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Evaluating Blood Perfusion of the Corpus Luteum in Beef Cows during Fescue Toxicosis

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Fescue toxicosis is a common problem in beef cattle grazing endophyte-infected (E+) tall fescue. Symptoms include decreases in feed intake, weight gain, and reproductive efficiency along with vasoconstriction. The mechanisms by which fescue toxicosis affects the bovine reproductive tract have yet to be discovered. The objective of this study was to determine if the onset of fescue toxicosis conditions would alter the blood perfusion observed in the CL and peripheral concentrations of progesterone in cattle. We hypothesized that during fescue toxicosis, the vasoconstrictive symptoms would lead to a reduction in CL blood perfusion thus decreasing peripheral concentrations of progesterone. Overall, fescue toxicosis was induced as cows fed an E+ treatment diet had greater rectal temperatures and reduced pulse pressure and mean arterial pressure measurements; however, total blood perfusion of the CL and peripheral concentrations of progesterone did not differ in cattle under fescue toxicosis compared to those without.

DEDICATION

I would like to dedicate this thesis to my family: my parents, Jeff and Colleen Chaffin, and my siblings, Logan and Ashley Cline and Maggie Chaffin. I cannot thank you enough for the love, support, guidance and encouragement that you provide to me on a daily basis. I would not have been able to do this without all of you behind me at every step. I love you all more than I could ever express! Thank you for everything!

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CHAPTER I

REVIEW OF LITERATURE

Tall Fescue

Tall fescue *(Schedonorus arundinaceus)* is a primary forage in the eastern United States, specifically in the "transition zone" between the temperate climate of the northeast and the subtropical climate of the southeast (Aiken and Strickland, 2013). Tall fescue is a cool-season (C-3), perennial forage that originated in Europe. It is considered an extremely hardy grass and is tolerant of acidic soils and areas prone to poor drainage or drought (Ball et al., 2007). The discovery of tall fescue in the United States occurred in Kentucky in the early 1900s. The first cultivar, Festuca arundinacea 'Kentucky 31' (KY31), was adapted and commercially introduced in 1943 (Buckner et al., 1977). Tall fescue, on an as-fed basis, has a crude protein content of approximately 12 to 15%, a dry matter digestibility content of 69 to 74% and total digestible nutrients range from 62 to 68%. These amounts depend on the season and stage of growth (Mississippi State University, 2014).

Ergotism/Fescue Toxicosis

In the late 1940s and early 1950s, cattle producers began reporting foot and leg problems in cattle grazing Kentucky 31 pastures (Cunningham, 1948). Researchers in New Zealand were the first to attribute the problems to a type of ergotism known commonly as “fescue foot” (Cunningham, 1948). Several studies conducted in the United States shortly after Cunningham’s discovery supported those results and authors attributed herd lameness to the fescue foot condition. Plants that did not have a seed head did not cause the same issues, ruling out a species of *Claviceps* being the culprit (Pratt and Haynes, 1950; Goodman, 1952; Merriman, 1955; Maag and Tobiska, 1956).

In 1956, scientists linked the problems in cattle to certain strains of ergot alkaloids (Maag and Tobiska, 1956). Focus shifted to determining the mechanism of manufacturing these ergot alkaloids to better understand how to remedy the problem. The seed head was infected with an endophyte, originally named *Epichloe typhina* and presently known as *Neotyphodium coenophialum*, which is the cause of the problematic ergot alkaloids (Bacon et al., 1977). Hoveland et al. (1983) fed tall fescue infected with either lesser concentrations of endophyte or greater concentrations of endophyte and confirmed these findings. Three different types of alkaloids were discovered: pyrrolizidines, peramines and ergot alkaloids (Siegel et al., 1990). Each of these types plays a symbiotic role in the grass. Pyrrolizidines increase resistance to moisture stress and insects (Belesky et al., 1989; Schardl and Panaccione, 2005), peramine also counteracts insect predation (Dumas, 1988) and ergot alkaloids reduce insect herbivory (Strickland et al., 2011). However, the ergot alkaloids have similar effects on herbivory in mammals, which could explain lessened feed intakes by cattle grazing tall fescue. Ergovaline is the foremost ergot alkaloid found in Kentucky 31, accounting for 85 to 97% of all ergopeptines present (Lyons et al., 1986).

The effects of fescue toxicosis have been estimated to cost beef producers over $500 million a year in the United States alone (Hoveland, 1993). The first noticeable symptom in cattle with fescue toxicosis was reduced feed intake and decreased productive gains. Average daily gain of steers was reduced from 30 to 100% in steers grazing endophyte-infected (E+) pastures compared to endophyte-free (E-) pastures; this response was observed year round (Hoveland et al., 1984). For every 10% increase in endophyte infestation, a reduction of 0.045 kg/d was observed (Garner et al., 1984). Once removed from the infected pastures, these animals have compensatory gain suggesting that the negative effects of KY31 on feed intake and average daily gain are not permanent (Cole et al., 1987).

The greatest reduction in performance occurred during the hotter months and became known as “summer slump.” Environmental conditions did affect the degree to which feed intake and daily gains were altered by endophyte infection (Hemken et al., 1981; Peters et al., 1992). Ergovaline was most prevalent in May and authors suggested that a relationship between the endophytes and ambient temperature did exist (Hemken et al., 1981; Peters et al., 1992). A more recent study, where ground fescue seed was fed to beef steers, supported the previous findings. The reduction in feed intake for steers under fescue toxicosis was 25% at thermo-neutrality and an additional 46% when under toxic conditions and heat stress (Spiers et al., 2012).

Reproduction is also affected by fescue toxicosis. Beers and Piper (1987) reported calving rates decreased by 10% while calf weights decreased by 15% due to fescue toxicosis. In a multi-year study, calving rates were 95% in cattle grazing E- tall fescue and 55% in cattle grazing E+ tall fescue (Gay et al., 1988). A similar result was observed in another trial as pregnancy rates decreased from 96 to 55% (Schmidt et al., 1986). Decreased pregnancy rates were observed in cows grazing E+ tall fescue as opposed to E.

In cows that did become pregnant, early embryonic losses and abortions were more common among those cows grazing E+ pastures (Brown et al., 1997; Brown et. al., 2000). Weanling heifers grazing endophyte-infected pastures had a 45% increase in cases of ovarian dysfunction including reduced estrous activity such as standing heat after synchronization and a greater percentage of luteal dysfunction (Mahmood et al., 1994). The specific cause or mechanism of these problems was unknown, but authors listed loss of body weight and body condition score, and changes in hormonal profiles as indicators of the problems. In cows fed E+ diets, the diameter of the largest follicle was smaller and throughout the estrous cycle there were a reduced number of class 2 (6 to 9 mm) follicles (Burke and Rorie, 2002). Cows in a control group, administration on ergotamine tartrate, produced a greater number of embryos viable for transfer and a greater percentage of embryos that developed to at least the compacted morula stage or compared to cows administered ergotamine tartrate, simulating toxicosis. In a connected study, pregnancy rates of recipients treated with ergotamine tartrate or receiving the negative control were similar; suggesting that while uterine competency was not affected, embryonic development was (Schuenemann et al., 2005). Finally, large amounts of ergot alkaloids can reduce plasma concentrations of hormones, specifically prolactin and luteinizing hormone (LH; Browning et al., 1998).

Ergot alkaloids serve as a vasoconstrictor. Blood flow to both periphery and core tissues were reduced in steers and lambs consuming E+ tall fescue, leading to a decrease in the animal's natural thermoregulation ability and an increase in body temperatures (Rhodes et al., 1991). Due to the decrease in blood flow to both extremities and core tissues, the authors speculated that a reduction in total cardiac output could be a symptom of fescue toxicosis. Aiken et al. (2009) reported decreased caudal artery area, heart rate, and blood flow to the caudal artery in heifers on an E+ treatment compared to heifers on either an E- or combination treatment. Foote et al. (2011) reported that both ergovaline and ergotamine increased contractility of ruminal arteries and veins, suggesting alteration of blood flow to tissues is an effect of fescue toxicosis.

Alternative Tall Fescue Varieties

Initial research into alternative varieties aimed to eliminate the causative endophyte. However, when the first E- cultivars were released in the 1980s they severely lacked the persistence of KY31. The stands of E- tall fescue succumbed to drought conditions that E+ persisted through (Aiken et al., 2013). Also, grazing pressure caused a more significant toll on the persistence of the stand. Most stands of the E- tall fescue only lasted 2 to 4 years compared to the 10+ years expected from KY31 stands. Although the endophyte caused the problems associated with tall fescue, it was also responsible for the positive attributes (Aiken et al., 2013). Thus, researchers aimed to find strains of the endophyte that produced very small amounts of the ergot alkaloids. These strains of tall fescue still seemed to retain the resistance to drought and pests as well as persistence of stands under grazing pressure (Hill et al., 1991). The endophyte AR542 was obtained by scientists at Ag Research, a company located in New Zealand. Pennington Seed, Inc purchased this new endophyte, commonly known as MaxQ and released seeds commercially (Gunter and Beck, 2004).

Corpus Luteum

During a bovine estrous cycle, ovulation and the surge of gonadotropins, especially LH, triggers the beginning of luteinization and the formation of a corpus luteum (CL). The luteinization process primarily targets the granulosal and thecal cells, causing a shift in the steroidogenic pathway making progesterone the primary hormone produced by both cell types (Niswender et al., 2000). The primary cell types found after luteinization include the steroidogenic small and large luteal cells, and the nonsteroidogenic endothelial cells and fibroblasts. Small luteal cells are the direct derivative of the thecal cells while the large luteal cells are the product of the luteinization of the granulosal cells (Niswender et al., 2000).

Two unique characteristics of the CL are the rapid pace of growth and vascularization. Vascular endothelial growth factor (VEGF), and specifically the isoforms $VEGF_{120}$ and $VEGF_{164}$, are the primary regulation factors in the angiogenesis of the bovine CL (Miyamoto et al., 2009). During the first 5 days of the CL formation, the rapid rate of angiogenesis builds a capillary network structure that causes the CL to have the greatest blood perfusion rate in a ratio to mass of any structure within the body (Skarzynski et al., 2013). By the mid-luteal phase, almost every steroidogenic cell within the CL is in close proximity to at least one capillary (Bollwein et al., 2013). During CL formation, the growth rate is unusual compared to most adult cells and tissues. This results in a mitotic rate that closely resembles that of rapidly-growing tumors, making the CL a model that has been used frequently in tumor research (Reynolds et al., 2000).

One of the primary functions of the CL is its secretion of progesterone and the role this plays in the biochemical pathways that signals the maternal recognition of pregnancy (Niswender et al., 2000). Luteal dysfunction including interrupted angiogenesis, interrupted progesterone production, a persistent CL, and early luteolysis can cause irregular estrous cycles as well as embryonic death (Reynolds et al., 2000).

Luteolysis, or the structural and functional lysis of CL, is initiated by the secretion of PGF_{2a} by the ipsilateral uterine horn. The first event of luteolysis is functional lysis. $PGF_{2\alpha}$ binds to receptors on the large luteal cells, triggering a cascade of events including the activation of protein kinase-C resulting in the loss of the cell's ability to secrete progesterone. This is closely followed by the death and loss of the luteal cells structural integrity in the presence of cytokines produced by macrophages and lymphocytes. After completion of luteolysis the body is able to begin a new estrous cycle (Niswender et al., 2000; Senger, 2003).

Progesterone

Progesterone is a vital steroid hormone in the bovine reproductive system. Progesterone, in its crystalline form, was first isolated by Wintersteiner and Allen in 1933 (Wintersteiner and Allen, 1934). Progesterone has 2 primary roles in cattle, the first being maintenance of pregnancy as well as being serving a partial role in the hormonal regulation of normal estrous cycle length (McDonald et al., 1952). Progesterone is secreted primarily from the CL, located in the ovary, and later in pregnancy by the placenta (Short, 1960).

The synthesis of progesterone is dependent on the presence of several compounds, namely LH, cholesterol, and both high-density lipoproteins (HDL) and low-density lipoproteins (LDL). The cholesterol esters are first transported into the luteal cell primarily using LDL that form an LDL-cholesterol complex. The LDL-cholesterol receptors are internalized after signaling from messengers produced by adenylate cyclase, a compound activated by a G-protein created after the activation of LH receptor complexes in the presence of LH (Senger, 2003). Cholesterol esters are then transported from the outer mitochondria to the inner mitochondria by the steroidogenic acute regulatory protein (StAR; Stocco et al., 2000). Following transportation, the P-450 side-chain cleavage enzyme (P-450scc) is responsible for the conversion of the cholesterol into pregnenolone. Pregnenolone is then transported back outside the mitochondrial wall where 3β-hydroxysteroid dehydrogenase (3β-HSD) is responsible for the final conversion of the pregnenolone into progesterone. Progesterone is then transported outside of the cell wall and into the blood stream for travel to the target tissues (Niswender et al., 2000; Senger, 2003).

The target tissues on which progesterone acts include the hypothalamus, pituitary gland, reproductive tract (primarily the uterus) and mammary glands. Maslar et al. (1986) reported in a human study that progesterone's influence on the uterus include the differentiation of stromal cells into epitheloid decidual cells, initiating glandular secretions and differentiation of the endometrium and protein secretion, a vital step for successful embryonic attachment and the maintenance of pregnancy. Lack of adequate concentrations of progesterone can result in reproductive failures including increases in embryonic death and a failure to maintain pregnancy (Wiltbank et al., 2006). Primary causes of inadequate concentrations of progesterone include the dysfunction of the CL as well as an up-regulation in the liver enzymes, such as Cytochrome P450 3A (CYP3A), that are responsible for the inactivation of progesterone (Reynolds et al., 2000; Inskeep and Dailey, 2005; Lemley and Wilson, 2010).

Ultrasonography

Pierson et al. (1988) first reported the use of ultrasonography in cattle and horses as a reproductive management tool. An ultrasound machine utilizes the feedback from high frequency sound waves to produce images and maps of non-visible internal organs based on how the sound waves are reflected back to a probe. This technique is visualized on a monitor attached to the probe in a black-white-gray scale depending on the density of the tissue that is being monitored. More dense tissues, such as bone and other connective tissues, will reflect most of the sounds waves, creating a white image. On the other end of the spectrum, liquids such as blood, follicular fluid and urine will not reflect sound waves, resulting in a black image. Finally, tissues that fall between these densities, such as the uterus, cervix and ovaries will appear as a range of gray based upon their own specific density. This allows for specific target tissues, such as the ovaries and more specifically CL and follicles, to be clearly seen and recorded by a technician. This early, 2-dimensional form of ultrasonography that utilizes only the white-black color scale to produce an image is known as B-mode or brightness imaging (Pierson et al., 1988).

Technological advancements have since been able to incorporate color scales into ultrasonography to assess such things as blood flow characteristics. Early versions of color Doppler were limited as they could only assess the direction of blood flow and the velocity at which blood was traveling. These 2 assessments are created by the detection of red blood cells by the probe, whether they are moving toward or away from the probe and at what velocity they are travelling using frequency shifts, this image is then depicted on the ultrasound screen, using a color scale that utilizes red and blue (Martinoli et al., 1998a; Ginther, 2007). However, utilizing the velocity and direction of blood is limited because of restrictions such as its dependence on consistent probe angle to be able to accurately compare results, making it unfeasible for unique obervations such as a CL (Ginther, 2007).

A further advancement of color Doppler technology, primarily known as power Doppler but also referred to as color amplitude imaging and ultrasound angiography, has overcome these limitations. This has further expanded the usefulness of color Doppler ultrasonography as a tool for reproductive research. Power Doppler's independence from probe angle restriction has proven to make it more sensitive and accurate when measuring and quantifying the blood flow of small or irregular tissues, vessels and organs (Martinoli et al., 1998b; Ginther, 2007; Ginther and Utt, 2004).

The results of Bollwein et al. (2002) verified that power Doppler could be a non-invasive, accurate and repeatable means of studying the blood perfusion of a CL. In their study, 3 separate images were taken of a mare's CL and analyzed for number of color pixels, and intra-class correlation coefficient of 0.9 was observed. Acosta et al. (2002) determined that the amount of blood perfusion was correlated to concentrations of progesterone in plasma, as a reduction in luteal blood flow mirrored a reduction in plasma concentrations of progesterone when administered varying doses of PGF_{2a}. Herzog et al. (2010) supported these findings with a correlation coefficient of 0.71 between luteal blood flow and concentrations of progesterone over the entirety of a bovine estrous cycle. Voelz et al. (2015) also reported a tendency for a positive correlation between total luteal volume and plasma concentrations of progesterone with a correlation coefficient of 0.39. These authors also reported that there was a positive correlation between pixel counts from image analyzing software and visual perfusion scoring by technicians, verifying an alternative technique for measuring blood perfusion in the CL (Voelz et al., 2015). A review by Matsui and Miyamoto (2009) concluded that color Doppler ultrasonography was an accurate and reliable tool for the measurement of blood flow and ovarian mapping, and that as accessibility and affordability increased, color Doppler will become a popular reproductive diagnostic tool for cattle.

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CHAPTER II

EVALUATING BLOOD PERFUSION OF THE CORPUS LUTEUM IN BEEF COWS DURING FESCUE TOXICOSIS

Abstract

The aim of this study was to determine if fescue toxicosis altered blood perfusion in the corpus luteum (CL), and peripheral concentrations of progesterone (P4) in cattle. In two replicates, the estrous cycles of 36, non-pregnant Angus or Charolais cows were synchronized using the CO-Synch+CIDR protocol. Seven days after initiation of the protocol, cows were assigned (d 0) to 1 of 2 treatments: 2.5 kg of 1) Kentucky-31 endophyte-infected (E+; n = 14), or 2) MaxQ novel endophyte (NE; n = 12) tall fescue seed. On d 7 ovaries were examined using ultrasonography and only cows that had 1 CL present remained on the study (n = 26). Images of blood perfusion of CL, blood samples, rectal temperatures, and blood pressure of tails were collected on d 10, 13, 15 and 18. Images of CL blood perfusion were analyzed using ImageJ software for pixel density, and scored visually (0 to 9 with 0 = no perfusion, 9 = complete perfusion) by 2 independent technicians. The MIXED procedure of SAS was used with day as a repeated measure. LSMeans and pooled SEM are reported. Cows receiving E+ had greater rectal temperatures (P = 0.02; 38.73 ± 0.08°C) than those receiving NE (38.46 ± 0.08°C), providing evidence the cows treated with E+ were influenced by fescue toxicosis. Pulse pressure and mean arterial pressure were decreased (P < 0.01) in cows receiving E+

(54.08 ± 3.21 and 80.06 ± 2.71 mmHg, respectively) than NE (67.12 ± 3.11 and 91.37 ± 2.93 mmHg, respectively). Concentrations of P4 were similar (P = 0.54) among cows receiving E+ (6.04 ± 0.53 ng/mL) or NE (6.36 ± 0.63 ng/mL). Pixel densities (P = 0.14) and visual perfusion scores were similar (P = 0.11) between cows receiving E+ (1,477.20 ± 655.62 pixels and 2.23 ± 0.34, respectively) or NE (2,934.70 ± 718.20 pixels and 3.00 ± 0.36, respectively). Mean CL volume was similar (P = 0.39) between treatments. In conclusion, blood perfusion of CL or peripheral concentrations of P4 were not altered at the onset of fescue toxicosis, indicating that a decrease in blood perfusion of the CL may not be a primary mechanism involved in decreased reproductive efficiency of cattle during fescue toxicosis.

Introduction

While tall fescue *(Schedonorus arundinaceus)* is widely utilized as forage in the southeast’s beef cattle industry, a condition known as fescue toxicosis may result. Fescue toxicosis is caused by the consumption of an endophyte *(Neotyphodium coenophialum)* present in the seed head of the grass (Bacon et al., 1977). The symptoms of fescue toxicosis include decreased feed intake, decreased weight gain, increased body temperature, and decreased reproductive efficiency. The ergot alkaloids produced by this endophyte also cause vasoconstriction and a reduction in blood flow to tissues throughout the body (Rhodes et al., 1991). The decreased productivity has been estimated to cost the U.S. beef industry over $600 million annually (Paterson et al., 1995). Elucidation of mechanisms causing the decrease in reproductive efficiency could facilitate the development of mitigation strategies to assist producers in reducing the economic loss from the toxicosis (Porter and Thompson, 1992).

The corpus luteum (CL) is a primary source of progesterone and plays a vital role in the maintenance of pregnancy (Niswender et al., 2000). The CL is unique in its rapid growth rate and intense vascularization (Miyamoto et al., 2009). Due to these characteristics, the CL is dependent on a large volume of blood perfusion and has the greatest blood perfusion to mass ratio in the body (Skarzynski et al., 2013). Luteal dysfunction can cause irregular estrous cycles and embryonic mortality which lead to decreased pregnancy rates (Reynolds et al., 2000). Power Doppler ultrasonography allows the quantification of blood perfusion of small and irregular tissues, such as the CL (Martinoli et al., 1998). Bollwein et al. (2002) determined that power Doppler was a reliable, accurate, and repeatable method of quantifying luteal blood perfusion.

Therefore, the objective of this study was to determine if the onset of fescue toxicosis conditions would alter the blood perfusion observed in the CL and peripheral concentrations of progesterone in cattle.

Materials and Methods

All procedures in this study were approved by the Institutional Animal Care and Use Committee of Mississippi State University.

Animals and Experiment

A total of 36 non-pregnant, cows at the H.H. Leveck Animal Research Center-Beef Unit (Mississippi State, MS) were used. Ten cows did not respond appropriately to estrus synchronization and have a CL at the start of the measurements collection period and thus were removed from the study. Of the 26 that continued all were either Charolais (n = 7) or Angus (n = 19), the average body weight was 617 ± 70.5 kg (range of 470 to

753 kg). Cows were also grouped into two ages groups either younger than 7 years (n = 10) or 7 years and older (n = 16). From 0600 to 1800, cows were housed in a dry-lot pen with ad libitum access to water and hay, which was a mixture of Bermudagrass, Johnsongrass, and Dallisgrass. From 1800 to 0600, cows were individually housed in covered pens with ad libitum access to water and access to specified treatment diets for each 18-d repetition. Repetition 1 began on July 18, 2014 and repetition 2 began on August 30, 2014. Cows' estrous cycles were synchronized using the CO-Synch + CIDR protocol (Lamb et al., 2001) with the first day of the synchronization protocol corresponding to d -7 of the study period. Thus, on d -7, an injection of GnRH (2 mL i.m.; Factrel, Fort Dodge Animal Health, Fort Dodge, IA) and a vaginal insert of progesterone (1.38 g; Eazi-Breed CIDR, Zoetis, Inc., Kalamazoo, MI) were given. On d 0, an injection of $PGF_{2\alpha}$ (5 mL i.m.; Lutalyse) was given and the vaginal insert was removed. On d 3, an injection of GnRH (2 mL i.m.; Factrel, Fort Dodge Animal Health) was given.

Feeding of treatment feedstuffs began on d 0 of the period and was continued for 18 d. The positive (E+) treatment was fed to cows at a rate of 2.5 kg of Kentucky-31 (KY31; The Wax Company, LLC, Amory, MS) tall fescue seed per day so that each cow received a calculated 15 to 20 μg of ergovaline per kg of body weight per day to induce fescue toxicosis. This concentration was designed to simulate a typical case of symptomatic fescue toxicosis cattle may experience while grazing pasture with infected tall fescue. The negative (NE) treatment was fed to cows at a similar rate using Novel-Endophyte MaxQ (MaxQ; Pennington Seed, Inc., Madison, GA) tall fescue seed. Treatments were randomly assigned in repetition 1 and for all new cows in repetition 2, cows who remained on the study throughout both repetitions were administered the opposite treatment for repetition 2. The tall fescue seed for both treatments was mixed with approximately 3 kg of corn silage to help improve palatability and intake. Nutritional values of all feedstuffs were analyzed by wet chemistry (Dairy One, Inc., Ithaca, NY) and are shown in Table 1.

On d 7, ovaries were examined by transrectal ultrasonography (10.0 to 5.0-MHz linear-array transducer, MicroMaxx, SonoSite, Inc., Bothell, WA). Cows that had responded to the CO-Synch + CIDR protocol and had a single CL remained in the study and continued to receive treatment, while those that did not respond appropriately to the synchronization protocol were excluded from the ongoing repetition. Those cows excluded from repetition 1 due to improper response to synchronization were eligible for reenrollment in repetition 2.

On d 10, 13, 15 and 18 ovaries were examined using transrectal ultrasonography beginning at 0600. During the ultrasonography exam, the power function was used to detect blood perfusion of the CL. Three still images were taken and saved for later analysis. The diameter of each CL was recorded and volume was calculated using a method previously published (Vasconcelos et al., 2001). Rectal temperature was taken. Three blood pressure measurements at the base of the tail using a standard wrist cuff sphygmomanometer were obtained and averaged (Omron Healthcare, Inc., Lake Forest, IL). Pulse pressure (systolic - diastolic) and mean arterial pressure ((pulse pressure/3) + diastolic value) were calculated for each cow. Blood samples were also obtained by venipuncture of the tail in a spray-coated K2 EDTA tube (Becton, Dickinson, and Company, Franklin Lakes, NJ) and immediately placed on ice. Blood collection tubes were centrifuged for 20 min at 2,000 × *g* at 4°C. Plasma was then removed and frozen at -20°C for later analysis. An Immuchem Double Antibody kit (MPBiomedicals, Inc., Costa Mesa, CA) was used to determine concentrations of progesterone and the manufacturer's protocol was followed.

Image Analysis

ImageJ software (version 1.47, US National Institutes of Health, Bethesda, MD) was used to analyze images for count of perfusion locations, total area of perfusion, average area of perfusion per location and raw integrated density for measurement of perfusion by pixel analysis. Images were also visually scored on a scale of 0 (0% perfusion) to 9 (100% perfusion) using previously published methods (Voelz et al., 2015) by presenting each image to two independent technicians with no knowledge of treatment. When the ultrasound failed to detect any perfusion, the observations were removed from the data set rather than assuming that blood perfusion was zero since some amount of perfusion is necessary to maintain the tissue.

Statistical Analysis

Rectal temperature, pulse pressure, mean arterial pressure, total perfusion of the CL, integrated density, luteal volume, visual perfusion scores, and concentrations of progesterone were analyzed using the Mixed procedure of SAS (SAS software version 9.3, SAS Institute Inc., Cary, NC) with day as a repeated measure, cow as a random variable, and an autoregressive-1 covariate. Repetition, breed, and age (categorized younger than 7 and 7 and older) were considered as covariates. Means were separated with the LSMEANS statement and PDIFF option. The CORR procedure of SAS was used to analyze Pearson correlation coefficients. Statistical significance was declared at $P < 0.05$. Least squares means and pooled SE are reported.

Results

The response rate to the CO-Synch + CIDR protocol for repetition 1 was 58% (11 of 19 cows) and for repetition 2 was 100% (17 of 17 cows), with a total response rate of 78% (28 of 36 cows). Of the 28 cows who responded, 2 were determined to not be suitable for the study, due to structural unsoundness and injury, and a total of 26 cows received treatment.

Rectal temperature was greater ($P = 0.02$; Table 2) in cows receiving KY31 than in those receiving MaxQ. There was also a difference in rectal temperature ($P = 0.03$) between breeds with Angus cows (38.73 ± 0.06°C) being warmer than Charolais cows (38.46 ± 0.10°C). Repetition 1 was greater ($P = 0.0002$; 38.80 ± 0.15 °C) than repetition 2 (38.39 ± 0.13 °C). Finally, there was a repetition by age interaction ($P = 0.02$) for rectal temperature (Figure 1) with older cows in repetition 2 being cooler than all other categories. Pulse pressure and mean arterial pressure were lesser (Table 2) in cows receiving KY31 than in those receiving MaxQ, but were similar between repetitions, breeds, and age categories of cows.

Total area of perfusion within the CL were similar ($P = 0.13$; Table 3) between cows receiving KY31 or MaxQ, as was luteal volume ($P = 0.95$). A treatment by repetition by breed by age ($P = 0.0005$) interaction for luteal volume was observed. Total area of perfusion as a ratio of total luteal volume (relative area of perfusion) was also similar ($P = 0.90$; Table 3) between cows receiving KY31 or MaxQ but was different ($P = 0.04$) between breeds with Charolais cows being greater (3.05 ± 0.66 score/cm^3) than

Angus cows (1.36 ± 0.39 score/cm^3). Relative area of perfusion was similar between repetitions and age categories.

Integrated densities were similar (*P* = 0.14; Table 3) between cows receiving KY31 (1,477.20 ± 655.62 pixels) and cows receiving MaxQ (2,934.70 ± 718.20 pixels). Additionally, visually-scored perfusion scores were similar (*P* = 0.11; Table 3) between cows fed KY31 and those fed the MaxQ treatment.

Total area of perfusion within the CL was positively correlated with total luteal volume (*P* = 0.0054; Table 4), integrated density (*P* < 0.0001; Table 4), and visually-scored perfusion scores (*P* < 0.0001; Table 4). Total luteal volume was also positively correlated with integrated density (*P* = 0.04; Table 4). Integrated density was positively correlated with visually scored perfusion (*P* < 0.0001; Table 4).

Plasma concentrations of progesterone were similar (*P* = 0.54; Table 3) between cows receiving KY31 (6.04 ± 0.53 ng/mL) or MaxQ (6.36 ± 0.63 ng/mL). Plasma concentrations of progesterone were different by age (*P* = 0.03) as younger cows (7.07 ± 0.49 ng/mL) had greater concentrations than older cows (5.28 ± 0.49 ng/mL).

Discussion

Fescue toxicosis is a condition caused by the consumption of ergot alkaloids produced by an endophyte in the seed of tall fescue (Bacon et al., 1977). The incidence of fescue toxicosis is negatively associated with several reproductive efficiency measures as well as reduced feed intake and weight gain by cattle as quickly as a week after initiation of feeding. Effects of toxicosis include a reduction in pregnancy rates (Schmidt et al., 1986; Brown et al., 1997), an increase of luteal dysfunction (Mahmood et al., 1994) and a negative effect on embryonic development (Schuenemann et al., 2005) leading to negative economic impacts for cattle producers. Better understanding the way these ergot alkaloids may affect the ovary, and specifically the CL, has the potential to lead to development of interventions to decrease the potential negative ramifications of the use of this prominent forage. This would make a positive economic impact on beef cattle producers in the U.S.

The CL structure in cattle is characterized by rapid rates of growth and vascularization (Miyamoto et al., 2009). Incidences of luteal dysfunction including interrupted angiogenesis or early luteolysis causes the interruption of normal estrous cycles and increases embryonic mortality (Reynolds et al., 2000), suggesting it is critical that the young CL be supplied with a normal and consistent blood supply for normal development and function. This is concerning because fescue toxicosis causes vasoconstriction. Rhodes et al. (1991) found that blood flow to both periphery and core tissues was reduced in steers under fescue toxicosis conditions suggesting a reduction in total cardiac output. The current study aimed to determine if blood perfusion of the CL was altered in cows experiencing the onset of fescue toxicosis. This was an investigatory experiment to identify a potential culprit in causing a greater incidence of luteal dysfunction and thus reduced reproductive efficiency in cattle grazing endophyte-infected tall fescue.

Another important factor in regards to the circulating concentrations of progesterone could be the participation of cytochrome P450 3A(CYP3A). CYP3A has been shown to play a role in the inactivation of progesterone (Lemley and Wilson, 2010) as well as in the clearance of ergot alkaloids from the body (Moubarak and Rosenkrans, 2000). The interaction of both progesterone and ergot alkaloids being cleared by similar enzymes could play a role in both the severity of the symptoms of fescue toxicosis as well as the circulating concentrations of progesterone. Also, the vasoconstriction caused by fescue toxicosis could affect the blood flow to the liver, further affecting the clearance of either the alkaloids or progesterone.

Rectal temperature, pulse pressure and mean arterial pressure values were obtained to verify that the cows in the KY31 treatment group were actually experiencing fescue toxicosis conditions. Vasoconstriction, a symptom of fescue toxicosis, will lead to a decrease in the animal's ability to regulate internal temperature and a decrease in blood flow to extremities (Rhodes et al., 1989). The significant effects of the KY31 treatment on both increasing rectal temperature and decreasing pulse pressure and mean arterial pressure imply that there were adequate amounts of ergot alkaloid-producing endophytes being fed to the KY31 cows to induce symptoms of fescue toxicosis.

Repetition 1 was conducted from late July to early August and repetition 2 was conducted primarily in September. Ambient temperatures were warmer during repetition 1 compared to repetition 2, which explains why rectal temperatures were also warmer during repetition 1 compared to repetition 2. There was no apparent reason why a group by age interaction was observed. There may be an increased ability to quickly recover from heat stress conditions in more mature cows as the older cows in repetition 2 were significantly cooler than any other repetition by age group. Angus cows were warmer than Charolais cows, likely as a result of differences in color of hair coat and body surface area.

The use of image analyzing software has been reported as a consistent way to measure pixel quantity for over a decade (Ginther and Utt, 2004), while the reliability of visually scoring blood perfusion has only recently been published (Voelz et al., 2015). The strong correlation between integrated pixel density and visual scoring of CL perfusion in the current study agrees with the findings of Voelz et al. (2015), indicating that either image analyzing software or visual scoring can be used as an effective and repeatable mechanism to assess detected luteal blood perfusion. Although these methods of quantifying blood perfusion have been verified, it is still unclear whether using luteal blood perfusion as an indicator of concentrations of progesterone in circulation or luteal function are accurate. Acosta et al. (2002) reported that luteal blood perfusion was positively correlated with concentrations of circulating progesterone. Herzog et al. (2010) supported these findings. However, Voelz et al. (2015) reported no correlation between CL perfusion and concentrations of progesterone, similar to the findings in the current study. In the current study, there was a large variation in CL blood perfusion scores which could indicate that there is a large variation among cows during the luteal phase. Neither blood perfusion characteristics nor concentrations of progesterone were affected by treatment, indicating the onset of fescue toxicosis did not alter blood perfusion of the CL or the amount of progesterone in circulation.

There was a 4-way interaction with treatment by repetition by breed by age present when analyzing total luteal volume. There was no apparent scientific cause for this interaction but 1 cow had a very large, potential cystic, CL, which may have contributed to this statistical interaction. However, there was no immediate identifiable cause to justify removing her from the experiment and thus she remained. Luteal volume was not affected by treatment indicating that the development of the CL was not affected by the onset of fescue toxicosis.

Conclusion

In conclusion, the onset of fescue toxicosis did not have a significant effect on blood perfusion of the CL or concentrations of progesterone in circulation within the current study. This could indicate alterations in blood profusion of the CL are not a primary underlying mechanism to causing luteal dysfunction and subsequent decrease in pregnancy rates observed in cattle grazing endophyte-infected tall fescue. Overall, more research is necessary to elucidate these mechanisms and identify mitigation techniques.

Table 1 Nutritional analysis of components of diets on a dry matter basis.

	Feedstuff			
Component	Hay	Silage	KY31Seed	MaxQ Seed
CP, %	8.2	8.5	14.8	13.0
ADF, %	47.2	21.1	12.9	12.1
NDF, %	77.4	37.8	29.5	29.9
NFC, %	4.4	45.2	45.1	46.6
TDN, %	53.0	71	65	65
NE_l, Mcal/kg	0.14	0.34	0.32	0.32
NE_m, Mcal/kg	0.19	0.34	0.30	0.30
NE_g, Mcal/kg	0.08	0.21	0.18	0..18
Ca, %	0.50	0.29	0.19	0.19
P, %	0.24	0.25	0.37	0.35
Mg, %	0.14	0.11	0.19	0.16
K, %	1.60	0.79	0.43	0.54
Na, %	0.059	0.006	0.005	0.005
Fe, ppm	575	127	120	94
Zn, ppm	34	27	28	28
Cu, ppm	8	5	6	6
Mn, ppm	88	35	64	32
Mo, ppm	0.5	0.8	0.9	0.1

Table 2 Rectal temperature and cardiac measurements of cows fed novel endophyte (MaxQ) or endophyte-infected (KY31) tall fescue seed.

Variable	Treatment MaxQ	Treatment KY31	*P*-Value
Rectal Temperature, °C	38.46 ± 0.08	38.73 ± 0.08	0.02
Pulse Pressure, mmHg	67.12 ± 3.11	54.08 ± 3.21	0.0046
Mean Arterial Pressure, mmHg	91.38 ± 2.93	80.06 ± 2.72	0.0059

Table 3 Blood perfusion characteristics, corpus luteum (CL) volume, and concentrations of progesterone between cows fed novel endophyte (MaxQ) or endophyte-infected (KY31) tall fescue seed.

Variable	Treatment MaxQ	Treatment KY31	*P*-Value
Total area of perfusion, score	19.63 ± 4.65	10.12 ± 4.20	0.13
Total CL volume, cm^3	4.90 ± 0.48	4.86 ± 0.47	0.95
Relative area of perfusion, score/cm^3	2.25 ± 0.53	2.15 ± 0.54	0.90
Integrated density, pixels	2,934.70 ± 718.20	1,477.20 ± 655.62	0.14
Visual scored perfusion, score	3.00 ± 0.3	2.23 ± 0.34	0.11
Concentration of progesterone, ng/mL	6.36 ± 0.63	6.04 ± 0.53	0.54

Table 4 Pearson correlations (r) between blood perfusion and CL characteristics.

	Total area of perfusion	Total CL volume	Visual score
Total CL volume	0.34*		0.22
Integrated density	0.91*	0.22*	0.76*
Visual score	0.89*	0.22	

* $P < 0.05$

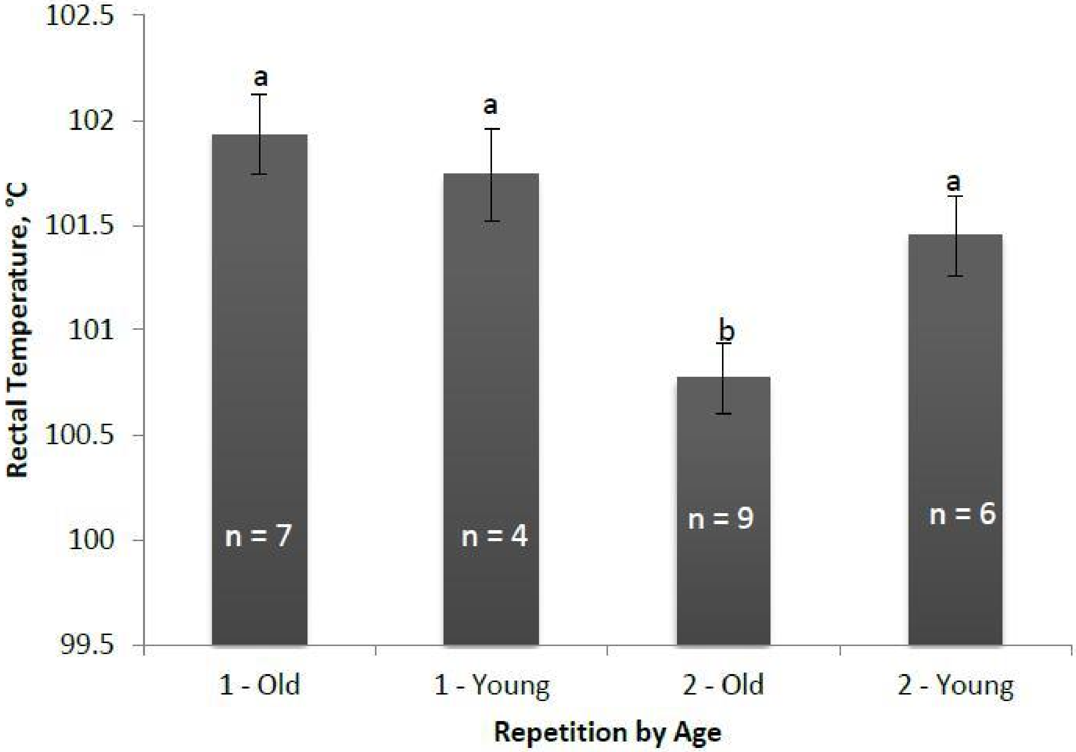

Figure 1 Interaction between repetition and age on rectal temperature.

[a,b] – Means with different superscripts differ; $P < 0.05$.

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