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Fescue toxicosis and its influence on the rumen microbiome: mitigation of production losses through clover isoflavones

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ABSTRACT

This review highlights concerns with endophyte-infected tall fescue as a primary forage base in the southeastern United States and discusses specific physiological and ruminal effects caused by consumption of ergot alkaloids. Additionally, in an effort to promote various mitigation strategies to abate production limitations caused by fescue toxicosis, this review discusses the use of cool-season legumes, specifically red clover, with the already established forage base. Clovers are often mixed into pastures and help improve animal performance. Clovers contain phytoestrogenic compounds known as isoflavones that may be beneficial in reducing physiological limitations with consumption of endophyte-infected tall fescue.

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I. Introduction

Tall fescue [Lolium arundinaceum (Darbyshire)] is a cool-season perennial grass and is the most common forage utilized by beef cattle operations in the mid-South region of the United States. The plant is known for its hardiness in disease and insect resistance as well as prominent stand persistence throughout seasons of drought. Despite the nutritive benefits tall fescue provides, negative impacts on livestock growth and reproduction can occur when infected with the endophytic fungus Epichloë coenophiala. In ruminants, consumption of the endophyte often results in the condition of fescue toxicosis. This condition is characterized by several symptoms including decreased weight gain, reduced conception, depressed feed intake, and increased blood pressure and body temperatures. Periods of extreme environmental stress from heat or cold will cause animals to exhibit more pronounced symptoms leading to an unthrifty appearance. These symptoms manifest in decreased productivity which costs beef producers across the United States over \$1 billion annually (Hoveland 1993; Kallenbach 2015). To meet the nutritional demands of a growing global population, cattle producers are under increased pressure of the costs associated with efficiently producing beef for consumers. As many cow-calf and stocker operations utilize endophyte-infected tall fescue, it is critical to identify economical and novel methods and management strategies that will reduce losses incurred by symptoms of fescue toxicosis.

While the physiological effects of the tall fescue endophyte are well known from decades of research, the effects of the endophyte upon the ruminant system as a whole have yet to be determined. Behavioural changes from fescue toxicosis symptoms have been shown to negatively affect the health and nutrient intake of cattle (Howard et al. 1992). In an effort to cope with heat stress that is amplified by consumption of the endophyte, animals may spend increased amounts of time in shady areas of a pasture, or wade in pools of water or mud to alleviate this stress. As extreme environmental conditions exacerbate the effects of fescue toxicosis, the resultant behaviours should be elucidated more clearly to increase awareness of potential with tall fescue consumption in a pasture. Due to observances of these behaviours alongside physiological stressors, historically producers have used various mitigation methods, particularly the use of coolseason legumes such as red and white clover within these pastures to reduce these effects. Legumes are unique in their ability to fix nitrogen in the soil, as well as containing compounds known as isoflavones. Isoflavones found in several legume species may promote vasodilation of tissues, reducing the effects of vasoconstriction caused by ergot alkaloids.

The ruminal microbiome is critical to the health and nutritional status of the ruminant, as fermentation provides vital energy precursors. The presence of bacteria, protozoa, fungi, and archaea within the rumen act synergistically to ferment plant compounds, although the abundance of each group may vary depending on environment and diet composition. Without the extensive phylogenetic (Myer et al. 2017) and functional diversity of the rumen microbiome, the animal's ability to ferment forages like tall fescue, and other feedstuffs would be impaired. Yet, as concerning as the effects of endophyteinfected tall fescue are for reproduction and other physiological production parameters, little research exists as to the effect of the tall fescue endophyte on the rumen microbiome.

To that end, this review discusses fescue toxicosis caused by the *Epichloë* spp. derived ergovaline, its effects on the rumen microbial populations, as well as mitigation through grazing management, focusing on animal responses associated with

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II. Tall fescue toxicosis

A. Physiological effects of consumption

The ability of tall fescue to remain tolerant to stressors lies within the presence of the fungal endophyte Epichloë coenophiala (Leuchtmann et al. 2014; Young et al. 2014). The endophyte and tall fescue plant form a mutualistic relationship, improving the plant's competitive advantage over surrounding forages even in harsh environmental conditions such as drought, and temperature extremes. The fungus persists throughout the plant and produces ergot alkaloid compounds which are often concentrated in the seed head of tall fescue. Despite the advantages, the endophyte provides the plant, cattle, and horses grazing endophyte-infected tall fescue will often develop syndromes that reduce production, lactation and reproductive capacity due to these ergot alkaloids (Ball et al. 1991; Waller 2009). These concerns for reduced animal performance first gained attention in the 1950s, where Merriman reported a loss of appetite, reduced body weight and increased respiration rates (1955a). Reduced average daily gain, increased respiration rates and reduced reproductive efficiency became noted in cattle consuming endophyteinfected tall fescue compared to other forages and legumes (Blaser et al. 1956; Forney et al. 1969; Jacobson et al. 1970; Williams et al. 1972). Improved research efforts unearthed that the endophyte itself is not harmful, but is merely responsible for the production of ergot alkaloids throughout the plant. When consumed by livestock, these compounds bind to receptors found throughout the body. Most namely of these alkaloids is ergovaline, which is well known for its dopamine agonist (Yates et al. 1985; Lyons et al. 1986; Campbell et al. 2014), prolactin regulation (Strickland et al. 1994) and vasoconstrictive effects (Klotz et al. 2007; Foote et al. 2012).

In cattle, reduced overall gains decreased reproductive performance, reduced neuroendocrine function and increased heat stress are exhibited when ergot alkaloids are consumed. It has been reported that adverse effects with gain may be related to reduced ruminal and gastrointestinal function and motility. Dry matter intake has been reduced in cattle and sheep consuming endophyte-infected tall fescue (Hemken et al. 1979; Hemken et al. 1981). Appetite suppression may be the result of poor thermoregulation due to high ambient temperature stress (Beede and Collier 1986), as well as the possible interaction of the ergot alkaloid with serotonin receptors (Dyer 1993). Other researchers have suggested that the reduced weight gains exhibited by cattle consuming endophyte-infected tall fescue could not be completely accounted for by only reduced intake (Schmidt and Osborn 1993). Although differences in weight gain may not be entirely explained by heat stress or intake; altered rumen kinetics (Hannah et al. 1990) or reduced blood flow to the digestive tract (Rhodes et al. 1991; Klotz et al. 2007; Foote et al. 2012) will decrease gain, nutrient uptake and subsequent utilization.

The effects of vasoconstriction occur with both arteries and veins, becoming most apparent with extreme heat or cold stress. In periods of heat stress, ergot alkaloids interfere with the thermoregulatory mechanism by impeding heat dissipation that is dependent on circulating blood to the extremities and skin surface to be cooled (Walls and Jacobson 1970). The combination of vascular constriction throughout the animal's body and the reduced ability to shed a winter hair coat provide the opportunity for cattle to undergo heat stress during the warmer summer months in the mid-South transition region. Hoveland et al. (1983) reported elevated rectal temperatures and increased respiration rates when cattle consuming endophyte were subjected to high ambient temperature. In a study conducted by Klotz et al. Klotz et al. (2016), steer vasculature remained constricted even after removal from toxic endophyte-infected tall fescue for 28 days, suggesting that reduced susceptibility to heat stress and increasing vascular activity requires at least 35 days on non-toxic tall fescue or another forage before returning to baseline levels. Additionally, ergot alkaloids may be stored in adipose tissue (Realini et al. 2005), and thus can disrupt normal metabolism after animals are removed from the endophyte-infected pastures.

These studies provide evidence that the constriction of the animal's vasculature may be most responsible for the characteristic symptoms of heat stress with fescue toxicosis. In addition to experiencing more pronounced heat stress, when cattle consume endophyte-infected tall fescue in periods of extreme cold temperatures, the vasoconstrictive effects of ergovaline may cause cattle to experience lameness, commonly known as fescue foot, as well as sloughing off the tips of tails and ears (Spiers et al. 1995) due to impaired vascular function in the extremities.

The reproductive effects of ergot alkaloid consumption in ruminants have focused a great deal on female reproductive physiology, of which ergot alkaloid consumption has been demonstrated to reduce conception, and lactation (Gay et al. 1988; Peters et al. 1992). Male reproductive concerns with ergot alkaloids are not as clearly defined, with much research focusing on limitations of spermatozoa production. Schuenemann et al. (2005) observed reduced fertility potential in bulls that were exposed to ergot alkaloids compared to bulls grazing novel/non-toxic endophyte tall fescue varieties. Reductions in overall sperm concentrations and the increased presence of abnormal sperm have been observed when bulls grazed endophyte-infected tall fescue (Pratt, Stowe, et al. 2015). The subsequent consequence of these observations is reduced bull fertility, however, this may be a trait that is not shared by all species. In a study conducted using rats as a model for livestock grazing endophyte-infected tall fescue, testicular, spermatozoa and sperm production potential parameters were measured (Zavos et al. 1986) but no significant differences were observed with respect to motility, sperm count or weight.

Prolactin receptors are found throughout the male reproductive organs, and prolactin has been observed in the seminal fluid of cattle (Pratt, Calcatera, et al. 2015). Testicular prolactin receptors that are influenced by ergot alkaloids may provide further insight on the reduced reproductive capacity of males. Reduced serum prolactin concentrations in both males and females is often a sign of ergot alkaloid pressure and fescue toxicosis. Localized vasoconstriction of the testes due to ergot alkaloid presence may reduce the temperature of the scrotum, and further reduce spermatogenesis and motility (Schuenemann et al. 2005).

Consumption of endophyte-infected tall fescue alters several neuroendocrine pathways. The endophyte has been shown to reduce serum prolactin concentrations across species, in cattle (Hemken et al. 1979), sheep (Bond et al. 1981), and horses (McCann et al. 1992). Dopamine is the major prolactininhibiting neurotransmitter and the ability of the endophyte to reduce serum prolactin levels is due in part to the actions of ergot alkaloids on pituitary dopamine receptors (Goldstein et al. 1980; Civelli et al. 1993; Campbell et al. 2014). Administration of a dopamine antagonist to animals affected by fescue consumption increases the circulating levels of prolactin and demonstrates the dopaminergic effect of the alkaloids produced by the endophyte (Lipham et al. 1989). Prolactin, while pivotal for milk production and growth of mammary tissue, is also associated with the shedding or retention of a winter hair coat. Cattle grazing endophyte-infected tall fescue frequently maintain their winter coat, which is known to be a classic symptom of animal's experiencing summertime fescue toxicosis.

B. Ergot alkaloids and rumen microbiome

Microbial populations in the rumen are able to degrade many of the ergot alkaloids consumed by the animal (Moyer et al. 1993), and in the earthworm intestine (Rattray et al. 2010). However, it is unknown which organisms within the rumen microbiome are responsible for the degradation. Tryptophan is essential for the biosynthesis of ergovaline (Garner et al. 1993; Roylance et al. 1994). The structure of tryptophan allows for the formation of the ergovaline ring structure (Figure 1) which has a similar backbone to several catecholamines, including dopamine and serotonin. The presence of the detrimental ergot alkaloids is not unique to the tall fescue plant; perennial ryegrass produces an endophyte containing ergopeptides that provide similar symbiotic traits to the ergot alkaloids from tall fescue as well as in *Claviceps purpurea* and related species (Clay 1988; Bush et al. 1997). Where fescue toxicosis impacts production



Figure 1. Structure of ergovaline, courtesy of Klotz et al. (2007).

throughout the United States, ryegrass toxicity is found commonly throughout Australia and New Zealand (Van Heeswijck and McDonald 1992; Easton 1999), with detrimental effects persisting through consumption of a similar endophyte that infects tall fescue, *Neotyphodium lolii*. Ryegrass staggers have been well documented throughout literature, caused by consumption of endophyte-infected perennial ryegrass that produces the neurotoxin lolitrem B (Figure 2). Lolitrem B acts as a calcium-activated potassium channel inhibitor, causing muscle tremors, stiff movements, and lateral recumbency (Tor-Agbidye et al. 2001; Imlach et al. 2008). Although endophyte-infected perennial ryegrass contains ergovaline, Fletcher and Harvey (1981) determined the symptoms of ryegrass staggers were attributed to lolitrem B concentrations rather than ergovaline.

In both ryegrass and tall fescue, the endophyte is not uniformly distributed throughout the plant, and grazing management strategies to reduce seed heads and limit maturation of the plant have proven effective at reducing instances of these toxicities. Klotz (2015) described several levels of ergot alkaloids consumed and the effect of each dose, with 0.009 mg/kg BW ergovaline resulting in symptoms of fescue foot, and higher doses of 0.016 mg/kg BW resulting in inflammation of the hoof. Nicol and Klotz (2016) further described a maximum ergovaline concentration of typical ryegrass pastures in New Zealand at 0.7 mg/kg DM, considered significantly higher than allowed concentrations in the United States.

In a recent in vitro study conducted by Harlow, Goodman et al. (2017), it was determined that ruminal hyper-ammoniaproducing bacteria (HAB), which are responsible for the deamination of amino acids and breakdown of peptides, could be responsible for the breakdown of ergovaline and ergopeptide compounds. HAB are unique in that they produce ammonia at very high rates from amino acids, such as tryptophan, which is necessary for ergovaline synthesis. Specifically, it was concluded that five bacteria found in the rumen were able to degrade ergovaline, all of which had characteristics of hyperammonia-producing bacteria, lending support that bacteria which degrade tryptophan, also have the ability to degrade ergovaline. Specifically, these bacteria were gram-positive and rod-shaped, phylogenetically similar to Clostridium botulinum, a gram-positive organism, but non-toxic when ruminant animals consume this orally (Allison et al. 1976). While the majority of ruminal bacteria are designated gram-negative, an increase in the proportion of gram-positive bacteria may be observed during the transition from forage-based diets to increased concentrate diets. Higher concentrate diets and



Figure 2. Structure of lolitrem B found commonly in perennial ryegrass. Image adapted from Saikia et al. (2008).

more readily digestible forages can cause an increase in bacterial numbers of 10–100 fold compared to when animals are fed a less digestible forage diet (Nagaraja 2016), often improving feed efficiency and volatile fatty acid production from microbial populations. Harlow et al. also concluded that some *Prevotella* species contribute to the metabolism of ergovaline (2017). *Prevotella* species are relatively abundant in the rumen (Jami and Mizrahi 2012), and thus inducing a ruminal environment favourable to *Prevotella* and other HAB production could be beneficial in reducing instances of fescue toxicosis.

Ergovaline can be degraded into lysergic acid (Figure 3), where it is then excreted in urine and faeces, but where this degradation specifically occurs has not been ascertained (Hill et al. 2001; Schultz et al. 2006). The mechanism behind the conversion between ergovaline and lysergic acid has yet to be understood. De Lorme et al. suggest that ergovaline is first liberated from feed material and thus microbial action degrades it further to lysergic acid, acting on tissues throughout the body where it will eventually be excreted in urine and faecal material (2007). Merrill et al. (2007) examined the influence of yeastderived cell wall preparation on high-ergot alkaloid tall fescue straw provided to steers and cows, where alkaloids included both ergovaline and lysergic acid. The authors determined that including yeast-derived cell wall improved serum prolactin post-calving in cows, and may alleviate concerns with high-alkaloid tall fescue, however, excretion of lysergic acid and ergovaline were not affected by the yeast-derived cell wall treatment.

Klotz et al. (2006) concluded that ergovaline is exponentially more powerful in stimulating vasoconstriction within in vitro bovine vasculature compared to lysergic acid, and more potent throughout host physiology as a whole. Ruminal microorganisms interact with feedstuffs in three established ways: those within the ruminal fluid, those loosely attached to feed particles and those firmly attached to feedstuffs (Cheng and McAllister 1997), thus breakdown of feedstuffs is essential for several types of microorganisms to access nutrients. Consequently, potentially detrimental compounds such as ergot alkaloids including ergovaline and subsequently lysergic acid are also accessible by microorganisms.

When tall fescue seed was incubated with rumen fluid in an *in vitro* fermentation study conducted by Westendorf et al. (1992), the diet that had been previously incubated with fluid was less toxic to rats consuming the whole seed than those that were fed a non-ruminal fluid incubated fescue seed. In the same study, authors determined that rats consuming the rumen fluid inoculated endophyte-infected seed had improved



gain: feed conversion compared to those with non-incubated seed (1992). This suggests that the rumen microbiota are responsible for reducing some of the toxicity of the endophyte, in agreement with results by De Lorme et al. (2007). However, the results from Westendorf et al. (1992), De Lorme et al. (2007) and the bacteria isolated by Harlow et al. (2017), were accessed from rumen fluid conducted as an *in vitro* or *ex vivo* experiment, rather than *in vivo* where additional physiological parameters can be measured for efficacy.

As endophyte-infected tall fescue will continue to persist throughout the United States as an important forage base, it is paramount to understand its benefits as well as limitations. The impacts of ergot alkaloids on livestock production have been problematic to producers for decades but also have prompted the development of several successful mitigation methods.

III. Mitigation strategies: novel endophyte, clovers, and isoflavones

A. Novel endophyte-infected tall fescue

Research on the effects associated with the consumption of endophyte-infected fescue has led to the investigation of management strategies to improve production where replacement of tall fescue forage is not economically appropriate. As costs of replacing existing stands of endophyte-infected tall fescue can exceed \$600/ha, additional management alternatives can be sought out (Kallenbach 2015). Some of these strategies have included supplementation of vitamin E (Jackson et al. 1997), thiamine (Dougherty et al. 1991), and protein (Aiken et al. 2001) to rectify many of the concerns with fescue toxicosis.

The use of a novel endophyte-infected tall fescue plant provides similar nutritional value as the endophyte-infected plant, but without the deleterious effects of the endophyte. Cattle grazing novel endophyte-infected tall fescue experience increased growth rates and increased average daily gain without reduced prolactin, increased respiration rates and rectal temperatures (Nihsen et al. 2004; Beck et al. 2008; Hancock and Andrae 2009). While the novel endophyte proves useful in managing and reducing fescue toxicosis, it is not always the most economical solution. Gunter and Beck (2004) estimated at least a three-year span between introductions of the novel endophyte before yields were enough to become economically profitable. Zhuang et al. (2005) estimated that until a pasture is 70% or more infected with the endophyte, it is not economically beneficial to utilize tall fescue with the novel endophyte.

B. Inclusion of legumes into pastures

Grazing management strategies including rotational grazing and inclusion of legumes and other grasses within a tall fescue pasture have been used for decades to reduce the instance of fescue toxicosis. Legumes have a distinctive benefit compared to other forage species, due to their ability to harness and fix nitrogen into the soil, improving soil fertility and nutrient value. Vincent (1980) and Young and Johnston (1989) describe the relationship between legumes and nitrogen

fixation as a result of the symbiotic soil microorganism Rhizobium spp, The bacteria form a symbiotic relationship with the legumes, creating small nodes on the root system of the legume. The plant will supply carbohydrates for the bacterium. and the *Rhizobium* spp. provide nitrogen fixation to the plant. In addition to benefitting both the bacterium and soil by this nitrogen fixation property, legumes are high in digestible nutrients and protein, increasing the guality of the pasture when included alongside grasses (Chestnut et al. 1991; Duranti and Gius 1997). When conducting a study with sheep, Thornton and Minson calculated voluntary intake of legumes was 28% higher when compared to equally digestible grasses (1973). As they are more digestible and palatable, the use of lush pastures of legumes is cause for speculation on their use, as livestock can have a tendency to bloat with the consumption of such nutritive forages, particularly pastures of legumes with alfalfa and ladino clover (Bryant et al. 1960; Lees et al. 1981). Despite these concerns, the inclusion of legumes into forage stands presents a great opportunity for improving forage nutritive value. Particularly in the mid-South region of the United States, forage recommendations may include planting red and white clover or other cool-season legumes alongside tall fescue stands in a pasture, for the previously mentioned benefits.

In particular, red and white clover have proven to be most successful at increasing growth and overall gains and mitigating fescue toxicosis (McLaren et al. 1983; Lusby et al. 1990; Chestnut et al. 1991; Beck et al. 2012). Lusby et al. (1990) found that the inclusion of red clover into stands of tall fescue not only reduced the severity of fescue toxicosis (reduced rectal temperatures and respiration rates) but it improved average daily gain and carcass guality compared to cattle on tall fescue alone. Broderick (1995) noted that the protein content of legumes provided an excellent source of nitrogen for ruminants, citing that red clover compared to alfalfa was more efficient as a source of rumen un-degradable protein (RUP). Previous literature has indicated that the inclusion of legumes in tall fescue pasture will reduce the occurrence of fescue toxicosis by a dilution or competitive selection against tall fescue. However, recent research is indicating the legumes may reduce the severity of fescue toxicosis by other mechanisms.

C. Isoflavones

Legumes, including clovers and soybeans, contain phenolic, phytoestrogen compounds known as isoflavones. These compounds are considered secondary metabolites and act as antimicrobial and antioxidant agents in the plant (Wink 2013). Isoflavones are also known for their use in human medicine for mitigation of menopausal symptoms in women (van de

 Table 1. Averages of the total isoflavone concentration (mg/g) among plant parts and clover species (adapted from Butkute et al. (2014)).

Plant Part	Trifolium spp.®				
	T. medium	T. pretense	T. repens	T. pannonicum	T. rubens
Stems	3.62	3.62	0.204	0.230	0.780
Leaves	7.54	2.74	0.191	0.274	0.493
Flowers	2.31	2.22	0.171	0.98	0.38

^aConcentration of total isoflavones in mg/g

Weijer and Barentsen 2002). While the isoflavones are considered nonsteroidal, they maintain a weak-moderate affinity for estrogen receptors, specifically estrogen receptor β (ER- β). Receptor ER-B is most widely expressed in non-reproductive tissues such as bone and blood vasculature where it mediates some of the growth-promoting effects of estrogen on nonreproductive tissues (Sunita and Pattanayak 2011). However, Millington et al. (1964) noted an estrogenic effect of wethers grazing red clover pastures. Adams (1995) suggested that fertility is only affected by cows and ewes consuming legumes, while males remain unaffected. When cows consumed solely red clover silage, a significant reduction in conception was noted until the silage was removed from the diet, and consequently the cows were able to conceive (Kallela et al. 1984), contrasting unaffected heifer fertility rates reported by Austin et al. (1982). In males, Lightfoot et al. (1967) noted a failure of sperm transport from rams to ewes previously grazing legume pastures. Various reports indicate species differences of subterranean clover varieties contributing to these fertility differences rather than all clover or legume varieties (Trifolium subterraneum L.) (Cox and Braden 1974; Hughes 1988). Nevertheless, the nutritive value of red and white clover varieties add to pastures should be taken into account when addressing fertility concerns. Waghorn and McNabb (2003) suggested that reducing specific isoflavone consumption from clover varieties will have a beneficial effect on fertility. Contrary to a specific area of tall fescue where the endophyte is concentrated, isoflavones are found throughout the entire plant, rather than concentrated in the bloom or leaves (Table 1).

Four major forms of isoflavones found in red clover include biochanin A, formononetin, genistein, and daidzein (Figure 4). In ruminants, formononetin is metabolized to daidzein and further metabolized to equol. Biochanin A is metabolized to genistein and then further to p-ethylphenol (Braden et al. 1967; Dickinson 1988). Equol has been suggested to have a greater influence on bovine blood vasculature than its precursors (Dickinson et al. 1988; Beck et al. 2005). Red clover, the most commonly used commercial clover, contains biochanin A in the highest quantity. When dairy cows were fed on a white clover, red clover, lucerne, or chicory pastures, equol from red clover was found in the highest concentrations throughout the body and discovered in the milk (Andersen et al. 2009). Sheep are more susceptible to formononetin



Figure 4. Molecular structure of the four major isoflavones: formononetin, daidzein, genistein and biochanin A. Image adapted from Wu et al. (2010).

compared to larger ruminants, having reduced fertility from lingering estrogenic effects. Wu et al. (2010) determined that formononetin, identified to have a similar magnitude of vasorelaxation as biochanin A, may possess a nitric-oxidedependent and endothelium-independent relaxation effect on vasculature unrelated to progesterone or estrogen receptor activation.

It has been reported that when exposed to isoflavones, the vasculature will relax (Nevala et al. 1998; Simoncini et al. 2005), whereas when ergovaline from tall fescue is consumed vasculature will constrict due to binding on amide receptors (Rhodes et al. 1991; Aiken et al. 2001). Due to the isoflavones found in red clover, it has been suggested that red clover may contribute to an antimicrobial effect within ruminal bacteria communities (Flythe and Kagan 2010) and reduce production of ammonia from ruminal bacteria at a concentration of 30 ppm (Flythe et al. 2013). Interestingly, the hyperammonia-producing bacteria are reduced in the presence of isoflavones, which are among the bacteria needed for ergovaline degradation as elucidated by Harlow et al. (2017). It was further determined that when 30 mg of biochanin A per litre of rumen was infused into the rumen of goats receiving endophyte-infected tall fescue seed, vasorelaxation and return to normal pulse were observed (Aiken et al. 2016). This has been associated with agonist activity at β-adrenergic receptors within the endothelium of the blood vessels which stimulates synthesis of nitric oxide that in turn will promote vasorelaxation. As vasoconstriction contributes to heat stress, promoting vasorelaxation with the inclusion of legumes may be a solution to understanding tall fescue toxicosis mitigation.

Jia et al. (2015) examined mesenteric vasculature contractility in response to ergotamine after prior incubation with ergovaline tall fescue seed extract, isoflavones formononetin and biochanin A and subsequent combinations. The combination of the isoflavones provided improved relaxation compared to individual isoflavones alone, lending support that a combination of isoflavones found in plant tissue may promote mitigation of vasoconstriction from ergot alkaloids.

In an effort to greater understand the influence of these compounds on rumen microflora, Harlow et al. (2017) used biochanin A in combination with dried distiller's grains to determine amino acid degradation and performance of beef steers. They observed that biochanin A reduced HAB and had an additive effect on steer weight gain. Additionally, studies conducted by Harlow et al. targeted specific amylolytic (2017) or cellulolytic (2018) bacterial species and their sensitivities to biochanin A. Three cellulolytic bacteria (Fibrobacter succinogenes S85, Ruminococcus flavefaciens 8, and Ruminococcus albus 8) and four amylolytic bacteria (Strep. bovis JB1, Strep. bovis HC5, Lactobacillus reuteri, Selenemonas ruminatium) were reduced with exposure to biochanin A. However, the minimum inclusion amount of biochanin A in a diet has not been determined, and may have a much lower threshold than what has been previously used in diet formulation. Biochanin A may be the primary isoflavone to improve the performance of production animals. This adds further evidence that the use of isoflavones improves beef cattle efficiency, and future studies should be conducted to determine a minimum inclusion rate for positive results.

Isoflavones, although described as phytoestrogens, maintain a weak affinity to bind to estrogen receptors compared with naturally produced or synthetic estrogen. Previous research by Davenport et al. (1993) indicated that estradiol implants on steers grazing high endophyte-infected tall fescue improved overall average daily gain. Similarly, Beconi et al. (1995) indicated that estradiol implants on steer calves grazing stockpiled high endophyte-infected tall fescue significantly improved average daily gain and gain: feed efficiency compared to other forage types. However, these beneficial effects may be limited to stocking density of pastures, as indicated by Aiken et al. (2006).

The utilization of soybean hulls to mimic beneficial estrogen/ progesterone implant effects was documented by Carter et al. (2010) and Shappell et al. (2015). Of the four previously described isoflavones, genistein is considered to have the most estrogenic activity in soybeans that may act as a β -adrenergic receptor antagonist (Grossini et al. 2008) to promote vasodilation. Carter et al. (2010) determined steers receiving pelleted soybean hulls had increased ADG and serum prolactin while having reduced rough hair coats than on a steroid hormone implant alone. However, the authors indicated this response may be from dilution of ergot alkaloids rather than an active role in mitigating tall fescue toxicosis.

Shappell et al. (2015) supplied soybean hulls at estradiol equivalents similar to the implant and noted improved serum prolactin in steers receiving soybean hulls alone, and when combined with steroid implant became comparable to steers consuming endophyte-free tall fescue. The estrogenicity and potency of soy-based feed supplements and isoflavones have not previously been reported, and concerns about the bioavailability of isoflavones in the rumen should be considered. Additionally, further studies should be conducted comparing estrogenicity of legumes and isoflavones with estradiol/progesterone implants and efficacy of reducing tall fescue toxicosis.

IV. Conclusion and future directions

These preliminary studies indicate a change in vasculature and rumen microbial communities in response to red clover sourced isoflavones, but further studies need to be conducted to determine the effect on the entire rumen bacterial community and physiology in cattle with that specific concentration of isoflavones. It has yet to be elucidated if clover isoflavones can mitigate symptoms of fescue toxicosis in cattle and if daily administration of isoflavones will cause any microbial dysbiosis within the rumen. Therefore, due to the prevalence of fescue toxicosis throughout the Southeast, there is a critical need to determine microbial and host-physiological responses to clover isoflavones in cattle, possibly resulting in additional mitigation approaches to fescue toxicosis. Reducing any potential dysbiosis in rumen microbial populations that may occur due to consumption of endophyte-infected tall fescue may contribute to increased nutritional efficiency and subsequent health of the animal. Therefore, determining the effect of clover isoflavones on rumen microbial health and host physiology can provide further insights into combating fescue toxicosis.

As the inclusion of legumes into pastures provides many benefits for both soil fertility and forage quality, determining the minimum amount of isoflavones in a diet to sufficiently reduce symptoms of fescue toxicosis can be key to determining forage management practices. For example, not over- or underseeding pastures with legumes can result in reduced productivity for the producer due to competition of other plants and upkeep expenses for these broadleaf plants. Determining the minimum amount of isoflavones in red clover and other legume species that will reduce instances of fescue toxicosis, could improve forage as well as grazing management recommendations to producers.

Disclosure statement

No potential conflict of interest was reported by the authors.

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