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Using Condensed Tannin to Mitigate Tall Fescue Toxicosis

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Abstract

Endophyte-infected (*Neotyphodium coenophialum*) tall fescue, [*Schedonorus arundinaceus* (Shreb.)] produces ergot alkaloids. Condensed tannins could bind to ergot alkaloids and render them ineffective. The first objective was to improve the health of sheep offered endophyte-infected tall fescue by binding toxins in the rumen with condensed tannins. Non-toxic novel-endophyte infected tall fescue (NE) or toxic endophyte-infected tall fescue forage was harvested, baled at targeted moisture of 55%, wrapped within 8 hours of baling with 2 layers of net wrap and 20 layers of plastic, and ensiled for at least 60 days. Prior to feeding, forages were chopped and packed into plastic trash bins (167 L) lined with 2 plastic bags (3 mil). Non-pregnant, non-lactating ewes (n = 20; 57 ± 1.3 kg initial BW) were housed in individual 1 × 1.5-m pens with metal grate flooring and ambient temperature maintained at 27 to 29 °C. Sheep were offered ad libitum access to either NE or toxic endophyte-infected tall fescue baleage with no condensed tannin (E0), condensed tannin at 10 g/kg DM (E10), or 30 g/kg DM (E30). All ewes received 4 g/kg BW liquid molasses, serving as the carrier for the condensed tannin supplement in E10 and E30. The study consisted of two 21-d periods with a 14-d adaptation followed by 7-d total fecal and urine collection, and a 30-d washout between periods. Dry matter intake (DMI; $P < 0.05$) was greater for NE than E10 and E30. Dry matter digestibility (DMD; $P < 0.05$) and OM digestibility (OMD; $P < 0.05$) were greater for NE than E30. Digestible DMI ($P < 0.05$) and organic matter intake (OMI; $P < 0.05$) were greater for NE than treated groups. Prolactin concentrations were greater for ewes offered NE (P <0 .01). Nitrogen retained did not differ among treatments ($P \geq 0.31$). The second objective evaluated the effects on forage components and silage fermentation products by ensiling chopped tall fescue with quebracho tannin. Tall fescue was harvested with a Carter harvester at 1000 h, spread on concrete, mixed

thoroughly, and dried for 1 or 2 h to achieve 67 and 44% moisture [high moisture (HM) and low moisture (LM), respectively]. Quebracho tannin was either not applied or mixed with distilled water 7 d prior to packaging and applied at 1 and 2% of the total silage DM to both moisture treatments prior to ensiling. Tall fescue and fescue-tannin mixtures were pressed into PVC pipes (10.2 cm × 29.2 cm) to achieve a packing density of 192 kg/m^3 and stored at room temperature (23.7 ℃) for 60 d. Sub-samples were removed and analyzed for fermentation parameters and forage chemical components. Pre-ensiled ergovaline was not different across treatments ($P \geq$ 0.19). Pre-ensiled acid detergent fiber (ADF) and OM were affected by the moisture × tannin interaction ($P = 0.01$). Pre-ensiled ADF was greatest in LM silages with 1% tannin compared to all other moisture-tannin treatments. Pre-ensiled OM was greatest in HM with 0% tannin compared to HM with 2% tannin and all LM treatments. Changes between pre- and post-ensiled WSC were greater from HM vs. LM ($P < 0.05$). Final pH was greater in LM silage with 0% and 1% tannin compared to all HM treatments ($P < 0.05$). Total acids were greatest in HM ($P < 0.05$) but did not differ ($P = 0.54$) among tannin treatments. Ammonia concentrations decreased as tannin levels increased ($P < 0.05$). Condensed tannin inclusion with toxic, tall fescue did not mitigate tall fescue toxicosis in these studies.

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Chapter 1

Review of Literature

Introduction

Pastures with forage cover significant acreage worldwide and are managed for grazing animals or hay production (Young et al., 2013). Because of the need for forages by grazing animals year-round, ensiling forages has become a common practice (Jayanegara et al., 2018). Ensiling forages is considered to be a diverse practice due to the ability to be made in hot and cold regions, fed to livestock either in summer or winter, and stored outside since the ensiled bale is completely wrapped (Bernardes et al., 2018; Keogh et al., 2009; Weinberg et al., 2011; Woodward et al., 2006). A wide variety of forages can be ensiled, including tall fescue.

Tall fescue [*Schedonorus arundinaceus* (Shreb.)] is a prevalent forage in the United States and grows well in the east-central and southeastern United States (Franzluebbers and Poore, 2021). It was first introduced in the 1800s with the variety Kentucky-31 released in the mid-20th century and is popular for many reasons (Franzluebbers and Poore, 2021). Tall fescue has a wide range of establishment and adaptation, long grazing season, pest resistance, and seed production (Stuedemann and Hoveland, 1988). However, these agronomic benefits have also come with challenges as tall fescue has a symbiotic relationship with a fungal endophyte which produces ergot alkaloids. When ergot alkaloids are consumed by livestock, they can produce adverse effects commonly known as fescue toxicosis. These negative impacts have driven producers to explore ways to mitigate tall fescue toxicosis.

Condensed tannins are secondary plant metabolites and can bind to nutrients, specifically protein (Naumann et al., 2013). Protein that is bound by condensed tannins could bypass the rumen and shift digestion to the intestines (Woodward et al., 2009). Condensed tannins can form

a complex with alkaloids since alkaloids are nitrogen-based secondary compounds (MacAdam and Villalba, 2015). Therefore, condensed tannins forming complexes with secondary compounds could minimize the negative effects of individual components. The objective of this literature review is to discuss tall fescue, condensed tannins, and ensiling characteristics.

Tall Fescue

Ergot Alkaloids in Tall Fescue

Tall fescue is a popular forage because of its symbiotic relationship with the fungal endophyte *Neotyphodium coenophialum* (Young et al., 2013). Because of this relationship with the endophyte, tall fescue is more tolerant to different stressors (Leuchtmann et al., 2014). The fungal endophyte produces ergot alkaloids, which have deleterious effects on the central nervous system of Mammalia (Ball et al., 1991; Tudzynski and Tenberge, 2003; Waller, 2009). These mycotoxins tend to be heavily concentrated in the seed, but also have a presence in the leaf and stem tissue (Rottinghaus et al., 1991). Ergot alkaloids reach peak concentrations in pastures in the late spring when the forages go to seed, and concentrations can decrease during the summer before increasing again in the fall (Rottinghaus et al., 1991). Environmental conditions may also impact the level of ergot alkaloids synthesized (Yates, 1962).

Tall fescue ergot alkaloids are distributed throughout the leaf blade, sheath, and seed. These alkaloids are not found in non-infected tall fescue (Lyons and Bacon, 1985). Many ergot alkaloids produced by tall fescue are ergoline alkaloids or ergopeptine alkaloids, including ergovaline and ergotamine (Roberts et al., 2009). The ergot alkaloids produced by *N. coenophialum* can cause problems collectively referred to as fescue toxicosis (Aiken and Strickland, 2013).

Fescue Toxicosis

Tall fescue toxicosis was first observed by producers reporting reduced performance and reproductive efficiency in livestock consuming tall fescue compared to other forages and legumes (Blaser et al., 1956; Forney et al., 1969; Jacobson et al., 1970). Tall fescue toxicosis is generally grouped into three main problems or syndromes (Stuedemann and Hoveland, 1988), and livestock typically present with symptoms grouped into four different categories: 1) decreased weight gain and pregnancy rate; 2) decreased feed and increased water intake; 3) physiological responses; and 4) changes in sera or plasma levels (Bouton et al., 2002).

The first syndrome was referred to as the "summer slump" and resulted in decreased animal performance (Stuedemann and Hoveland, 1988). During the summer slump, animals can have rough hair coats (Aiken and Strickland, 2013), elevated core body temperatures, labored respiration, and decreased prolactin concentrations (Bouton et al., 2002). Sheep consuming endophyte-infected tall fescue exhibited appetite suppression and poor thermoregulation (Beede and Collier, 1986; Hemken et al., 1979). Livestock experiencing summer slump tend to spend excessive time in shade or ponds during the summer months (Ferguson et al., 2021).

The second syndrome, "fescue foot", presented with symptoms like foot rot or frozen feet (Yates, 1962). During the winter months, livestock presented with lameness in the hindquarters, specifically in the left hind foot (Guerre, 2015). This eventually led to livestock losing tail switches, ears, and hooves (Ferguson et al., 2021). Low environmental temperatures and obstructed blood flow were determined to cause the loss of extremities (Jensen et al., 1956).

The third syndrome, fat necrosis, occurred when high rates of nitrogen fertilizer were applied to the tall fescue. Ergot alkaloids are thought to be stored in adipose tissue, which can disrupt normal metabolism even after animals are no longer grazing endophyte-infected pastures

(Realini et al., 2005). Hard fat will accumulate in the abdominal cavity, and no symptoms are seen until vital body processes are affected (Ferguson et al., 2021). Changes in behavior from fescue toxicosis have been shown to negatively impact livestock health and nutritional status, and these negative impacts have cost the forage-based livestock industry in the United States over a billion dollars each year (Aiken and Strickland, 2013; Howard et al., 1992).

Nontoxic vs Toxic Endophytes

Endophyte-infected tall fescue can persist in environmental conditions that would weaken other grasses (Kallenbach, 2015). The symbiosis between the tall fescue and the endophyte is why tall fescue is an ideal forage, and this relationship produced the beneficial agronomic traits but also compromised animal production (Aiken and Strickland, 2013; Kallenbach, 2015). Endophyte-free cultivars were commercially released in the 1980s with the hopes of alleviating the challenges of endophyte-infected tall fescue, but unfortunately, endophyte-free cultivars lacked persistence and the grazed stands deteriorated rapidly even though animal performance improved (Aiken and Strickland, 2013). Endophyte-infected tall fescue persists better under drought, poor soil fertility, and intensive grazing versus the endophyte-free cultivars (Kallenbach, 2015).

Since endophyte-free cultivars lacked persistence, different tall fescue cultivars were developed with novel, nontoxic endophytes that produce little to no detectable ergot alkaloids (Bouton et al., 2002; Nihsen et al., 2004). The novel cultivars presented an equal stand survival to the endophyte-infected tall fescue (Ferguson et al., 2021). Animal performance was not negatively affected by novel cultivar (Ferguson et al., 2021). Cattle experienced increased growth rates and average daily gain without reduced prolactin or increased rectal temperatures

and respiration rates when grazing novel endophyte-infected tall fescue (Nihsen et al., 2004; Beck et al., 2008; Hancock and Andrae, 2009; Ferguson et al., 2021).

Tannins

Condensed vs Hydrolysable Tannins

Tannins are polyphenolic polymers of varying molecular weight and complexity and are divided into two subgroups: condensed tannins and hydrolysable tannins (Piluzza et al., 2014). Tannins are secondary plant metabolites and provide a defense against herbivory (Salminen and Karonen, 2011). They are synthesized to meet physiological demands but also as a response to biotic and abiotic stresses (Alonso-Amelot et al., 2007). Both classes can have adverse or beneficial effects depending on different factors, such as tannin concentration and structure, plant source, animal species, and physiological state (Piluzza et al., 2014).

Hydrolysable tannins are esters of gallic acid which are linked to a sugar core, like glucose (Buzzini et al., 2008; Okuda and Ito, 2011; Tharayil et al., 2011). Hydrolysable tannins are often referred to as potentially toxic to ruminants when consumed in large quantities (Waghorn, 2008). Condensed tannins are polyphenolic compounds, also known as polyhydroxy-flavan-3-ol oligomers (Tharayil et al., 2011). They are typically described as high molecular weight and astringent polyphenolic compounds which characteristically bind and precipitate proteins as well as carbohydrates and minerals (Feeny and Bostock, 1968; Hagerman and Butler, 1981; Makkar, 2003). Condensed tannin expression depends on the plant species and plant parts (Chezem and Clay, 2016). Tannin concentrations are typically greater in new leaves and flowers of the plants, and a particular type of condensed tannin may be predominant in a specific plant (Frutos et al., 2004; Waghorn, 2008). For example, birdsfoot trefoil, *Lotus corniculatus,* is predominantly procyanidin-type condensed tannin, whereas big trefoil, *Lotus pedunculatus,*

contains prodelphinidin-type condensed tannin (Foo et al., 1996, 1997). Tannins can have anti-herbivory effects in insects (Salminen and Karonen, 2011), but herbivory effects in ruminants are thought to be marginal since forages containing condensed tannins are consumed (Waghorn, 2008).

Why use condensed tannin in ruminant nutrition?

Condensed tannins are considered to have nutritional benefits to a ruminant animal (Barry, 1989; Wang et al., 1994). Most proteins are rapidly degraded and release nitrogen in the rumen in high-quality forage diets (Min et al., 2003). However, condensed tannins could reduce proteolysis by rumen microbes and improve nitrogen retention in the animal (Min et al., 2003) by their ability to bind proteins with a high affinity (Waghorn, 2008) and increase amino acid availability for small intestine absorption (Wang et al., 1996). Condensed tannins can bind to more than one site on a protein which changes the conformation of the substrate and protects the protein in the ruminant digestive tract, but the affinity of tannins for proteins can be variable (Piluzza et al., 2014). Typically, condensed tannins at concentrations greater than 50 g/kg DM can reduce intake and inhibit protein digestibility (Barry, 1989). The specific type of condensed tannin can also influence voluntary feed intake and animal performance greatly (Piluzza et al., 2014). However, these nutritional benefits have been variable in responses and led to contradictory reports (Min et al., 2003; Mueller-Harvey, 2006; Waghorn, 2008).

Reports of the nutritional benefits of condensed tannins include improved growth (MacAdam and Villalba, 2015), tolerance to some intestinal parasites (Hoste et al., 2015; Terrill et al., 2012; Waghorn, 2008), and bloat prevention due to tannins reducing foam stability which traps the fermentation gases (Wang et al., 2012). Condensed tannins in birdsfoot trefoil, *Lotus corniculatus,* and sulla, *Hedysarum coronarium*, can be used to increase essential amino acid

absorption from the small intestine (Min et al., 2003). Condensed tannin at 80 g/kg from sainfoin, *Onobrychis viciifolia,* was considered beneficial in sheep. (Mueller-Harvey, 2006). For instance, Douglas and colleagues (1999) found that when lambs were offered sulla at 72 g/kg DM, growth rate was not affected in the lambs. Additionally, Terrill and others (1992) found that sheep consuming sulla at 40 to 50 g/kg DM had increased live weight gains compared to those consuming alfalfa or perennial pasture.

Different condensed tannin-containing plants have complex mixtures of tannins and not all have the same feeding effects (Piluzza et al., 2014). Condensed tannins in big trefoil and sainfoin did not increase essential amino acid absorption in the small intestine (Min et al., 2003). Diets fed to lambs containing carob pulp, *Ceratonia siliqua*, at 26 g/kg DM exhibited a decrease in growth rate (Priolo et al., 2000). A different study showed carob at 25 g/kg of condensed tannin can be harmful and depress growth rates in sheep (Mueller-Harvey, 2006). Sheep consuming birdsfoot trefoil had reduced carcass fatness when compared to those consuming alfalfa without condensed tannin (Piluzza et al., 2014). Nutritional effects of condensed tannins are complicated due to the diversity between their structure and biological properties and this diversity has led to confusion when determining benefits or toxicity (Mueller-Harvey, 2006). Therefore, condensed tannins need to be studied individually to determine any benefits or toxicity (Piluzza et al., 2014).

Ensiling Forages

Fermentation Qualities

The silage making process is typically divided into 4 phases: initial aerobic phase in the silo following harvest; the fermentation phase; the stable storage phase in the silo; and when the silo is open for the ensiled material to be exposed to air (Wilkinson and Davies, 2013). The

fermentation phase of the ensiling process is thought to last 7 to 45 days (Pahlow et al., 2003). Fermentation of baled silages is inherently restricted and dependent on the integrity of the polyethylene film remaining sealed and creating an anaerobic environment (Coblentz and Akins, 2018; Vough and Glick, 1993). Fermentation profiles can be used to determine whether silage underwent good or bad fermentation, and quality can be impacted by the rate of packing, packing density, additive used, and length of forage (Kung et al., 2018). When evaluating fermentation quality, there are certain components to look at which include pH, the concentration of organic acids, alcohols, and ammonia-nitrogen, and different microbial populations (Pahlow et al., 2003). A distinct odor can also be noted due to the fermentation end products (Kung et al., 2018).

The pH of ensiled samples is an indication of its acidity, and the final pH of baled silages tends to be more basic than suggested values (Coblentz and Akins, 2018; Kung et al., 2018). The final pH of silage is affected by numerous factors including moisture level, water-soluble carbohydrates, and the buffering capacity (Jaster, 1995; Kung et al., 2018; Pitt et al., 1985). Lactic acid, which is typically the acid found in the greatest concentration in silages, contributes to a drop in pH during fermentation (Kung et al., 2018). As dry matter content increases above 40%, the silage pH increases as well due to the lack of water available for the growth of lactic acid bacteria (Whiter and Kung, 2001). The pH and oxygen concentration within a silo can create favorable conditions for clostridial growth, which can leave visible evidence of mold and relatively low concentrations of lactic acid and high pH values (McDonald et al., 1991; Wilkinson and Davies, 2013).

The quality of silage is determined by the lactic acid content, as lactic acid is roughly 10 to 12 times stronger than any other major acids found in ensiled forages (Kung et al., 2018). However, baled silages restrict the availability of sugars to lactic acid-producing bacteria

because forages are baled without particle size reduction which forces the sugars to diffuse from inside the plant to the outside of the plant (Muck et al., 2003). Baled silages produce less lactic and acetic acid, but more butyric acid, which is indicative of greater clostridial activity (Coblentz et al. 2016; McEniry et al., 2006). The lactic to acetic acid ratio is a common indicator of fermentation quality, and a ratio below 1 is typically an indication of abnormal fermentation (Kung et al., 2018). Baled tall fescue silage had a ratio of 3.1 (Coblentz et al., 2021) which is similar to the suggested values of 2.5 to 3 (Kung et al., 2018).

Silage, which is well fermented, should not have a strong odor because lactic acid is nearly odorless, but most silages will present a mild vinegar smell due to acetic acid and its volatility. If the silage contains high levels of acetic acid, one will often experience a burning sensation in the eyes and nose and the silage will be more yellowish in color, specifically at the bottom of the silo (Kung et al., 2018). Silages that emit a fruity, sweet odor are often thought to be well-fermented and stable. However, the sweet smell is typically due to high alcohol concentrations that are mainly produced by yeast as well as many bacteria. The alcohols can react with acids in the silage, which will produce esters and add to the fruity smell (McDonald et al., 1991). Silages that are aerobically unstable can present a musty or moldy smell and may contain visible mold growth (Korosteleva et al., 2009).

Effect on Ergovaline Concentrations

Ergovaline is the most prevalent ergot alkaloid produced by the endophyte and can be affected by numerous variables (Guerre, 2015; Lea et al., 2014). Fresh forage, generally, contains the highest levels of ergot alkaloids while ammoniated hay contains the lowest (Norman et al., 2007; Roberts et al., 2002, 2009, 2011). There are limited reports on the ergot alkaloid concentrations in tall fescue silage, but Turner and colleagues (1993) concluded ergovaline

concentrations did not change during ensiling. Also, Roberts and others (2002) found that ergovaline concentrations in ensiled tall fescue were similar to green chop tall fescue. However, this was in fall grown silage and not spring. Recent reports of spring grown silage indicated ergot alkaloids can change in tall fescue during ensiling (Roberts et al., 2011). Ergovaline concentration decreases by 57 to 60% during ensiling due to degradation by microbial activity, especially in high moisture silages (Roberts et al., 2014).

Conclusion

Tall fescue is a common forage in pastures across the United States, primarily in the "Fescue Belt" in the east-central and southeastern United States. Livestock grazing endophyte-infected tall fescue are susceptible to fescue toxicosis, presenting symptoms that affect performance and overall animal health. Fescue toxicosis costs the forage-based livestock industry billions of dollars each year and negatively impacts animal performance. Mitigation strategies for fescue toxicosis are necessary to improve animal performance and well-being. While novel endophyte-infected tall fescue is a popular mitigation strategy, it may not be feasible for producers to completely reseed their fields. Condensed tannins, which are present in many legumes, could offer a feasible mitigation strategy and do not require complete reseeding of pastures. Condensed tannins can bind to proteins and ergot alkaloids which could help mitigate tall fescue toxicosis and improve animal performance. However, the benefits of condensed tannins have been contradictory and require more research. Therefore, the objective is to determine the effect of condensed tannins in mitigating tall fescue toxicosis.

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Chapter 2

Using supplemental condensed tannin to mitigate tall fescue toxicosis in non-pregnant, non-lactating ewes consuming tall fescue baleage

Abstract

Tall fescue is a prevalent forage in the United States and has a symbiotic relationship with an endophytic fungus which produces ergot alkaloids and can lead to fescue toxicosis. Condensed tannins can bind to these ergot alkaloids and render them ineffective. The objective was to improve the health and well-being of sheep offered tall fescue [*Schedonorus arundinaceus* (Shreb.)] infected with the endophytic fungus (*Neotyphodium coenophialum*) by binding toxins in the rumen with condensed tannins. Non-toxic novel-endophyte infected tall fescue (NE) or toxic endophyte-infected tall fescue forage was harvested, baled at targeted moisture of 55%, and wrapped within 8 hours of baling with 2 layers of net wrap and 20 layers of plastic and allowed to ensile for at least 60 days. Prior to feeding, forages were chopped and packed into plastic trash bins (167 L) lined with 2 plastic bags (3 mil). Non-pregnant, non-lactating ewes (n = 20; 57 ± 1.3 kg initial BW) were housed in individual 1 × 1.5-m pens with metal grate flooring and were offered ad libitum access to either NE or toxic endophyte-infected tall fescue baleage with no condensed tannin (E0), or condensed tannin at 10 (E10), or 30 g/kg baleage DM (E30). Additionally, ewes received 4 g/kg BW liquid molasses, which served as the carrier for the condensed tannin supplement in the E10 and E30 treatments. The study consisted of 2, 21-d periods with a 30-d washout between periods. In each period, a 14-d adaption was followed by 7 d of total fecal and urine collection. Blood samples for prolactin analysis were taken on the last day of each period. Ambient temperature was maintained at 27 to 29 °C. Data were analyzed using PROC MIXED of SAS where treatments were fixed effects, period and pen

were random effects, and the individual ewe within period was the experimental unit. Dry matter intake (DMI, g/kg BW) was greater ($P = 0.05$) from NE vs. the other treatments, and organic matter intake (OMI, g/kg BW) tended to be greater ($P = 0.09$) from NE compared to E30. Silage DMI (g/kg BW) was greater ($P < 0.05$) from NE vs E10 and E30. Dry matter digestibility (DMD, g/kg DMI) and OM digestibility (OMD, g/kg OMI) were greater ($P < 0.05$) from NE vs E30, and OMD was also greater ($P < 0.05$) from NE compared to E0. Digestibility of NDF was greater ($P < 0.05$) from NE vs E0, E10, and E30. Digestible DMI and OMI (g/kg BW) were greater ($P < 0.05$) from NE vs the other treatments. Apparent N absorbed (g/kg N intake) was greater ($P < 0.05$) by ewes offered E0 and E10 compared with E30. Nitrogen retained (g/kg N intake and N absorbed) did not differ ($P \geq 0.31$) among treatments. Prolactin concentrations were greater ($P < 0.01$) in ewes offered NE. Therefore, the overall impacts of ensiled toxic fescue on lambs were negative, and the impact of adding condensed tannins at 30 g/kg DM was negative on most of the DM and OM digestibility measurements.

Introduction

Forages cover significant acreage worldwide and are managed for grazing or hay production (Young et al., 2013). Tall fescue [*Schedonorus arundinaceus* (Shreb.)] is a prevalent forage in pastures in the United States (Franzluebbers and Poore, 2021). It is popular due to its wide range of establishment and adaptation, long grazing season, pest resistance, and seed production (Studemann and Hoveland, 1988).

Tall fescue has a symbiotic relationship with *Neotyphodium coenophialum*, a fungal endophyte found throughout the plant that produces ergot alkaloids and allows the forage to remain tolerant to stressors (Ball et al., 1991; Leuchtmann et al., 2014; Waller, 2009; Young et al., 2013). While ergot alkaloids are primarily concentrated in the seed, they also exhibit some

presence in the leaf and stem tissue (Rottinghaus et al., 1991). Ergovaline is the most prevalent ergot alkaloid produced by the endophyte and is considered the primary cause of fescue toxicosis (Guerre, 2015; Lea et al., 2014).

Reports of reduced body weight gain, loss of appetite, and increased respiration rates gained attention in the 1950s. Livestock consuming endophyte-infected tall fescue exhibited reduced performance and reproductive efficiency compared to other forages and legumes (Blaser et al., 1956; Forney et al., 1969; Jacobson et al., 1970; Williams et al., 1972). Fescue toxicosis produces various symptoms, including reduced dry matter intake, low blood prolactin, and reduced weight gain (Roberts et al., 2002). Livestock with fescue toxicosis can also exhibit intolerance to heat, rough hair coat, elevated body temperature (Stuedemann and Hoveland, 1988), increased water intake (Bouton et al., 2002), and excessive panting (Ferguson et al., 2021). Adverse impacts from fescue toxicosis cost the forage-based livestock industry in the United States over a billion dollars each year (Aiken and Strickland, 2013).

Salminen and Karonen (2011) describe tannins as secondary plant metabolites. They are classified into two subgroups, condensed tannins and hydrolysable tannins (Piluzza et al., 2014). Condensed tannins are polyphenolic compounds synthesized by herbaceous legumes (Naumann et al., 2013). Condensed tannins are associated with an ability to combine with dietary proteins, which delay or prevent their digestion and can lead to reduced proteolysis and improve nitrogen retention in the animal (McSweeney et al., 2001; Min et al., 2003). Nutritional benefits in a ruminant animal from condensed tannins include the prevention of pasture bloat (Wang et al., 2012), improved growth (MacAdam and Villalba, 2015), and tolerance to some intestinal parasites (Hoste et al., 2015; Terrill et al., 2012; Waghorn, 2008). Recently, tannins were

reported to potentially bind to ergot alkaloids since ergot alkaloids are nitrogen-based secondary compounds (MacAdam and Villalba, 2015).

The objective of the study was to determine if tannin supplementation could be used to bind fescue toxins in the rumen of sheep and thereby mitigate tall fescue toxicosis. We hypothesized the tannin would bind to tall fescue toxins in the rumen and improve animal performance, including increased serum prolactin concentrations.

Materials and Methods

Forage Harvest, Silage Making, and Storage

Non-toxic, novel endophyte-infected tall fescue was harvested at the University of Arkansas Milo J. Shult Research and Extension Center in Fayetteville, AR, USA, and endophyte-infected tall fescue was harvested at the Savoy Research Center located near Savoy, AR, USA on the same day and at the early bloom vegetative stage. Both forages were baled with a John Deere 460M baler (John Deere US, Moline, IL), then wet-wrapped with an RB200 Single Round Bale Wrapper (Groupe Anderson Inc., Chesterville, QC, Canada.) with 4 layers of silage wrap (1-mil thickness; Sunfilm Gold) and stored outside for a minimum of 60 d prior to the digestion study. Forages were chopped (< 5 cm particle size) by a plot harvester machine (Wintersteiger, Cibus S., Wintersteiger Inc., Salt Lake City, UT, USA), followed by packing the forages in plastic trash containers (167 L) lined with two, 3-mil plastic bags (0.07619 mm thickness). Forages were packed by walking on the forage as it was placed into the containers. After packing the silage into the containers, head-space air was removed using a Wet/Dry Vacuum (Mod. L 250, Shop-Vac. Corporation, Williamsport, Pennsylvania, USA), and plastic bags were tied individually with strings.

Digestion Study

Animals and Treatments

The study protocol was approved by the Institutional Animal Care and Use Committee at the University of Arkansas (Protocol #18118) and was carried out at the University of Arkansas Milo J. Shult Research and Extension Center located in Fayetteville, AR, USA. Twenty non-pregnant, non-lactating ewes of predominantly Dorper breeding were stratified by body weight (57 ± 1.3 kg initial BW) and assigned randomly to diets in a 2-period study with each period consisting of 21 d. Diets consisted of non-toxic, novel-endophyte infected tall fescue baleage (NE) with no tannins, or toxic, endophyte-infected tall fescue baleage offered with no tannins (E0), or tannins at 10 (E10), or 30 (E30) g/kg baleage DM as quebracho (*Schinopsis quebracho-colorado)* condensed tannin (Actifor®, Delacon Biotechnik GmbH, Engerwitzdorf, Austria). The same 20 ewes were used in both periods. However, the ewes were allocated randomly to treatments with the stipulation that no ewe received the same diet in both periods.

Housing

Ewes were housed in individual 1 × 1.5-m pens with metal grate flooring within an enclosed building with 14 h of light and 10 h of darkness each day, and the temperature was maintained at 27 to 29 °C. Ewes were removed from their pens for a 3 h exercise period with access to water but no feed after a 14-d adaptation period. During the exercise period, pens were cleaned thoroughly prior to the 7-d total collection period. Following period 1, ewes were removed from their pens and placed on a mixed pasture of bermudagrass and crabgrass for 30 d to allow toxic effects to subside. For the second period, ewes were returned to the same pen they were housed in period 1.

Feeding and Sample Collection

Chopped baleage was weighed twice daily at 0800 h and 1600 h with approximately half offered immediately, and the remaining portion offered 4 h later to minimize spillage. Orts were collected prior to the 0800 h feeding and weighed. All ewes received 0.4% of BW as liquid molasses (as-fed basis) daily, split equally. Condensed tannins were divided equally between feedings. Condensed tannins and molasses were weighed and mixed together by hand prior to 0800 and 1600 h feedings. The molasses and tannin mixture was top-dressed at the first portion of the 0800 and 1600 h feedings.

Feed samples were taken from each feeding beginning on d 13. A subsample was placed in a gallon freezer bag and stored in a freezer (-20 °C). A second subsample was weighed and placed in a paper bag then dried to a constant weight at 50 °C. Beginning on d 14, orts were sampled and dried to a constant weight at 50 °C. Total feces and urine were collected for 7 d in the following manner beginning on d 15. Trays with a solid corrugated polyvinyl chloride (PVC) sheet on the underside and covered on the top with fiberglass screening were placed underneath each individual pen. Total feces were gathered from the screens into plastic gutters at the end of each screen once daily, then weighed and dried to a constant weight at 50° C. Urine was collected directly into plastic, 1-L containers surrounded by icepacks (Freez Pak (LF4941), Lifoam, Waxahachie, TX). Total urine was transferred from the 1-L containers 4 times daily at 0700, 1100, 1500, and 2100 h into 3.8-L containers and stored frozen (-20°C) for later analyses. Icepacks were replaced with frozen ones after each collection. Blood samples were taken on d 21 at 0800 h from the jugular vein into blood collection tubes (BD Vacutainer – SST, Becton, Dickinson and Company, Franklin Lakes, NJ). Samples were centrifuged (1,303 × g), and serum was extracted and stored frozen (-20 °C) for later prolactin analysis.

Chemical analysis

Dried forage, orts, and fecal samples were allowed to equilibrate to atmospheric moisture, then ground through a Wiley mill (Arthur H. Thomas, West Washington Square, PA, USA) to pass through a 1-mm screen. Frozen urine samples were thawed and composited by pen number within each period.

A portion of composited frozen forage samples was analyzed by Dairy One Forage Testing Laboratory (Ithaca, NY) for silage fermentation profiles. Forage pH was determined by blending 50 g samples at 20,000 rpm for 2 min in 750 mL of deionized water then filtering the mixture through cheesecloth. The pH was analyzed using Thermo Orion Combination Sure-Flow pH Electrode and Thermo Orion 410 A meter (Thermo Fisher Scientific, Waltham, MA) which was calibrated with buffers referenced to NIST SRMs: pH 4 buffer contained potassium hydrogen phthalate and pH 7 buffer contained sodium phosphate dibasic and potassium phosphate monobasic.

Ammonia-N was analyzed by using a peristaltic pump (Timberline Instruments, 1880 S. Flatiron Ct. Suite I, Boulder, CO) which directs the sample, caustic, and absorbing solutions into a diffusion cell (Carlson, 1978). Overall acids were determined by taking an aliquot of extract mixed 1:1 ratio with 0.06 M oxalic acid containing 100 ppm trimethyl-acetic acid (internal standard). Samples were injected into a Perkin Elmer Clarus 680 Gas Chromatograph containing a Supelco packed column with the following specifications: 2 m × 2 mm Tightspec ID, 4% Carbowax 20M phase on 80/120 Carbopack B-DA. To analyze lactic acid, an aliquot of extract was used with YSI 2950D-1 or 2700 SELECT Biochemistry Analyzer equipped with an L-Lactate membrane (YSI Incorporated Life Sciences, Yellow Springs, Ohio).

A lyophilized portion of forage was analyzed by the Veterinary Medical Diagnostic Laboratory (University of Missouri, Columbia, MO) for ergot alkaloid and ergovaline concentrations. Total ergot alkaloids and ergovaline concentrations were determined by HPLC according to Rottinghaus et al. (1991) with modifications reported by Hill et al. (1993).

Serum samples were analyzed at the University of Tennessee – Knoxville for prolactin concentrations using the modified procedures of Bernard et al. (1993). Intra- and inter-assay coefficients of variation were 5.03% and 9.01%, respectively.

Ash concentrations were determined by combusting forage samples at 500 °C in a muffle furnace, and organic matter (OM) was calculated as DM weight - ash weight. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) content in forage, orts, and feces were analyzed sequentially using a 200 Ankom Fiber analyzer (ANKOM Technology Corporation, Macedon, NY, USA; Vogel et al., 1999) and expressed inclusive of residual ash. Forage lignin content was analyzed using the sulfuric acid method (method 973.18; AOAC, 2000). Nitrogen was analyzed on feed, ort, fecal, and liquid urine samples using the Dumas total combustion method (Fison's NA2000 Nitrogen/Protein Instrument; method 990.03; AOAC, 2000). Urine samples were also diluted 50-fold and analyzed for urea nitrogen using a blood urea N kit (Teco Diagnostics, Anaheim, CA). All laboratory analyses on dried, ground samples were corrected to a DM basis (method 934.01; AOAC, 2000).

Statistical Analysis

Data were analyzed using SAS 9.4 Mixed Procedure (SAS Institute, Cary, NC) using the individual ewe within period as the experimental unit. Treatments were considered fixed effects, and pen and period were considered random effects. When a significant ($P < 0.05$) treatment effect occurred, means were separated using an F-protected t-test.

Results

Forages contained similar concentrations of NDF, ADF, and lignin (Table 1). The E+ baleages contained 193 µg/kg ergovaline, whereas the NE+ baleages contained no detectable ergovaline concentrations. Total acids along with lactic acid and acetic acid concentrations were greater, and pH was lower in NE compared with E+ baleages (Table 2).

Dry matter intake (DMI, g/day) was greater ($P < 0.05$) from NE compared to E10 and E30 treatments but not different from E0. Organic matter intake (OMI, g/day) was greater from NE compared with E30. When expressed as per unit of BW (g/kg BW), ewes offered NE consumed more ($P = 0.05$) DM than those offered the other treatments and tended ($P = 0.09$) to consume more OM than those offered E30. Intake of NDF was greater ($P < 0.05$) from NE compared to all E+ treatments, but intake of ADF did not differ ($P = 0.38$) among treatments.

Intake, digestibility, and digestible intake are reported in Table 3. Dry matter digestibility (DMD, g/kg DMI) was greater ($P < 0.05$) from NE and E10 than E30, and E0 was intermediate. Organic matter digestibility (OMD, g/kg OMI) was greater ($P < 0.05$) from NE than E0 and E30, whereas sheep consuming E10 did not differ ($P \geq 0.09$) from other treatments. Digestibility of NDF (g/kg DM) was greater ($P < 0.05$) from NE than from the other treatments, but ADF digestibility tended to be greater ($P = 0.08$) from NE, E0, and E10 compared to E30. Hemicellulose digestibility (g/kg DMI) tended to be greater ($P = 0.10$) from NE compared to E30. Digestible DMI and OMI (g/kg BW) were greatest ($P < 0.05$) from ewes consuming NE compared to all other treatments. Serum prolactin concentrations were greater ($P < 0.01$) from ewes offered NE versus all other treatments (Figure 1).

Nitrogen intake and N apparently absorbed (g/d) were greater ($P < 0.01$) from NE, E0, and E10 compared to E30 (Table 4). Apparent N absorption (g/kg N intake) was lesser ($P <$

0.05) from E30 compared to E0 and E10. Nitrogen retained (g/d, g/kg N intake, g/kg N absorbed) did not differ ($P \geq 0.31$) among treatments. Total N excreted (g/d) tended to be greater ($P = 0.08$) from ewes offered E0 diets than E10 and E30 treatments and NE was intermediate. Fecal N output (g/N Intake) was greater ($P < 0.05$) for E30 compared to E0 and E10 treatments, but NE was intermediate. Urine N (g/N intake and g/kg N excreted) and fecal N (g/kg N excreted) did not differ ($P \geq 0.14$) among treatments. Urine hippuric acid N (g/kg total urine N) and urine urea N (g/kg total urine N) did not differ ($P = 0.63$) among any treatments. However, urea N (g/kg urine) tended to be greater ($P = 0.08$) in NE and E0 compared to E10.

Discussion

The intent of this study was to evaluate the effects of reducing fescue toxicosis with a condensed tannin supplement. Fescue baleage fermentation profiles indicate that NE bales fermented to a greater extent than E+, as demonstrated by greater pH and lower lactic acid and total acids concentrations from E+. The NE baleage had a sweeter smell than the E+ baleage. This is mistakenly associated with good fermentation, but the sweet smell could be due to a high concentration of alcohol produced by yeasts and bacteria (Kung et al., 2018). Average ergovaline concentrations were 193 ppb for endophyte-infected baleage. There was no detection of ergovaline in NE baleage. Stamm et al. (1994) suggested ergovaline concentrations of more than 150 ppb could produce symptoms associated with fescue toxicosis.

For this study, DMI tended to be lower for E0, E10, and E30 than NE. However, intake could have been suppressed since animals were in confinement. Lower intake from sheep consuming E+ treatments regardless of tannin treatment could be due to post-ingestive effects caused by the presence of ergot alkaloids (Villalba et al., 2016). Because DMI tended to be greater from NE than the treatments with E+, the tannins did not appear to alleviate fescue

toxicosis. Organic matter intake decreased numerically from NE to endophyte-infected treatments with the only significant difference between NE and E30. This is consistent with another study that reported decreased OMI numerically but not statistically in treatments of tall fescue seed with no ergovaline, 1.5 ppm ergovaline, or 3.0 ppm ergovaline (Hannah et al., 1990). Tannin supplementation can cause post-ingestive responses, and greater levels of tannin can cause a loss of appetite and decrease intake (Catanese et al., 2014). The post-ingestive response could explain the difference in NE and E30 treatments.

The current study had similar DMD for E+ baleage when condensed tannin levels were equal to 10 g/kg DM compared to NE baleage. Endophyte-infected treatments were lower for DMD than NE when condensed tannin levels were 30 g/kg DM. This is consistent with results reported by Matthews et al. (2005) which saw greater DMD when condensed tannin levels were less than or equal to 20 g/kg DM. However, DMD was lower in the current study when compared to Matthews et al. (2005). The lower DMD in this study could be because of ad libitum intake instead of restricted intake (Matthews et al., 2005). Organic matter digestibility decreased numerically from NE to E30, but OMD for NE and E10 was not different. In this study, E30 treatment had the lowest OMD. This could be explained by tannin's effect of depression on digestive tract enzyme activities (Silanikove et al., 1994). Dry matter digestibility and OMD in this study decreased numerically with increasing addition of condensed tannin. The difference in DMD and OMD could be due to the population of fiber-degrading bacteria associated with the activity of cellulolytic enzymes because the metabolism of gram-positive bacteria can be impaired by the absorption of condensed tannins (Abdullah et al., 2018; Silva et al., 2017).

Nitrogen retained was greater in both NE and E+ treatments in the current study when compared to previous studies (Koontz et al., 2014; Matthews et al., 2005). Similar to the present study, Koontz and colleagues (2014) found that nitrogen retained as a percent of N intake did not differ between endophyte-infected and endophyte-free treatments. Nitrogen retained was numerically lowest from sheep consuming the E30 treatment but numerically greatest from those consuming the E10 treatment. According to Costa and others (2021), diets containing condensed tannins reduce the rate and extent of protein degradation by rumen microbes which also reduces the excess release of ammonia-N. This can explain the low amount of retained N from sheep consuming the E30 treatment.

Decreased prolactin levels are a definitive indicator of fescue toxicosis. Previous studies have shown reduced prolactin levels in ruminants offered endophyte-infected tall fescue (Parish et al., 2003; Koontz et al., 2012; Matthews et al., 2005). In this study, the prolactin concentrations were significantly lower from sheep offered E0, E10, and E30 treatments compared to sheep offered NE. This is different from a previous study which had no difference in serum prolactin concentrations between steers consuming fescue with the toxic endophyte and non-toxic endophyte (Matthews et al., 2005). However, ergovaline concentrations in this study (120 ppb) were lower than Matthews and others (2005) reported. The low serum prolactin concentrations in the present study are a clear indicator the fescue was toxic, and condensed tannins did not appear to alleviate fescue toxicosis.

Conclusion

Dry matter intake tended to be greater in NE treatment than E+ baleage with any level of supplemental tannin. Furthermore, OMI only differed between NE and E30 treatments. Sheep consuming NE and E+ treatments had very few differences in nitrogen utilization. Prolactin

concentrations decreased significantly from NE to E+ treatments indicating fescue toxicosis in E+ treatments.

Based on this study, the condensed tannins did not properly bind to the ergot alkaloids to alleviate fescue toxicosis. Overall, condensed tannin supplementation did not improve indicators of animal performance.

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Tables and Figures

Table 1. Forage composition and ergovaline concentrations of non-toxic, novel endophyte infected (NE) or toxic, wild-type infected (E+) tall fescue baleage

	Baleage	
Item[1]	NE	E+
Moisture, g/kg	548	448
OM, g/kg DM	922	936
NDF, g/kg DM	649	625
ADF, g/kg DM	343	339
Hemicellulose, g/kg DM	324	306
Lignin, g/kg DM	26	31
Ergovaline, ppb	0.00	193
Total ergot alkaloids, ppb	< 25	193

[1]DM= dry matter, OM = organic matter, NDF = neutral detergent fiber with inclusive ash, ADF = acid detergent fiber

Table 2. Fermentation profiles of non-toxic, novel endophyte infected (NE) or toxic, wild-type infected (E+) tall fescue baleage

	Baleage	
Item[1]	NE	E+
Lactic acid, g/kg DM	38.2	21.7
Acetic acid, g/kg DM	6.6	2.7
Lactic/Acetic ratio	6.01	8.41
Propionic acid, g/kg DM	0.3	1.0
Butyric acid, g/kg DM	0.0	0.8
Isobutyric, g/kg DM	0.0	0.2
Total acids, g/kg DM	45.0	26.3
pH	4.55	5.90
CP, g/kg DM	112.6	114.9
Ammonia, CP equivalent	0.62	0.54
Amm-N, g/kg N	55.1	47.0

[1]DM = dry matter, CP = crude protein, N = nitrogen

Table 3. Intake and digestibility by sheep offered non-toxic, novel endophyte tall fescue silage or toxic, wild-type infected tall fescue silage supplemented with different levels of condensed tannin

	Treatments[1]					
Item[2]	NE	E0	E10	E30	Standard Error	*P* - value
Intake						
DMI, g/day	1404^{a}	1325^{ab}	1328^{b}	1260^{b}	147.2	0.03
DMI, g/kg BW	25.0^{x}	23.5^{y}	23.6^{y}	22.6^{y}	3.42	0.05
OMI, g/day	1290^{a}	1242^{ab}	1237^{ab}	1171^{b}	132.9	0.05
OMI, g/kg BW	23.0^{x}	22.0^{xy}	22.0^{xy}	21.0^{y}	3.10	0.09
NDF Intake, g/kg BW	15.6^{a}	14.1^{b}	14.0^{b}	13.3^{b}	2.16	<0.01
ADF Intake, g/kg BW	7.9	8.0	7.9	7.5	1.40	0.38
Digestibility						
DMD, g/kg DMI	588.0^{a}	566.7^{a}	567.6^{a}	542.4^{b}	9.31	0.01
OMD, g/kg OMI	611.0^{a}	581.7^{b}	581.9^{ab}	561.9^{b}	9.13	0.01
NDFD, g/kg NDF	591.6^{a}	527.4^{b}	533.4^{b}	506.3^{b}	13.93	<0.01
ADFD, g/kg ADF	549.5^{x}	544.2^{x}	544.0^{x}	506.8^{y}	12.99	0.08
Hemi digestibility, g/kg hemi intake	597.5^{x}	569.8^{xy}	586.0^{xy}	549.6^{y}	15.87	0.10
Digestible intake						
DDMI, g/day	834.1^{a}	757.2^{b}	749.4^{b}	682.2^{b}	89.84	0.01
DDMI, g/kg BW	14.8^{a}	13.4^{b}	13.3^{b}	12.3^{b}	2.04	0.01
DOMI, g/kg BW	14.1^{a}	12.9^{b}	12.7^{b}	11.8^{b}	1.88	0.01

[1]Treatments consisted of non-toxic, novel-endophyte infected tall fescue baleage (NE) with no tannins, or toxic, endophyte infected tall fescue baleage offered with no condensed tannins (E0), or condensed tannins at 10 (E10), or 30 (E30) g/kg baleage DM from Actifor.

[2]BW= bodyweight, DMI= dry matter intake, OMI= organic matter intake, DMD = dry matter digestibility, OMD = organic matter digestibility, NDFD = neutral detergent fiber digestibility with inclusive ash, ADFD = acid detergent fiber digestibility, Hemi = hemicellulose, DDMI= digestible dry matter intake, DOMI = digestible organic matter intake.

$^{a, b, c}$Within a row, means with different subscripts differ at $P < 0.05$.

$^{x, y}$Within a row, means with different subscripts tended to differ at $0.05 < P < 0.10$.

Table 4. Nitrogen intake, absorption, retention, and excretion from sheep offered non-toxic, novel endophyte tall fescue or toxic, wild-type infected tall fescue supplemented with different levels of condensed tannin

	Treatments[1]					
Item[2]	NE	E0	E10	E30	Standard Error	*P* - value
N intake, g/day	24.6[a]	25.5[a]	24.8[a]	22.4[b]	2.04	0.01
Apparent N absorbed, g/day	13.0[a]	14.3[a]	13.1[a]	11.1[b]	0.72	0.01
Apparent N absorption, g/kg N intake	527.4[ab]	554.6[a]	536.5[a]	497.8[b]	26.06	0.03
N retained, g/day	7.1	6.9	8.6	6.3	1.19	0.32
N retained, g/kg N intake	284.2	266.4	351.5	289.8	38.21	0.36
N retained, g/kg N absorbed	535.7	481.7	657.4	580.5	75.54	0.31
N excreted, g/day	17.86[xy]	18.83[x]	16.05[y]	15.70[y]	1.48	0.08
Fecal N, g/kg N intake	472.6[ab]	445.4[b]	463.5[b]	502.2[a]	26.06	0.03
Fecal N, g/kg N excreted	677.3	624.4	725.4	723.4	44.22	0.14
Urine N, g/kg N intake	242.9	289.2	181.1	211.3	47.49	0.23
Urine N, g/kg N excreted	322.8	375.6	274.6	276.6	44.22	0.14
Hippuric N, g/kg urine	0.34	0.45	0.23	0.22	0.12	0.40
Hippuric N, g/kg total urine N	0.61	0.79	0.67	0.65	0.18	0.78
Urea N, g/kg urine	2.3[x]	2.3[x]	1.3[y]	1.7[xy]	0.29	0.08
Urea N, g/kg total urine N	459.3	483.1	451.1	523.8	51.63	0.63

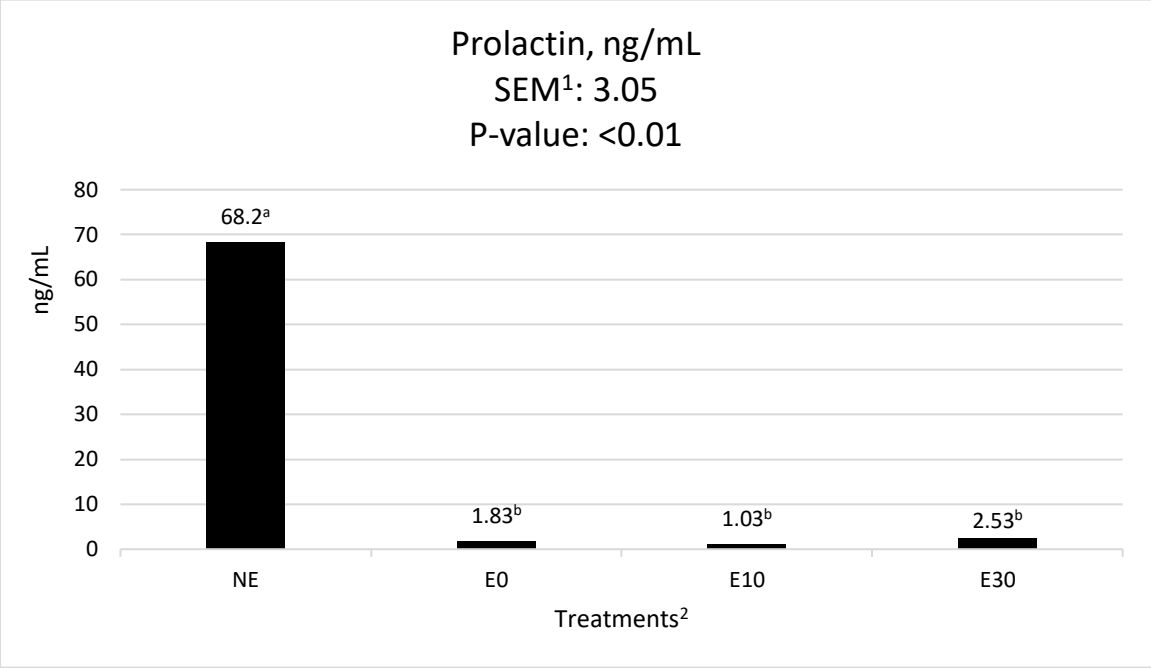
[1]Treatments consisted of non-toxic, novel-endophyte infected tall fescue baleage (NE) with no tannins, or toxic, endophyte infected tall fescue baleage offered with no condensed tannins (E0), or condensed tannins at 10 (E10), or 30 (E30) g/kg baleage DM from Actifor

[2] N= nitrogen.

[a, b]Within a row, means with different subscripts differ at $P < 0.05$.

[x, y]Within a row, means with different subscripts tended to differ at $0.05 < P < 0.10$.

Figure 1. Serum prolactin concentrations in sheep offered non-toxic, novel endophyte tall fescue or toxic, wild-type infected tall fescue supplemented with different levels of condensed tannin



[1]Standard error of the mean

[2]Forage treatments consisted of non-toxic, novel-endophyte infected tall fescue baleage (NE) with no tannins, or toxic, endophyte-infected tall fescue baleage offered with no condensed tannins (E0), or condensed tannins at 10 (E10), or 30 (E30) g/kg baleage DM from Actifor

[a, b, c]Means with different subscripts differ at $P < 0.05$.

Chapter 3

Inclusion of quebracho tannin as a silage additive in tall fescue silage

Abstract

Tall fescue [*Schedonorus arundinaceus* (Shreb.)] is commonly grazed in the mid-south and has potential use for spring silage. During ensiling, protein can be converted to ammonia, and condensed tannins, such as those in quebracho (*Schinopsis quebracho-colorado*), can reduce this conversion by binding to dietary protein. However, acid production may also be reduced. Condensed tannins have the potential to bind to ergot alkaloids and render them ineffective. Quebracho tannin was added to chopped endophyte-infected tall fescue and ensiled at different moisture concentrations to determine those effects on forage components and silage fermentation products. Tall fescue was harvested with a Carter harvester at 1000 h, spread on concrete, mixed thoroughly, and dried for 1 or 2 h to achieve 67 and 44% moisture [high moisture (HM) and low moisture (LM), respectively]. Quebracho tannin was either not applied or was mixed with distilled water 7 d prior to packaging and applied at 1 and 2% of the total silage dry matter (DM) to both moisture treatments immediately prior to packing into mini-silos. Tall fescue and tall fescue-tannin mixtures were pressed into PVC pipes (10.2 cm × 29.2 cm) to achieve a packing density of 192 kg/m^3 and stored at room temperature (23.7 ℃) for 60 d, after which sub-samples were removed and analyzed for fermentation parameters and forage chemical components. Pre-ensiled ergovaline was not different across treatments ($P \geq 0.19$). Pre-ensiled acid detergent fiber and OM were affected by the moisture × tannin interaction ($P = 0.01$). Pre-ensiled ADF was greatest in LM silages with 1% tannin compared to all other moisture-tannin treatments. Pre-ensiled OM was greatest in HM with 0% tannin compared to HM with 2% tannin and all LM treatments. Changes between pre- and post-ensiled WSC were greater ($P < 0.05$) from HM vs.

LM. Moisture × tannin interaction affected final pH ($P < 0.05$), with the lowest pH in HM silages with 0 and 1% tannin. Lactic acid (g/kg DM) was greater ($P < 0.05$) in silages with 0 compared to 2% tannin, but 1% did not differ from either 0 or 2% tannin. Moisture × tannin interaction affected acetic acid, with greater concentrations in HM silages with 0 and 2% tannin compared to LM with 2% tannin. Overall, total acids (g/kg DM) were greatest ($P < 0.05$) in HM, but did not differ ($P=0.54$) among tannin treatments. Ammonia concentrations (g/kg DM) decreased ($P < 0.05$) as tannin level increased and ammonia-N expressed as a proportion of total N tended to be greater ($P < 0.10$) in HM. Ensiling tall fescue with tannins reduced proteolysis regardless of moisture concentration and did not affect ergovaline concentrations up to 2% tannin inclusion.

Introduction

Since silage can be made in hot and cold regions, ensiling forages has become a common preservation practice (Jayanegara et al., 2019). The ensiling process requires the absence of air which allows the fermentation of soluble carbohydrates and various end products (Kung et al., 2018). The silage-making process is generally divided into 4 phases: the initial aerobic phase in the silo following harvest; the fermentation phase; the stable storage phase in the silo; and when the silo is open and material is exposed to air (Wilkinson and Davies, 2013). The fermentation phase of the ensiling process is thought to last 7 to 45 d and fermentation profiles can be used to determine whether silage underwent good or bad fermentation (Kung et al., 2018; Pahlow et al., 2003). Silage quality can be impacted by the rate of packing, packing density, an additive used, and the chop length of the forage (Kung et al., 2018).

Tall fescue, [*Schedonorus arundinaceus* (Shreb.)], is a prevalent forage in the United States and is popular for many reasons, including a wide range of establishment and adaption as well as a long grazing season and pest resistance (Stuedemann and Hoveland, 1988). This forage

has a symbiotic relationship with the fungal endophyte, *Neotyphodium coenophialum*, which produces ergot alkaloids (Young et al., 2013). Ergot alkaloids are heavily concentrated in the seed with some presence in the leaf and stem tissue (Rottinghaus et al., 1991). Fresh, tall fescue generally contains the greatest concentrations of ergot alkaloids, but there are few reports of ergot alkaloid concentrations in tall fescue silage (Roberts et al., 2014).

Condensed tannins, like quebracho (*Schinopsis quebracho-colorado*), are polyphenolic compounds and are synthesized by plants (Tharayil et al., 2011). They are typically described as having a high molecular weight and astringent compounds which characteristically bind to proteins (Feeny and Bostock, 1968; Hagerman and Butler, 1981; Makkar, 2003). Tannins may also bind to ergot alkaloids since ergot alkaloids are nitrogen-based secondary compounds (MacAdam and Villalba, 2015). Furthermore, condensed tannins do not appear to affect the overall fermentation of silages in terms of pH and lactate concentration but can reduce plant protein degradation into non-protein nitrogen during the ensiling process (Jayanegara et al., 2019). Therefore, the objective of this study was to determine the effect of quebracho tannin on fermentation and ergovaline levels in tall fescue silage.

Materials and Methods

Forage Harvest, Silage Making, and Storage

Tall fescue was harvested at USDA-ARS Dale Bumpers Small Farms Research Center near Booneville, AR. A field of predominantly *Neotyphodium coenophialum*-infected tall fescue was mowed, and the forage was removed on April 6. The field was fertilized with 36 kg N on April 21. Forage was harvested (10 cm particle size) using a Carter 3-chute forage harvester (Carter Mfg. Co. Inc., Brookston, IN, USA) on June 16 at 1000 h and hand-chopped (<5 cm particle size) with scissors prior to ensiling. Forage was spread on concrete and allowed to dry

for either 1 h to 67% moisture (high moisture (HM)) or 2 h to 44% (low moisture (LM)). Quebracho tannin was mixed with distilled water 7 d prior to ensiling at 0.17 and 0.34 g/mL. There were a total of 24 silos with 4 silos for each moisture-tannin treatment.

At the time of ensiling, distilled water or the tannin solution was mixed with the chopped tall fescue to provide either 0%, 1%, or 2% tannin in the total silage DM. Forage was weighed to achieve a packing density of 192 kg/m^{3}DM, and quebracho tannin was measured then added to the forage and mixed thoroughly by hand. A subsample was taken and stored frozen (-20°C) in a gallon Ziploc bag for pre-ensiled analyses. After mixing, forage was packed into mini-silos made of PVC pipe (10.2 cm × 29.2 cm). Both ends were capped with cap PVC fitting (10.2 cm × 12.7 cm; Fernco – US, Davison, MI), and the top end was fitted with a one-way valve (ZRM&E, China). They were stored at room temperature (23.7 °C) for 60 d. After 60 d, laboratory silos were opened, and two post-ensiled samples were taken and stored frozen (-20 °C) in gallon Ziploc bags. The pre-ensiled sample and one post-ensiled sample were lyophilized and then ground through a Wiley mill (Arthur H. Thomas, West Washington Square, PA, USA) to pass through a 1-mm screen. The second post-ensiled sample was analyzed for fermentation profiles.

Chemical analysis

Ash concentrations were determined by combusting forage samples at 500 ºC in a muffle furnace, and organic matter (OM) was calculated as OM = DM weight – ash weight. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) content in forage, orts, and feces were analyzed sequentially using a 200 Ankom Fiber analyzer (ANKOM Technology Corporation, Macedon, NY, USA; Vogel et al., 1999) and expressed inclusive of residual ash. Forage lignin content was analyzed using the sulfuric acid method (method 973.18; AOAC, 2000).

A sample of each of the composited frozen forage samples was analyzed by Dairy One Forage Testing Laboratory (Ithaca, NY) for silage fermentation profiles. Forage pH was determined by blending 50 g samples at 20,000 rpm for 2 min in 750 mL of deionized water and then filtering the mixture through cheesecloth. The pH was measured using Thermo Orion Combination Sure-Flow pH Electrode and Thermo Orion 410 A meter (Thermo Fisher Scientific, Waltham, MA) which was calibrated with buffers referenced to NIST SRMs: pH 4 buffer contained potassium hydrogen phthalate and pH 7 buffer contained sodium phosphate dibasic and potassium phosphate monobasic.

Ammonia-N was analyzed by using a peristaltic pump (Timberline Instruments, 1880 S. Flatiron Ct. Suite I, Boulder, CO) which directs the sample, caustic, and absorbing solutions into a diffusion cell (Carlson, 1978). Overall acids were determined by taking an aliquot of extract mixed 1:1 ratio with 0.06 M oxalic acid containing 100 ppm trimethylacetic acid (internal standard). Samples were injected into a Perkin Elmer Clarus 680 Gas Chromatograph containing a Supelco packed column with the following specifications: 2 m × 2 mm Tightspec ID, 4% Carbowax 20M phase on 80/120 Carbopack B-DA. To analyze lactic acid, an aliquot of the extract was used with YSI 2950D-1 or 2700 SELECT Biochemistry Analyzer equipped with an L-Lactate membrane (YSI Incorporated Life Sciences, Yellow Springs, Ohio).

Nitrogen was analyzed on forage samples using the Dumas total combustion method (Fison's NA2000 Nitrogen/Protein Instrument; method 990.03; AOAC, 2000). For condensed tannin (CT) analyses, purified self-standards were prepared from dried herbage (Wolfe et al., 2008). Biologically active CT was analyzed as protein precipitable phenolics as reported by Hagerman and Butler (1978) and modified by Naumann et al. (2014); Total CT was assayed as described by Terrill et al. (1992). Quantities of ergovaline were determined using high-pressure

liquid chromatography (HPLC) with fluorescence detection as described in Carter et al. (2010) with modifications described by Klotz et al. (2018).

Water-soluble carbohydrates (WSC) were determined from 0.25-g ground subsamples from each pre- and post-ensiled sample with a 2-h extraction in 150 mL of deionized water, followed by gravity filtering through Whatman #1 filter paper. The phenol-sulfuric acid reaction was used to quantify WSC with a spectrophotometric procedure using dextrose to formulate standards (DuBois et al., 1956). All laboratory analyses were corrected to a DM basis (method 934.01; AOAC, 2000).

Statistical Analysis

Silage chemical composition and fermentation parameters were analyzed using SAS 9.4 PROC GLIMMIX (SAS Inst. Inc., Cary, NC, USA) procedure for a 2 × 3 factorial arrangement. Changes in chemical composition that occurred between pre- and post-ensiling were analyzed as stated for the individual components and whether the changes differed from zero using the LSMEANS statement in SAS. Significance detected at $P < 0.05$.

Results

Pre- and post-ensiled forage chemical components are reported in Table 1. Condensed tannins were not detected in any of the pre- and post-ensiled silages. Pre-ensiled NDF (g/kg DM) tended ($P < 0.10$) to be greater in fescue silage with 1% tannin compared with 2% added tannin, but NDF concentrations of pre-ensiled fescue without tannin were not different from either the 1% ($P = 0.55$) or 2% ($P = 0.10$) added tannin. A moisture × tannin interaction ($P < 0.05$) was found for pre-ensiled ADF (g/kg DM), as LM with 1% tannin was greater than any other treatment. Hemicellulose (g/kg DM) pre-ensiled concentrations tended ($P < 0.10$) to be greater in HM tall fescue silage than in LM silage. Pre-ensiled OM (g/kg DM) was greatest ($P < 0.05$) in

HM with 0% tannin and lowest ($P < 0.05$) in HM with 2% tannin (moisture × tannin interaction; $P = 0.01$). Water-soluble carbohydrates (g/kg DM) in pre-ensiled samples tended ($P < 0.10$) to be greater in HM silage than in LM silage. Pre-ensiled ergovaline concentrations were not different ($P \geq 0.19$) among moisture or tannin treatments.

Post-ensiled NDF and hemicellulose (g/kg DM) tended ($P \leq 0.09$) to be affected by the moisture × tannin interaction, and concentrations of OM and N were affected ($P \leq 0.05$) by the moisture × tannin interaction. While post-ensiled NDF tended ($P < 0.10$) to be affected by a moisture × tannin interaction, no differences ($P \geq 0.07$) were detected between any pair-wise comparisons. The tendency for the interaction could be due to an increase in NDF as tannin addition increased within HM silages but declined slightly with increasing tannin addition within LM silages. Hemicellulose concentrations tended to be lowest ($P < 0.10$) in HM silage with 0% added tannin compared to all other treatments except LM with 1% added tannin ($P = 0.10$). Post-ensiled ADF and ADL concentrations were not different ($P \geq 0.33$) among moisture or tannin treatments. Post-ensiled OM (g/kg DM) was greatest ($P < 0.05$) in HM with 0% tannin and lowest in HM with 2% tannin. Nitrogen (g/kg DM) in post-ensiled silage was greatest ($P < 0.05$) in HM with 0% tannin when compared to all other treatments. Post-ensiled WSC (g/kg DM) were greater ($P < 0.05$) in LM silage vs HM silages but not affected by tannin concentration ($P = 0.14$). Ergovaline concentrations in post-ensiled fescue were greatest in 1% tannin when compared to 0% ($P < 0.05$), but fescue ensiled with 2% tannins had ergovaline concentrations that were intermediate and not different from those of either 0% tannin ($P = 0.21$) or 1% tannin ($P = 0.17$).

The change in forage chemical components between pre-ensiling and post-ensiling can be found in Table 2. Concentrations of NDF were different from zero ($P < 0.05$) for HM and LM

silages with 2% added tannin, indicating that these concentrations increased between pre- and post-ensiling, whereas the other treatments did not increase. However, changes in NDF concentrations were not affected ($P \geq 0.41$) by moisture, tannin level, or their interaction. Concentrations of ADF increased ($P < 0.05$) in all moisture and tannin treatments between pre- and post-ensiling except LM with 1% added tannin but were not affected ($P \geq 0.15$) by the main effects or their interaction. Hemicellulose concentrations decreased ($P < 0.05$) in HM silages with 0% and 1% tannin, but not the other moisture and tannin combinations. This resulted in a tendency for a greater ($P < 0.10$) decrease in hemicellulose concentrations in HM vs LM. Organic matter concentrations decreased ($P < 0.05$) in HM silages with 0% added tannin and LM silages with 2% added tannin, but OM changes were not affected by moisture, tannin level, or their interaction ($P \geq 0.26$). Nitrogen concentrations increased ($P < 0.05$) in HM silages with 0% and 2% added tannin but did not differ in moisture, tannin treatments, or their interaction ($P \geq 0.44$). Concentrations of WSC decreased ($P < 0.05$) in all moisture and tannin treatments during the ensiling process but to a greater extent ($P < 0.01$) in HM silages than the LM silages. Ergovaline concentrations decreased ($P < 0.05$) in LM with 2% added tannin but did not differ in moisture, tannin treatments, or their interaction ($P \geq 0.22$).

Silage pH, acetic acid (g/kg DM), and the lactic to acetic acid ratio were affected ($P < 0.05$) by the moisture × tannin interaction, and silage moisture (%), ammonia (g/kg DM), propionate (g/kg DM), and butyrate tended ($P \leq 0.10$) to exhibit a moisture × tannin interaction (Table 3). The pH of LM silage with 0% and 1% tannin was greater ($P < 0.05$) than HM with any level of tannin. High moisture silage pH with 2% tannin was greater ($P < 0.05$) than the HM silage with either 0% or 1% tannin. Acetic acid (g/kg DM) was greater ($P < 0.05$) in HM with 0% and 2% tannin than LM with 2% tannin. The lactic to acetic acid ratio was greater ($P < 0.05$)

in LM with 0% tannin compared to LM with 1% and HM with 2% tannin. Moisture, butyrate, and total silage acids were greater ($P < 0.01$) in HM silages than in LM. Ammonia-N as a proportion of total N tended ($P < 0.10$) to be greater from HM compared with LM silages.

Ammonia concentrations (g/kg DM) were greater ($P < 0.05$) from 0% tannin compared with 1% and 2% tannin. Lactic acid (g/kg DM) concentrations were greater ($P < 0.05$) in 0% tannin compared with 2% added tannin. Propionate concentrations tended to be greater ($P < 0.10$) in HM with 1% tannin compared to all other treatments. Ammonia-N (% N) tended ($P < 0.10$) to be greater in HM silage.

Discussion

The HM silage had an average of 72% moisture across all tannin treatments vs LM, which had an average of 58% moisture. In this study, many nutritive values were similar regardless of moisture or tannin levels in pre- and post-ensiled forages. Post-ensiled NDF increased to 630 g/kg for HM silage with 2% added tannin and to 617 g/kg for LM with 2% added tannin, but these were the only two treatments that increased in NDF concentration. Feng and others (2018) reported that post-ensiled NDF of tall fescue ensiled for 3 months decreased from 738 g/kg DM to 650 g/kg DM. In this study, pre-ensiled ADF was 355 g/kg DM in LM silages with 1% added tannin, which was the highest ADF value among all forage treatments. Richard and colleagues (2020) reported a similar value for ADF of 353 g/kg DM from tall fescue silage at 65% moisture. Concentrations of hemicellulose decreased the greatest by 19.5 and 16.9 g/kg DM in HM silage with 0% and 1% added tannin, and HM silage with 2% tannin exhibited a greater numerical decrease than LM silages regardless of added tannin. Loss of hemicellulose during ensiling can occur because of acid or enzymatic hydrolysis of the hemicellulose (Yahaya et al., 2002).

In this study, total acids were 77.9 g/kg DM in HM silage compared to 66.2 g/kg DM in LM silage. This was expected since WSC concentrations decreased in all moisture and tannin treatments, but the decrease was greater in HM silage regardless of tannin treatment. Ensiling silage at moistures greater than 76% can increase the losses of WSC, and lactic acid-producing bacteria convert WSC into organic acids during the fermentation phase which can also reduce silage pH (Pang et al., 2012; Yahaya et al., 2002).

Ergot alkaloid concentrations can change in spring-grown tall fescue during ensiling (Roberts et al., 2011). Roberts and colleagues (2014) reported that ergovaline concentrations decreased by up to 60% during the ensiling process in spring, high moisture silages. In the current study, ergovaline concentrations in LM with 2% added tannin decreased by 24% between pre- and post-ensiled treatments, but changes among the other treatment combinations were not different from zero. Ergovaline concentrations should be similar between high and low moisture treatments, but numerically greater in HM silages due to less exposure to light, heat, and oxygen because of shorter wilting time (Fajardo et al., 1995; Garner et al., 1993; Roberts et al., 2009). However, in this study, ergovaline concentrations were very similar between LM (138 ppb) and HM (126 ppb) silages pre-ensiling.

Silage fermentation parameters were unaffected by moisture × tannin interaction except for pH, acetic acid, and lactic to acetic acid ratio. The pH of silage is a measure of acidity and can be affected by many factors (Kung et al. 2018). In this study, pH ranged from 4.2 (HM with 0% and 1% tannin) to 4.6 (LM with 0% and 1% tannin). Kung and others (2018) suggested that normal pH values for grass silages between 75 to 65% moisture are between 4.3 and 4.7. Lactic acid ranged from 55.0 to 63.8 g/kg DM across all moisture-tannin treatments in this study. The greatest lactic acid concentrations, numerically, were in HM with 0% added tannin (63.8 g/kg

DM) and LM with 0% added tannin (62.8 g/kg DM) and the lowest concentration, numerically, was in LM with 2% added tannin (55.0 g/kg DM). Lactic acid concentrations were close to suggested concentrations (60 to 100 g/kg DM) for grass silage suggested by Kung and others (2018) and were close (54.2 g/kg DM) to pea and wheat bi-crop silage treated with quebracho tannin reported by Salawu and colleagues (2001). Acetic acid was lower than suggested by Kung et al. (2018) for silo acetic acid concentrations (10 to 30 g/kg DM). While low acetic acid levels can indicate unstable silages when exposed to air because of insufficient acetic acid to inhibit lactate-assimilating yeasts, high acetic acid levels can indicate unwanted fermentation by clostridia bacteria (Kung et al., 2018; McDonald et al., 1991).

Ammonia-N concentrations in silage reflect the level of protein degradation, and high-moisture silages typically have greater concentrations of ammonia-N (Driehuis, et al., 2001; Kung et al., 2018). In a previous study, ammonia-N of ryegrass silage was extremely high (260 g/kg DM) after 90 days indicating the involvement of proteolytic clostridia (Davies et al., 1998). Ammonia-N (g/kg N) was lower in the present study than suggested concentrations by Kung et al. (2018), indicating lower proteolytic activity.

Conclusion

Tall fescue ensiled with quebracho tannin had few effects on nutritive quality and fermentation parameters. Furthermore, ergovaline concentrations only decreased in low moisture silages with a 2% inclusion of Quebracho tannin. Tall fescue ensiled with up to 2% quebracho tannin appears to reduce proteolysis regardless of moisture concentrations without affecting other fermentation parameters. However, more research should be conducted to determine the effects of ensiling under different conditions on the ergovaline concentrations and fermentation parameters of tall fescue silage.

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Tables

Table 1. Pre- and post-ensiled forage chemical components of tall fescue ensiled in mini-silos at different moistures and with quebracho tannin at 0%, 1%, or 2% total silage DM

Item	Forage treatment combinations[1]							Effect *P*-values		
	High			Low						
Pre-ensiled[2]	0	1	2	0	1	2	SEM[3]	Moisture	Tannin	Moisture × Tannin
NDF, g/kg DM	612	616	608	612	614	598	5.2	0.38	0.07[4]	0.64
ADF, g/kg DM	339^{b}	337^{b}	339^{b}	339^{b}	355^{a}	334^{b}	3.4	0.17	0.03[5]	0.01
Hemicellulose, g/kg DM	272	279	268	272	259	265	5.2	0.08[6]	0.58	0.17
ADL, g/kg DM	33	36	35	34	38	35	1.4	0.52	0.13	0.80
OM, g/kg DM	929^{a}	923^{ab}	898^{c}	916^{b}	911^{b}	914^{b}	4.2	0.45	<0.01[7]	0.01
N, g/kg DM	17	17	16	17	17	16	0.4	0.96	0.13	0.57
WSC, g/kg DM	149	149	121	128	127	119	10.5	0.09[6]	0.16	0.56
Ergovaline, ppb	122	117	140	123	154	137	10.6	0.19	0.31	0.15
Post-ensiled										
NDF, g/kg DM	616^{x}	626^{x}	630^{x}	628^{x}	627^{x}	617^{x}	5.0	0.93	0.66	0.07
ADF, g/kg DM	364	364	368	365	367	355	4.3	0.38	0.62	0.15
Hemicellulose, g/kg DM	253^{y}	262^{x}	262^{x}	263^{x}	260^{xy}	262^{x}	2.8	0.24	0.27	0.09
ADL, g/kg DM	38	39	37	37	42	38	2.5	0.61	0.33	0.69

Table 1 (Cont.). Pre- and post-ensiled forage chemical components of tall fescue ensiled in mini-silos at different moistures and with quebracho tannin at 0%, 1%, or 2% total silage DM

Item	Forage treatment combinations[1]							Effect *P*-values		
	High			Low						
Post-ensiled[2]	0	1	2	0	1	2	SEM[3]	Moisture	Tannin	Moisture × Tannin
OM, g/kg DM	918[a]	914[ab]	889[d]	914[ab]	905[bc]	899[cd]	3.8	0.70	<0.01[7]	0.05
N, g/kg DM	18[a]	17[b]	17[b]	17[b]	17[b]	17[b]	0.2	0.23	0.02[8]	0.04
WSC, g/kg DM	67	57	44	88	87	85	6.5	<0.01[9]	0.14	0.35
Ergovaline, ppb	100	127	128	109	133	105	9.3	0.68	0.04[10]	0.17

[1]Forage treatment combinations included tall fescue ensiled at either high moisture (HM) or low moisture (LM) with quebracho tannin at 0%, 1%, or 2% total silage DM.

[2]OM = organic matter; NDF = neutral-detergent fiber with inclusive ash; ADF = acid detergent fiber; hemicellulose = NDF – ADF analyzed sequentially; ADL = acid detergent lignin; WSC = water soluble carbohydrates; DM = dry matter; N = nitrogen.

[3]Standard error of the mean.

[4]Means for 1% tended to be greater than 2% ($P < 0.10$).

[5]Means for 1% were greater than 2% ($P < 0.05$).

[6]Means for HM tended to be greater than those for LM ($P < 0.10$).

[7]Means for 2% differed from 0% and 1% ($P < 0.05$).

[8]Means for 0% were greater than those for 2% ($P < 0.05$).

[9]Means for LM were greater than those for HM ($P < 0.05$).

[10]Means for 1% were greater than for 0% ($P < 0.05$).

[a, b, c, d]Within a row, means with different superscripts differ at $P < 0.05$.

[x, y]Within a row, means with different superscripts tended to differ at $P < 0.10$.

Table 2. Change in forage chemical components between pre-ensiling and post-ensiling from tall fescue ensiled in mini-silos at different moistures with quebracho tannin at 0%, 1%, or 2% total silage DM

	Forage Treatment Combinations[1]									
	HM			LM				Effect *P*-values		
Item[2]	0	1	2	0	1	2	SEM[3]	Moisture	Tannin	Moisture ×Tannin
NDF, g/kg DM	4.4	10.0	22.5*	15.8	13.0	18.3*	8.2	0.61	0.41	0.64
ADF, g/kg DM	23.9*	26.9*	28.9*	22.5*	12.0	21.2*	5.7	0.15	0.57	0.39
Hemicellulose, g/kg DM	-19.5*	-16.9*	-6.4	-9.3	1.0	-2.9	6.4	0.06[4]	0.32	0.55
ADL, g/kg DM	5.1	3.3	1.2	2.6	0.0	3.1	2.9	0.89	0.76	0.70
OM, g/kg DM	-10.7*	-8.0	-8.5	-1.9	-6.4	-14.5*	4.3	0.69	0.46	0.26
N, g/kg DM	1.5*	0.4	1.1*	0.6	0.7	0.9	0.4	0.49	0.54	0.44
WSC, g/kg DM	-82.3*	-92.4*	-77.0*	-39.8*	-40.2*	-34.7*	9.65	<0.01[5]	0.56	0.84
Ergovaline, ppb	-21.9	9.52	-11.6	-14.5	-20.9	-32.6*	14.2	0.22	0.49	0.40

[1]Forage treatment combinations included tall fescue ensiled at either high moisture (HM) or low moisture (LM) with quebracho tannin at 0%, 1%, or 2% total silage DM.

[2]OM = organic matter; NDF = neutral-detergent fiber with inclusive ash; ADF = acid detergent fiber; hemicellulose = NDF – ADF analyzed sequentially; ADL = acid detergent lignin; WSC = water soluble carbohydrates; DM = dry matter; N = nitrogen.

[3]Standard error of the mean.

[4]Changes for HM tended to be greater than those for LM ($P < 0.10$).

[5]Changes for HM were greater than those for LM ($P < 0.05$).

*Means differed from zero ($P \leq 0.05$) indicating that there was a statistical change in concentration between pre-ensiling and post-ensiling.

Table 3. Post-ensiled fermentation parameters of tall fescue ensiled in mini-silos at different moistures with quebracho tannin at 0%, 1%, or 2% total silage DM

Item[2]	Forage treatment combinations[1]							Effects *P*-values		
	HM			LM						
	0	1	2	0	1	2	SEM[3]	Moisture	Tannin	Moisture × Tannin
Moisture, %	71.7^{x}	72.8^{x}	72.2^{x}	55.0^{z}	58.7^{y}	61.1^{y}	1.17	<0.01[4]	0.03[5]	0.08
pH	4.2^{c}	4.2^{c}	4.4^{b}	4.6^{a}	4.6^{a}	4.5^{ab}	0.05	<0.01[6]	0.88	0.02
Ammonia, g/kg DM	8.3^{x}	7.0^{y}	7.3^{y}	7.6^{xy}	7.6^{xy}	7.1^{y}	0.3	0.52	0.02[7]	0.05
Lactic Acid, g/kg DM	63.8	58.2	59.4	62.8	58.4	55.0	2.4	0.38	0.04[9]	0.61
Acetic Acid, g/kg DM	6.4^{a}	5.2^{ab}	6.5^{a}	5.1^{ab}	6.1^{ab}	4.7^{b}	0.5	0.07[8]	0.95	0.02
Lactic/Acetic Ratio	10.4^{abc}	11.2^{abc}	9.1^{c}	12.3^{a}	9.6^{bc}	11.7^{ab}	0.78	0.14	0.40	0.03
Propionate, g/kg DM	0.0^{y}	0.3^{x}	0.0^{y}	0.0^{y}	0.0^{y}	0.0^{y}	0.1	0.11	0.08[10]	0.08
Butyrate, g/kg DM	7.2^{y}	10.4^{y}	15.2^{x}	1.0^{z}	2.0^{z}	3.4^{z}	1.2	<0.01[4]	<0.01[11]	0.10
Total Acids, g/kg DM	78	74	81	68	66	63	2.8	<0.01[4]	0.54	0.17
AmmN, g/kg N	67	60	65	60	60	60	2.8	0.09[8]	0.42	0.41

[1]Forage treatment combinations included tall fescue silage at either high moisture (HM) or low moisture (LM) ensiled with quebracho tannin at 0%, 1%, or 2% total silage DM.
[2]DM = dry matter, N = nitrogen.
[3]Standard error of the mean.
[4]Means for HM were greater than those for LM ($P < 0.05$).
[5]Means for 2% were greater than means for 0% ($P < 0.05$).
[6]Means for LM were greater than those for HM ($P < 0.05$).
[7]Means for 0% differed from 1% and 2% ($P < 0.05$).
[8]Means for HM tended to be greater than those for LM ($P < 0.10$).

[9]Means for 0% were greater than those for 2% ($P < 0.05$).
[10]Means for 1% tended to be greater than those for 0% and 2% ($P < 0.10$).
[11]Means for 2% were greater than those for 0% and 1% ($P < 0.05$).
[a, b, c]Within a row, means with different superscripts differ at $P < 0.05$.
[x, y, z]Within a row, means with different superscripts

Conclusion

In the first experiment, decreased prolactin levels from the non-toxic, novel endophyte tall fescue treatment to any of the toxic, endophyte-infected tall fescue treatments is a clear indicator of fescue toxicosis in the toxic treatments. Therefore, the condensed tannins did not properly bind to the ergot alkaloids and alleviate fescue toxicosis. Dry matter intake tended to be greater from ewes consuming the non-toxic, novel endophyte tall fescue treatment compared to any toxic, endophyte-infected tall fescue treatments. Nitrogen utilization from ewes consuming non-toxic, novel endophyte and toxic, endophyte-infected tall fescue exhibited very few effects. Condensed tannin supplementation did not improve indicators of animal performance in experiment one.

Tall fescue ensiled with quebracho tannin had few effects on forage chemical composition and fermentation parameters. Changes in ergovaline concentrations from pre- and post-ensiled silages only decreased significantly in LM silages with 2% tannin inclusion, and the inclusion of 2% quebracho tannin in ensiled tall fescue appears to reduce proteolysis. More research should be conducted to determine the effects of ensiling tall fescue with differing conditions on ergovaline concentrations and fermentation parameters. Based on these two studies, condensed tannin inclusion with toxic, endophyte-infected tall fescue did not affect fescue toxicosis in ewes or silage.

Appendix

DIVISION OF AGRICULTURE
RESEARCH & EXTENSION
University of Arkansas System

To:	Kenneth Coffey
Fr:	Billy Hargis - Ag-IACUC Chair
Date:	September 14th, 2020
Subject:	IACUC Approval
Expiration Date:	April 30th, 2021

The Division of Agriculture Institutional Animal Care and Use Committee (Ag-IACUC) has APPROVED your personnel addition(s) of Sarah Shelby and Ally Grote to protocol # **18118:** *Effects of dietary modifications on digestion and nutrient balance in sheep and goats*.

In granting its approval, the Ag-IACUC has approved only the addition of the personnel listed. Should there be any further changes to the protocol during the research, please notify the Ag-IACUC in writing (via the Modification form) prior to initiating the change.By policy the Ag-IACUC cannot approve a study for more than 3 years at a time.

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

BMH/tmp