Consumption of Endophyte Infected Fescue during Gestation in Beef Cows

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Abstract

Tall fescue is a widely grown, cool season grass prevalent in the eastern United States that is known for its resistance to abiotic and biotic stresses. A main reason for tall fescue’s resistance to these stresses is attributed to the presence of a fungal endophyte. Unfortunately, this endophyte also adversely affects cattle production. Cows consuming the ergot alkaloids produced by these endophytes can exhibit decreased feed intake, growth performance, organ vasoconstriction, and increased rectal temperature. This work is interested in examining how endophyte toxin exposure impacts pregnancy in cattle. Reduced blood flow to the fetus and inadequate maternal nutrition contributes to intrauterine growth restriction (IUGR), and this work proposed that fescue endophyte toxicity affects the gestating cow and fetus. Three studies were completed. In experiment 1, gestating cows grazed high or low endophyte fescue pastures during late gestation to determine if exposure to ergot alkaloids in utero results in IUGR and if calves from these pregnancies have altered growth performance. Creep feeding was evaluated as a mitigation strategy for impaired calf growth due to fescue toxicity, and feedlot performance was evaluated to determine if consuming fescue during gestation and creep feeding would affect feedlot performance. Calf BW was different \( (P < 0.01) \) by treatment x time. Birth weights of calves were similar, prior to creep feeding calves exposed to high endophyte fescue were lower, and post-supplementation creep fed calves had increased BW. Days on feed and dressing percentage were decreased in the supplemented group, and marbling score was decreased for both the supplemented and
unsupplemented groups following the completion of the feedlot phase ($P < 0.05$). The second study was setup similar to study one, however cows were exposed to fescue pastures from d 170 of gestation until calving. Calf birth weights did not differ, but weights were increased in the supplemented group post creep feeding ($P < 0.05$). Average daily gains (ADG) of supplemented calves were greater during the supplementation period ($P < 0.01$). In the third study, indwelling vaginal temperature probes were used to evaluate differences in body temperature of cows fed fescue seed with high or low levels of ergot alkaloids during early gestation, and in varying environmental conditions. In the winter trial, body temperature was measured hourly from days 0-14 of gestation. In the summer trial, body temperature was measured hourly from days 0-32 of gestation. Body temperatures were different ($P < 0.01$) between treatments during both trials.
Dedication

This thesis is dedicated to my family, my parents David and Teresa Oliver, and my fiancé Jacob VanValin. Their hard work and sacrifice has allowed me to chase my dreams, and make them a reality.
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Chapter 1. Introduction and Literature Review

Introduction

Tall fescue (Festuca arundinacea) is a widely grown cool-season forage grass in the United States, with origins in Europe. (Cowan, 1956). Cool season grasses, including tall fescue can be grazed through cold winter months (Yates, 1962). Positive agronomic characteristics including drought tolerance, pest resistance, contribute to tall fescues’ overall hardiness, and popularity (Hoveland, 1993b). Tall fescue is widely adaptable and is found growing across the world including parts of South America, Australia, New Zealand, China, and Africa, as well large portions of the eastern United States (Rudgers and Clay, 2007).

Kentucky 31 (KY-31), is a popular cultivar of tall fescue, which was first discovered growing in Eastern Kentucky by Dr. E.N. Fergus in 1931 (Hoveland 1993). Kentucky 31 became commercially available in 1942 and quickly became the leading cool season forage grass grown in the southeastern United States (Stuedemann and Hoveland, 1988). Livestock consuming KY-31 showed signs of decreased performance soon after the cultivar’s release. (Hoveland et al., 1983). The cause of these issues is now attributed to the presence of a fungal endophyte (Epichloë coenophiala) (Hoveland 1993). Endophytes are fungi that live their entire life within a host grass, where they form nonpathogenic and asymptomatic relationships with the host (Hyde and Soytong, 2008). The endophyte in tall fescue produces ergot alkaloids, which are a group of chemicals with similarities in chemical structure to biogenic amines (Lyons et al., 1986). Similarities in chemical structure between ergot alkaloids and biogenic amines are responsible for the decreased performance of animals consuming endophyte infected tall fescue (Berde et al., 1978).
The relationship between tall fescue and its fungal endophyte is complex, however it is this relationship that is responsible for the productivity of tall fescue (Yurkonis et al., 2012). Consumption of ergot alkaloids produced by the fungal endophyte, results in a variety of symptoms collectively known as fescue toxicity (Schmidt et al., 1982). Decreased livestock performance due to fescue toxicity is estimated to cost the United States livestock industry one billion dollars annually (Smith et al., 2012).

**Fescue and Endophyte Symbiosis**

Ergot alkaloid production by the endophyte decreases animal performance while increasing the performance of tall fescue. (Pedersen, 1990). Tall fescue and its’ endophyte have an asymptomatic symbiosis (Wilkinson and Schardl, 1997). *Epichloë coenophiala* relies on vertical reproduction via dissemination through tall fescue seeds (Tadych et al., 2007).

Mature endophyte infected grasses are better able to adapt to abiotic stresses than non-infected grasses (Lewis, 2004). Metabolic requirements of the endophyte come at a cost to the plant, and young endophyte infected plants may have decreased performance and growth when exposed to environmental stress (Cheplick et al., 1989). However endophyte infected grasses are more adaptable to abiotic stresses including drought, mineral imbalance, and soil acidity (Bacon, 1993; Malinowski et al., 1999). The endophyte affects the mechanisms responsible for drought avoidance in the host (Malinowski and Belesky, 2000). Endophyte infected grasses have increased ability to avoid drought through improved water uptake from the soil, reduced transpiration loss, and increased water storage ability (Simpson, 1981). The root systems of endophyte infected grasses have increased dry matter, root hair length, and decreased root diameter, which increases the surface area of the root system, and increases water uptake (De
Battista et al., 1990; Malinowski and Belesky, 1999a). Endophyte infected plants also have reduced water loss via transpiration (Neill et al., 2008). During drought conditions, an increase in the rate of stomatal closure results in decreased water loss in endophyte infected grasses (Turner, 1986). Water content of endophyte infected grasses is higher than non-infected grasses during drought conditions, which suggests an ability for endophyte infected grasses to store increased amounts of water (Elbersen and West, 1996).

Minerals are inorganic substances necessary for the maintenance of normal physiological conditions in living matter including plants (Soetan et al., 2010). Stress conditions occur when the mineral concentration of soils are too high or too low for a given plant. Endophyte infected plants are better able to avoid or adapt to these stress conditions (Belesky and West, 2009). Endophyte infection of tall fescue improves tolerance to increased aluminum (Al) concentrations in soil through a mechanism which increases phenolic compound production. These compounds are then able to bind to the free Al in the soil (Malinowski and Belesky, 1999a). Increased concentrations of soil nitrogen (N) due to fertilization with N based fertilizers results in increased plant DM (Clay, 1987). However, when endophyte infected tall fescue is grown at low N levels, DM production is similar to that of non-endophyte infected fescue with high levels of N. This suggests that the endophyte allows for better plant performance at less than optimum N levels (Arechavaleta et al., 1992). Dry matter production is also increased in endophyte infected plants grown in a P deficient soil (Rahman et al., 2003).

Decreased soil pH results in limited plant growth due to alterations in soil mineral availability (Marschner and Marschner, 2012). Endophyte infected tall fescue can increase soil pH more quickly than non-endophyte infected varieties of fescue, resulting in more favorable growing conditions for the plant (Malinowski and Belesky, 1999b). Additionally, endophyte
infected fescue can produce root systems with increased DM compared to non-infected varieties when grown in acidic conditions, and this increased root DM can lead to increased nutrient uptake by the plant (Belesky and Fedders, 1995).

**Ergot Alkaloid Concentrations**

Two methods can be used to describe the amount of endophyte infection in tall fescue. The first method is measuring the percentage of infected plants in a given stand of fescue. The general trend with infectivity is that as the rate of infection increases within a stand, animal performance and reproductive potential decreases. Average daily gains of growing cattle are reduced by 45g for every 10% increase in infection rates of endophyte-infected fescue (Thompson et al., 1993). Also, conception rates for cows is decreased by 3.5% for every 10% increase in infection rate (Schmidt and Osborn, 1993).

The second method used to describe the amount of endophyte infection is by determining the amount of ergot alkaloids present in a stand of fescue. Concentrations of ergot alkaloids ranging from 400-750 µg/L or higher cause fescue toxicity symptoms in cattle (Tor-Agbidye et al., 2001). The presence or amount of ergot alkaloids in plant tissues can be measured by mass spectrometry (Yates et al., 1985) or near-infrared spectroscopy (Roberts et al., 2005). Enzyme-linked immunosorbent assays (ELISA) also have been developed to quantify the concentrations of ergot alkaloids (Hill and Agee, 1994; Schnitzius et al., 2001). Mass spectrometry and near-infrared spectroscopy methods require costly analytical equipment. These techniques also are time consuming (Schnitzius et al., 2001). ELISA assays are limited to determining total ergot alkaloid concentration, however mass spectrometry assays are able to quantify concentrations of individual ergot alkaloids and their epimers (Crews, 2013).
Fescue Toxicosis

Fescue toxicosis is the condition that develops when livestock consume tall fescue infected with high levels of the fungal endophyte (Aiken et al., 2013). Ergot alkaloids in tall fescue, bind to neurotransmitter receptors throughout the body, including dopamine, serotonin, and norephenpherine (Scharld, 2015). Ergot alkaloids are able to bind to multiple receptor sites and act as agonist, partial agonist, and antagonists, and this action results in the wide variety of adverse physiological outcomes (Klotz, 2015). Livestock consuming tall fescue suffer from gangrenous infections, decreased growth and reproductive performance, and fat necrosis (Thompson and Stuedemann, 1993).

Fescue foot is a gangrenous infection of the extremities, commonly seen when livestock consume endophyte infected fescue in the late fall and early winter months (Jacobson et al., 1970). Ergot alkaloids cause vasoconstriction resulting in decreased blood flow to the hoof, tips of the ears, and tail (Egert et al., 2014; Klotz, 2015). Symptoms of fescue foot include dry gangrenous infections, reduced hoof and horn growth, swelling in the limbs near the fetlock and lameness (Jacobson et al., 1970; Yates et al., 1985).

Summer Fescue toxicosis, or ‘summer slump’, is responsible for significant economic losses for the cattle industry in the United States (Schmidt and Osborn, 1993). Symptoms of summer fescue toxicosis include reduced reproductive efficiency of both males and females, reduced feed intake and weight gain, increased salivation, rough hair coat that fails to shed in the spring, and intolerance to increased environmental temperatures (Schmidt et al., 1983; Porter and Thompson, 1992).

Cattle consuming endophyte-infected fescue also are more susceptible to heat stress during the summer months than those consuming an endophyte free diet (Hemken et al., 1981b).
Heat stress occurs when an animal is exposed to ambient temperatures exceeding the upper limits of their thermoneutral zone. This normally ranges from that is -15 and 28°C for mature adult cattle, although it is highly dependent on lactation status, growth and other parameters (Armstrong, 1994). When animals are subjected to heat stress conditions, the normal physiological response is for blood flow to increase to peripheral tissues for enhanced heat dissipation (Cheeke and Shull, 1985). Vasoconstriction occurring because of ergot alkaloid consumption (Oliver et al., 1993) reduces peripheral blood flow and this compromises heat dissipation, thus increasing core body temperature. (Rhodes et al., 1991).

Cattle consuming endophyte infected tall fescue have rough hair coats. This occurs because hair follicles fail to cycle through their normal growth cycles (Aiken et al., 2011). The mammalian hair follicle cycle includes the anagen (active phase), catagen (shortening phase), telogen (resting phase) and exogen phases (shedding phase; (Hardy, 1992; Higgins et al., 2009). The shedding of the winter hair coat in cattle is needed to facilitate the growth of a sleek summer hair coat. However, in cattle consuming high endophyte fescue, rough hair coats are present during the summer months even if the winter coat has been shed (McClanahan et al., 2008). The possible mechanism behind the retention of a rough winter hair coat is the marked decrease in prolactin seen in cattle consuming endophyte infected fescue (Forsyth et al., 1997). Disruptions in prolactin concentrations are associated with changes in hair follicle cycles (Dicks et al., 1994). Other endophyte toxicity events that are manifested by altered prolactin production will be discussed in upcoming sections.
**Fat Necrosis**

Fat necrosis is an underreported symptom of fescue toxicosis. It is defined as the presence of hard masses of necrotic fat located in the mesentery of the intestinal tract (Buckner and Bush, 1979). This hardening can cause difficulties with normal passage of digesta, parturition, and renal function (Thompson and Stuedemann, 1993). Histologic examination of these lesions has revealed that along with the necrotic lesions, an increase in macrophages and lymphocytes numbers also occurred (Smith et al., 2004). The necrotic regions have increased inflammatory responses, along with increased inflammatory responses in the abomasum, small intestine, and large intestine (Greatorex and Mantle, 1974). These findings could be a result of increased intestinal permeability to microflora, which support the movement of bacteria normally restricted within the lumen of the intestine to areas where lesions were found (Klotz, 2015).

**Decreased Prolactin**

Prolactin is a polypeptide hormone secreted from lactotroph cells in the anterior pituitary gland. The original role of prolactin was linked to be promotion of lactogenesis, but prolactin now is also known for mediating more than 300 additional biological functions (Freeman et al., 2000). Hypoprolactemia, or decreased serum prolactin (PRL), is a common symptom of fescue toxicosis (Brown et al., 2009; Aiken et al., 2013; Parish et al., 2013). This decrease in PRL concentration is used as a means to determine if the endophyte has induced a physiological change in animals consuming endophyte infected fescue (Hurley et al., 1980b; Bernard et al., 1993; Coffey et al., 2001).
Ergot alkaloids interfere with the normal neuroendocrine regulation of prolactin secretion. Under normal physiological conditions, prolactin secretion is regulated by dopamine acting on D2-dopamine receptors located on the lactotroph cells of the anterior pituitary, thus inhibiting prolactin secretion (Lamberts and Macleod, 1990). Endophyte infected fescue consumption in animals could lead to hypoprolactemia. The ergoline ring of ergot alkaloids is similar in structure to dopamine, which allows many ergot alkaloids to bind D2-dopamine receptors thus limiting prolactin secretions (Berde et al., 1978).

The marked decrease in PRL seen during fescue toxicity may in part lead to a variety of fescue toxicity symptoms including decreased feed intake and average daily gains (Cheeke and Shull, 1985). For example, PRL is involved in the maintenance of lactation and milk secretion by acting on luminal epithelial cells (Neville et al., 2002). Serum prolactin concentrations are correlated with the size of the largest follicle following estrous synchronization, thus implicating reduced prolactin in decreased reproductive performance (Flores et al., 2008). An increase in PRL levels seen by the dopamine antagonist, Domperidone, leads to an increase in esophageal peristalsis and facilitation of gastric emptying, thus suggesting a correlation between PRL concentrations and gut motility (Ahmad et al., 2006). Dry matter intake and appetite are increased with hyperprolactemia, which is a means to maintain a positive energy balance especially during lactation (Noel and Woodside, 1993).

To summarize up to this point, the importance of tall fescue as a forage grass is well established. Additionally, ergot alkaloid consumption from infected tall fescue reduces performance in cattle. The following section will focus on examining how ergot alkaloid toxins adversely impact reproductive performance of both the male and the female.
Effects on Male Reproductive efficiency

The full extent of fescue’s role in the reproductive performance of the bull is still unclear (Schuenemann et al., 2005b; Looper et al., 2009; Stowe et al., 2013). Marked changes in common measurements of bull fertility exist when bulls consume endophyte-infected fescue. One common measure of a bull’s reproductive performance is the breeding soundness exam (BSE). A BSE accounts for scrotal circumference and the percentage of motile and mobile sperm. Fescue toxicity affects the bulls’ ability to pass a breeding soundness exam (Stowe et al., 2013). Consumption of endophyte-infected fescue can decrease sperm motility and morphology, while scrotal circumference and plasma testosterone are unaffected (Looper et al., 2009). However, other studies failed to show these effects (Schuenemann et al., 2005a). With that said, there is evidence that sperm from bulls fed endophyte-infected fescue are inferior at fertilizing oocytes (Schuenemann et al., 2005b). Therefore, there are apparent adverse effects of endophyte-infected fescue consumption on bull reproductive performance but these adverse effects seem to be difficult to detect in some circumstances.

Effects on Female Reproductive Efficiency

Most cattle consuming endophyte infected fescue in southeastern United States are in cow-calf operations (Schmidt and Osborn, 1993). Several profound outcomes of grazing endophyte-infected fescue exist in cow-calf operations. Cows in poor body condition score upon calving will exhibit estrus later than those in proper body condition score (Richards et al., 1986). Cows grazing endophyte infected fescue during lactation produce lighter weaned calves (Schmidt et al., 1983). This likely occurs because cow grazing in infected fescue have a prolonged state of negative energy balance, which reduces their milk production (Peters et al.,
Cows weaning lighter calves, and failing to conceive after weaning leads to significant economic losses for cow-calf producers (Hoveland, 1993b).

Cows grazing endophyte infected pastures have reduced conception rates (Tucker et al., 1989). One possible explanation is that cows in negative energy balance have reduced conception rates due to a delay in normal ovarian function, which is related to a decrease in luteinizing hormone concentrations (Butler and Smith, 1989). In cattle luteinizing hormone is critical for ovulation and proper progesterone production from the corpus luteum. Progesterone is critical the maintenance of pregnancy (Garverick et al., 1992).

Heifers exposed to endophyte infected fescue have reduced serum progesterone levels and fewer occurrences of large follicles during the estrous cycle, (Burke et al., 2001). An increase in size of the ovulatory follicle may influence oocyte quality, ovulation, and uterine environment and this will lead to improvements in pregnancy rate (Baruselli et al., 2012). Cows in proper body condition do not show differences in the diameter of the large follicle or estradiol concentrations at ovulation (Burke and Rorie, 2002).

**Fescue Toxicosis Mitigation Strategies**

Several methods have been developed to lessen the negative effects of fescue toxicity. One strategy is to use endophyte-tolerant fescue strains. Conventional selection has yielded several notable strains. These include non-endophyte infected varieties of fescue (GA-5) and varieties containing a novel endophytes (Max Q; (Nihsen et al., 2004; Watson et al., 2004b). Novel endophyte varieties of fescue contain an endophyte that benefits the plant but does not produce ergot alkaloids, making these forage varieties safer for livestock consumption (Gunter and Beck, 2004). Novel endophyte fescue varieties were produced by infecting endophyte free
fescue seeds with non-ergot alkaloid producing endophytes (Bouton et al., 2002). While livestock performance is increased, establishing endophyte free or novel endophyte tall fescue varieties in pastures previously containing endophyte infected tall fescue can be costly, and endophyte free fescue is less persistent under grazing conditions, and less drought tolerant than endophyte infected varieties (Camp, 1986).

An additional solution for managing endophyte infected fescue pastures is to dilute the endophyte infected fields by inter-seeding nontoxic forages like legumes (e.g. red and white clover) (Roberts and Andrae, 2004). This is a popular method for reducing the occurrence of fescue toxicity in livestock. However, the southeastern United States legumes must compete with warm season grasses and be resistant to environmental factors such as heat, drought, and pests (Hoveland, 1989). When adding clover to endophyte infected pastures, average daily gains are most improved in fields with moderate infection rates as opposed to those with high levels of endophyte infection (Thompson et al., 1993).

Seaweed (Ascophyllum nodosum) can be used to increase antioxidant activity when applied to fescue. It also is effective when included as a supplement to ruminants consuming endophyte infected fescue (Allen et al., 2001; Fike et al., 2001; Saker et al., 2001). Antioxidant activity is defined as how well an antioxidant delays or prevents oxidation of an oxidizable substrate (Tirzitis and Bartosz, 2010). When animals consume endophyte-infected fescue, there is an increase in activity of the mixed function oxidase system, which increases reactive oxygen metabolites (Zanzalari et al., 1989). As reactive oxygen metabolites are formed faster than they can be neutralized, oxidative stress occurs (Jackson et al., 1997). In steers, Tasco-forage (a product produced from seaweed) does not increase weight gains of steers grazing endophyte infected or endophyte free fescue (Fike et al., 2001), but steers grazing pastures sprayed with
Tasco-forage have increased marbling scores, improved color stability, and an extended shelf life (Allen et al., 2001; Montgomery et al., 2001).

Vitamin E is an antioxidant that has been evaluated for its ability to improve milk production in cattle consuming of ergot alkaloids (Jackson et al., 1997). Although Vitamin E has known antioxidant properties, there is no positive responses in milk production, serum prolactin, and alkaline phosphatase between cows consuming high endophyte fescue and high endophyte fescue supplemented with either 1,000 or 2,000 IU of vitamin E/day (Jackson et al., 1997).

Genotype may also alter an individual animals susceptibility to fescue toxicity symptoms (Smith and Cassady, 2015). Single nucleotide polymorphisms (SNP) are found in the genome where variations in a single nucleotide exist across individuals (Syvänen, 2001). Much of the work in cattle consuming endophyte infected fescue has focused around genes that influence the control of prolactin. Two SNPs have been found in the enhancer region of the bovine prolactin gene, at c1286t and a1167g. Cows with TT (thymine-thymine) genotype at c1286t and AA (adenine-adenine) genotype at a1167g have decreased lifetime calving rates when consuming endophyte infected fescue (Looper et al., 2010).

Another genotype marker for endophyte resistance is contained in the Kell blood group complex subunit-related family member 4 (XKR4) gene (Porto Neto et al., 2012). Cattle with genotypes containing adenine maintain physiological normal concentrations of prolactin when consuming endophyte infected fescue (Bastin et al., 2014). XKR4 also affects residual feed intake, average daily gains, and average daily feed intake in cattle, and in addition to decreased prolactin, a reduction in these phenotypes is commonly seen in animals with fescue toxicity (Bolormaa et al., 2011a; Bolormaa et al., 2011b; Lindholm-Perry et al., 2012).
A polymorphism in the dopamine receptor D2 gene (DRD2) is also associated with decreased serum prolactin concentrations and increased hair scores (Civelli et al., 1993). The more favorable adenine-adenine genotype is more prevalent in spring calving cows (Campbell et al., 2014), which have decreased reproductive and growth performance when consuming endophyte infected fescue (Caldwell et al., 2013). The increased prevalence of the favorable AA genotype in spring calving cows suggests that natural selection may occur for animals more resistant to fescue toxicosis symptoms (Smith and Cassady, 2015).

Multiple genetic markers have been found to show that individual animals maybe more susceptible to fescue toxicity, and as a result a commercially available test has been developed to provide producers with guidance when selecting animals for their operation (Masiero et al., 2016). Many mitigation strategies exist for fescue toxicity, thus multiple mitigation strategies may be needed to improve animal performance when consuming endophyte-infected fescue. A combination of pasture management, and animal selection strategies that have been discussed in this review may help to alleviate the detrimental effects of fescue toxicity.

**Intrauterine Growth Restriction**

Intrauterine growth restriction (IUGR) is defined as impaired growth and development of the mammalian embryo/fetus or its organs during pregnancy as a result of limitations of uterine capacity, under- and over nutrition of the dam, and insufficient utero placental blood flow (Wollmann, 1998). There is a lack of understanding of how environmental factors such as: maternal nutrition, stress, disease, and toxins affect fetal growth and development, and as a result IUGR remains a major problem in animal agriculture (Wu et al., 2006).
The swine model has been used to study IUGR as it occurs spontaneously as a result of limitations in uterine capacity, due to industry demands for increased litter size (Town et al., 2004). IUGR can also be detected in cattle as a result of uterine overcrowding. When cattle are selected for increased ovulation rates, where twin and triplet pregnancies are more common, there is also an increase in fetal mortality as a result of uterine overcrowding (Echternkamp et al., 2007). Even in single offspring pregnancies, uterine capacity can limit development, as demonstrated by birth weight differences of genetically similar embryos that were placed in donor cows of different frame sizes (Ferrell, 1991).

Maternal undernutrition is another factor that can result in IUGR. While nutrient partitioning during gestation favors fetal development over maintenance of the dam, this results in a negative maternal energy balance (Barron, 1946). In spite of nutrient partitioning, prolonged maternal undernutrition could result in IUGR. Undernutrition during early gestation can cause IUGR that can be recovered from by late gestation or time of calving. Calves can go through a period of undetected IUGR but can be born with normal birth weights (Long et al., 2009). Some studies focus on early to mid-gestation nutrient restriction as this is the time when placental development and organogenesis occurs (Vonnahme et al., 2007; Funston et al., 2010) Since 75% of fetal ruminant growth occurs during the last two months of gestation, other studies examine late gestational undernutrition as a cause for IUGR (Robinson et al., 1977).

Alterations in maternal nutrient status results in potential changes in immune response of the progeny. When maternal nutrition is altered, changes in colostrum IgG concentrations, and utilization efficiency (Hammer et al., 2007; Swanson et al., 2008). Metabolic changes including an inefficiency in glucose metabolism can occur in offspring as a result of maternal
undernutrition (Ford et al., 2007). Maternal nutrition changes may also result in altered feedlot and carcass performance (Stalker et al., 2006; Larson et al., 2009).

**IUGR and Fescue Toxicosis**

A number of factors that can result in IUGR are also seen in fescue toxicity. These include decreased uteroplacental blood flow (Poole et al., 2016) and maternal undernutrition due to decreased DMI (Hemken et al., 1981b). IUGR has not been widely studied in fescue toxicity, however some studies show animals exposed to endophyte infected fescue have decreased birthweights (Watson et al., 2004b; Duckett et al., 2014). Intrauterine growth restriction can be caused by insufficient uteroplacental blood flow (Reynolds and Redmer, 1995). Under normal physiological conditions, umbilical blood flow increases during gestation to maintain the needs of the growing fetus (Molina et al., 1991). Due to the vasoconstrictive nature of ergot alkaloids, uterine and placental blood flow is reduced with maternal ingestion of endophyte infected fescue during gestation (Dyer, 1993). Recently reductions in both uterine and ovarian artery and vein diameter have been shown as a result of ergot alkaloid consumption in fescue seed (Poole et al., 2016).

**Fetal Programing**

IUGR may cause permanent changes in the growth and development of the embryo/fetus, and the adverse effects of IUGR may be expressed permanently in postnatal life. This concept is known as fetal programing or the Barker hypothesis (Barker, 1995). Fetal programming during the prenatal period causes lasting effects on the offspring’s physiology in postnatal life in ways that alter their health/disease status. Various mechanisms cause alterations in fetal development
and postnatal outcomes. These include 1) alteration in hormonal regulation of fetal genes, 2) deviations in organogenesis, 3) modifications in placental function that alter fetal growth trajectory, and 4) epigenetic modifications in genes that impact postnatal health (Merlot et al., 2008).

One major target for fetal programming in cattle that is especially concerning in production agriculture are alterations in muscle development. The development of muscle fibers occurs in utero and plays a major role in the growth and development in postnatal life. If this process is disrupted due to poor maternal nutrition, permanent post-natal differences in the number of muscle fibers will occur (Russell and Oteruelo, 1981; Zambrano et al., 2006). Prenatal muscle fiber growth is due to hyperplasia of muscle fibers, and the number of muscle fibers is determined in utero (Du et al., 2010b). Additionally, the ratio of secondary to primary muscle fibers is reduced due to maternal undernutrition in ruminants (Zhu et al., 2004). Postnatal muscle growth is largely due to the hypertrophy of existing muscle fibers. Thus, a reduction in the number of muscle fibers developed in utero can have lasting impacts on post-natal muscle growth (Duckett et al., 2014).

Maternal nutrition also affects intramuscular fat (marbling) of the offspring. During fetal development, both muscle cells and adipocytes originate from the same mesenchymal stem cells (Du et al., 2010b). While the majority of mesenchymal stem cells develop into myogenic cells, a small number differentiate into adipocytes (Tong et al., 2008). Adipogenesis (formation of adipocytes) is initiated around mid-gestation (Gnanalingham et al., 2005), which occurs concurrently with the second myogenesis period (Du et al., 2010b). When maternal nutrition is limited, offspring have a limited number of mesenchymal cells thus limiting the number of cells
that differentiate into adipocytes, resulting in a decrease in intramuscular adipocytes and subsequently a reduction of marbling in beef cattle (Du et al., 2010b).

Summary

Fescue toxicity is a condition with a variety of symptoms resulting in decreased animal performance (Aiken et al., 2013). Of special interest for this work, fescue toxicity results in reduced reproductive efficiency in both the male (Schuenemann et al., 2005a; Schuenemann et al., 2005b) and the female (Baruselli et al., 2012; Tucker et al., 1989). While a variety of mitigation strategies have been evaluated (Jackson et al., 1997; Masiero et al., 2016; Thompson et al., 1993), fescue toxicity still results in economic losses of one billion dollars annually to the livestock industry (Smith et al., 2012).

Vasoconstriction of uterine and ovarian blood vessels results from the consumption of ergot alkaloids in endophyte infected fescue (Poole et al., 2016). Reductions in uteroplacental blood flow can result in IUGR (Reynolds and Redmer, 1995). While much work has been conducted to study fescue toxicity, it is not well defined if in utero exposure to ergot alkaloids from fescue would affect postnatal calf growth. This led us to conduct research investigating the effect of in utero exposure to ergot alkaloids from endophyte infected fescue on calf growth and development. Another aim of this work was to evaluate whether ingesting endophyte infected fescue seeds affects the thermoregulatory ability of the cow in Southwestern Virginia, which has a more temperate climate than most of the rest of the Southeast United States.
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Chapter 2. Use of an Indwelling Vaginal Temperature Probe for Determining Body Temperature in Cows Fed High or Low Ergot Alkaloid Fescue Seed
Abstract

Two studies were conducted to evaluate changes in body temperature due to the consumption of high (HE) or low (LE) ergot alkaloid fescue seed using an indwelling vaginal temperature probe. These studies were conducted from January to February, 2015 and mid-May to mid-July, 2015 to simulate both cool and warm environmental conditions. Multiparous Angus cross cows were used for experiment one (n = 6) and experiment two (n = 9). Cows were fed 0.90 kg of endophyte-infected seeds (HE seeds contained 1,320 µg/L ergot alkaloids, LE seeds contained 11 µg/L ergot alkaloids) and silage for 30 d before being bred using timed AI (d 0). During experiment one, cow BW was recorded on d -30, -14, -8, and 6 and was different ($P < 0.05$) across time but not between treatments. In experiment two BW was recorded on d -30, -8, 0, 18, and 32 and were-different ($P < 0.01$) across time but not between treatments. In experiment one body temperatures were recorded from d 0-14, in experiment two temperatures were recorded from day 0-32. Temperatures were recorded every h. Body temperatures were different ($P < 0.05$) between treatments for both the winter (HE= 38.49± 0.010°C, LE= 38.43 ± 0.010°C) and the summer (HE= 38.48 ± 0.007°C, LE= 38.41 ± 0.007°C). Area under the curve of the body temperatures for each cow was calculated and showed no difference ($P ≥ 0.40$) between treatments. Consumption of ergot alkaloids in fescue seed had no physiologically significant effect on the body temperature of mature cows under the environmental conditions of these studies.
Introduction

Tall fescue (*Festuca arundinacea*) is the primary cool season grass grown in the eastern United States, covering nearly 14 million Ha, and serves as a feed source for nearly 8.5 million head of cattle (Ball et al., 1996). Tall fescue is known for its drought and pest resistance which can be attributed to the presence of a fungal endophyte (*Epichloë coenophiala*; Hoveland, 1993a). The fungal endophyte found in tall fescue produces chemical compounds known as ergot alkaloids, which share a similar chemical structure with biogenic amines (dopamine, epinephrine, norepinephrine, and serotonin; Weber, 1980). This similarity in structure allows ergot alkaloids to bind to biogenic amine receptors and disrupt normal physiological processes in livestock consuming tall fescue (Strickland et al., 2011). The symptoms seen in cattle as a result of ergot alkaloid consumption is collectively known as fescue toxicity, which has been estimated to cost the United States cattle industry one billion dollars annually due to decreased animal performance (Smith et al., 2012).

Animals with fescue toxicity have increased difficulty dissipation body heat, resulting in an increase in core body temperature (Al-Haidary et al., 2001). In addition to disruption in body temperature regulation, vasoconstriction caused by fescue toxicity has been linked to decreased fertility, as well as gangrenous infection of the extremities (Tor-Agidye et al., 2001; Rensis and Scaramuzzi, 2003). The inability to dissipate heat is due to the vasoconstrictive properties of ergot alkaloids and is exacerbated with high and low ambient temperatures (Hemken et al., 1981b; Oliver et al., 1998).

Animals have a specific range of ambient temperatures within which they can effectively regulate their body temperature, this range is known as the thermoneutral zone (TNZ; Robertshaw, 1981). When ambient temperatures rise above the upper limits of the TNZ, animals
can become susceptible to heat stress (St-Pierre et al., 2003). In addition to increased ambient temperatures, the compounded effect of relative humidity can also add to heat stress; thus, the temperature humidity index (THI) equation was developed as an additional way to measure heat stress in animals (Bouraoui et al., 2002). When animals are exposed to ambient temperatures below the lower limits of the TNZ, animals become susceptible to cold stress (Young, 1981).

In previous studies, body temperature has been recorded using handheld digital thermometers to record rectal temperature at pre-determined time points (Aldrich et al., 1993b; Nihsen et al., 2004; Aiken et al., 2006). Measuring rectal temperature using rectal thermometers in a research setting has limitations such as errors in measurements due to handling cattle as well as high labor and facilities requirements. Thus use of an indwelling temperature probe can provide more robust temperature data for cattle research (Reuter et al., 2010).

The objective of these studies was to evaluate changes in body temperature as a result of ergot alkaloid consumption, during times of both cool and warm ambient temperature, using an indwelling vaginal temperature probe.

**Materials and Methods**

**Animals and Diets.** The experimental protocol was approved by the Institutional Animal Care and Use Committee at Virginia Polytechnic Institute and State University. Two experiments were conducted, in the winter and summer to study the effect of ergot alkaloid consumption and environmental temperature on cow body temperature. Experiment one was conducted from January - February, 2015. Experiment two was conducted from mid-June to mid-July, 2015.

In experiment one, 12 Angus cross cows were randomly assigned to two treatment diets, High endophyte fescue seed (HE) and low endophyte fescue seed (LE), (n = 6 LE, n = 6 HE). In
experiment two, 18 Angus cross cows were randomly assigned to the same dietary treatments (n = 9 LE, n = 9 HE). Cows were housed at the Shenandoah Valley Agricultural Research and Extension Center dry lot facility located in Raphine, VA. The dry lot facility was located in a three sided barn, and allowed animals access to both shade and sunlight.

Prior to the beginning of each trial, cows were trained to the Calan gate system (American Calan, Northwood, NH). Cows were fed silage and hay daily to meet NRC requirements, in addition cows were fed 0.90 kg of treatment seeds daily. Fescue seeds were analyzed for ergot alkaloid content by Agrinostics Ltd. (Watkinsville, GA). High endophyte variety seeds (Kentucky 31Tall Fescue Southern States Cooperative, Inc., Richmond, VA) contained 1,320 µg/L ergot alkaloids. The low endophyte seed (Tall Fescue “Forage Type”, Southern States Cooperative, Inc.) contained 11 µg/L ergot alkaloids. Cows were fed seed for 30 d prior to the start of the study and throughout the trial period. During experiment one cow BW was recorded on d -30, -14, -8, 6. During experiment two, cow BW was recorded on d -30, -8, 0, 18, 32.

**Estrus Synchronization and Artificial Insemination.** Estrus was synchronized in cows using a 5-day Select Synch plus CIDR protocol (Bridges, 2011). Heat mount detection patches (Kamar Products, Inc., Zionsville, IN) were used to detect cows in estrus prior to d 0 of the study, and any cows in estrus were bred by artificial insemination (AI). Cows were given GnRH (100 ug) 56 h after Lutalyse, and cows were inseminated 12-16 h later. All cows were detected in estrus either before or 12-16 h after GnRH (I think this is correct). The day of timed AI is indicated as day 0. For both studies, semen from four bulls were randomly assigned to cows in both treatment
groups (generously provided by Select Sires, Plain City, OH), in order to minimize any potential sire effect.

**Body Temperature Recording.** Temperature data loggers (Star-Oddi, DST-Micro T, Gardabaer, Iceland) were inserted vaginally using a blank controlled internal drug release device (CIDR) (Zoetis, Florham Park, New Jersey) according to the protocol by (Burdick, 2012). Temperature probes were inserted vaginally. At least one study indicates that vaginal and rectal temperature are comparable in cows (Suthar et al., 2013). Data loggers were placed in cows on day zero of each trial and recorded vaginal temperature once every hour. Temperature data was averaged over 4 hour periods for 14 (experiment one) or 28 (experiment two) days.

**Environmental Weather Recordings.** Environmental temperature and relative humidity was recorded by the USDA, Natural Resources Conservation Service, SCAN site at the Shenandoah Valley Agricultural Research and Extension Center. Data from the SCAN site was averaged over the same 4 hour period as the body temperature data. The THI was calculated using the equation: 

\[
\text{THI} = (0.8 \times \text{temperature}) + \left( \frac{\% \text{ relative humidity}}{100} \times (\text{temperature} - 14.4) \right) + 46.4 
\]

(Mader, 2003).

**Statistical Analysis.** Data was analyzed using the MIXED procedure in SAS (version 9.4, SAS Institute, Cary, NC), and recorded as least square means ± SEM. Body weight and body temperature were analyzed using repeated measures in the MIXED procedure. Area under the curve (AUC) was calculated for body temperatures using GraphPad Prism (version 6.00 for Windows GraphPad Software, La Jolla, CA) and then analyzed using the MIXED procedure in
SAS. The statistical model included seed treatment and time point as fixed effects. Differences were considered significant at $P \leq 0.05$.

**Results**

*Body Weight.* Body weights for cows showed no difference between treatment, but were different ($P < 0.01$) over time for both experiment one (Figure 1) and experiment two (Figure 2).

*Body Temperature.* During both trials, there were significant treatment ($P < 0.0001$) (Table 1) and time ($P < 0.0001$) effects. Area under the curve was calculated for each cow, and showed no significant differences between treatments for both trial periods (Table 1).

**Discussion**

In cattle, obtaining rectal temperature with a handheld thermometer can be difficult and inaccurate as animals may become excited or stressed when approached by humans, causing physiological responses including changes in body temperature (Voisinet et al., 1997; Burdick et al., 2010). Using an indwelling temperature probe allows for a more robust measure of body temperature, with the ability to obtain data from multiple animals simultaneously without human interference (Burdick, 2012).

Elevated rectal temperatures are often seen in cattle consuming ergot alkaloids from tall fescue due to an inability to affectively dissipate body heat (Hurley et al., 1980a; Parish et al., 2003a). In previous studies, cow body temperature was measured at set time points during the day (Read and Camp, 1986; Matthews et al., 2005) which may have missed some fluctuations in the core body temperature. In this study, body temperature of cows consuming either high or low
endophyte fescue seeds, were recorded continuously using an indwelling vaginal temperature probe.

Since tall fescue is a cool season grass that grows throughout much of the year in the southern United States, both spring and fall calving herds can utilize fescue as a feed source (Bagley et al., 1987). Traditionally spring calving operations have seen greater decreases in animal performance and profitability when compared to fall calving operations, while consuming endophyte infected tall fescue (Smith et al., 2012; Caldwell et al., 2013). This study was conducted over winter and summer trials in order to simulate conditions during breeding seasons for fall and spring calving operations.

Paragraph to tell readers about the diets. Why did we pick 0.9 kg of seed? Did others find differences in physiological outcomes with similar differences in ergot alkaloid loads?

In previous studies, increases in rectal temperature in animals consuming ergot alkaloids from fescue compared to animals receiving a control diet ranged from 0.3°C to 0.8°C (Read and Camp, 1986; Parish et al., 2013). In these studies there was a significant difference between the LE and HE treatment groups, however this difference was only 0.06°C for experiment one and 0.02°C for experiment two. While this difference was statistically significant, it is not expected to result in physiological differences between the two treatment groups. Animals are able to maintain their core body temperature when ambient temperatures fall within the TNZ for that animal. In mature beef cows consuming a maintenance diet the TNZ is between -15 and 28°C (Armstrong, 1994). During experiment one, ambient temperatures were below the cows’ thermoneutral zone for periods of time, but this seemed to have no effect on the body temperature. The lower limits of an animals TNZ can be altered based on animals dry matter intake (Mader, 2003), hair coat, and housing conditions (windbreaks; Young, 1981). In these
studies, animals were housed in a dry lot within a three-sided barn, which provided a wind break for the animals, which would allow for animals to experience ambient temperatures below the TNZ; yet, still maintain thermoregulation.

During experiment two, ambient temperatures rose above the thermoneutral zone for mature cows, however this occurred only for short periods of time and did not seem to have an effect on the cows’ body temperature. Although the upper limit of cows’ thermoneutral zone is 28°C, studies have shown that ergot alkaloid consumption did not cause an increase rectal temperature until ambient temperatures reached at least 31°C (Hemken et al., 1981b; Al-Haidary et al., 2001). During experiment two, ambient temperatures at our trial site did not rise above 31°C. When animals are exposed to higher ambient temperatures during the day, but night time temperatures fall below 21°C for 3-6 h, animals are able to dissipate heat gained during the day (Muller et al., 1994). During the summer trial, nighttime temperatures dropped below 21°C for at least 3-6 h during most nights, which helps explain why greater increases in body temperature were not seen.

In addition to ambient temperature, relative humidity can also play a role in the severity of heat stress, thus the THI was developed to calculate the effect of ambient temperature and relative humidity, and to quantify the level of heat stress that livestock species are exposed to (Bohmanova et al., 2007) Conditions are considered comfortable for cattle when THI values are 70 or less (Silanikove, 2000), values less than 74 are classified as alert, whereas THI values ranging from 74 to 79 are classified as danger, and from 79 to 84 are classified as emergency according to the livestock weather safety index (Hahn et al., 2009). When THI values are greater than 73 animals respiration rate increases as a means to dissipate heat, and when THI values increase to 80 or higher rectal temperature increases in addition to increased respiration rates.
(Lemerle and Goddard, 1986). During the summer trial, the THI remained within the comfortable range for the majority of the time. While THI values did rise into the danger and emergency ranges for short periods of time the ability of the cows to dissipate heat was not different across treatments as seen by similar vaginal temperatures. Other studies have used THI in a fescue toxicity model (Boling et al., 1989; Dougherty et al., 1991), however the use of different equations for calculating THI and scales for interpreting THI, make comparing results from these studies with the results of this study unfeasible.

Under the conditions of this study, the vasoconstrictive properties of ergot alkaloids do not seem to decrease the thermoregulatory ability of mature cows during both winter and summer conditions. Ambient temperatures residing in the thermoneutral zone of mature cows, as well as THI values within a comfortable range provided an environment that did not challenge the animals’ ability to thermoregulate due to ergot alkaloid consumption.
References


Table 2-1. Body temperature and Area under the curve for body temperature during both trials

<table>
<thead>
<tr>
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<th>Experiment One</th>
<th></th>
<th>Experiment Two</th>
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<tr>
<td></td>
<td>¹HE</td>
<td>¹LE</td>
<td>P-value</td>
<td>HE</td>
</tr>
<tr>
<td>Body Temperature</td>
<td>38.49&lt;sup&gt;a&lt;/sup&gt; ± 0.01</td>
<td>38.43&lt;sup&gt;b&lt;/sup&gt; ± 0.01</td>
<td>0.0001</td>
<td>38.48&lt;sup&gt;a&lt;/sup&gt; ± 0.007</td>
</tr>
<tr>
<td>Temperature AUC</td>
<td>3,079 ± 3.85</td>
<td>3,074 ± 4.22</td>
<td>0.4101</td>
<td>29,501 ± 29.54</td>
</tr>
</tbody>
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¹Treatments: HE= high ergot alkaloid, LE= low ergot alkaloid
²Temperature AUC: Area under the curve of body temperatures
Experiment One Body Weights

HE  LE  Time $P < 0.01$

Figure 2-1: The body weights (kg) of cows were recorded on day -30, -14, -8, and 6 relative to breeding. Significant differences are shown by differing superscripts ($P < 0.05$). (HE= High endophyte and LE= Low endophyte).
Figure 2-2: The body weights (kg) of cows were recorded on day -30, -8, 0, 18, and 32 relative to breeding. Significant differences are shown by differing superscripts. (HE= High endophyte and LE= Low endophyte).
Figure 2-3: Body temperatures for all cows and ambient temperatures were averaged over a 4 hour period, and each point on the graph is one four hour period. The x-axis shows days of the trial. The y-axis shows degree in °C for both ambient and body temperatures. (HE= High endophyte and LE= Low endophyte).
Figure 2-4: Body temperatures, Temperature humidity index (THI), and ambient temperature were averaged over 4 hour periods, each point on the graph represents one for hour period. The x-axis shows days of trial. The left y-axis shows temperature in °C for both ambient temperature and body temperature. The right y-axis shows the THI. (HE= High endophyte and LE= Low endophyte).
Chapter 3. Grazing Tall Fescue during Late Gestation Does Not Result in Long Lasting Deficits in Calf Growth and Carcass Characteristics
Abstract

The objective of this study was to determine if consumption of endophyte infected fescue with high levels of ergot alkaloids, during late gestation results in intrauterine growth restriction (IUGR) and impaired postnatal growth and development. Additionally creep feed supplementation was evaluated as a mitigation strategy for decreased calf growth and development and to determine if feedlot performance is affected by in utero exposure to fescue and creep feeding. Seventeen (n = 5 or 6 per treatment) Angus cross cows were divided into 3 treatments with the supplemented and unsupplemented cows placed on high endophyte infected pastures and the control group placed on low endophyte infected pastures for an average of 51 ± 4 days prior to calving. Post-calving all cow-calf pairs were moved to the same pastures and remained together until weaning, at which time all steers remained on the same pastures until the feedlot phase. There was a treatment × time interaction for calf body weights (P < 0.01). Although BW of calves at birth was similar, weights of calves born to cows exposed to high endophyte infected pastures were lower prior to creep feeding. Calves were weaned at an average of 219 ± 4 days of age, following 60 days of creep feed supplementation at which time the supplemented calves had increased body weights, and maintained that advantage until entry into the feedlot. Plasma amino acid concentrations of cows post calving showed no differences. Plasma amino acid concentrations of calves pre- and post- supplementation were different for isoleucine (P < 0.05), lysine (P < 0.05), leucine (P < 0.05), phenylalanine (P < 0.05), proline (P < 0.01), and glutamate (P < 0.01). Days on feed, marbling score, and dressing percentage were lower (P < 0.05) in the supplemented group compared to the other two groups. Backfat thickness, KPH, Rib-eye are (REA), HCW were not different among treatment groups. Exposure to endophyte infected pastures during late gestation did not result in intrauterine growth.
restriction, however creep feed supplementation did improve performance of calves exposed to high endophyte fescue during gestation. Some parameters of feedlot performance were affected, by the combination of in utero exposure to high endophyte fescue and creep feeding.
**Introduction**

Tall Fescue (*Festuca arundinacea*) is a prevalent cool season grass in the United States, and is estimated to be grown on at least 35 million acres (Ball et al., 1993). A natural symbiotic relationship between Tall Fescue and an endophytic fungi (*Epichloë coenophiala*) allows for increased persistence during drought and heat conditions observed in the southern United States (Bouton et al., 1993). While benefiting the plant, the fungal endophyte produces ergot alkaloids, that when consumed by livestock results in a condition known as fescue toxicosis (Hemken et al., 1979; Lyons et al., 1986). Ergot alkaloids are a group of chemical compounds that contain a tetracyclic ergoline ring, making them structurally similar to the biogenic amines serotonin, dopamine, norepinephrine, and epinephrine (Berde, 1980; Weber, 1980). These structural similarities allow ergot alkaloids to bind to biogenic amine receptors resulting in symptoms of fescue toxicity (Strickland et al., 2011). Symptoms of fescue toxicity include vasoconstriction, decreased growth and reproductive performance, and decreased circulating prolactin (Thompson et al., 1987; Jones et al., 2003; Parish et al., 2003b; Klotz et al., 2007).

Minute amounts ($1 \times 10^{-8}$ M - $1 \times 10^{-7}$ M) of ergot alkaloid consumption can result in vasoconstriction of bovine blood vessels (Oliver et al., 1993; Klotz et al., 2007). In animals with fescue toxicity, vasoconstriction reduces blood flow to both peripheral and core body tissues and organs including the uterus and placenta via the uterine and umbilical cord arteries, which can result in decreased blood flow to the fetus (Rhodes et al., 1991; Poole et al., 2016). Adequate utero placental blood flow is essential for normal fetal growth, and reduced blood flow to the fetus results in intrauterine growth restriction (IUGR; (Rigano et al., 2001; Reynolds et al., 2006).
In IUGR, the development of organs and tissues critical for survival including the brain and heart are spared, while the growth and development of less important organs and tissues is limited (Hales and Barker, 2013), especially skeletal muscle (Bauman et al., 1982). It is well established that skeletal muscle development during the fetal period, determines postnatal growth potential since the number of muscle fibers is set at birth (Greenwood et al., 2000; Nissen et al., 2003). Not only does IUGR impede muscle growth but it could also reduce fat deposition. Muscle cells and adipocytes (which develop into intramuscular fat) originate from the same mesenchymal stem cells (Caplan and Bruder, 2001). When fetal nutrition is limited as a result of poor maternal nutrition, a limited number of mesenchymal cells develop thus limiting the number of cells that can differentiate into intramuscular adipocytes subsequently reducing the ability of the animal to accumulate intramuscular fat (Du et al., 2010a).

Prolactin a protein hormone, is known for the role it plays in lactogenesis (Tanaka et al., 1980; Mann and Bridges, 2001), and is also involved in over 300 other processes throughout the body (Bole-Feysot et al., 1998). Reductions in prolactin as a result of consuming endophyte infected fescue results in decreased milk production, leading to decreased calf weaning weights (Thompson et al., 1987; Peters et al., 1992a). This could lead to decreased returns for cow-calf producers raising cattle on endophyte infected fescue and marketing their calves at weaning.

As a method to increase calf weaning weights, creep feeding can be used to supply additional nutrients to the calf prior to weaning (Tarr et al., 1994). Creep fed calves have increased average daily gains compared to non-creep fed calves (Loy et al., 2002). While creep feeding has been evaluated as a means to increase weaning weights of spring born calves raised on tall fescue (Tarr et al., 1994), the use of creep feeding to increase the performance of calves exposed to ergot alkaloids in utero as not been evaluated.
The objectives of this study were to: 1) determine if consuming high or low levels of ergot alkaloids in tall fescue during late gestation would result in IUGR, and impaired postnatal calf growth and development and 2) Evaluate creep feeding prior to weaning as a mitigation strategy for impaired calf growth due to IUGR, and 3) to determine whether consuming fescue during gestation and creep feeding would affect feedlot performance.

**Materials and Methods**

*Animals and Diets.* The experimental protocol was approved by the Institutional Animal Care and Use Committee at Virginia Polytechnic Institute and State University. Seventeen fall calving gestating beef cows were divided into three groups: control ($n=6$), supplemented ($n=5$) and unsupplemented ($n=5$). The control group grazed low endophyte fescue pastures (ergot alkaloid concentrations of 0-41 ppb), while the supplemented and unsupplemented treatment groups grazed high endophyte infected pastures. Pastures were sampled to determine the concentrations of ergot alkaloids in the low endophyte pastures (Agrinostics LTD. CO. Watkinsville, GA).

All cows were placed on treatment pastures 30-75 days prior to calving. After the final calf was born, cow/calf pairs were placed on the same pastures for the remainder of the study. All calves used in this study were bull calves and castrated at birth. The supplementation period began 60 days prior to weaning, and calves were weaned at an average age of $219 \pm 3.52$ days of age. The steers entered the feedlot period at an average of $411 \pm 3.52$ days of age. All calves were maintained in the feedlot until meeting a subcutaneous fat thickness of 1.0 cm measured between the 12th and 13th ribs by ultrasonography using an Aloka 500V real-time ultrasound machine with a 17.2 cm, 3.5-MHz linear transducer (Hitachi Aloka Medical America, Inc.,
Wallingford, CT 06492) as previously described (Brethour, 1992). In this study the animals’ hair was clipped prior to performing the ultrasound. Calves were weighed at birth, pre-supplementation, post supplementation (weaning), prior to feedlot entry, and at harvest.

**Blood Samples and Analysis.** Blood samples were collected by jugular venipuncture all cows after all cows had calved, and from calves at birth, pre-supplementation, and post supplementation. A 10 mL of blood was collected in heparinized blood collection tubes (Covidien, Monoject, Minneapolis, MN). Plasma was separated by centrifugation at 3,500 × RPM for 10 min at 4°C. Plasma was stored at -20°C until used. Plasma was analyzed for amino acid concentrations by isotope dilution gas chromatography-mass spectrometry as previously described (Calder et al., 1999).

**Creep Feeding.** A grain based creep feed (Table 1) was supplied ad libitum to calves in the supplemented treatment group for 60 days prior to weaning. Ration was formulated to meet NRC requirements for growing steer calves (NRC, 1999)

**Feedlot Management.** Steers were moved to the Shenandoah Valley Agricultural Research and Extension Center for the feedlot trial. Steers were housed in the dry lot facility, and trained to use a calan gate system (American Calan, Northwood, New Hampshire). Feed intake was measured from all steers. The steers received a total mixed ration (TMR) consisting of 78% concentrate pellet (Table 2) and 22% corn silage.
**Carcass Characteristics.** Carcass characteristics were recorded after harvest at the Virginia Tech Meat Center. Hot carcass weight (HCW) was recorded immediately following harvest. Carcasses were then chilled for 24-hours before recording backfat thickness, rib eye area (REA), percentage of kidney, pelvic and heart fat (KPH), and marbling scores. Marbling scores were assigned a numerical value (Wertz et al., 2002). Dressing percentages were calculated using the pre-harvest live weight and hot carcass weight from all steers. All carcasses were evaluated by a trained panel of three people, and the average of those results was used for analysis.

**Data Analysis:** All data was analyzed using the PROC MIXED procedure in SAS (Version 9.4, SAS Inst. Inc., Cary, NC). Calf body weights and plasma amino acid concentrations were analyzed using repeated measures. The statistical model included treatment group and time point as fixed effects. When a significant effect was determined by ANOVA, means were compared using Tukey’s post hoc test. All results were considered significant at the $P < 0.05$.

**Results**

**Calf growth and performance.** There was a significant treatment × time interaction for calf body weight ($P < 0.01$). Birth weights were similar between treatments. At pre-supplementation calves born to cows exposed to high endophyte fescue weighed on average 21.46 kg less than calves from cows exposed to low endophyte fescue during gestation. At the end of the supplementation period, at weaning, the calves in the supplemented treatment group had heavier body weights ($252 \pm 14.40$ kg) than calves in the control ($229 \pm 14.40$ kg) or unsupplemented groups ($213 \pm 18.43$ kg). Yet all calves had similar body weights upon entering the feedlot and at harvest (Figure 1).
**Plasma Amino Acids.** Plasma amino acids were determined from blood samples taken from cows right after calving. There were no differences ($P \geq 0.15$) between treatment groups for all amino acids that were analyzed (Figure 2). In calves isoleucine had a significant treatment × time interaction ($P < 0.01$), at weaning the supplemented group was higher than the control and unsupplemented groups (Supplemented 172.7 µmol/L, Unsupplemented 135.9 µmol/L, Control 113.7 µmol/L) (Figure 3). Lysine had a treatment × time interaction ($P < 0.05$). The supplemented group at weaning was significantly higher than the control group, and unsupplemented group (supplemented 164.6 µmol/L, unsupplemented 137.2 µmol/L, control 110.2 µmol/L) (Figure 4). Leucine had a significant treatment × time interaction ($P < 0.05$) with the supplemented group being significantly higher than the control and slightly higher than the unsupplemented groups at weaning (supplemented 245.5 µmol/L, unsupplemented 193.0 µmol/L, control 171.8 µmol/L) (Figure 5). Phenylalanine concentrations were higher ($P < 0.05$) in the unsupplemented group (91.3 µmol/L) compared to the supplemented and control groups (supplemented 84.6 µmol/L, control 71.4 µmol/L) (Figure 6). Concentrations of proline were increased ($P < 0.05$) pre-supplementation than at weaning (pre-supplementation 153.3 µmol/L, weaning 128.1 µmol/L) (Figure 7). Concentrations of glutamate were increased ($P < 0.05$) at pre-supplementation, compared to weaning (pre- Supplementation 200.8 µmol/L, weaning 171.9 µmol/L) (Figure 8). Concentrations of glutamine were increased ($P < 0.01$) at weaning compared to pre-supplementation (pre-Supplementation 195.9 µmol/L, weaning 279.8 µmol/L) (Figure 9) No differences were observed between treatment groups for valine (Figure 10), threonine (Figure 11), methionine (Figure 12), histidine (Figure 13), alanine (Figure 14), glycine (Figure 15), serine (Figure 16), aspartate (Figure 17), and tyrosine (Figure 18).
Feedlot Performance and Carcass Characteristics. The supplemented steers needed fewer days on feed ($P < 0.05$) to reach the same back fat thickness when compared to the other two treatment groups (Supplemented 114, unsupplemented 143, control, 150) The control group had increased DMI compared to the unsupplemented group. There were no differences for average daily gain, and G: F (Table 3-3).

Marbling Scores were also different between treatment groups with the supplemented group having significantly lower marbling scores ($P < 0.05$) than the other two treatment groups (Supplemented 1147.06, Unsupplemented 1143.67, Control 1225.32) (Figure 20). Dressing percentage was significantly decreased ($P < 0.05$) in the supplemented group when compared to the control group (Supplemented 59.22, Unsupplemented 60.95, Control 61.75). Since all steers were kept on feed until reaching a backfat thickness of approximately 1.0 cm, no differences ($P \geq 0.40$) were seen between treatment groups when carcass backfat thickness was measured. Additionally there were no differences ($P \geq 0.77$) across treatment groups for HCW, REA and KPH (Table 3-3).

Discussion

The negative effects of fescue toxicity on cow-calf performance has been previously documented (Stricker et al., 1979; Peters et al., 1992a; Watson et al., 2004a), however these studies did not evaluate the effects of pre-partum ergot alkaloid consumption on postnatal calf growth and development. Thus it was unclear whether exposure to fescue toxicity in utero could have long lasting effects on beef cattle raised to market. In this study, pregnant cows were allowed to graze either infected or non-infected tall fescue during late gestation (30-75 days prior to calving), in order to determine if fescue results in IUGR, and in order evaluate if creep feeding
can be utilized to mitigate impaired growth due to fescue consumption during gestation. Lastly this study evaluated feedlot performance of steers exposed to fescue in utero and creep feed before weaning.

Although we expected calves born to cows consuming endophyte infected fescue pastures to weigh less than those consuming non-infected fescue, calves weights were similar across all groups at birth. The majority of bovine fetal growth occurs after day 150 of gestation (Evans and Sack, 1973), thus the lack of differences in calf birth weights may have resulted from the duration of time (30-75 days) that the cows were exposed to the treatment pastures prior to calving. This length of time may not be a long enough time to restrict fetal growth. In other studies that show a reduction in fetal growth in sheep and cattle due to fescue consumption, dams consumed their treatment diets for the entire length of gestation (Watson et al., 2004a; Duckett et al., 2015).

As a result of increased fetal growth, pregnancy imposes a strain on maternal nutrition, however maternal nutritional repartitioning of body reserves allows for the continued growth of the fetus (Bauman and Currie, 1980). Another possible reason for the lack of difference in birth weights in this study, was that maternal nutrition supply remained adequate, enough to sustain normal fetal growth, regardless of ergot alkaloid consumption. This is supported by the similar amino acid concentrations seen in cow plasma in this study which would suggest that all cows were receiving adequate nutrition.

It had been shown that cows grazing endophyte infected fescue not only lose more weight but also produce lighter calves at weaning than cows grazing endophyte free pastures (Paterson et al., 1995). In previous studies calves from endophyte infected pastures grow at a rate of 0.73 kg/day compared to 0.91 kg/day for calves grazing endophyte free grass, and as a result have
decreased weaning weights (228 vs 196 kg; Peters et al., 1992a; Paterson et al., 1995). In this study, calves whose dams consumed high concentrations of ergot alkaloids during gestation had only numerically lower body weights pre-supplementation. The lack of differences may suggest that the calves were consuming adequate nutrition from their dams. Though a decrease in calf growth and performance may be due to decreased milk production as a result of ergot alkaloid consumption (Brown et al., 1993), cows in this study likely were able to produce an adequate milk supply for the growing calves.

The lack of decreases in plasma concentrations of the essential amino acids suggests that while the calves were growing, they were maintained on an adequate level of nutrition from the combination of milk and grazing, as well as creep feed supplementation for the supplemented treatment group. Our results were similar to another study that found that steers with the same feed intake, also have consistent plasma amino acid concentrations (Davenport et al., 1990).

As expected the supplemented treatment group had higher body weights post supplementation (at weaning) than the other treatment groups. These results are similar to other studies that reported increased weaning weights in calves given creep feed supplementation (Tarr et al., 1994; Loy et al., 2002). The increased body weights of supplemented calves at weaning likely is a direct result of the creep feed supplement provided to these calves. The supplemented calves were able to maintain their slight advantage in growth performance upon entering the feedlot, as shown by the numerically higher pre-feedlot body weight. During the feedlot trial, the supplemented calves spent fewer days on feed before reaching the terminal end point of 1 cm of backfat. Our findings are supported by another study, where calves receiving creep feed supplementation spent fewer days in the feedlot (Myers et al., 1999). If the supplemented calves entering the feedlot at a higher body weight also had an increased backfat thickness, this would
explain their ability to reach the terminal endpoint for this study sooner than the other treatment groups. These findings are supported by other results that show steers with more backfat upon feedlot entry spent fewer days on feed (Brethour, 2000).

Since these steers were fed to the same terminal endpoint based on back fat thickness, it is no surprise that there were no differences in back fat thickness between treatments. The similarities in ribeye area, KPH, and HCW maybe a result of feeding the steers to a similar terminal endpoint instead of to a specific number of days on feed. Due to the similarities in live harvest weight and HCW, it is was not expected that dressing percentage would be statistically significant, but this may be due in part to the low standard error in the dressing percentage calculations.

The supplemented treatment group had decreased marbling scores, that was likely due to the decreased number of days on feed, other studies show similar findings with similarities in days on feed and marbling score (Horn et al., 1995; Stalker et al., 2006). Since there were no differences in birthweight, it is not likely that the reduction in marbling score is a result of decreased intramuscular adipocytes that is sometimes seen in intrauterine restricted calves (Du et al., 2010a).

Further studies are necessary to determine if ingesting high endophyte fescue during late gestation results in IUGR calves, or to determine if the cow is able compensates to produce a normal birthweight calf. It would be helpful to study ergot alkaloid ingestion for varying lengths of gestation to determine how much, if any amount of prolonged ergot alkaloid exposure will result in intrauterine restricted calves. Placing cows on treatment pastures earlier in gestation would help to provide a more real world setting, as producers do not often have the means to choose between high or low endophyte pastures.
Although this study did not show that consuming endophyte infected fescue during late gestation would result in intrauterine restricted calves, it did show that providing creep-feed supplementation maybe able to benefit the producer that is raising calves on endophyte infected fescue. The benefit of creep feeding have been well studied (Tarr et al., 1994; Loy et al., 2002), and the results from this study support the findings that providing additional nutrition to the growing calf can help to improve growth performance. In order to better evaluate the effects of exposure to fescue in utero and creep feeding on feedlot performance, it would be necessary to evaluate feedlot performance when animals are kept on feed for the same amount of time. This would allow for determination if differences in marbling score are a result of decreased days on feed, in utero exposure to fescue, or creep feeding.
References


Nissen, P., V. Danielsen, P. Jorgensen, and N. Oksbjerg. 2003. Increased maternal nutrition of sows has no beneficial effects on muscle fiber number or postnatal growth and has no impact on the meat quality of the offspring. J Anim Sci 81: 3018-3027.


Table 3-1: Creep feed formulation and nutrient composition

<table>
<thead>
<tr>
<th>Item</th>
<th>%DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingredient</td>
<td></td>
</tr>
<tr>
<td>Soybean hull</td>
<td>40.00</td>
</tr>
<tr>
<td>Whole kernel corn</td>
<td>20.50</td>
</tr>
<tr>
<td>Soybean meal-44</td>
<td>15.00</td>
</tr>
<tr>
<td>Cottonseed meal</td>
<td>15.00</td>
</tr>
<tr>
<td>Cane molasses</td>
<td>5.00</td>
</tr>
<tr>
<td>Cottonseed hulls</td>
<td>4.50</td>
</tr>
</tbody>
</table>

Nutrient composition

<table>
<thead>
<tr>
<th>NE&lt;sub&gt;m&lt;/sub&gt; kcal/Kg</th>
<th>1,683</th>
</tr>
</thead>
<tbody>
<tr>
<td>NE&lt;sub&gt;g&lt;/sub&gt; kcal/Kg</td>
<td>1,086</td>
</tr>
<tr>
<td>Fat</td>
<td>2.21</td>
</tr>
<tr>
<td>CP</td>
<td>21.31</td>
</tr>
</tbody>
</table>

<sup>1</sup>Creep feed was provided to the supplemented treatment group for 60 days prior to weaning.
Table 3-2: Concentrate pellet formulation for feedlot diet fed to steers during the feedlot trial.

<table>
<thead>
<tr>
<th>Item</th>
<th>%DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingredient&lt;sup&gt;1,2&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Ground corn</td>
<td>60.78</td>
</tr>
<tr>
<td>Dried corn gluten feed</td>
<td>20.26</td>
</tr>
<tr>
<td>Wheat middlings</td>
<td>14.96</td>
</tr>
<tr>
<td>Limestone</td>
<td>2.59</td>
</tr>
</tbody>
</table>

<sup>3,4</sup>Diet Composition

<table>
<thead>
<tr>
<th>Item</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NE&lt;sub&gt;g&lt;/sub&gt; kcal/kg</td>
<td>0.626</td>
</tr>
<tr>
<td>CP</td>
<td>13.04</td>
</tr>
<tr>
<td>EE</td>
<td>3.81</td>
</tr>
<tr>
<td>NDF</td>
<td>20.84</td>
</tr>
<tr>
<td>ADF</td>
<td>8.47</td>
</tr>
</tbody>
</table>

<sup>1</sup>Ingredients in concentrate pellet

<sup>2</sup>Vitamins, mineral, and feed additive inclusion (%DM) in concentrate pellet: 1.00% Sodium bicarbonate, 0.25% Sodium chloride, 0.125% Zinpro Availa4, 0.017% Rumensin 90, 0.004% Vitamin E premix (20,000 IU/g), 0.001% Vitamin D premix (30,000 IU/g), 0.001% Ethylenediaminodihydroiodide

<sup>3</sup>Nutrient composition of total mixed ration (TMR) fed to steers during the feedlot trial (TMR= 78% concentrate pellet, 22% corn silage).

<sup>4</sup>TMR Vitamin and mineral concentrations: 0.6% Ca, 0.5% P, 0.2% S, 6,855 IU/kg Vitamin A, 308 IU/kg Vitamin D, 739 IU/kg Vitamin E

<sup>5</sup>Back calculated based on performance data using the iterative formula in NRC (1996)
Table 3-3: Influence of dam exposure to high or low endophyte infected pastures during late gestation on progeny feedlot performance and carcass characteristics.

<table>
<thead>
<tr>
<th>Item</th>
<th>1,2Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Con</td>
</tr>
<tr>
<td><strong>Feedlot Performance</strong></td>
<td></td>
</tr>
<tr>
<td>Initial weight, kg</td>
<td>359 ± 13.1</td>
</tr>
<tr>
<td>Final live weight, kg</td>
<td>584 ± 10.2</td>
</tr>
<tr>
<td>Days on feed</td>
<td>144 ± 7.6&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>ADG, kg/d</td>
<td>1.3 ± 0.10</td>
</tr>
<tr>
<td>DMI, kg/d</td>
<td>14.1 ± 0.24&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>G:F</td>
<td>0.04 ± 0.003</td>
</tr>
<tr>
<td><strong>Carcass Characteristics</strong></td>
<td></td>
</tr>
<tr>
<td>HCW</td>
<td>359 ± 8.5</td>
</tr>
<tr>
<td>Dressing Percentage</td>
<td>61.7 ± 0.55&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Backfat thickness, cm</td>
<td>1.2 ± 0.09</td>
</tr>
<tr>
<td>KPH, %</td>
<td>2.7 ± 0.25</td>
</tr>
<tr>
<td>Rib eye area, cm&lt;sup&gt;2&lt;/sup&gt;</td>
<td>34.8 ± 1.57</td>
</tr>
<tr>
<td>Marbling score</td>
<td>1225 ± 29.6</td>
</tr>
</tbody>
</table>

1Treatments: Control, Supplemented (Supp), Unsupplemented (Unsupp)
2All results are reported as mean ± SEM, values with differing superscripts are significantly different at \( P < 0.05 \)
3Weight at beginning of feedlot trial
4Dressing percentage was calculated using final live weight and HCW
5Slight=900, Small=1,000, Modest= 1100, Moderate= 1200, Slightly abundant= 1300, Moderately abundant= 1400, Abundant= 1500.
Figure 3-1: Calf BW in the control, supplemented (supp), unsupplemented (unsupp) groups, at birth, pre-supplementation, post-supplementation, pre-feedlot, and harvest. Values represent mean ± SEM.
Figure 3-2: Essential amino acid concentrations in the plasma of cows in the control, supplemented (supp), unsupplemented (unsupp) groups post calving. Values represent mean ± SEM.
Figure 3-3: Plasma concentrations for isoleucine of calves in the control, supplemented (supp), unsupplemented (unsupp) groups. Values represent mean ± SEM
Figure 3-4: Plasma concentrations for lysine of calves in the control, supplemented (supp), unsupplemented (unsupp) groups. Values represent mean ± SEM.
Figure 3-5: Plasma concentrations for phenylalanine of calves in the control, supplemented (supp), unsupplemented (unsupp) groups. Values represent mean ± SEM.
Figure 3-6: Plasma concentrations for proline of calves in the control, supplemented (supp), unsupplemented (unsupp) groups. Values represent mean ± SEM.
Figure 3-7: Plasma concentrations for glutamate of calves in the control, supplemented (supp), unsupplemented (unsupp) groups. Values represent mean ± SEM.
Figure 3-8: Plasma concentrations for glutamine of calves in the control, supplemented (supp), unsupplemented (unsupp) groups. Values represent mean ± SEM.
Figure 3-9: Plasma concentrations for valine of calves in the control, supplemented (supp), unsupplemented (unsupp) groups. Values represent mean ± SEM.
Figure 3-10: Plasma concentrations for threonine of calves in the control, supplemented (supp), unsupplemented (unsupp) groups. Values represent mean ± SEM.
Figure 3-11: Plasma concentrations methionine of calves in the control, supplemented (supp), unsupplemented (unsupp) groups. Values represent mean ± SEM.
Figure 3-12: Plasma concentrations histidine of calves in the control, supplemented (supp), unsupplemented (unsupp) groups. Values represent mean ± SEM.
Figure 3-13: Plasma concentrations for alanine of calves in the control, supplemented (supp), unsupplemented (unsupp) groups. Values represent mean ± SEM.
Figure 3-14: Plasma concentrations for glycine of calves in the control, supplemented (supp), unsupplemented (unsupp) groups. Values represent mean ± SEM.
Figure 3-15: Plasma concentrations for serine of calves in the control, supplemented (supp), unsupplemented (unsupp) groups. Values represent mean ± SEM.
Figure 3-16: Plasma concentrations for aspartate of calves in the control, supplemented (supp), unsupplemented (unsupp) groups. Values represent mean ± SEM.
Figure 3-17: Plasma concentrations for tyrosine of calves in the control, supplemented (supp), unsupplemented (unsupp) groups. Values represent mean ± SEM.
Chapter 4. Grazing Tall Fescue During mid to Late Gestation, Evaluation of Deficits in Calf Growth Performance
Abstract

The objective of this study was to determine if ergot alkaloid consumption during mid to late gestation by cows would result in intrauterine restricted (IUGR) calves. Cows \( n = 36 \) were placed on treatment pastures containing either high ergot alkaloid concentrations (average 552 ppb) or low ergot alkaloid concentrations (average 72 ppb). The use of creep feed supplementation for 60 days prior to weaning was also evaluated as a means to mitigate deficiencies in calf growth due to in utero exposure to ergot alkaloids. Body condition score (BCS) of the cow was recorded prior to the beginning of the study, pre-calving, pre-weaning, and at weaning. BCS increased during the grazing trial, decreased from calving to pre-weaning, and increased \( (P < 0.05) \) at weaning for both treatments. Birth weights of the calves did not differ \( (P < 0.05) \) between groups at birth or pre-supplementation, but were increased at weaning in the supplemented group. Average daily gain (ADG) of the calves did not differ from birth to pre-weaning, but was increased \( (P < 0.01) \) in the supplemented calves from pre-supplementation to weaning. Plasma amino acid concentrations were measured to determine differences in feed intake. There were no differences between treatments for the cows’ plasma concentrations prior to the beginning of the trial and pre-calving. In calves, plasma concentrations of valine, leucine, isoleucine \( (P < 0.01) \), and Tyrosine \( (P < 0.05) \) were increased in the supplemented treatment post supplementation at weaning.
Introduction

Tall fescue (*Festuca arundinacea*) is a widely grown cool-season grass found in the eastern United States, and serves as a food source for 8.5 million head of cattle (Hoveland, 1993b). Tall fescue contains a fungal endophyte (*Epichloë coenophiala*) which provides the plant with the ability to better tolerate both abiotic and biotic stress (Arachevaleta et al., 1989; Latch, 1993). The fungal endophyte found in tall fescue produces ergot alkaloids, which when consumed by livestock species results in a syndrome known as fescue toxicity (Oliver et al., 2000; Franzluebbers and Hill, 2005). Symptoms of fescue toxicity include decreased circulating prolactin, reduced growth performance, and vasoconstriction of both peripheral and central blood vessels (Schmidt and Osborn, 1993; Klotz et al., 2007; Egert et al., 2014). The ability for ergot alkaloid consumption to result in the symptoms of fescue toxicity is due to similarities in chemical structure between ergot alkaloids and biogenic amines (dopamine, epinephrine, norepinephrine, and serotonin), which allows ergot alkaloids to bind to biogenic amine receptors (Weber, 1980; Strickland et al., 2011).

Vasoconstriction as a result of fescue toxicity results in decreased utero placental blood flow in the bovine uterine and umbilical cord arteries, which can result in decreased blood flow to the fetus (Poole et al., 2016). A reduction in utero placental blood flow could reduce fetal growth (Lang et al., 2003) and consequently results in intra uterine growth restriction (IUGR) (Resnik, 2002). In addition, decreased maternal feed intake can lead to IUGR (Lesage et al., 2002). In ruminant species, IUGR alters postnatal growth rates, and in some cases decreased carcass quality (Ford et al., 2007; Long et al., 2010).

In mammals including ruminants the majority of fetal growth occurs during the last two-thirds of gestation (Evans and Sack, 1973). During this period of high growth, exposure to ergot
alkaloids could reduce fetal growth. For example, sheep consuming endophyte infected fescue seeds during mid to late gestation decreases fetal growth (Duckett et al., 2014). Similarly, the exposure to ergot alkaloids during gestation has also been shown to reduce birthweight in spring born calves (Watson et al., 2004b).

One possible mitigation strategy for the reduction in growth seen in calves as a result of fescue toxicity is creep feeding. Creep feeding serves as a way to supply additional nutrients to the calf prior to weaning, and results in an increased weaning weight (Tarr et al., 1994). The objective of this study was to determine if consumption of high or low ergot alkaloid producing fescue during gestation would result in IUGR offspring in a fall calving production system. Additionally this study evaluated the use of creep feeding to mitigate any effects of in utero fescue consumption on the calf.

**Materials and Methods**

*Animals and Diets.* The experimental protocols in this study were approved by the Institutional Care and Use Committee at Virginia Polytechnic Institute and State University. In this study 35 multi parous Angus cross cows were randomly assigned to two treatment diets ($n = 24$ high endophyte, $n = 12$ low endophyte). All cows were bred by artificial insemination (AI) on the same day, and cows were placed on their treatment pastures at day 170 of gestation. Pastures used in this study were located at the Middleburg Agricultural Research and Extension Center (MAREC). Two weeks prior to the expected calving date, cows were transported from the experiment station to the Virginia Tech Beef Cattle Center in Blacksburg, VA. Upon arrival to Blacksburg, cows remained on either high or low endophyte fescue pastures until after calving.
Following calving, all cow-calf pairs were moved to the same tall fescue pastures at the Virginia Tech Kentland Farm. All cow-calf pairs remained on the same pastures for the remainder of the study. Sixty days prior to weaning the high endophyte cow-calf pairs were divided into two treatment groups (supplemented $n = 12$ and un-supplemented $n = 12$). The cow-calf pairs were blocked by calf weight and sex of the calf. Calves in the supplemented group received a creep feed supplementation for 60 days until weaning.

**Ergot Alkaloid Concentrations.** Forage samples were analyzed for ergot alkaloid concentration using commercial services (Agrinostics Ltd. Watkinsville, GA). Samples were collected from pastures at both farms used in this trial, with pastures from MAREC being samples in mid-April and pastures at the beef center being measured in mid-August. The cows grazing high endophyte fescue were exposed to pastures with an average ergot alkaloid concentration of 551 ppb. The cows grazing low endophyte infected pastures were exposed to ergot alkaloid concentrations of 72 ppb.

**Body Condition Scores and Weights.** Body condition scores were recorded for cows at day 170 of gestation and before calving. Additionally body condition scores of cows were taken prior to the beginning of the calf supplementation period and at weaning. Body weights for the calves were recorded at birth, pre-supplementation, and weaning using a Tru-Test XR50001 scale (Tru-Test Group., Auckland, New Zealand.)

**Blood Collection and Analysis.** Blood samples were collected via jugular venipuncture from all cows at day 170 of gestation and prior to calving and from the calves within 12 hours after birth,
at pre-supplementation, and at weaning. Blood samples were collected via jugular venipuncture in EDTA tubes (Covidien, Monoject., Minneapolis, MN). Plasma was separated by centrifugation at 1200 x G for 10 min at 4°C. Plasma was stores at -20°C until analysis. The plasma samples were analyzed for plasma amino acid concentrations by gas chromatography - mass spectrometry (Calder et al., 1999).

**Creep feed supplementation.** A grain creep feed (Table 1) was supplied ad libitum to calves in the supplemented treatment group for 60 days prior to weaning. The ration was formulated to meet NRC requirements for growing steer and heifer calves (NRC, 1999).

**Weather Data.** Temperature data were collected from the beginning of the experiment until after calving using United States Department of Agriculture, Natural Resources Conservation Service, Soil Climate Analysis Network (SCAN) sites. The sites chosen were based on their elevation, and geographical proximity to the grazing sites in this study. For weather data at the MAREC the North Piedmont AREC SCAN site was chosen. The distance between the two locations is approximately 100 kilometers and the change in elevation is approximately 12 meters. For weather data at the Virginia Tech Beef Center in Blacksburg, VA the Shenandoah SCAN site was chosen. The distance between the two locations is approximately 160 kilometers and the difference in elevation is approximately 96 meters.

**Statistical Analysis.** All data was analyzed using the PROC MIXED procedure in SAS (Version 9.4, SAS Inst. Inc., Cary, NC). All results are shown as mean ± SEM. Weather data is shown as
the mean daily temperature. Results were considered significant at $P < 0.05$. Means were separated using Tuckey post-hoc test.

**Results**

*Cow Performance.* There was a significant treatment × time interaction ($P < 0.05$) for body condition scores (BCS). Scores for both treatment groups increased while on the treatment pastures from d170 of gestation until prior to calving. The high endophyte treatment had a mean BCS at the beginning of the grazing period of 5.08 and a mean BCS pre-calving of 5.62. The low endophyte treatment had a mean BCS at time beginning of the grazing period of 4.83, and a mean BCS of 6.08 pre-calving. Both Treatment groups had a decrease in body condition score from pre-calving to pre-weaning (60 days prior to weaning). The mean BCS for the high endophyte treatment cows pre-weaning was 4.58 while the low endophyte treatment cows had an average BCS of 4.42. Both treatment groups had increased BCS at the time of weaning with the high endophyte treatment have an average BCS of 5.57 and the low endophyte treatment having a BCS of 5.41 (figure 1).

Days Gestation was calculated for all cows, based on day of breeding and day of calving. There were no significant differences between treatment groups (Table 2).

*Calf Growth Performance.* Calf body weight had a treatment × time interaction ($P < 0.01$). Calf birth weight was not different across treatment groups (Control= 32.59 ± 8.80 kg, Supplemented= 33.01 ± 8.42 kg, Unsupplemented = 33.31 ± 8.42 kg). Calves were weighed prior to the beginning of the supplementation period which began 60 days prior to weaning. The pre-supplementation weights were significantly higher than at birth but there were no differences
among treatments (Control= 184.85 ± 8.99 kg, Supplemented= 172.07 ± 8.42 kg, Unsupplemented = 157.4 ± 8.59 kg). At weaning and post supplementation the supplemented group had significantly heavier body weights than the unsupplemented and control treatment groups (Control= 231.4 ± 8.79 kg, Supplemented= 283.16 ± 8.66 kg, Unsupplemented = 210.83 ± 8.42 kg) (Figure 2).

Average daily gains were calculated for the calves with the time from birth to pre-supplementation representing the first time period, and the time from pre-supplementation to weaning representing the second time period. The control calves had lower average daily gains during the second compared to the first period (\(P < 0.01\)) (Control time 1 = 1.0164 ± 0.07763 kg/day vs. time 2 = 0.8078 ± 0.07763 kg/day). The unsupplemented calves did not grow at significantly different rates during the two treatment periods (unsupplemented time 1= 0.9001 ± 0.07432 kg/day vs. unsupplemented time 2= 0.8495 ± 0.07432 kg/day). The supplemented treatment grew at a faster rate during the second time than during the first period (Supplemented time 1= 0.9477 ± 0.07432 kg/day vs. supplemented time 2= 1.7159 ± 0.7977 kg/day).

**Plasma Amino Acid Concentrations.** Plasma amino acid concentrations of the cows did not differ significantly between treatments when measured before placement on treatment pastures and prior to calving. Plasma concentrations of methionine and threonine (\(P < 0.05\)) were higher pre-calving than they were at the beginning of the grazing trial. Plasma concentrations of leucine, valine, and isoleucine were significantly higher (\(P < 0.05\)) for the supplemented calves post supplementation, at weaning.
Weather Data. The average daily temperature during the grazing trial was 22.1°C with the minimum average daily temperature of 13.8 which occurred on the very first day of the grazing trial. The highest average daily temperature was 28.6°C which occurred on day 34 and again on day 68 of the grazing trial.

Discussion

While studies have shown that fescue consumption during gestation results in decreased birth weights (Watson et al., 2004b; Duckett et al., 2014), it is not well established if in utero exposure to endophyte infected fescue alone, would result in decreased birth weights, with long lasting deficits in calf growth performance. In this study gestating cows were placed on either high or low endophyte fescue pastures during gestation, and post calving maintained on the same pastures. Thus any differences in calf growth performance after birth would be related to in utero-exposure to endophyte infected fescue. Additionally, creep feeding is used to increase weaning weights (Tarr et al., 1994), and in this study creep feeding was evaluated as a means to mitigate deficits in calf growth due to in utero exposure to endophyte infected fescue.

The pastures used in this study were analyzed for ergot alkaloid concentrations during different times of the year, with the first pastures being measured for ergot alkaloid concentration in April. The level of endophyte infection in tall fescue plants is not static, and the lowest rates of infection are found during winter and spring (Ju et al., 2006). Along with the rate of endophyte infection seasonal variations in the amount of ergot alkaloids produced by the endophyte exist as well, with the highest concentrations of alkaloids found in the summer months (Rottinghaus et al., 1991). This suggests that the fields that were sampled in mid-April, may have had higher concentrations during the experimental period which lasted from mid-May until late-August.
The minimum concentration needed to produce fescue toxicity symptoms in cattle ranges from 450-700 ppb (Tor-Agbidye et al., 2001). While the high endophyte fields that were sampled in April had minimum ergot alkaloid concentrations below the range needed to produce fescue toxicity, by the start of the experimental period these concentrations may have increased. The low fescue variety fields that were sampled in either April or August showed ergot alkaloid concentrations well below the concentration needed to induce fescue toxicity symptoms in cattle.

In this study a fall calving system was chosen due to the increased ergot alkaloid content present during the summer months, while the cows were in gestation. However fall calving herds have shown increases in birth weights and weaning weights when compared to spring calving herds grazing the same endophyte infected fescue pastures (Smith et al., 2012; Caldwell et al., 2013).

A common symptom of fescue toxicity is decreased feed intake (Hemken et al., 1981b; Aldrich et al., 1993a; Spiers et al., 2005). In addition, body condition score is known to correlate well with feed intake (Roche et al., 2006). Accordingly, body condition scoring was used as a surrogate measure for feed intake. Based on the increase in BCS of cows grazing both treatments, our data suggested that dry matter intake although lower in LE was still adequate for both treatment groups. Moreover, plasma essential amino acid concentrations is tightly correlated with feed intake (El-Kadi et al., 2008). Since no differences were observed in essential amino acid concentrations across treatments, large differences in feed intake were not likely.

In addition to decreased utero placental blood flow being a cause for decreased birth weight in livestock consuming ergot alkaloids, decreased birth weights may also be due to decreases in BCS (Porter and Thompson, 1992; Watson et al., 2004b). Endophyte infected fescue seed consumption reduces birth weights and decreases gestational length in sheep and cattle.
(Long et al., 2010; Duckett et al., 2014). In this study gestational length and birth weights did not differ between treatment groups, suggesting that feed intake was adequate and that gestational length was not adversely affected by ergot alkaloid consumption.

Animals consuming ergot alkaloids have a decreased ability to dissipate heat, especially when ambient temperatures are greater than 32°C (Hemken et al., 1981a; Sessler et al., 1990; Aldrich et al., 1993a). In this study the daily ambient temperature did not rise above 30°C. After calving, all of the cow-calf pairs were moved to the same pastures post calving, this was done so that any changes in calf performance for the remainder of the study would be due to events that occurred in utero. Since there were no differences in body weights and average daily gains between the different groups at pre-supplementation, it is not likely that ergot alkaloid consumption during gestation caused decreases in calf performance when heat stress is not an issue.

Calf performance was increased by creep feed supplementation as seen by the increased body weight and average daily gain of the supplementation treatment group when compared to the other treatment groups. The results of this study follow results of a study that showed creep feeding calves for 56 days prior to weaning increases the growth performance of calves consuming fescue (Tarr et al., 1994).

Calves in the supplemented group had increased plasma concentrations of valine, tyrosine, leucine, and isoleucine. The higher plasma concentrations of amino acids in the animals receiving supplementation would be expected since these animals were consuming more nutrients. Other studies have also shown changes in plasma amino acids based on nutrient intake (Kwon et al., 2004).
The results of this study suggest that ergot alkaloids exposure during mid to late gestation does not result in IUGR, as long as the cows have adequate body reserves. Cows with adequate body reserves can maintain sufficient supply of nutrients to the growing fetus in spite of possible minor reduction in feed intake. While differences in birth weight were not seen in this study, one possible explanation for the decreased weights prior to weaning of calves exposed to high endophyte fescue during gestation is fetal programming. Fetal Programming or the Barker hypothesis suggests that events that occur during prenatal life may result in altered growth and development later in life (Barker, 1995). In cattle changes in maternal nutrition can result decreased feedlot and carcass performance (Larson et al., 2009).

Not only exposure to ergot alkaloids may play a role in fescue toxicity symptoms, but also environmental temperature that may likely exacerbate those symptoms. In the current study, environmental temperatures were well below 30°C which is within the thermoneutral zone of the cows. Lastly, creep feeding of calves whose dam consumed ergot alkaloids during gestation have increased weaning weights, however no differences are seen in calf performance prior to the initiation of creep feeding.
Belesky, D. P., and C. P. West. 2009. Abiotic stresses and endophyte effects. Tall Fescue for the Twenty-First Century: 49-64.


Nissen, P., V. Danielsen, P. Jorgensen, and N. Oksbjerg. 2003. Increased maternal nutrition of sows has no beneficial effects on muscle fiber number or postnatal growth and has no impact on the meat quality of the offspring. J Anim Sci 81: 3018-3027.


Table 4-1: Creep feed formulation and nutrient composition

<table>
<thead>
<tr>
<th>Item</th>
<th>% DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soybean hull</td>
<td>40.00</td>
</tr>
<tr>
<td>Whole kernel corn</td>
<td>20.50</td>
</tr>
<tr>
<td>Soybean meal-44</td>
<td>15.00</td>
</tr>
<tr>
<td>Cottonseed meal</td>
<td>15.00</td>
</tr>
<tr>
<td>Cane molasses</td>
<td>5.00</td>
</tr>
<tr>
<td>Cottonseed hulls</td>
<td>4.50</td>
</tr>
</tbody>
</table>

Nutrient composition

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NE&lt;sub&gt;m&lt;/sub&gt; kcal/Kg</td>
<td>1,683</td>
</tr>
<tr>
<td>NE&lt;sub&gt;e&lt;/sub&gt; kcal/Kg</td>
<td>1,086</td>
</tr>
<tr>
<td>Fat</td>
<td>2.21</td>
</tr>
<tr>
<td>CP</td>
<td>21.31</td>
</tr>
</tbody>
</table>

<sup>1</sup>Creep feed was provided to the supplemented treatment group for 60 days prior to weaning.
Table 4-2: Days of gestation showed no significant differences ($P > 0.05$). Results are shown as mean ± SEM.

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HE</td>
</tr>
<tr>
<td>Days of Gestation</td>
<td>280.9 ± 1.49</td>
</tr>
</tbody>
</table>

¹Treatment: HE = high endophyte; LE = low endophyte.
Figure 4-1: Cow body condition score (BCS) of cows in the high endophyte (HE) and low endophyte (LE) groups. Values represent mean ± SEM.
Figure 4-2: Body weights of calves in the high control, supplemented (supp), and unsupplemented (unsupp) groups. Values represent mean ± SEM.
Figure 4-3: Average daily gains (ADG) of calves in the high control, supplemented (supp), and unsupplemented (unsupp) groups. Values represent mean ± SEM.
Figure 4-4: Plasma concentrations of valine for cows in the low endophyte (LE), and high endophyte (HE) groups. Values represent mean ± SEM.
Figure 4-5: Plasma concentrations of phenylalanine for cows in the low endophyte (LE), and high endophyte (HE) groups. Values represent mean ± SEM.
Figure 4-6: Plasma concentrations of threonine for cows in the low endophyte (LE), and high endophyte (HE) groups. Values represent mean ± SEM.
Figure 4-7: Plasma concentrations of tyrosine for cows in the low endophyte (LE), and high endophyte (HE) groups. Values represent mean ± SEM.
Figure 4-8: Plasma concentrations of histidine for cows in the low endophyte (LE), and high endophyte (HE) groups. Values represent mean ± SEM.
Figure 4-9: Plasma concentrations of leucine for cows in the low endophyte (LE), and high endophyte (HE) groups. Values represent mean ± SEM.
Figure 4-10: Plasma concentrations of Isoleucine for cows in the low endophyte (LE), and high endophyte (HE) groups. Values represent mean ± SEM.
Figure 4-11: Plasma concentrations of lysine for cows in the low endophyte (LE), and high endophyte (HE) groups. Values represent mean ± SEM.
Figure 4-12: Plasma concentrations for valine of calves in the control, supplemented (supp), unsupplemented (unsupp) groups. Values represent mean ± SEM.
Figure 4-13: Plasma concentrations for phenylalanine of calves in the control, supplemented (supp), unsupplemented (unsupp) groups. Values represent mean ± SEM.
Figure 4-14: Plasma concentrations for threonine of calves in the control, supplemented (supp), unsupplemented (unsupp) groups. Values represent mean ± SEM.
Figure 4-15: Plasma concentrations for tyroside of calves in the control, supplemented (supp), unsupplemented (unsupp) groups. Values represent mean ± SEM.
Figure 4-16: Plasma concentrations for histidine of calves in the control, supplemented (supp), unsupplemented (unsupp) groups. Values represent mean ± SEM.
Figure 4-17: Plasma concentrations for leucine of calves in the control, supplemented (supp), unsupplemented (unsupp) groups. Values represent mean ± SEM.
Figure 4-18: Plasma concentrations for isoleucine of calves in the control, supplemented (supp), unsupplemented (unsupp) groups. Values represent mean ± SEM.
Figure 4-19: Plasma concentrations for lysine of calves in the control, supplemented (supp), unsupplemented (unsupp) groups. Values represent mean ± SEM.
Figure 4-20: Ambient temperature during grazing trial of experiment. Values represent daily average temperature.
Overall Summary

Fescue toxicity results in production losses for cattle producers due to decreased animal performance. In spite of much work done in this area, it has not been well established whether in utero exposure to ergot alkaloids from fescue would affect postnatal calf growth. The aim of this research was to investigate the effect of ingesting endophyte infected fescue during gestation on calf growth and development. A secondary aim was to evaluate whether ingesting endophyte infected fescue seeds affects the thermoregulatory ability of the cow. With this research we were better able to study the role that fescue consumption and environmental temperature play in the ability of cows to maintain thermoregulation. Furthermore, we were able to evaluate if fescue consumption during gestation would result in IUGR, and the consequences this would have on calf growth and development have not been previously studied.

Thermoregulation was investigated in cows through the use of indwelling temperature probes. Ambient temperatures during both experiment one and two were not extreme, and did not exceed the cows’ thermoneutral zone. Thus body temperatures were not altered to a degree that would cause major physiological consequences. The results of this study further highlight the important role that ambient temperatures play in increasing core body temperatures during fescue toxicity. While disruption of thermoregulation has been previously reported in cows, it is important to highlight that these studies were conducted in geographical areas that have more extreme ambient temperatures and temperature humidity index values than those recorded in our studies.

The second study aimed at establishing whether pregnant cow exposure to ergot alkaloids infected fescue pastures during late gestation would result in low birth weights of the calves, and to assess creep feeding as a means to mitigate any effects of fescue toxicity on the calves. There
were no differences in birth weights among the treatment groups. In addition, the calves went through a feedlot period, and were evaluated at slaughter. While there were differences in marbling score, days on feed, and dressing percentage, these differences were likely not due to in utero exposure to ergot alkaloids and may be explained in part by the use of creep feed supplementation, and by the design of the experiment. Since all animals were kept on feed until reaching a similar backfat thickness, versus being fed for a set amount of time, days on feed was expected to be different. Additionally differences in marbling score can be associated with more or less time on feed, and since these animals were of similar genetic makeup, it is not likely that genetic differences alone would result in the significant differences in marbling score.

The short duration of cow exposure to ergot alkaloids during gestation in this study may have played a role in dampening the effects of that exposure to ergot alkaloids in fescue on calve birth weight and growth performance. Thus a second study was conducted where cows were placed on their treatment pastures at day 170 of gestation and remained until after calving. However even with this extended period of endophyte infected fescue consumption during gestation there were no differences in birthweight. This is likely due to the fact that cows used in this study were mature and in adequate body condition.

The results of these studies underline the complexity of fescue toxicity in cattle. There are some observations that need to be highlighted. The first is that the age of the dam may be inversely related to the severity of the symptoms. Previously published data suggest that heifers are more susceptible to fescue toxicity than more mature cows. This may relate to continued growth of the heifers during pregnancy which could limit nutrient availability for the conceptus. Thus cows may have the ability to mobilize body reserves to maintain a proper supply of nutrients to sustain adequate fetal growth. The second, is that severe environmental temperatures,
especially in hot climates, is a prerequisite for symptoms of fescue toxicity to develop and not
ergot alkaloid consumption alone.

One important point to note is that in both studies while no differences were seen at birth, calves that were exposed to high endophyte fescue had decreased weights compared to the low endophyte controls prior to weaning. While this in interesting observation, there was not enough evidence to conclude if this was due to decreased reduction in milk production of the cow or to other reasons that may have affected the calves in utero. Regardless, calf weaning weights were improved by creep feed supplementation in both studies, however these differences were lost as the animals got older.