages based on morphological events. The meristem or growing point of grass during the vegetative stage is located at the shoot apical meristem at the base and translocated to an above-ground meristem for the elongation and later stages (Brommert and Whipple, 2018). This change is regulated by several internal and environmental factors (Brommert and Whipple, 2018). The plant's response to defoliation is dependent on the location of the meristem. When defoliation is above the meristem, growth continues as normal but when the meristem is elevated and defoliation occurs below the growing point, that shoot will not re-grow and new growth will have to come from new tillers (Hyder 1972).

Prescribed fire is also a strategy that could be used to achieve seedhead suppression. Probbasco and Bjugstad (1977) conducted controlled burns at various times of the year and found that burning in April resulted in a 50% reduction in seedheads.

Limited research is available describing effects of burning on tall fescue. Prescribed fire is used in renovation of Kentucky 31 tall fescue to other forage types. Madison et al. (2001) significantly decreased canopy cover of tall fescue one-month post burn for March, July and November burns with November burn (8.5%) experiencing the greatest decrease, then July (25.5%) and March burn (51%) compared to control (91.7%). Differences were no longer significant at one-year post burn indicating no long-term impact on tall fescue canopy cover. Probasco and Bujugstad (1977) conducted burns on tall fescue in February, April, August and November, all of which resulted in no difference in herbage accumulation compared to no burn. There was however, an increase in yield of red clover in response to April and February burns.

Forage response to fire

Prescribed burning of rangeland has many different effects on ecology and grazing animal performance. A common incentive for burning range is increased stocker cattle weight gain. Svejcar (1989) found burning tallgrass prairie in April increased stocker weight gain by 11.2 kg/ha or 17% over the grazing season compared to stockers grazing unburned pastures. This increased gain was also demonstrated on Kansas bluestem range and the Edwards Plateau in Texas (Anderson et al., 1970; McGinty et al., 1983). Increased gains were observed only in early months following burn (Anderson et al., 1970; McGinty et al., 1983). This is likely due to greater forage quality in response to burning as concluded by Anderson et al. (1970), as burned forage in the plots remained in vegetative state in May and June following the burn and senesced material was excluded from the diet. This was also exhibited by increased in vitro digestible organic matter

(IVDOM) the month following the burn compared to unburned plots (McGinty et al., 1983; Svejcar, 1989).

Effects of fire on forage production have produced mixed results. Burning has decreased (Owensby and Anderson, 1967; Anderson et al., 1970; Brockway et al., 2007), not affected (Owensby and Anderson, 1967; Anderson et al., 1970; Probasco and Bjugstad, 1977), or increased (Sharrow and Wright, 1977; Svejcar, 1989) herbage accumulation when compared to no burn. Change in production is mainly attributed to removal of surface litter (Sharrow and Wright, 1977). Without litter to cover and insulate the ground soil temperature and moisture are affected. Sharrow and Wright (1977) reported an increase in temperature in the first 8 cm of soil in response to removal of litter from both burning and clipping. This allowed soil to reach an optimum temperature for growth earlier than plots with remaining litter cover.

Soil moisture, a significant factor of herbage accumulation, was decreased on burned plots versus unburned (Anderson, 1965; Anderson et al., 1970; Sharrow and Wright, 1977). These differences were thought to be due to greater evaporation and runoff on exposed ground. Time at which the burn is conducted is also an important factor in soil moisture and forage production. Anderson (1965) and Anderson et al. (1970) described how time of burn in changed soil moisture and subsequently yield. Burns were conducted in the early, mid, and late spring periods, all of which decreased soil moisture. Early and mid-spring burns produced a greater reduction in soil moisture than late spring. These decreases resulted in a decreased herbage accumulation in early and mid-spring plots and no difference in late spring and control plots. These authors (Anderson, 1965; Anderson et al., 1970) hypothesized the lack of difference in spring was due to similar soil moisture. Difference in moisture among burn dates can be explained by the extended period of bare soil exposure leading to greater runoff and reduced infiltration (Anderson et al., 1970). Anderson (1965) recommended burning at initiation of spring growth to limit bare ground exposure and therefore maximize soil moisture and yield.

Prescribed fire may be an effective management tool for tall fescue systems. The reduction of toxic seedheads and improved forage quality could increase the productivity of tall fescue forage systems.

Chapter 2

PRESCRIBED BURNING OF ENDOPHYTE INFECTED TALL FESCUE PASTURES: EFFECTS ON FORAGE PRODUCTION, ALKALOID CONCENTRATIONS, AND BOTANICAL COMPOSITION

ABSTRACT

Prescribed fire may be a non-chemical alternative for seedhead suppression in endophyte-infected tall fescue forage systems. We evaluated the effects of prescribed fire on seedhead production, ergot-like alkaloids, forage production, forage nutritive value, and stand composition in K31 tall fescue plots (endophyte infection = 96%). We randomly applied treatments of an undisturbed control (CON), March mow (MOW), March burn (EARLY), and April burn (LATE) to 56 m^2 plots with 10 replicates per treatment. Plots were sampled for forage nutritive value and alkaloid concentrations monthly from May to October. Forage accumulation and botanical composition were recorded in June and October. Tall fescue seedhead count was conducted in May. After June sampling, plots were clipped to a height of 7.62 cm and litter was removed. Spring forage accumulation was greatest (P<0.01) in CON plots and MOW was greater than EARLY and LATE. Fall forage accumulation did not differ (P=0.30) across treatments; thus, CON had greater (P<0.01) total forage accumulation over the entire growing season. The LATE burn tended to reduce (month × trt; P=0.11) ergovaline concentration in June but all treatments were above the established threshold for fescue toxicosis. Crude protein in LATE was greater (month x trt; P<0.01) than other treatments in May and both LATE and CON had greater CP (month x trt; P<0.01) than EARLY and MOW in June.

Neutral detergent fiber concentrations were less (month x trt; $P<0.01$) in LATE compared to other treatments in May and June. Tall fescue seedhead production was decreased ($P<0.01$) by 49% in LATE plots. There was no treatment effect ($P\geq0.22$) on forb and non-tall fescue grass frequency in May and warm season grass frequency was greater ($P<0.01$) in LATE plots in October. Under conditions of this experiment, April burns decreased seedhead count and alkaloid concentration in endophyte-infected tall fescue plots.

INTRODUCTION

Endophytic fungi (*Epichloë coenophiala*) associated with Kentucky 31 tall fescue (*Schedonorus arundinaceus*) enhance plant productivity and hardiness (Bacon et al., 1977; Clay, 1987; Arachevaleta et al., 1989); however, the endophyte produces ergot-like alkaloids and a toxicosis results when ruminants consume alkaloid-impregnated forage (Lyons et al., 1986). A myriad of plant- and animal-based management strategies have been developed in effort to mitigate fescue toxicosis. A salient feature to nearly all plant and animal-based management strategies designed to mitigate fescue toxicosis is increases in labor and costs and transient reductions in forage production.

Alkaloids typically concentrate in tall fescue seedheads in the spring (Rottinghaus et al., 1991). Thus, several authors have suggested suppression of seedhead development in tall fescue stands may be a viable mitigation strategy to tall fescue toxicosis, along with more intensive strategies (e.g., sward replacement). Chemical seedhead suppression has been effectively used to reduce toxicosis and resulted in a 39% increase in ADG

(Aiken et al., 2012), but a drawback to chemical seedhead suppression is a 54% decrease in spring forage yield (Israel et al., 2016).

Prescribed fire is a management tool commonly used in the tallgrass prairie and may be an effective management tool in mitigating impacts of alkaloid consumption on cattle performance and health. Benefits of prescribed fire include increased cattle weight gain (Svejcar, 1989), forage nutritive value (McGinty et al., 1983; Svejcar, 1989), and brush control (Stritzke and Bidwell, 1990). Probasco and Bjugstad (1977) evaluated prescribed fire in a tall fescue sward and found that burning in April reduced tall fescue seedheads by 50%. Hall et al. (2014) subjected tall fescue to fire in a greenhouse experiment and reported a reduction in reproductive tillers, but no difference in total biomass produced.

Our objective was to evaluate the effects of a late winter prescribed burn on herbage accumulation of tall fescue and concentration of alkaloids in tall fescue swards across a growing season.

MATERIALS AND METHODS

Site description

The study was conducted on a 0.8 ha tall fescue stand located at the University of Missouri Southwest Research Center (37˚1’ N, 93˚53’ W, elevation 400 m) near Mount Vernon, MO. The site contained an established stand of Kentucky 31 tall fescue (*Schedonorus arundinaceus*) that was historically used for hay production. A sample of 100 tillers were tested using a Western blot assay (Hiatt et al., 1999; Agrinostics Ltd. Co., Watkinsville, GA) for the presence of the fungal endophyte (*Epichloë coenophiala*) and shown to be 96% infected. The soil type was a sandy loam and precipitation totals are shown in Table 1. Each individual plot was 6.10 × 9.14 meters. Each plot was separated by a 1.5 m mowed alley.

Experimental design and treatment structure

Four treatments were applied in a complete randomized design with ten plots per treatment. The treatments were 1) no burn control (CON), 2) March mowed (MOW), 3) March burn (EARLY), and 4) April burn (LATE). Response variables were 1) biomass yield, seedhead production, and botanical composition, recorded once for the spring (June 19) and fall (October 18) growing seasons and 2) alkaloid concentration and forage nutritive value collected monthly.

Plot management and sampling

Plots assigned to MOW were mowed on March 10, 2018 using a flail style mower with a bagger to clip grass to a height of 7.62 cm. The EARLY burn treatment was applied on the same day as the MOW treatment by creating a head fire using a propane torch. Once the fire passed through the plot the torch was used to ignite any patches left unburnt. The same procedure was used to conduct the LATE burn on April 10, 2018.

Beginning in May and repeated monthly until October, tall fescue tillers were hand clipped at ground level while walking an “X” pattern through plots until 50 g of DM was collected. Samples were stored on ice until frozen at -20˚C and later lyophilized. Forage samples were collected for nutritive value analysis using a push mower with a bagger attached. Two strips on the short side of the plots were mowed to a 5 cm height starting and ending 1 meter from plot edge. At least 100 g of DM were then subsampled out of the bagger to be dried in a 55˚C forced-air oven and later analyzed for CP, NDF and ADF.

In May, seedhead numbers and species frequencies were recorded. A measuring tape was strung from opposite corners of the plot to create an X pattern. Each 1.83 m a 0.09 m^2 quadrat was placed parallel to one side of the tape and seedhead number, non-tall fescue grass and forb frequency were recorded. Quadrats (n=12) were placed and the side of the tape at which the quadrat was placed alternated after each placement. This procedure was repeated in October but only recording species frequency. Forage accumulation was measured in June and October by mowing a 6.1 × 0.8 m strip through each plot using a flail mower. Forage collected was weighed and sampled for measurement of CP, NDF and ADF by collecting 200 g of mixed clippings. After samples were collected in June, grass was clipped to a height of 8 cm and litter was removed over the entirety of the plots to remove spring growth.

Forage analysis

Ground samples were analyzed for DM by drying samples in a 105˚C oven overnight. Analyses of non-sequential ADF, NDF (Van Soest et al., 1991), DM and CP were conducted on forage samples. Sodium sulfite and α-amylase was used with the NDF analyses. Nitrogen content was determined by combustion (LECO FB-428, LECO Corporation, St. Joseph, MI). 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 by HPLC for ergovaline using procedures described by Rottinghaus et al. (1991).

Statistical Analyses

Data were analyzed as a complete randomized design using SAS version 9.4 (SAS Institute, Inc., Cary, NC). The CP, NDF, ADF and alkaloid 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. Plot was included as a random term. The repeated term was month with plot × 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 ($P \leq 0.05$).

Seedhead frequency, botanical composition and forage accumulation data points were averaged within plots and the means of each were analyzed using the MIXED procedure with seedheads, botanical composition and accumulation as dependent variables, treatment and plot as independent variables. The LSMEANS function was used to separate means and calculate SEM. The PDIFF function was used when the *F*-statistic was significant ($P \leq 0.05$). Tendencies were declared at ($P \leq 0.15$).

RESULTS AND DISCUSSION

During the time of the study (March-October) precipitation was 76% of the historical average (53 years; Table 2.1). From April to July, precipitation was 46% of the historical average. Drought conditions during the experiment may have played a role in the results reported below.

Forage accumulation

Forage accumulation was reduced (*P*<0.01; Figure 2.1) by MOW, EARLY and LATE treatments for the spring growth season measured in June with EARLY and LATE having a greater reduction than MOW. We did not remove residual forage from plots prior to initiating this experiment, thus it is possible that greater forage yield in June for CON compared to MOW, EARLY and LATE was reflective of residual forage rather than greater forage production in spring for CON. However, it is possible that removal of photosynthetic area from mowing or burning would require regeneration of leaf material and may have decreased growth rate for a period in MOW, EARLY and LATE. Forage accumulation was not different (*P*=0.30) in October among treatments. After June collections, all plots were clipped to simulate grazing of the spring growth and a lack of difference in forage yield in fall supports the hypothesis that greater forage yields in CON in spring were reflective of residual forage mass instead of greater forage production. Yield in the fall was greater than yield in the spring (1647 and 820 kg/ha, respectively) which differs from the typical tall fescue yield distribution (Lacefield et al., 2003). Our observations of seasonal yield distribution were likely influenced by drought conditions in the spring that limited overall growth potential. Across treatments, CON had the greatest (*P* < 0.01) annual forage yield. Spring yield differences explain much of the difference observed for CON. The MOW tended to be greater than EARLY (*P*=0.07) and late (*P*=0.13) in total yield. Tall fescue yield was small over the growing year for the current experiment (2467 kg/ha). This was partially due to drought conditions early but may also be due to the lack of nitrogen input. Nitrogen fertilizer was omitted because of the known relationship between nitrogen fertilizer and alkaloid production in tall fescue forage systems (Rottinghaus et al., 1991). Wells (1983) increased tall fescue yield from 3800 kg/ha to 8595 kg/ha with 90 kg/ha of nitrogen fertilizer.

Seedhead frequency and botanical composition

Seedhead frequency was 49% less ($P<0.01$; Table 2.2) for LATE burn compared to all other treatments. This agrees with results observed in response to an April burn conducted by Probasco and Bjugstad (1977). The cause of the seedhead reduction may be due to fire damaging young, reproductive tillers. Tillers of most temperate perennial grasses require dual signaling to be able to produce and elongate a seedhead (Heide, 1994). The first signal comes from short day length and low temperatures that would occur over the winter. This gives the plant the ability to produce a seedhead in the following growing season. The second signal occurs during the growing season with long day length resulting in reproductive tiller elongation. Prescribed burns in the spring may damage the vegetative tillers that have gone through the first stage of signaling. The new tillers that grow to replace the damaged tillers may not have been vernalized and will not be capable of producing a seedhead. The result is fewer tillers of the tall fescue plant able to create seedheads. The seedhead suppression effect was not observed for the EARLY burn, possibly because these tall fescue plants were at an earlier developmental stage with the growing points less exposed compared to LATE.

No difference was observed in forb ($P=0.22$; Table 2.2) or non-tall fescue grass ($P=0.36$) frequency in May regardless of treatment. In October, no differences in forb frequency ($P=0.11$) were present but treatment affected non-tall fescue grass frequency ($P<0.01$). The LATE burn resulted in a greater frequency of non-tall fescue grasses compared to other treatments (81.6 for LATE compared to 45.8% for CON). Anderson et al. (1970) observed cool-season grasses decrease and warm-season grasses increase in response to spring fire in tallgrass prairie. Assessing botanical composition changes in the same year as the disturbance is challenging as quantifiable differences may not be observed until later years. Probasco and Bjugstad (1977) reported increases in clover biomass in response to a single April prescribed burn, but this was not observed in the current experiment. Changing of botanical composition in response to fire may be heavily influenced by the seedbank composition and environmental conditions. However, Hall et al. (2014) reported that fire had no impact on forage yield regardless of adequate or inadequate moisture availability for plant growth.

Ergot-like alkaloids

Differences in seedhead frequency across fescue swards may influence alkaloid concentrations. Ergovaline concentration in LATE tended to be less (treatment × month, P=0.11; Figure 2.2) compared to EARLY, MOW and CON in June when mature seedheads were present, but ergovaline concentration was not different in July after plots were clipped to simulate grazing and seedheads were removed. Israel et al. (2016) saw a reduction in ergovaline concentration in tall fescue that coincided with reduced seedhead production in response to herbicide application.

As expected, we observed temporal changes (P<0.01) in concentrations of ergovaline and ergopeptine alkaloids (ergovaline, ergosine, ergotamine, ergocornine, ergocryptine, ergocrostine). Specifically, total ergopeptine alkaloid concentration increased from May to June as seedheads matured, decreased in July after seedhead removal and slowly increased through October as plants became vegetative and matured.

Belesky et al. (1988) also reported that alkaloids increase through May, decrease in June and gradually increase to a maximum concentration in October.

Temporal changes in ergot alkaloid concentration differed in response to treatment (treatment × month, P=0.01; Figure 2.3). Specifically, ergot alkaloid concentration was least in LATE, intermediate in EARLY and MOW and greatest in CON in June but was not different in other months. Similar to difference in total ergot alkaloid concentration, ergovaline concentration tended (P=0.11) to be least in LATE, greatest in MOW and CON and EARLY were intermediate in June but were not different in other months. Biological effects of alkaloids outside of ergovaline is unknown but could be important to the etiology of fescue toxicosis.

Forage nutritive content

In May, LATE had the greatest (treatment × month, $P \leq 0.01$; Figure 2.4) concentration of CP compared to other treatments (14.1 vs 10.4%), and in June, both LATE and CON had greater concentrations of CP than EARLY and MOW (7.6 vs 5.5%). Greater June CP concentration observed for CON may contradict the hypothesis that residual growth contributed to greater forage yield as senesced residual growth would have less CP content. Allen et al. (1976) observed greater CP concentration in burned tallgrass prairie compared to unburned. As expected, overall CP concentration changed temporally; CP concentration decreased from May to June as plants matured, increased from July to September as plants experienced vegetative growth and decreased in October as fescue entered dormancy (P<0.01).

In May, CON had the greatest concentration of NDF (treatment × month, $P<0.01$; Figure 2.5), possibly due to the inclusion of the previous year's senesced leaf material. The LATE burn plots had the lowest NDF concentration in June, possibly due to the late burn keeping forage in a vegetative state. Allen et al. (1976) also reported reduced fiber in response to burning. Overall NDF increased from May to June as plants matured and decreased from June to July with plot clipping. Neutral detergent fiber remained constant in August until decreasing in September and October with vegetative regrowth. Overall ADF increased from May to June (treatment × month, $P<0.01$; Figure 2.6) as plants matured and decreased in June with plot clipping. Acid detergent fiber remained constant in August until decreasing in September and October with fall regrowth.

SUMMARY

A prescribed burn conducted in April reduced seedhead production, which in turn reduced alkaloid concentration in tall fescue compared to other treatments while seedheads were still present. The April burn also improved NDF and CP. Differences in forage accumulation were not observed across treatments designed to mimic industry practices (MOW, EARLY and LATE). The use of prescribed burns in April as tillers are starting to grow has the potential to increase grazing animal productivity through increased forage quality and reduced alkaloids.

Table 2.1. MU Southwest Center precipitation data, centimeters per month

Month	2018[1]	Historical average[2]
March	8.89	9.42
April	3.96	10.67
May	8.20	12.73
June	4.34	13.31
July	4.29	8.41
August	18.57	9.32
September	8.31	12.47
October	8.43	8.71
March-October precipitation	64.99	85.04
2018, % of historical avg.	76	
April-July precipitation	20.79	45.12
2018, % of historical avg.	46	

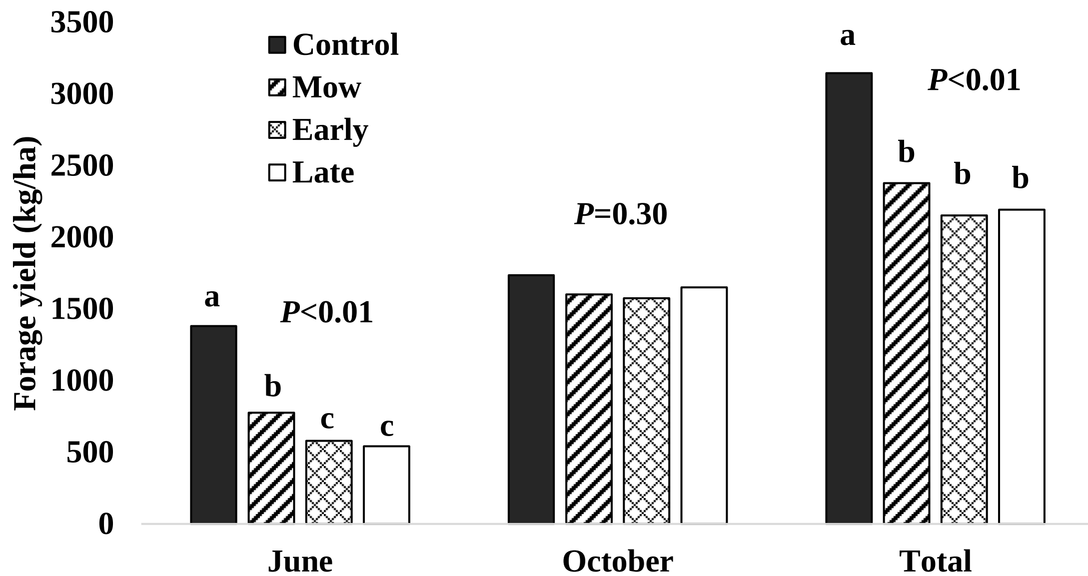
[1]http://agebb.missouri.edu/weather/stations/lawrence/index.htm
[2]https://wrcc.dri.edu/ (Period of record: 1960 – 2013)

Table 2.2. Seedhead and non-tall fescue forage frequency in K31 tall fescue plots[1] subjected to late winter herbage removal

Item	Control	Mow	Prescribed burn[2] Early	Prescribed burn[2] Late	SED[3]	*P*-Value
Fescue seedheads[4]	12.63[a]	11.94[a]	12.43[a]	6.34[b]	0.859	<0.01
May frequency[5]						
Forb	40.0	61.0	60.1	53.3	11.07	0.22
Non-tall fescue grass	15.0	16.7	12.5	8.0	5.08	0.36
Oct frequency[5]						
Forb	39.9	54.3	62.6	62.6	10.33	0.11
Non-tall fescue grass	45.8[a]	59.2[a]	55.8[a]	81.6[b]	9.75	<0.01

[1]6.10 x 9.14 m plots
[2]Control = Undisturbed, Mow = Mowed to 7.62 cm at time of Early burn, Early = Prescribed fire applied to plots on March 12, 2018, Late = Prescribed fire applied to plots on April 11, 2018
[3]SED = Standard error of the differences of least square means
[4]Fescue seedhead count represents an average of twelve 0.09 square meter quadrats (2% of plot area) recorded from within each plot
[5]Frequency represents proportion of twelve 0.09 square meter quadrats (2% of plot area) within each plot containing the forage of interest
[a,b]Means within rows lacking common superscripts differ ($P < 0.05$)

Figure 2.1. Forage accumulation from K31 tall fescue plots[1] subjected to late winter herbage removal



[1]6.10 x 9.14 m plots

[2]Control = Undisturbed, Mow = Mowed to 7.62 cm at time of March burn, March = Prescribed fire applied to plots on March 12, 2018, April = Prescribed fire applied to plots on April 11, 2018.

[a,b]Means without common superscripts differ ($P < 0.01$; SED = 119.8)

Figure 2.2. The effect of late winter prescribed burn on monthly ergovaline concentration in K31 tall fescue (96% endophyte infection). Plots were swathed after June forage collection. Treatment × month interaction; *P* = 0.11; SEM = 48.0

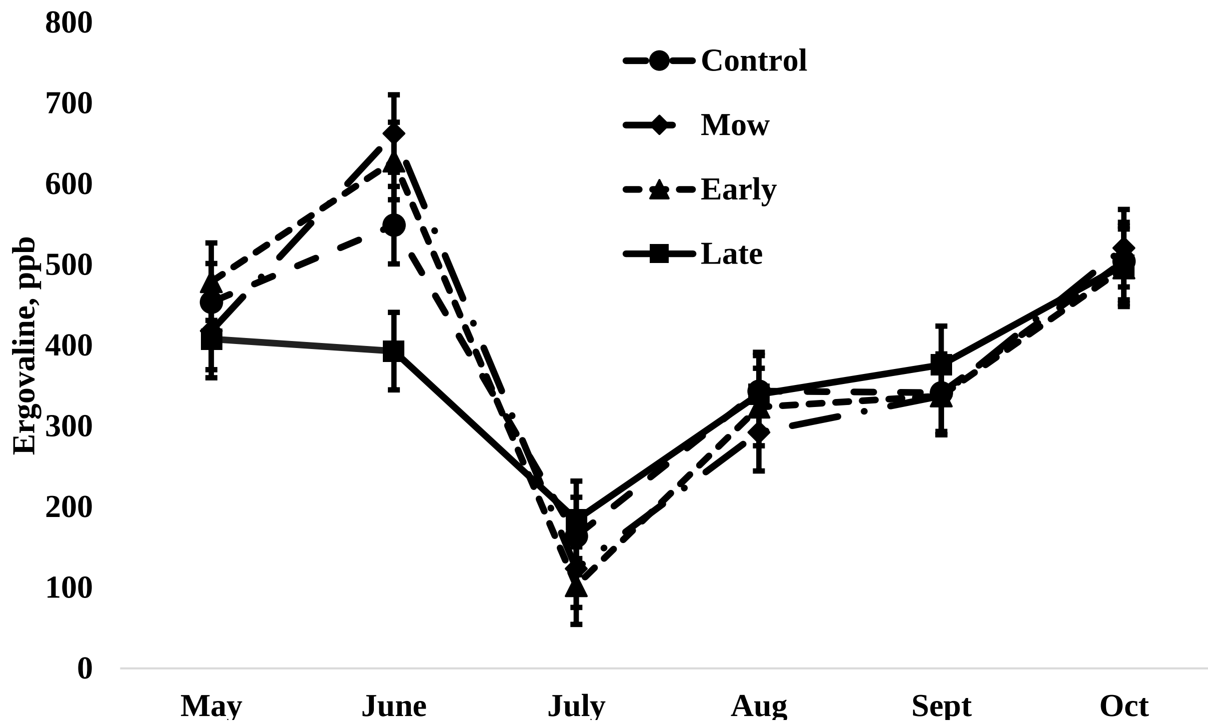
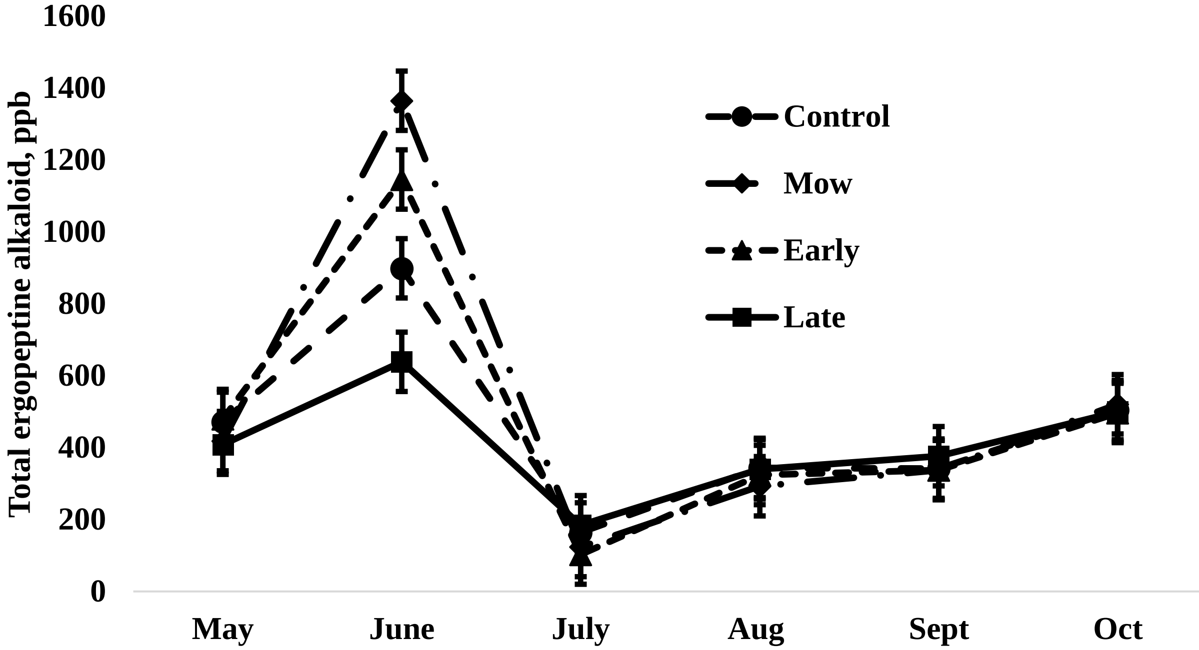


Figure 2.3. The effect of late winter prescribed burn on monthly total ergopeptine alkaloid[1] concentration in K31 tall fescue (96% endophyte infection). Plots were swathed after June forage collection. Treatment × month interaction; *P* < 0.01; SEM = 82.5



[1]Ergopeptine alkaloids: ergovaline, ergosine, ergotamine, ergocornine, ergocryptine, ergocrostine

Figure 2.4. The effect of late winter prescribed burn on monthly crude protein concentration in K31 tall fescue (96% endophyte infection). Plots were mowedafter June forage collection. Treatment × month interaction; *P* < 0.01; SEM = 0.36

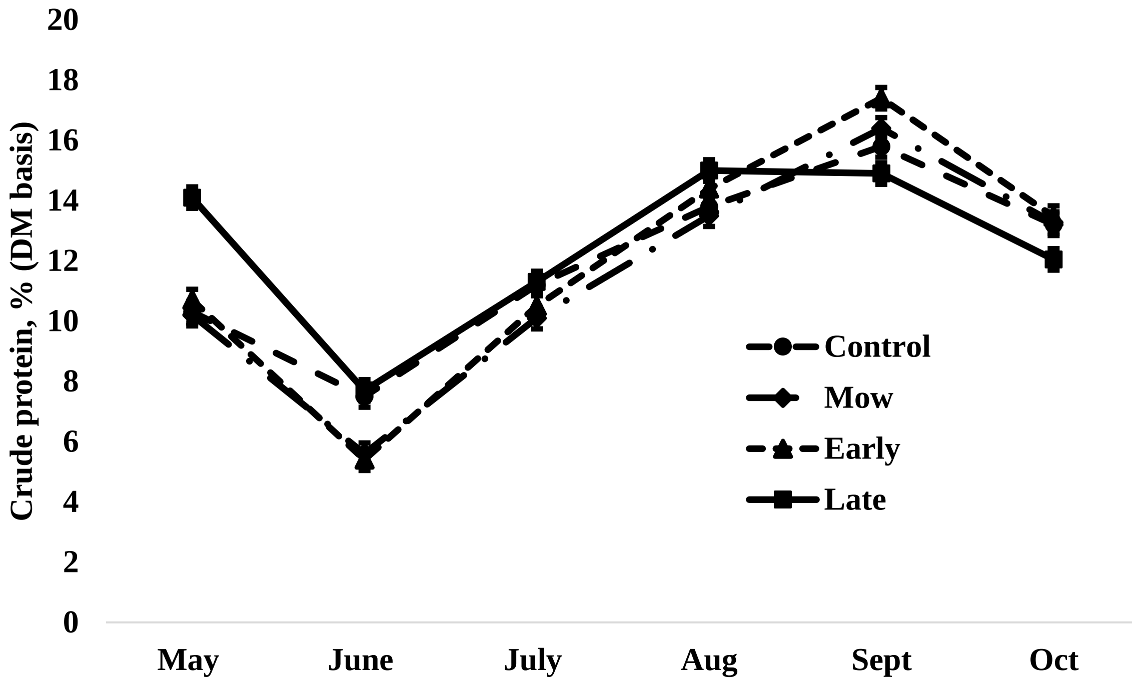


Figure 2.5. The effect of late winter prescribed burn on monthly neutral detergent fiber concentration in K31 tall fescue (96% endophyte infection). Plots were swathed after June forage collection. Treatment × month interaction; $P < 0.01$; SEM = 0.86

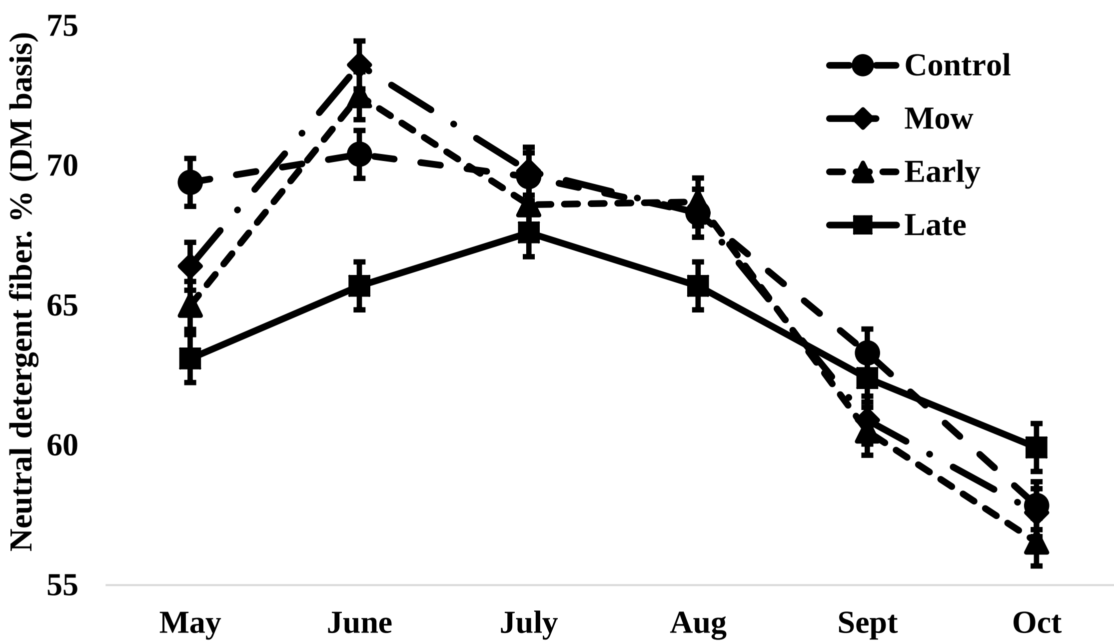
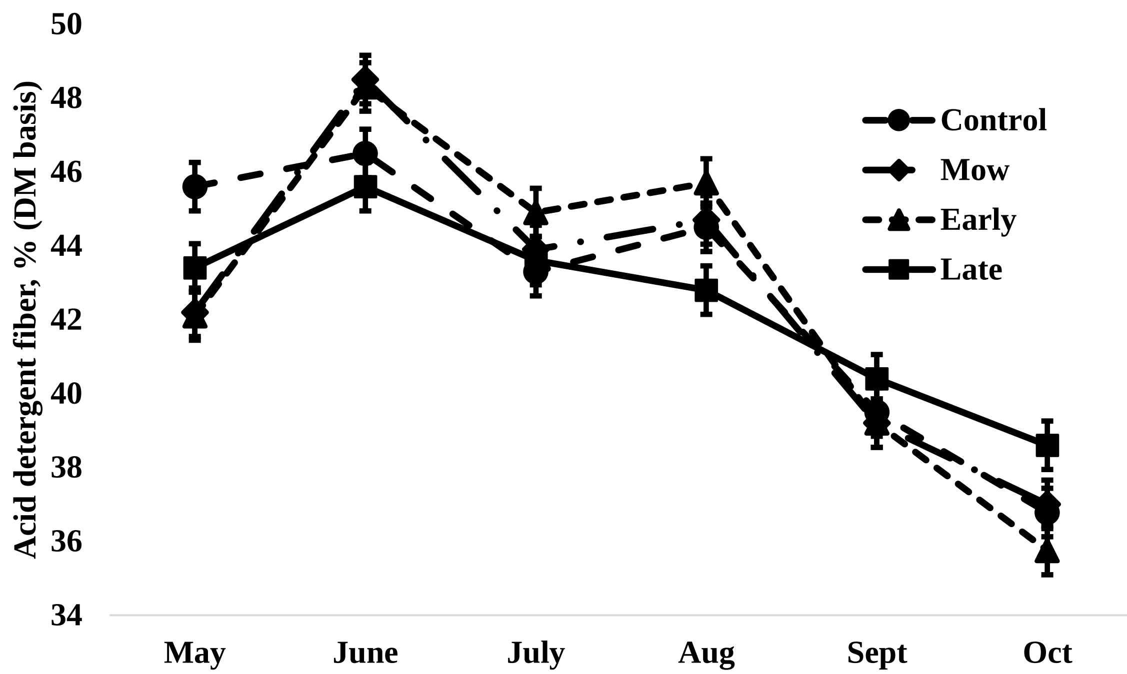


Figure 2.6. Effect of late winter prescribed burn on monthly acid detergent fiber concentration in K31 tall fescue (96% endophyte infection). Plots were swathed after June forage collection. Treatment × month interaction; *P* < 0.01; SEM = 0.66



CHAPTER 3

INTERACTION OF METSULFURON AND NITROGEN FERTILIZER IN TALL FESCUE FORAGE SYSTEMS

ABSTRACT

Chemical seedhead suppression of endophyte infected tall fescue (*Schedonorus arundinaceus*) improves stocker cattle performance but may decrease forage accumulation. Spring nitrogen application increases tall fescue growth with a concomitant increase in alkaloids produced by the symbiotic endophyte *Epichloë coenophiala*. We hypothesized that greater amounts of nitrogen applied to tall fescue would increase forage accumulation and offset losses in forage production from chemical suppression of seedheads with metsulfuron without effect on alkaloid concentration. Ninety-six steers (271±20 kg) were randomly assigned to one of sixteen pastures (1.8 ha) on April 18 and continuously grazed for 57 d. Pastures were blocked by previous use (n=4) and randomly assigned to one of four treatments; no metsulfuron, no nitrogen (NEGCON), metsulfuron with 0 (MET0), 67 (MET67), or 134 (MET134) kg/ha of ammonium nitrate, applied March 11th. Steers grazing MET0 pastures were removed 17 d early due to insufficient forage availability. Steer weight, forage accumulation, forage nutritive value and alkaloids in forage samples were measured monthly. Seedhead frequency and pasture botanical composition were determined in June. Metsulfuron application reduced ($P<0.01$) tall fescue seedheads by 80%. Metsulfuron application tended to decrease ($P=0.07$) ergovaline but ergovaline increased ($P<0.01$) at each monthly sampling across treatments. Nitrogen application had no impact ($P=0.50$) on ergovaline concentration measured after metsulfuron application. Forage accumulation tended (*P*=0.07) to be least for MET0, intermediate for NEGCON and MET67, and tended (*P*=0.08) to be greatest for MET134. Steer average daily gain was not affected (*P*=0.80) by treatment. Metsulfuron decreased (*P*=0.02) NDF concentration regardless of fertilization rate. Forage CP increased (*P*<0.01) with fertilization and no differences (*P*=0.45) were detected between NEGCON and MET0. Botanical composition was not impacted (*P*>0.07) by treatment. Metsulfuron decreased seedhead growth and ergovaline concentration in tall fescue. Nitrogen fertilizer ameliorated forage accumulation lost to metsulfuron application but did not impact steer gain.

INTRODUCTION

Tall fescue (*Schedonorus arundinaceus*) is a cool-season perennial grass planted across more than 14 million hectares in the United States (Buckner et al., 1979). The majority of tall fescue is the Kentucky 31 (K31) variety, known to have a symbiotic relationship with the fungal endophyte *Epichloë coenophiala*, which produces ergot-like alkaloids. This symbiotic relationship provides improved plant productivity and hardiness that tall fescue is known for (Clay, 1987; Arachevaleta et al., 1989). However, a toxicosis results when alkaloid-impregnated forage is consumed by ruminants (Lyons et al., 1986). Fescue toxicosis causes an estimated loss of over $1 billion annually in the US due to poor pregnancy rates, weaning weights and stocker gains (Strickland et al., 2011).

Rottinghaus et al. (1991) reported differences in alkaloid concentrations across plant parts with the highest concentration in the seedhead, followed by stems and leaves. Elevated alkaloid concentrations in the seedhead has led to the development of management strategies designed to reduce tall fescue seedhead production. Aiken et al. (2012) treated K31 tall fescue pastures with the herbicide metsulfuron. Tall fescue seedhead frequency was reduced by over 90% and steer ADG increased by 39%. However, metsulfuron decreased forage accumulation by 54% (Israel et al., 2016).

Spring nitrogen (N) fertilization increases tall fescue forage production (Bélanger et al., 1992). However, spring application of N is recommended at low rates in tall fescue pastures intended for grazing due to increases in alkaloid production by the endophyte (Rottinghaus et al., 1991). Use of metsulfuron with increased N fertilization rates may regain lost yield due to the herbicide. However, it is currently unknown how metsulfuron and N fertilizer may interact and impact alkaloid production in K31 tall fescue. Thus, our objectives were to evaluate the interaction of metsulfuron application and N fertilizer level applied in spring on tall fescue pastures grazed by beef calves.

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, MO. 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 1. Pastures (n=16; 1.8 ha per pasture) of established Kentucky 31 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).

Experimental design and 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. Blocks were determined based on usage in the previous growing season. Treatments consisted of a negative control with no metsulfuron or ammonium nitrate applied (NEGCON), metsulfuron with no nitrogen provided from ammonium nitrate (MET0), metsulfuron with 67kg/ha nitrogen provided from ammonium nitrate (MET67), and metsulfuron with 134kg/ha nitrogen provided from ammonium nitrate (MET134).

Pasture management and sampling

Ammonium nitrate was applied to MET67 and MET134 pastures on March 11. Metsulfuron (Chaparral, Corteva Agriscience, Wilmington, DE) was applied to the MET plots at a rate of 0.14 kg/ha on April 15 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 April 18 for 57 days. Animal measurement and forage collection dates occurred April 18 (day 0) at trial initiation, May 16 (day 28) and June 14 (day 57) at trial conclusion.

Tall fescue tillers were sampled from each pasture by walking in a W pattern and clipping the tall fescue tiller nearest to the technician every twenty paces. 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 and later lyophilized.

A rising plate meter (F400, Agricultural Supply Services, Whitminster, UK) was used to measure forage mass. Approximately 90 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 0.09 m^2 quadrat, air dried at 55˚C in a forced-air oven and weighed. A total of 240 calibration clippings were collected during 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. The regression: Forage mass(kg/ha) = 13,512 + 29.03(height) -196.01(date) + 0.68$(\text{date})^2$ with an adjusted R-square of 0.64 was used to calculate forage mass from mean pasture height measurement. Calibration clippings were composited within pasture and subsequently analyzed for CP, ADF, and NDF after weights for forage mass were recorded.

In June, 30 quadrats measuring 0.09 m^2 were randomly placed every 15 paces while walking an X pattern throughout each pasture. Tall fescue seedhead numbers were counted and the presence of tall fescue, Kentucky bluegrass, orchardgrass, crabgrass, clover, birdsfoot trefoil, and buckhorn plantain were recorded within each quadrat.

Animal management

Steers calves weighing 271 ± 20kg 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 (Revalor G, Merck Animal Health, Kenilworth, NJ) approximately 60 days before the start of the trial.

Steers were limit fed a 50:50 dried distiller's grains with solubles/soyhull pellet mix at 2% BW in a drylot for three days before the start of the trial to equalize gut fill. Cattle were individually weighed on 2 consecutive days at the end of the limit fed period. Before measures of BW were collected, the scale was calibrated to 454kg in 45.4kg increments. Six steers were assigned to each pasture based on a stratification of initial body weights. Steers weighed an average of 271 kg resulting in 1,626 kg of animal weight or 3.6 animal units per pasture. 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 May 16th (day 28) 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 28th (day 40) forage height was measured by a trained technician and pastures assigned to MET0 treatment had forage height less than 50mm. Forage availability was determined to be inadequate to support the steers and removed them from the experiment. Remaining steers were removed from pastures on June 14th (day 57), limit fed with the DDGS/ soyhull pellet mix at 2% BW for 4 days and weights recorded on the last 2 consecutive days.

Forage analysis

Plate meter calibration clippings (n=5) were dried in a forced air 55˚C oven to obtain partial 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), 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). 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 analyses

Data were analyzed as a randomized complete block design using SAS version 9.4 (SAS Institute, Inc., Cary, NC). Seedhead frequency and botanical composition data points were averaged within pastures and the means of each were analyzed using the MIXED procedure with seedheads and botanical composition as dependent variables and treatment and block as independent variables. Block was included as a random term. 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. Block 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

From March to June, 154% of the historical average precipitation was received on the experimental pastures (53 years; Table 3.1). With this large amount of rainfall, soil moisture was likely not a limiting factor of the forage system.

Seedhead and botanical composition

Metsulfuron application decreased ($P<0.01$; Table 3.2) tall fescue seedhead production was reduced by 80% compared to the negative control. Aiken et al. (2012) reported a 93% reduction in seedheads in response to metsulfuron. This herbicide acts to inhibit production of amino acids, which results in decreased plant development and bud death (Brown, 1990). Greater seedhead suppression occurs when metsulfuron is applied in the boot stage (Sather et al., 2013). Boot stage application also results reduced forage accumulation compared to vegetative stage application (Sather et al., 2013). Metsulfuron application to tall fescue pastures reduces alkaloid reservoirs and improves forage quality. Aiken et al. (2012) reported a 39% increase in stocker ADG in response to metsulfuron application and attributed it to those two factors.

Nitrogen fertilization had no impact (*P*>0.49) on seedhead production when metsulfuron was applied in April. Watson and Watson (1982) observed tall fescue seed yield increase as nitrogen fertilization rate increased. However, seedhead production decreased with defoliation dates later than March 30. Removing seedheads from K31 tall fescue enhances stocker cattle weight gain (Aiken et al., 2012), likely because ergovaline concentration in the tall fescue seedhead is greater than the rest of the plant (Rottinghaus et al., 1991). Nitrogen fertilization, in the presence of metsulfuron, did not increase seedhead production, the most toxic plant part.

Goff et al. (2014) reported a tendency for metsulfuron to increase density of non-fescue cool season species such as Kentucky bluegrass, orchardgrass and timothy after one application of metsulfuron. However, excluding other forbs, botanical composition measured in June did not change (*P*>0.20) in response to treatment. Frequency of tall fescue (*P*=0.38), Kentucky bluegrass (*P*=0.25), orchardgrass (*P*=0.41), other grasses (*P*=0.20), birdsfoot trefoil (*P*=0.43), and buckhorn plantain (*P*=0.43) were not affected by treatment with a tendency (*P*=0.07) for metsulfuron to decrease other forb frequency. This would be expected as metsulfuron largely targets forbs (Louhaichi et al., 2012).

Alkaloid production

Decreasing seedheads in stands of tall fescue influences alkaloid concentrations. Treatments tended (*P*=0.07; Figure 3.1a) to affect ergovaline concentration as the untreated control pastures had greater ergovaline concentrations than MET0 and MET134 and tended to be greater than MET67 (*P*=0.12). Israel et al. (2016) reported decreased ergovaline coinciding with seedhead suppression achieved with metsulfuron.

Ergovaline concentration did not increase (*P*=0.50) in response to nitrogen fertilizer, which differs from the data of Rottinghaus et al. (1991) and Lyons et al. (1986). Metsulfuron was not used in the aforementioned experiments. Nitrogen is an important nutrient for fungal endophyte growth (Naffa et al., 1998). Providing additional nitrogen to the plant and in turn the endophyte then likely would increase the fungi growth and alkaloid producing potential. Rottinghaus el al. (1991) observed ergovaline increase by 81% in response to 135 kg/ha of nitrogen (similar N level to the current experiment) with the greatest ergovaline concentration in the stems and seedheads. The decreased production of seedheads and stems caused by metsulfuron may have avoided large increases of ergovaline commonly observed in response to nitrogen.

Pasture ergovaline concentration increased (*P*<0.01; Figure 3.1b) across months with the largest increase in June. Belesky et al. (1988) similarly observed alkaloids increase through the end of May as seedheads developed but decrease in June. Alkaloid concentrations commonly peak in late spring with seedhead extension followed by a decrease in the summer as tall fescue goes dormant and a second peak occurs at the end of fall. Ergopeptine alkaloid concentration (ergovaline, ergosine, ergotamine, ergocornine, ergocryptine, ergocrostine) was not affected (*P*=0.48; Figure 3.2) by treatment but increased by month (*P*<0.01). Concentrations were low for April and May but great increases were observed in June, rising from 340 to 34,595 ppb. The biological effect of these alkaloids outside of ergovaline is unknown but could be impactful with such large concentrations.

Forage nutritive content and yield

In April, MET67 and MET134 had greater (treatment x month, P=0.02; Figure 3.3) biomass than NEGCON and MET0. Differences in forage biomass in April are likely a result of nitrogen fertilizer (Watson and Watson, 1982). The two treatments with nitrogen applied (MET67 and MET134) produced more herbage than the NEGCON and MET0 unfertilized treatments in April (4,192 vs 3,181 kg/ha). Metsulfuron was applied 3 days prior to measurement and would have had little effect on April forage production. However, nitrogen fertilizer was applied on March 11, giving 30 days of potential growth before metsulfuron application or initiation of grazing.

In May, herbage mass was not different (P=0.22) for NEGCON, and decreased for MET0, MET67 and MET134. The MET0 treatment had the least biomass out of all treatments while MET134 had a numerically greater biomass than NEGCON and MET67. In May, herbage mass decreased across treatments that had metsulfuron applied, indicating an inversion of forage growth rate and forage consumed by the steers. Herbage decreased by 34% between NEGCON and MET0 due to metsulfuron, which agrees with Israel et al. (2016). May forage mass decrease was overcome with the application of nitrogen.

Herbage mass increased (MET0, MET67, and NEGCON, P=0.02) or tended to increase (MET134, P=0.07) from May to June. A large increase in herbage was observed for MET0 in June as it was ungrazed for the last 17 days. The MET0 pastures had less biomass than MET134 (P=0.02) but neither were different than NEGCON and MET67 (P<0.10), which were intermediate.

Nitrogen fertilizer was only applied in conjunction with metsulfuron. A factorial arrangement of nitrogen and metsulfuron treatments would have better described the effects of metsulfuron on nitrogen use in K31 pastures. This was not the chosen experimental design due to a limitation of land to conduct the experiment. In addition, the effect on nitrogen alone on tall fescue has been documented previously (Rottinghaus et al., 1991; Bélanger et al., 1992). Increasing nitrogen fertilizer increased herbage mass linearly ($P<0.01$) in the presence of metsulfuron. Nitrogen fertilizer, when used in combination with metsulfuron, increased forage mass of pastures without the corresponding increase in ergovaline concentrations. Increased spring nitrogen fertilization of tall fescue may attenuate the depression in forage brought on by application of metsulfuron, without a concomitant increase in alkaloid production by the endophyte.

Neutral detergent fiber irrespective of month was greater ($P=0.02$; Figure 3.4) for NEGCON than MET134 and MET67 but not MET0. The MET0 treatment was greater than MET67 but not MET134. The MET0, MET67 and MET134 treatments had numerically lesser values than NEGCON in May as metsulfuron kept forage in a more vegetative state but this difference was not observed in June as herbage accumulated resulting in increased plant maturity. Sather et al. (2013) also reported lower NDF values in a spring harvest of tall fescue following metsulfuron application.

In April, MET67 had less ADF than NEGCON and MET0 but not MET134 (treatment × month, $P=0.04$; Figure 3.5). Acid detergent fiber increased in May for all treatments as plants matured, but MET134 and MET67 had a less ADF concentration than NEGCON but not MET0, and ADF was also less in MET134 than MET0 but not

MET67 in May (treatment × month, *P*=0.04). Concentration of ADF did not increase in June, but MET0, MET67 and MET134 were less than NEGCON (treatment × month, *P*=0.04). Less ADF content in June for MET0, MET67 and MET134 could indicate a reduction in lignin or cellulose in response to metsulfuron.

We did not observe an interaction of time and treatment (*P*=0.16) on CP content. As expected, crude protein concentration decreased with increasing plant maturity from April to May and stayed constant through June; however, MET67 and MET134 contained greater (*P*<0.01; Figure 3.6) amounts of CP than NEGCON and MET0. Concentration of CP was increased for nitrogen treatments, which is as expected (Wolf and Opitz von Boberfeld, 2003). Providing fescue with additional N can allow for greater protein synthesis when N limits plant growth. Metsulfuron did not appear to impact CP concentration as MET0 had similar concentrations to NEGCON. Similarly, Sather et al. (2013) reported no effect of metsulfuron on CP concentrations.

Steer performance

Steers grazing pastures assigned to the treatment MET0 were not included in the analysis of the second period as they were removed 17 d early due to insufficient forage availability.

Steer performance was not affected by treatment (*P*≥0.25; Table 3.3) throughout the experiment. Others (Aiken et al., 2012) reported an increase in steer gain in response to metsulfuron treated tall fescue pastures. Improved performance in their experiment was speculated to be related to improved forage quality and decreased alkaloids. Average daily gain was numerically greater (*P*=0.25) for MET0, MET67 and MET134 treatments during the first 28 days of the current experiment. Forage NDF was numerically (treatment × time, P=0.24) less for MET0, MET67, and MET134 compared to NEGCON in May, which may explain the numerical increase in ADG during the first 28 days. These numerical differences in NDF were no longer present in June. This possibly influenced similar the steer gains observed in the last 28 days as quality of grazed forage became similar between treatments. Irrespective of treatment, ADG decreased from the first to second 28 days (1.13 to 0.65 kg/d). Crude portion was lowest and NDF was at its highest in the last 28 days. This lower nutritive value of forage in the last 28 days could have influenced poor steer gain but increased alkaloids could have also played a role.

We observed no difference in ergovaline concentration across treatments in April and May (Figure 3.1b), but ergovaline concentration was above the toxic threshold (150 ppb; Stamm et al., 1994). Thus, it seems unlikely that alkaloids impacted steer gains during the first 28 days of this study. Ergovaline concentration was less for metsulfuron treatments in June but this did not appear to influence steer gain in the last 28 days. Ergovaline increased nearly 337% in June (340 to 1145 ppb), which likely reduced steer performance, during the final 28 days of the experiment. Indeed, Diaz et al. (2018) reported that cattle ADG decreased as ergovaline concentration in the diet increased. In the current experiment, despite decreased alkaloid concentrations, application of metsulfuron did not improve animal performance as seen by Aiken et al. (2012).

SUMMARY

The use of metsulfuron on K31 pastures decreased ergovaline concentration while nitrogen fertilization maintained forage mass relative to an untreated control. Nitrogen fertilization did not increase ergovaline concentrations when metsulfuron was also applied. Reduced ergovaline and enhanced forage growth should result in improved cattle performance and the ability to increase stocking rate of pastures. Overgrazing occurred in the METO plots while forage balance increased over time in pastures with nitrogen fertilizer applied. Overgrazing led to limited forage intake while undergrazing led to forage accumulation and increasing plant maturity, which could have impacted steer performance.

Table 3.1. MU Southwest Center precipitation data, centimeters per month

Month	2019[1]	Historical average[2]
March	8.36	9.42
April	12.34	10.67
May	34.67	12.73
June	15.47	13.31
March-June precipitation	70.84	46.13
2019, % of historical avg.	154	

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

Table 3.2 Seedhead and 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
Fescue seedheads, # per 0.09 m^2	10.36[a]	1.35[b]	1.97[b]	2.82[b]	1.43	<0.01
Frequency						
Tall fescue	100.0	97.5	99.3	99.3	0.98	0.38
Kentucky bluegrass	83.3	85.3	78.3	91.0	5.83	0.25
Orchardgrass	18.5	7.5	11.8	14.8	4.90	0.41
Other grass	3.3	3.3	1.8	0.8	1.92	0.20
Birdsfoot trefoil	1.8	0.8	0.0	0.0	0.95	0.43
Buckhorn plantain	0.8	1.8	0.0	0.8	1.02	0.43
Other forb	8.5	4.0	0.8	0.8	2.12	0.07

[1]88% endophyte infection rate
[2]Nitrogen applied March 11 and metsulfuron on April 15
[3]SEM = Standard error of means
[a b]Means within rows lacking common superscripts differ (P<0.05)

Table 3.3 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
ADG. Kg						
4/18 – 5/17	1.01	1.13	1.17	1.21	0.07	0.25
5/18 – 6/14	0.73	-[4]	0.64	0.59	0.09	0.62
Overall	0.86	-	0.90	0.90	0.05	0.80

[1]88% endophyte infection rate
[2]Nitrogen applied March 11 and metsulfuron on April 15
[3]SEM = Standard error of means
[4]ADG not included as MET0 steers were removed 17 d early in the 5/18 – 6/14 period

Figure 3.1a. Effect of metsulfuron herbicide and nitrogen application on ergovaline concentration in K31 tall fescue pastures. Treatment effect (*P*=0.07); SEM = 80.

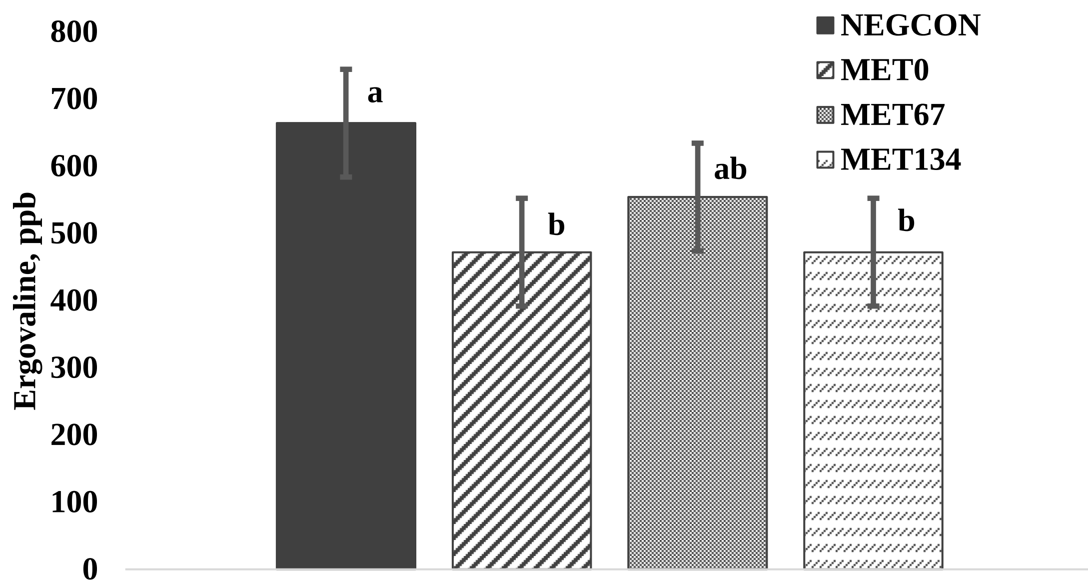


Figure 3.1b. Effect of metsulfuron herbicide and nitrogen application on ergovaline concentration in K31 tall fescue pastures. Treatment effect (*P*=0.07); month effect (*P*<0.01); SEM = 128.

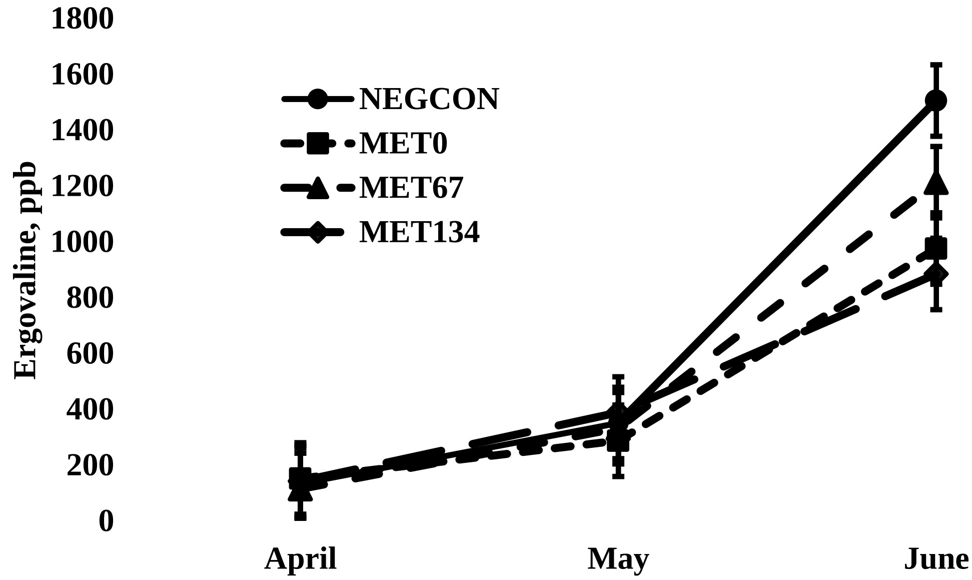
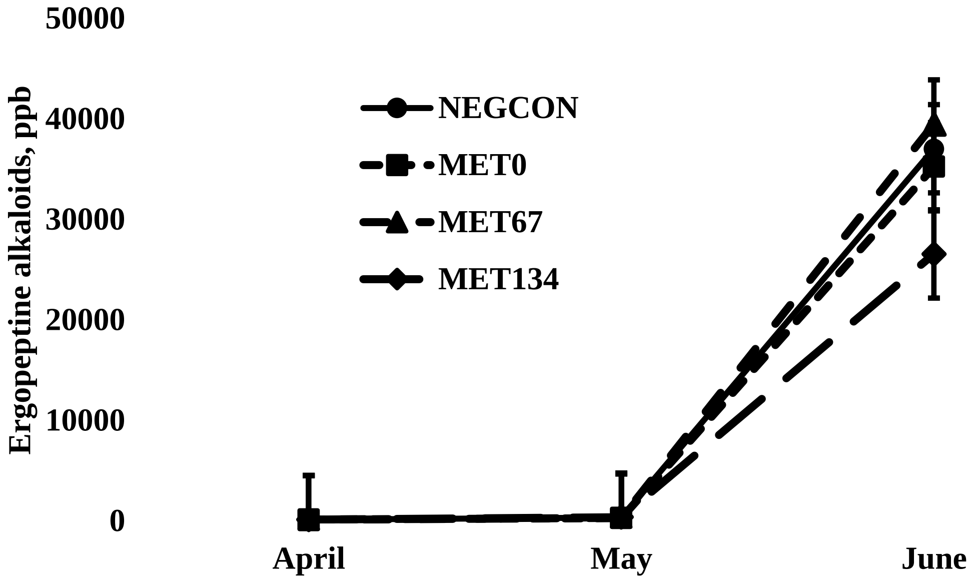
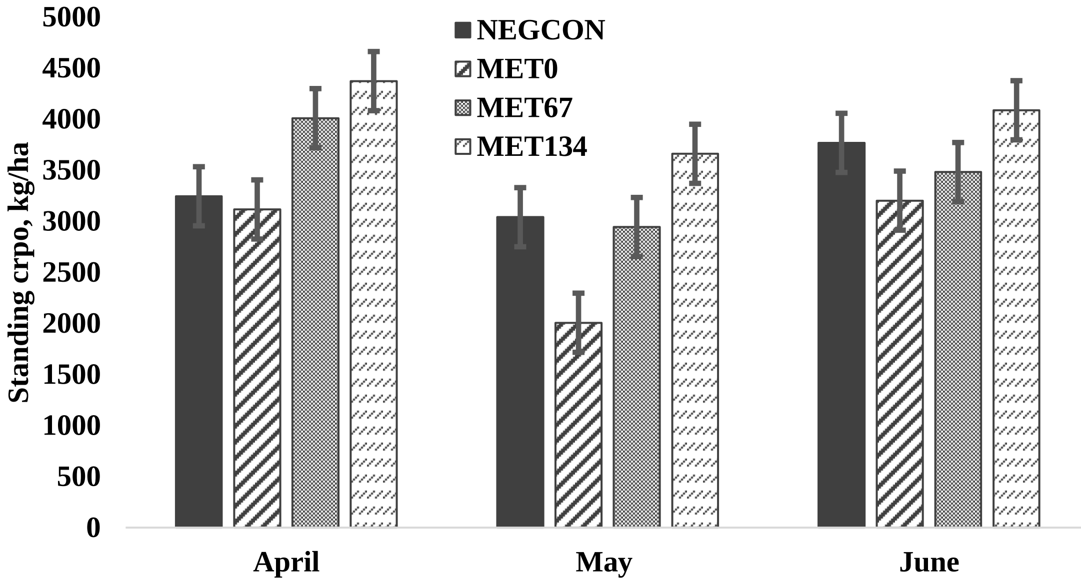


Figure 3.2. Effect of metsulfuron herbicide and nitrogen application on ergopeptine alkaloid[1] concentration in K31 tall fescue pastures. Treatment effect (*P*=0.48); month effect (*P*<0.01); SEM = 4775.



[1]Ergopeptine alkaloids: ergovaline, ergosine, ergotamine, ergocornine, ergocryptine, ergocrostine

Figure 3.3. Effect of metsulfuron herbicide and nitrogen application on standing forage crop in K31 tall fescue pastures[1]. Treatment x month interaction (*P*=0.02); linear nitrogen effect (*P*<0.01); SEM = 289.



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

Figure 3.4. Effect of metsulfuron herbicide and nitrogen application on monthly neutral detergent fiber concentrations in K31 tall fescue pastures. Treatment effect (*P*=0.02); month effect (*P*<0.01); SEM = 0.92.

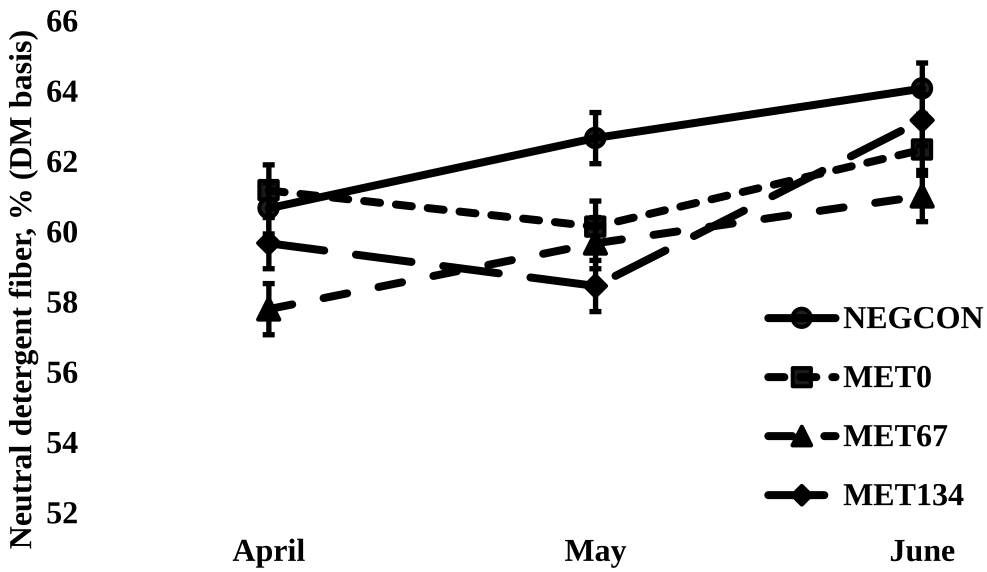


Figure 3.5. Effect of metsulfuron herbicide and nitrogen application on acid detergent fiber concentration in K31 tall fescue pastures. Treatment x month interaction (*P*=0.04); SEM = 0.86.

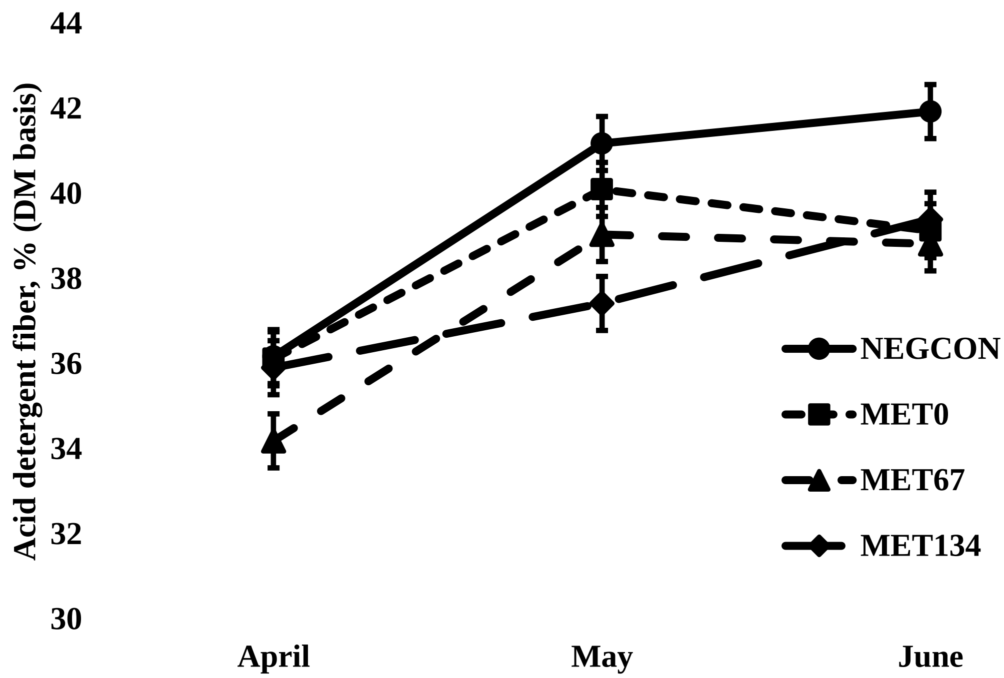
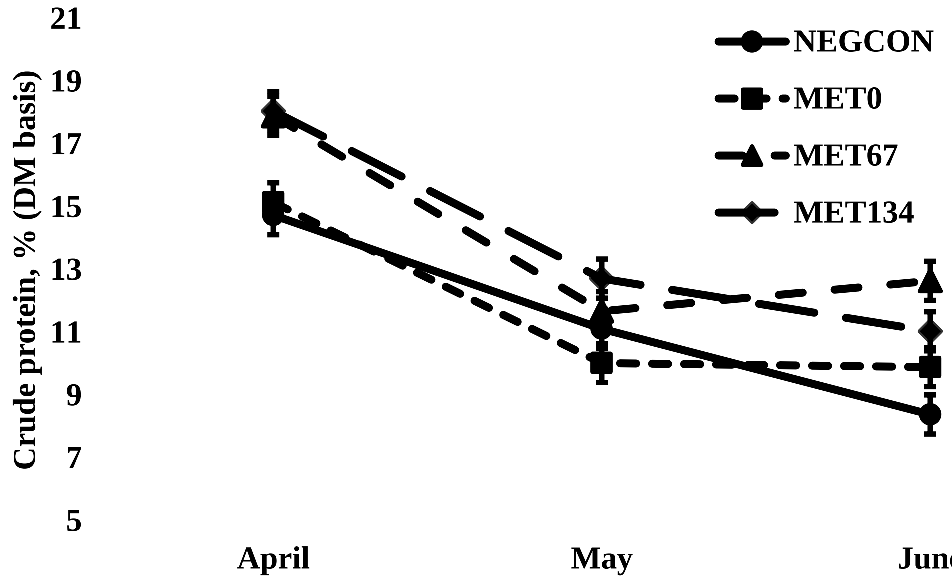


Figure 3.6. Effect of metsulfuron herbicide and nitrogen application on crude protein concentration in K31 tall fescue pastures. Treatment effect (*P*<0.01); month effect (*P*<0.01); SEM = 0.62.



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