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Influence of Pregnant Cattle Grazing Endophyte-Infected Tall Fescue on Offspring Immune Function

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Influence of Pregnant Cattle Grazing Endophyte-Infected Tall Fescue on Offspring Immune Function

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ABSTRACT

The objective was to evaluate the influence of pregnant cattle grazing endophyte-infected tall, toxic, fescue (E+) during gestation on innate immune response of male offspring to an endotoxin challenge. Crossbred multiparous cows (n = 36) were bred to Red Angus sires and housed in replicated E+ or novel endophyte-infected, non-toxic, fescue (NE+) pastures prior to breeding and throughout gestation. From this calf-crop, a subset of post-weaned steers (n = 8 E+; n = 8 NE+) were selected for a lipopolysaccharide (LPS) challenge. Blood was collected every 30 min beginning 2 h prior to (Pre-LPS) and continuing 9 h following (Post-LPS) the administration of LPS (0.5 ug/kg of body weight). Cortisol and glucose were analyzed every 30 min, while complete blood count (CBC) and rectal temperature (RT) were analyzed every h during the pre- and post-LPS periods. Pre- and post-LPS hematocrit were increased in calves born to dams that grazed E+ relative to NE+ during gestation ($P = 0.02$; $P < 0.01$). Post-LPS lymphocyte counts were decreased in calves born to dams consuming E+ relative to NE+ during gestation ($P = 0.01$). Post-LPS eosinophil counts were increased in calves born to dams consuming E+ relative to NE+ during gestation ($P = 0.05$). No other variables were influenced by treatment ($P > 0.05$). Preliminary results of altered hematology profiles in response to an endotoxin challenge suggest an altered innate immune response of male offspring born to dams consuming E+ relative to NE+ fescue during gestation and highlight the need of ongoing research to determine corresponding cytokine profiles.

Key words: Prenatal Programming, Toxic Fescue, Endotoxin, Lipopolysaccharide, Complete Blood Count

TABLE OF CONTENTS

CHAPTER I.

INTRODUCTION AND REVIEW OF LITERATURE

Introduction

Endophyte-infected, tall, toxic fescue covers approximately 37 million acres across the southeastern region of the United States known as the fescue belt (Bussard and Aiken, 2012). In 2011, 26% of the beef cows that calved in the United States were located within the fescue belt (Bussard and Aiken, 2012). Toxic fescue has a symbiotic relationship with a fungal endophyte that produces ergot alkaloids which play a role in making the grass better adapted to survive in stressful environmental conditions; however, ergot alkaloids have been associated with negative effects on livestock physiology (Coufal-Majewski et al., 2016; Strickland et al., 2011). Cattle consuming toxic fescue have been shown to have decreased growth rates, rough hair coats, decreased conception rates, inefficiency to dissipate body heat, altered immune function, and increased respiration rates (Coufal-Majewski et al., 2016; Strickland et al., 2011). Vasoconstriction is believed to play a primary mechanistic role in many of these negative effects. Specifically, consumption of toxic fescue has been associated with vasoconstriction and reduced blood flow in the uterine artery (Foote et al., 2012; Klotz et al., 2012, 2019; Poole et al., 2019; Littlejohn, unpublished). It has been established that the prenatal environment influences postnatal phenotypes (Nathanielsz et al., 2007). Decreased uterine artery blood flow has the potential to alter the prenatal environment and program postnatal phenotypes. Offspring born to livestock consuming toxic fescue during gestation have been reported to have lower birth and weaning weights (Duckett et al., 2014; Watson et al., 2014; Poole et al., 2018; Littlejohn, unpublished). Furthermore, offspring born to dams that were exposed to a psychological stressor during gestation have been found to have altered immune function (Llorente et al., 2002; Götz

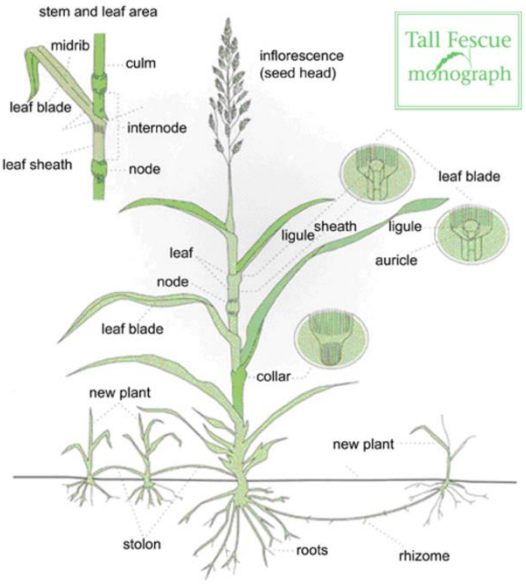
and Stefanski, 2007; Merlot et al., 2008; Littlejohn et al., 2019). However, there is limited research evaluating the influence of pregnant livestock grazing toxic fescue on offspring immune function. Alfaro and others reported greater neutrophil counts and decreased basophil counts in offspring born to fescue tolerant dams consuming toxic fescue seed relative to offspring born to fescue susceptible dams consuming toxic fescue seed (Alfaro et al., 2021). Characterizing differences in innate immune response of offspring born to dams consuming toxic fescue during gestation may provide novel insight regarding cattle health and performance in the fescue belt.

Endophyte-Infected Tall Fescue

Endophyte-infected tall fescue, commonly referred to as toxic fescue, is a well-adapted perennial grass grown in the southeastern part of the United States (Hemken, et al., 1984; Stuedemann and Hoveland, 1988; Strickland et al., 2011). Toxic fescue originated in Europe and was likely introduced to the United States through the contamination of other grass seeds imported from Europe (Bacon et al., 1986; Stuedemann and Hoveland, 1988). Kentucky 31, the most commonly grown cultivar, was found in eastern Kentucky in 1887 (Stuedemann and Hoveland, 1988). After its release as a cultivar in 1942, toxic fescue became incredibly popular during the 1940s and ‘50s, being used for pastures and vegetative cover on highway banks, parks, playgrounds, home lawns, and waterways (Stuedemann and Hoveland, 1988). The grass is well adapted to a large range of environmental conditions, and part of its popularity is due to its excellent agronomic characteristics, such as heat, drought, and pest resistance (Hemken, et al., 1984; Strickland et al., 2011; Aiken et al., 2013; Coufal-Majewski et al., 2016).

Over time, toxic fescue began to gain a reputation of poor animal performance, which was originally puzzling due to the high digestible dry matter, crude protein, amino acid, and mineral composition of high-quality, well-managed tall fescue (Hemken et al., 1984;

Stuedemann and Hoveland, 1988). Some of the negative effects of toxic fescue on livestock included fescue foot, bovine fat necrosis, and fescue toxicosis, which consists of animals exhibiting poor gains, intolerance to heat, rough hair coats, elevated body temperatures, reduced conception rates, excessive salivation, lower milk production, and nervousness (Stuedemann and Hoveland, 1988). Early hypotheses posed, and later confirmed, that a fungal endophyte, *Neotyphodium coenophialum*, residing in the plant was responsible for the negative effects of toxic fescue (Bacon et al., 1977; Stuedemann and Hoveland, 1988; Aiken et al., 2013; Poole et al., 2018). Researchers created a strain of the grass without the endophyte present, but the tolerance of the grass to heat, drought, and pests was compromised (Hoveland, 2009). Therefore, the presence of the endophyte itself was determined to be important in maintaining fescue's positive agronomic attributes. It was later discovered that the endophyte found in toxic fescue produces ergot alkaloids, which contribute both to the grass's hardiness as well as it's negative effects on cattle. The endophyte is located within the leaf sheath, and the ergot alkaloids produced can be found within the leaf blade, sheath, and seed of toxic fescue (Bacon et al., 1977).



(Fribourg et al., 2009)

Ergot alkaloids produced by the fungal endophyte in toxic fescue benefit the grass's agronomic performance. One indirect way it enhances drought tolerance is through deterring animal consumption; therefore, the plant will retain leaves, tillers, and roots, and be able to survive in challenging conditions (Belesky and West, 2009). Presence of the endophyte

decreases stomatal aperture, the opening and closing of the stomata pore that allows gas exchange; this decrease in stomatal aperture can limit the plant's water loss, contributing to drought tolerance (Belesky et al., 1987). In both chamber and field studies, presence of the endophyte within the seed has been associated with higher germination rates, greater tilling number, and increased dry weight compared to endophyte-free seeds (Pedersen et al., 1990). The mechanism of how the endophyte results in increased pest resistance is not clear; however, endophyte-induced insect resistance to various insects has been documented in laboratory settings (Pedersen et al., 1990). In stressful conditions, tall fescue plants containing the endophyte-producing ergot alkaloids tend to perform and survive better than fescue plants without ergot alkaloids (Belesky and West, 2009).

To combat the negative effects of ergot alkaloids, while maintaining the hardiness of the plant, researchers produced a variety known as novel non-toxic endophyte-infected fescue, “novel fescue” (Hemken et al., 1984). Unlike endophyte-free fescue, novel fescue produces little to no ergot alkaloids but still maintains the same attributes as the toxic fescue, such as heat, drought, and pest resistance (Hemken et al., 1984). It has been established that cattle consuming novel fescue have increased weight gains and improved pregnancy rates relative to those consuming toxic fescue (Coufal-Majewski et al., 2016). However, toxic fescue covers more than 37 million acres, and conversion of toxic to novel pasture is not practical for most producers due to the expense (Bussard and Aiken, 2012). Therefore, continuing research is essential to better understand how to improve management of animals grazing toxic fescue pastures.

Ergot alkaloids are amides of the terpenoid indole derivate D-lysergic acid and are formed from the condensation of isopentenyl diphosphate and tryptophan forming dimethylallyltryptophan, followed by consecutive methylation, oxidation decarboxylation, ring

closure and further oxidation (Kainulainen, 2003). There are more than 50 known ergot alkaloids, and their varying effects on animal physiology are largely thought to be due to their structural similarity to the tetracyclic ring of neurotransmitters (Kainulainen, 2003). The similarities in ring structures of ergot alkaloids and neurotransmitters results in the ability of ergot alkaloids to bind to different neurotransmitter receptors, such as dopamine, serotonergic, and α-adrenergic receptors, mimicking their binding (Strickland et al., 2012).

Toxic Fescue Consumption Effects on Animal Performance

Decreased feed intake, reduced weight gain, elevated respiration rates, inceased salivation, increased body temperatures, decreased reproductive efficiency, decreased serum prolactin, and vasoconstriction to the extremities are some of the common symptoms of fescue toxicosis observed in animals consuming toxic fescue (Strickland et al., 2011; Klotz et al., 2016).

Vasoactivity and ergot alkaloids

Changes in vasoactivity are known to play a mechanistic role in many of the symptoms of fescue toxicosis. The similar ring structures of ergot alkaloids and dopamine, allow ergot alkaloids to bind to dopamine receptors. Binding at dopamine receptors can inhibit prolactin production and result in decreased circulating prolactin concentrations (Strickland et al., 2011; Klotz et al., 2012). The reduction in prolactin has been associated with rough hair coat and poor milk production observed in cattle grazing toxic fescue (Strickland et al., 2011, 2012; Klotz et al., 2012). It was first reported that calves fed toxic fescue hay showed distension of small blood vessels, thickened walls, and small lumens compared to normal morphology (Williams et al., 1975). It was later found that ergot alkaloids can stimulate smooth muscle growth *in vitro,* and the thickening of blood vessels was attributed to hyperplasia of the smooth muscle layer

(Strickland et al., 1996). Ergot alkaloids can also serve as an agonist for serotonin and norepinephrine, specifically acting on S2-serotonergic and α-adrenergic receptors located within the vascular smooth muscle (Oliver et al., 1993). Binding of such an agonist with Gi-protein-coupled α_2-adrenergic receptors will reduce cAMP, resulting in contraction of the vascular smooth muscle (Oliver et al., 1993). Binding of such an agonist with Gq-protein-coupled α_1-adrenergic receptors will increase the IP_3 pathway, stimulating the release of Ca, and activating protein kinase C, also resulting in contraction of vascular smooth muscle (Oliver et al., 1993).

Ovine models have been utilized to evaluate the influence of toxic fescue on vasoconstriction. Klotz and others (2019) investigated uterine and umbilical artery vasoactivity in pregnant ewes consuming endophyte-infected (E+) seed or endophyte-free (E-) seed at days 35-85 and 86-133 during gestation. It was found that ewes consuming E+ seed at the time of tissue collection (d 133 of gestation) had uterine arteries with smaller inside and outside diameters compared to those receiving E- seed (Klotz et al., 2019). Ewes consuming E+ at time of tissue collection also had smaller outside diameters to the umbilical arteries compared to the ewes consuming the E- seed (Klotz et al., 2019).

Similar results have been reported in a bovine model. Poole and others (2018) examined growth and artery areas of non-pregnant heifers consuming diets containing E+ seed or E- seed. Heifers consuming E+ seed exhibited decreased caudal artery areas, uterine artery areas, and ovarian artery areas relative to heifers consuming E- seed (Poole et al., 2018). Furthermore, heifers consuming E+ seed exhibited decreased average daily gains and lower body weights relative to heifers consuming E- seed (Poole et al., 2018). Specifically, body weight began to be decreased in heifers consuming E+ seed relative to heifers consuming E- seed after 21 days (Poole et al., 2018). Klotz and others (2012) evaluated the lateral saphenous veins of steers

grazing either a low-endophyte-infected (LE) or a high-endophyte-infected (HE) tall fescue pasture. It was found that steers grazing the HE pastures had less serum prolactin and lighter body weight at slaughter relative to the steers grazing the LE pastures (Klotz et al., 2012). Furthermore, the lateral saphenous veins were smaller in steers grazing the HE pastures in comparison to steers grazing LE pastures (Klotz et al., 2012).

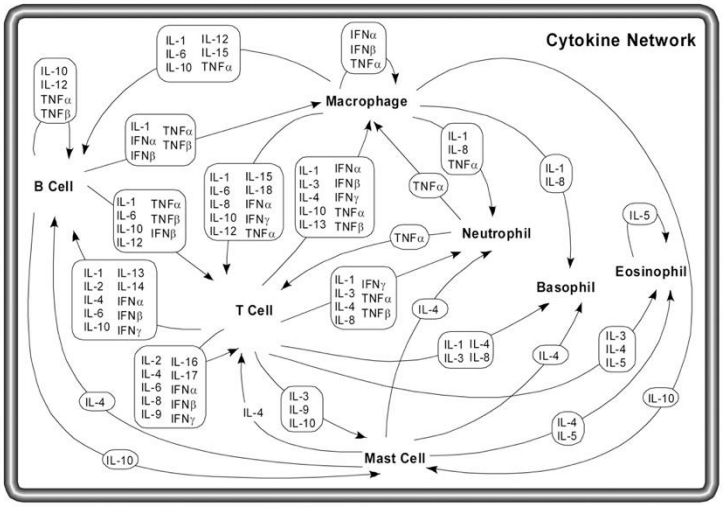
Immune system and ergot alkaloids

There is limited scientific literature evaluating the effects of toxic fescue on the immune system of livestock. The innate and adaptive immune systems work together to respond to a pathogen. The innate immune system provides the initial response and elimination of infection (Abbas et al., 2018). The adaptive immune system primarily functions by developing primarily memory T and B lymphocytes that are specific for antigens previously encountered by the individual (Abbas et al., 2018). The innate immune response is immediate and does not require prior exposure to the microbe or pathogen, while the adaptive immune response will develop T and B lymphocytes that have specific receptors for the pathogen (Abbas et al., 2018). The innate immune system has no memory, only being able to recognize a limited set of molecular structures, but the adaptive immune system uses memory to be able to recognize millions of pathogens and recruit cells with specific receptors for a pathogen (Abbas et al., 2018). This will enhance the magnitude, quickness, and effectiveness of the adaptive immune response.

One of the major mechanisms of the innate immune response is inflammation, which is characterized by the accumulation of leukocytes, plasma proteins, and fluid at the site of infection (Medzhitov and Janeway, 1997; Abbas et al., 2018). Leukocytes function to recognize the pathogen, engulf, and kill the invading pathogen (Ingvartsen and Moyes, 2013). Pattern recognition receptors (PRRs), which are proteins found in different locations throughout the

body, recognize pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) found on the surface of pathogens Abbas et al., 2018; Medzhitov and Janeway, 1997). When PRRs bind to PAMPs and DAMPs, they activate signal transduction pathways that initiate processes of innate immune response, such as inflammation (Abbas et al., 2018; Ingvartsen and Moyes, 2013; Medzhitov and Janeway, 1997). Toll-like receptors are an evolutionary family of PRRs expressed on many cell types that recognize a wide variety of microbes, including gram positive and gram negative bacteria (Abbas et al., 2018).

Leukocytes will also stimulate the production of cytokines. Cytokines are intercellular signaling polypeptides produced by activated immune cells (Maes et al., 1998; Gabay and Kushner, 1999; Dinarello, 2000). Cytokines are often produced in a cascade because one cytokine can stimulate its target cells to produce additional cytokines. There are two types of cytokines—proinflammatory and anti-inflammatory. Upon binding at PRRs, the mitogen-activated protein kinase signaling pathways also get activated and induce a cellular response, often resulting the induction of adhesion molecules, accelerating inflammation, ultimately leading to the production and release of pro-inflammatory cytokines (Dinarello, 2000). Pro-inflammatory cytokines, which can include IL-1, IL-6, and TNFα, regulate the inflammatory response, activating and recruiting other leukocytes to the site of infection (Zhang and An, 2007). Anti-inflammatory cytokines, which



(Zhang and An, 2007)

can include IL-1 antagonist, IL-4, IL-10, IL-11, and IL-13, work to regulate the pro-inflammatory cytokines (Zhang and An, 2007). Among the anti-inflammatory cytokines, IL-10 is very potent, repressing the expression TNFα, IL-6, and IL-1 by activated macrophages (Zhang and An, 2007).

Lipopolysaccharide (LPS) is an endotoxin from gram negative bacteria and can induce an immune response in livestock. Cattle that were injected with LPS exhibited decreased leukocyte counts at 2 and 6 h post-injection, but later returned to baseline or above baseline concentrations (Jacobsen et al., 2007). Cattle that were injected with LPS produced TNFα, IL-1β, IL-8, and IL-10 from a population of monocytes, and TNFα, IFNγ, IL-4, and IL-10 from a population of lymphocytes (Jacobsen et al., 2007). This study also suggested a shift in the type of cytokine stimulated, favoring anti-inflammatory cytokines in the initial response, and proinflammatory cytokines in the long-term response to LPS (Jacobsen et al., 2007). Filipov and others (1999) performed an LPS challenge on steers grazing either E+ or E- fescue for 8 months. Steers grazing E+ fescue had decreased body weight and average daily gains at the end of the 8-month grazing period prior to the challenge, and increased cortisol, TNFα, and IGF-1 in response to LPS relative to steers grazing E- fescue (Filipov et al., 1999).

Nutrition is a primary mediator of the body's innate immune response. Leukocytes use glucose, non-esterified fatty acids, beta-hydroxybutyrate, and glutamine as energetic fuel sources (Ingvartsen and Moyes, 2013). Specifically, glucose has been established as the preferred metabolic fuel for lymphocytes and macrophages during inflammation rather than fatty acids, amino acids, or ketones (Ingvartsen and Moyes, 2013). In an *in vitro* module using murine muscle tissue, there was an increased risk of infection due to inhibition of glucose uptake by macrophages and phagocytic cell types (Lang and Dobrescu, 1991; Barghouthi et al., 1995).

Therefore, maintaining leukocyte function is dependent on the efficient uptake of glucose (Ingvartsen and Moyes, 2013). Low glucose availability could limit immune cell function (Ingvartsen and Moyes, 2013). Supplemental crude protein (CP) has been reported to mitigate some of the reduced performance of livestock, such as animal growth and weight gain, due to the consumption of toxic fescue. Steers grazing E+ fescue supplemented with protein had increased average daily gains relative to steers grazing E+ fescue without supplemented protein (Elizalde et al., 1998). The effects of grazing E+ fescue supplemented with protein on immune function are more recently evaluated. Stocker steers that consumed E+ seed regardless of CP supplementation, had increased pro- and anti-inflammatory cytokines, such as TNFα, IFN-γ, IL-1α, IL-2, IL-13, 1L-15, and IL-21, compared to steers that consumed E+ (Poole et al., 2019). The increased concentrations of cytokines observed could suggest that chronic exposure to ergovaline could lead to a hyperactive innate immune response, potentially resulting in an immune-compromised animal (Poole et al., 2019).

Genetic and phenotypic considerations of fescue tolerance

Since cattle within the fescue belt have potentially been grazing fescue for their entire life and across many generations, it has been hypothesized that over time, cattle have adapted to overcome some symptoms associated with fescue toxicity. Efforts have been made to identify animals that are tolerant and susceptible to toxic fescue based on genetics. AgBotanic LLC, created a T-snip test using genotypic markers that have been associated with phenotypes of fescue toxicosis. The test evaluates DNA and gives a 1-5 star ranking with 1 being most susceptible and 5 being most tolerant to fescue toxicosis. Galliou and others (2020) compared T-snip test results and phenotypic data of cows grazing toxic fescue over a 13-week period. It was

observed that the 1-5 star ranking given from the test coincided with cow performance while on toxic fescue (Galliou et al., 2020)

Other reports have investigated immune system variables in relationship to fescue tolerance. Poole and others (2020) performed a study to determine if there were different cytokine concentrations present in cows who were more tolerant or susceptible to fescue toxicosis. Body weight, body condition scores, hair shedding scores, hair coat scores, rectal temperatures, and blood samples were collected weekly from cows grazing toxic fescue for 13 weeks. At the end of the grazing period, the phenotypic data was used to sort cows into fescue tolerant or fescue susceptible groups. Once cows were sorted in tolerant and susceptible groups, the blood collected during the grazing period was analyzed for pro- and anti-inflammatory cytokines. There were no differences in cytokine concentrations between fescue tolerant or fescue susceptible cows (Poole et al., 2020).

Influence of Ergot Alkaloid Consumption by Pregnant Cattle on the Developing Fetus

It has been established that an altered intrauterine environment can lead to altered postnatal phenotypes (Barker 1990, 2004). This idea became known as prenatal programming and can be further described as the process by which a fetus responds to a specific challenge during a critical developmental period of gestation that alters the developmental trajectory of that fetus (Nathanielsz et al., 2007). Toxic fescue has been associated with changes in bovine physiology, such as increased cortisol concentrations and vasoconstriction. Such changes in maternal physiology have the potential to alter the fetal environment and program postnatal phenotypes of the developing fetus.

Based on current literature reporting vasoconstriction and decreased blood flow specifically in the uterine artery of livestock consuming E+ fescue during gestation, there is potential to alter the fetal environment, thereby potential to program the postnatal phenotype of the fetus. Blood flow to the conceptus through the uterine artery and then the placentomes play a central role in fetal growth by delivering nutrients to the fetus (Reynolds et al., 2010). Fetal growth and development could be compromised if uterine or umbilical blood flow is reduced at any time during gestation (Reynolds et al., 2010). However, it is important to note that both uterine and umbilical artery blood flow increase exponentially throughout gestation, with the majority of fetal growth occurring in the last trimester of pregnancy. Anderson and others' (2005) utilized a rodent model with reduced uteroplacental perfusion pressure (RUPP) to evaluate the effects of reduced blood flow to the placenta on the fetus. It was found that fetuses that were born to dams that underwent the RUPP procedure experienced decreased placental weight, fetal weight, and litter size, compared to fetuses not exposed to RUPP (Anderson et al., 2005). Littlejohn (unpublished) observed reduced uterine artery blood flow in pregnant heifers consuming E+ seed for 70 d relative to pregnant heifers consuming E- seed for 70 d. Calves born to heifers consuming E+ seed for 70 d during gestation showed reduced weaning weights relative to calves born to heifers consuming E- seed for 70 d during gestation (Littlejohn, unpublished). Lambs born to ewes consuming E+ seed during gestation had reduced birth weight by 37% compared to lambs born to ewes consuming E- seed (Duckett et al., 2014). Organ and muscle weights were also lighter in lambs born to ewes consuming E+ seed during gestation compared to those born to ewes consuming E- seed during gestation (Duckett et al., 2014). Similar results have been reported in cattle. Watson and others (2014) evaluated pregnant cows grazing toxic fescue pastures during gestation compared to pregnant cows grazing novel fescue during

gestation. Cows grazing toxic fescue had lower body condition scores and average daily gains relative to cows grazing novel fescue (Watson et al., 2014). Calves born to cows grazing toxic fescue during gestation had reduced birth weights and weaning weights relative to calves born to cows grazing novel fescue during gestation (Watson et al., 2014).

Toxic fescue consumption may alter the intrauterine environment by stress-induced elevations in glucocorticoids in the dam. There is conflicting data evaluating toxic fescue consumption and cortisol concentrations. Cows in marginal body condition score and grazing E+ fescue prior to and during gestation, exhibited decreased cortisol relative to cows in good body condition scores grazing either E+ fescue or common Bermuda grass (Looper et al., 2010). Cortisol concentrations were similar between heifers fed E+ seed and E- seed (Aldrich et al., 1993). Ewes fed increased (0.75 mg/kg of ergovaline) or decreased (0.11 mg/kg of ergovaline) ergovaline exhibited similar cortisol concentrations (Looper et al., 2007). Steers with no prior exposure to toxic fescue and then injected with ergotamine tartrate exhibited increased cortisol concentrations compared to steers with no prior exposure to toxic fescue and then injected with saline or ergonovine (Browning et al., 1998). Primiparous, nursing cows with no prior exposure to toxic fescue injected with ergotamine tartrate exhibited increased cortisol concentrations compared to cows with no prior exposure to toxic fescue and then injected with saline or ergonovine (Browning et al., 1998). In both cycling heifers and cows that had no prior exposure to toxic fescue and then injected with ergotamine exhibited increased cortisol levels compared to cattle with no prior exposure to toxic fescue and injected with saline (Browning et al., 2000). Varying results of cortisol concentrations reported in livestock exposed to ergot alkaloids in toxic fescue is hypothesized to be due to different lengths of exposure as a chronic or acute stressor. However, the evidence of increased cortisol in response to the presence of an ergot

alkaloid suggests the potential for increased cortisol during gestation if the cow is consuming toxic fescue.

Under normal conditions, fetal exposure to maternal glucocorticoids is kept at low concentrations. Regulation of glucocorticoids to the fetus is due to the placental enzyme 11β-hydroxysteriod dehydrogenase type 2 (11β-HSD2; Benediktsson et al., 1997; Holmes et al., 2006; Merlot et al., 2008). The enzyme converts active cortisol to cortisone (Benediktsson et al., 1997). However, excessive increases in maternal glucocorticoids or the suppression of 11β-HSD2 activity, could lead to increased exposure for the fetus potentially causing DNA methylation/deacetylation (Benediktsson et al., 1997; Merlot et al., 2008). Mice born to 11β-HSD2 -/- matings had reduced birthweight and remained smaller throughout life compared to mice born to 11β-HSD2 +/+ matings (Benediktsson et al., 1997; Holmes et al., 2006).

Influence of Ergot Alkaloid Consumption by Pregnant Cattle on Offspring Health

Alterations to the fetal environment, such as cortisol exposure to the fetus or reduced blood flow to the conceptus potentially induced by consumption of toxic fescue by pregnant cattle, may have the potential to impact the offspring's immune system. Offspring born to dams that were exposed to a psychological stressor during gestation have been reported to exhibit impaired immune function (Merlot et al., 2008). Littlejohn and others (2019) performed an LPS challenge on Brahman bull calves born to dams that underwent transportation stress during gestation. Prior to LPS the prenatally stressed (PNS) bull calves exhibited reduced INF-γ, eosinophils, and basophils but greater concentrations of TNF-α, IL-6, and monocytes (Littlejohn et al., 2019). There were no differences in circulating concentrations of measured variables in response to LPS (post-LPS); however, PNS bull calves had a larger increase from baseline of INF-γ compared to controls (Littlejohn et al., 2019). Post-LPS, the PNS bull calves had larger

decreases from baseline for monocytes, eosinophils, and basophils compared to controls (Littlejohn et al., 2019). This study suggests altered innate immune response of offspring born to dams that experienced transportation stress during gestation (Littlejohn et al., 2019). Studies evaluating immune function in offspring born dams who were exposed to either a chemical or psychological stressor do have varying results. Carroll and others (2017) found that heifers born to cows that were exposed to LPS during gestation had increased concentrations of IL-6, but not TNF-α or INF-γ in response to an LPS challenge (Carroll et al., 2017). Thymic function has been reported to be altered in offspring born to mothers that were psychologically stressed during gestation (Llorente et al., 2002; Götz and Stefanski, 2007). Rodent models have reported decreased total lymphocytes, specifically $CD^{+}4$ and $CD^{+}8$, in response to a psychological stress stimulus in adult rats born to mothers psychologically stressed during gestation (Götz and Stefanski, 2007).

There is evidence linking toxic fescue consumption during gestation on offspring growth performance. However, there is limited research evaluating the effects of toxic fescue consumption during gestation on offspring health. Alfaro and others (2021) evaluated complete blood counts of offspring born to dams consuming E+ seed with or out rumen-protected niacin (RPN) during mid gestation for a 30-day period and were genetically susceptible or tolerant to fescue toxicity. After weaning, the calves were assigned to the same diet as their dam for a 30-day feeding period. The RPN supplementation was fed in attempt to counteract the vasoconstrictive effects of the E+ seed (Alfaro et al., 2021). Blood was collected at day 1 and 30 of the calves feeding period for analysis. Calves born to susceptible dams had lowered mean corpuscular hemoglobin and volume, showing signs of anemia after the 30-day feeding period relative to calves born to tolerant dams (Alfaro et al., 2021). Calves born to dams that were

tolerant and consumed the RPN had greater neutrophil to lymphocyte ratio at day 1 of the feeding period after weaning; this same treatment group also had increased neutrophils relative to calves born to tolerant dams without the RPN, susceptible dams with the RPN, and susceptible dams without the RPN (Alfaro et al., 2021). Offspring born to dams consuming the diets containing the RPN, regardless of genetic susceptibility, had decreased basophil concentrations (Alfaro et al., 2021). This study suggests the potential for altered immune function in offspring born to tolerant or susceptible dams consuming E+ seed for 30 d during gestation.

There is currently a gap in the scientific literature evaluating maternal consumption of toxic fescue during gestation on immune function of subsequent offspring. Increased research evaluating immune function of offspring born to dams consuming toxic fescue during gestation has the potential to lead to better management cattle in the fescue belt. Therefore, the objective of the present study was to evaluate the influence of pregnant cattle grazing toxic fescue on innate immune response of male offspring to an endotoxin challenge.

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CHAPTER II

INFLUENCE OF PREGNANT CATTLE GRAZING ENDOPHYTE-INFECTED TALL FESCUE ON INNATE IMMUNE RESPONSE OF MALE OFFSPRING

INTRODUCTION

Endophyte-infected tall fescue (E+) is a cool season perennial grass commonly referred to as toxic fescue (Aiken et al., 2013). Toxic fescue can be found in 15 states across 37 million acres within the United States, a region referred to as the fescue belt (Bussard and Aiken, 2012). It has been estimated that 26% of beef cows that calved in the U.S. in 2011 were located within the fescue belt and exposed to toxic fescue (Bussard and Aiken, 2012). Toxic fescue has a symbiotic relationship with the fungal endophyte, Neotyphodium coenphialum, that has been associated with contributing to the grass' heat, drought, and pest resistance by deterring animal consumption (Belesky and West, 2009; Aiken et al., 2013; Coufal-Majewski et al., 2016). The fungal endophyte produces ergot alkaloids, such as ergovaline, which have been associated with reductions in feed intake, growth rates, ability to dissipate body heat, altered immune function, and reproductive efficiency in cattle (Coufal-Majewski et al., 2016; Poole et al., 2019).

Due to the structural similarities of the ergoline ring portion of ergot alkaloids to dopamine, they are able to interact biogenic amine receptors, such as dopamine, serotonergic, and α-adrenergic receptors (Klotz, 2016; Strickland et al., 2012). This can mimic binding of dopamine and cause a decrease in prolactin secretions (Klotz, 2016; Strickland et al., 2011). This interaction and decrease in prolactin can contribute to the rough hair coat and decreased milk production that is seen in cattle consuming toxic fescue (Klotz, 2016). The binding that can occur at serotonergic and α-adrenergic receptors will mimic activity of serotonin and epinephrine, resulting in smooth muscle contraction and subsequently, vasoconstriction (Oliver

et al., 1993; Strickland et al., 2011, 2012; Klotz et al., 2012). Pregnant ewes consuming toxic fescue seed during gestation had smaller inside and outside uterine artery diameter and wall thickness than those consuming endophyte-free seed (Klotz et al., 2019). Poole and others (2018) showed heifers consuming toxic fescue seed prior to and during an estrus synchronization protocol had reduced uterine artery and vein cross section areas compared to those consuming endophyte-free seed. Our laboratory recently found that pregnant heifers consuming toxic fescue seed for a 70-d feed supplementation period during mid-late gestation exhibited decreased uterine artery blood flow relative to those consuming endophyte-free seed.

Altered maternal physiology, such as changes in uterine artery hemodynamics, as a result of consuming toxic fescue may impact the fetal environment. Prenatal programming can be defined as the fetal response to a specific challenge during a critical developmental window that alters the developmental trajectory of the fetus (Nathanielsz et al., 2007). Offspring born to dams consuming E+ during gestation had lower birth and weaning weights compared to offspring born to dams that consumed endophyte-infected, novel, non-toxic fescue (NE+) during gestation (Duckett et al., 2014; McCarty et al., 2020; Watson et al., 2014). While differences in offspring performance have been reported, there is limited research characterizing immune function of offspring born to dams consuming E+ during gestation. Alfaro and others (2021) evaluated complete blood count (CBC) variables in offspring born to dams that were genetically tested, using a T-snip test, as either tolerant or susceptible to fescue toxicity and consumed E+ with or without rumen-protected niacin (RPN) for 30 d during mid-gestation. The RPN was evaluated as a treatment to mitigate the vasoconstrictive effects of toxic fescue. The offspring were also fed the same diet as their dam for a 30-d period after weaning. Offspring born to susceptible dams had lower mean corpuscular volume relative to offspring born to tolerant dams (Alfaro et al.,

2021). Offspring born to tolerant dams that consumed diets with RPN had greater neutrophil to lymphocyte ratio at d 1 of the feeding period after weaning relative to the offspring born to tolerant dams not receiving RPN, susceptible dams receiving RPN, and susceptible dams not receiving RPN; this same treatment group also had increased neutrophils relative to the other three treatments throughout the feeding period (Alfaro et al., 2021). Offspring born to dams consuming diets with RPN and fescue, regardless of genetic susceptibility, had decreased basophil concentrations (Alfaro et al., 2021).

Immune function and animal growth can be related. For example, glucose consumption by leukocytes as an energy source becomes increased during infection utilizing most of the glucose provided by the diet (Colditz, 2002). Reduced feed intake, milk production and weight gain has been observed in lactating sows exposed to chronic stimulation of the immune system (Colditz, 2002). While there is growing evidence of negative effects of consumption of toxic fescue by pregnant cows on offspring birth and weaning weights, there is limited research evaluating its effects on offspring immune function. The objective of this study was to evaluate the influence of dams grazing endophyte-infected tall, toxic, fescue during gestation on the innate immune response of male offspring to an endotoxin challenge.

MATERIALS AND METHODS

All animal use, handling, and sampling techniques were approved by the University of Arkansas System Division of Agriculture Institutional Animal Care and Use Committee.

Animal Procedures

Thirty-six fall-calving crossbred multiparous cows were placed on replicated E+ or NE+ pastures (n = 20 E+; n = 16 NE+) at the University of Arkansas Division of Agriculture Forestry

and Livestock Research Station in Batesville, AR, 44 days prior to artificial insemination with semen from Red Angus sires. Ten days following artificial insemination, a Red Angus clean-up bull was placed with cattle for a 60-d period. Three days prior to the birth of the first calf, cows were moved from their respective replicated E+ or NE+ pastures to a common Bermuda grass pasture free of fescue, where they were maintained together as a single group until calves were weaned. Ergovaline concentrations of each pasture were measured in July. Ergovaline concentrations were >1337 ppb in E+ pastures and <100 ppb in NE+ pastures. Calves were born between September 4 - November 6. There were 8 bulls and 7 heifers born to dams that grazed NE+ during gestation and 14 bulls and 5 heifers born to the dams that grazed E+ during gestation. Calves were weaned at 213 ± 28 d of age.

From this calf crop, a subset of weaned steer calves (n = 8 E+; n = 8 NE+) of similar age and body weight were selected for a glucose tolerance test and immune challenge. This subset of steers was transported to a University of Arkansas research farm in Fayetteville, AR, two weeks after weaning. For the remainder of the study, calves were maintained together as a single group on mixed warm season grasses free of fescue and supplemented with corn gluten pellets. Upon arrival, calves were allowed a 30-d acclimation period. Following the acclimation period, a glucose tolerance test was conducted on all calves. Calves were then allowed to rest an additional 37 d, after which an immune challenge utilizing lipopolysaccharide (LPS) was conducted on all calves. A study timeline can be referenced in Figure 1.

LPS Challenge

Steers (301 ± 35 d of age; 261 ± 61 kg of body weight) were fitted with jugular vein catheters, placed in individual stalls, and allowed to rest a minimum of 7 h prior to blood sampling. Steers were given *ad libitum* access to water throughout the duration of the challenge

and coastal Bermuda hay until 2 h prior to the start of sampling. Blood samples and rectal temperatures (RT) were taken from each calf for a 2-h period prior to (Pre-LPS) and a 9-h period following (Post-LPS) intravenous LPS administration (0.5 ug/kg body weight; LPS from Escherichia coli O111:B4; Sigma-Aldrich, St. Louis, MO).

Blood Sampling Procedures

A 10-mL blood sample was collected from each steer and placed into an additive-free vacutainer (BD, Franklin Lakes, NJ) every 0.5 h, beginning 2 h prior to and continuing 9 h after the administration of LPS for determination of cortisol and glucose (Figure 2). Whole blood samples were allowed to clot overnight at 4°C. Whole blood samples were centrifuged at 1250 x g for 20 min at 4°C, and serum was collected. Serum was stored at -80°C until analysis. An additional 4-mL blood sample was collected from each steer and placed into a vacutainer containing EDTA (BD, Franklin Lakes, NJ) for determination of CBC variables every hour beginning 2 h prior to and continuing 9 h after the administration of LPS. Complete blood count variables were obtained using a Hemavet Hematology Analyzer (Drew Scientific, Miami Lakes, FL).

Rectal Temperature

Calf RT was recorded for each steer every hour, starting 2 h prior to and continuing 9 h following the administration of LPS (Figure 2). Rectal temperature was recorded using a M900 thermometer (GLA Agriculture Electronics, San Luis Obispo, CA) immediately following the collection of the blood sample at that timepoint.

Assays for Cortisol and Glucose

Serum cortisol concentrations were determined using a commercially available radioimmunoassay kit (MP Biomedicals, LLC, Solon, OH). Data were analyzed in a linear fit model. The minimum detectable cortisol was 0.948 ng/dL. The intra- and inter-assay coefficients of variation for the high control were 12.45% and 17.43% respectively. The intra- and inter-assay coefficients of variation for the low control were 19.62% and 27.89% respectively.

Serum glucose concentrations were determined using a commercially available colorimetric hexokinase assay kit (TECO Diagnostics, Anaheim, CA). Assays were performed in a 96 well plate, so a 10x dilution was used to bring sample concentrations within standard curve range. The intra- and inter-assay coefficients of variation were 3.6% and 11.5% respectively. Data are presented in mg/dL.

Statistical Analysis

Pre- and post-LPS serum cortisol, serum glucose, CBC variables, and RT were analyzed using the MIXED procedure of SAS specific for repeated measures with treatment, time, and the treatment x time interaction as fixed effects and sire as a random effect. Post-LPS maximum response and time at maximum response for RT were analyzed using the MIXED procedure of SAS with treatment as a fixed effect and sire as a random effect. Post-LPS area under the curve (AUC) maximum response, time at maximum response, and return to baseline for serum cortisol and serum glucose were analyzed using the MIXED procedure of SAS with treatment as a fixed effect and sire as a random effect. Time at return to baseline was determined as the time that the concentration reached baseline concentrations (baseline was calculated as the average of concentrations at -2 h – 0 h). If concentrations did not return to baseline by the end of sampling time, the time to return to baseline was assigned as 9.5 h for the purpose of the statistical

analysis. Post-LPS AUC was determined using the trapezoidal rule. Data are presented as the least square means ± SE.

RESULTS

Cortisol

There was no pre-LPS effect of treatment, time, or a treatment x time interaction on serum concentrations of cortisol ($P > 0.05$; Table 2). There was an expected post-LPS effect of time on cortisol with concentrations increasing in response to LPS and reaching peak concentrations at approximately 3 h ($P < 0.01$; Table 2). There was no post-LPS effect of treatment or a treatment x time interaction on cortisol ($P > 0.05$; Table 2). There was no post-LPS effect of treatment on cortisol AUC, maximum cortisol response concentration, time at maximum cortisol concentration, or time to return to baseline cortisol concentrations ($P > 0.05$; Table 2).

Glucose

There was no pre-LPS effect of treatment, time, or a treatment x time interaction on serum concentrations of glucose ($P > 0.05$; Table 3). There was an expected post-LPS effect of time on glucose with similar concentrations to the pre-LPS period through 3 h followed by a substantial decrease ($P < 0.01$; Table 3). There was no effect of treatment or a treatment x time interaction on post-LPS glucose ($P > 0.05$; Table 3). There was no post-LPS effect of treatment on glucose AUC, maximum glucose response concentrations, time at maximum glucose response concentration, or time to return to baseline glucose ($P > 0.05$; Table 3).

Rectal Temperature

There was a pre-LPS treatment x time interaction for RT, with calves born to dams consuming E+ during gestation exhibiting a slight increase relative NE+ in RT at 0 h ($P = 0.02$; Figure 3; Table 4). There was an expected post-LPS effect of time on RT with RT reaching peak temperatures at approximately 4 h ($P < 0.01$; Table 4). There was no post-LPS effect of treatment or a treatment x time interaction on RT ($P > 0.05$; Table 4). There was no post-LPS effect of treatment on maximum RT response or time at maximum RT response ($P > 0.05$; Table 4).

Complete Blood Count

There was no pre-LPS effect of treatment, time, or a treatment x time interaction on white blood cell (WBC) counts ($P > 0.05$; Table 6). There was an expected post-LPS effect of time on WBC counts with WBC counts decreasing within 1 h and begin to increase at 4 h ($P < 0.01$; Table 6). There was no post-LPS effect of treatment or a treatment x time interaction on WBC counts ($P > 0.05$; Table 6).

There was no pre-LPS effect of treatment, time, or a treatment x time interaction on neutrophil counts ($P > 0.05$; Table 6). There was an expected post-LPS effect of time on neutrophil counts with neutrophil counts decreasing within 1 h and begin to increase at 2 h ($P < 0.01$; Table 6). There was no post-LPS effect of treatment or treatment x time interaction on neutrophil counts ($P > 0.05$; Table 6).

There was no pre-LPS effect of treatment, time, or a treatment x time interaction on lymphocyte counts ($P > 0.05$; Table 6). There was a post-LPS effect of treatment on lymphocyte counts ($P = 0.01$; Table 6). Calves born to dams consuming E+ during gestation exhibited decreased lymphocyte counts relative to calves born to dams consuming NE+ during gestation.

There was an expected post-LPS effect of time on lymphocyte counts with lymphocyte counts decreasing within 3 h and beginning to increase at 7 h ($P < 0.01$; Table 6). There was no post-LPS treatment x time interaction on lymphocyte counts ($P > 0.05$; Table 6). However, there was a post-LPS tendency for a treatment x time interaction on lymphocyte counts with calves born to dams consuming E+ during gestation being decreased relative to calves born to dams consuming NE+ during gestation at 0 h, 8 h, and 9 h ($P = 0.09$; Table 6).

There was no pre-LPS effect of treatment, time, or a treatment x time interaction on monocyte counts ($P > 0.05$; Table 6). There was a post-LPS treatment x time interaction on monocyte counts with calves born to dams consuming E+ during gestation decreased relative to calves born to dams consuming NE+ during gestation at 0 h and then remain similar between treatment groups ($P \leq 0.01$; Figure 4; Table 6).

There was no pre-LPS effect of treatment, time, or a treatment x time interaction on eosinophil counts ($P > 0.05$; Table 6). There was a post-LPS effect of treatment on eosinophil counts with calves born to dams consuming E+ during gestation increased relative to calves born to dams consuming NE+ during gestation ($P = 0.05$; Table 6). There was an expected post-LPS effect of time on eosinophil counts with eosinophil counts decreased within 1 h and increasing at 2 h ($P = 0.01$; Table 6). There was no post-LPS effect of a treatment x time interaction on eosinophil counts ($P > 0.05$; Table 6).

There was no pre-LPS effect of treatment, time, or a treatment x time interaction on basophil counts ($P > 0.05$; Table 6). There was no post-LPS effect of treatment, time, or a treatment x time interaction on basophil counts ($P > 0.05$; Table 6). However, there was a post-LPS tendency for a treatment x time interaction on basophil counts with calves born to dams

consuming E+ during gestation being increased relative to calves born to dams consuming NE+ during gestation at 4 h and 6 h ($P = 0.09$; Table 6).

There was no pre-LPS effect of treatment, time, or a treatment x time interaction on lymphocyte:neutrophil ratio ($P > 0.05$; Table 6). There was no post-LPS effect of treatment, time, or a treatment x time interaction on lymphocyte:neutrophil ratio ($P > 0.05$; Table 6).

There was a pre-LPS effect of treatment on hematocrit percentage with calves born to dams consuming E+ during gestation increased relative to calves born to dams consuming NE+ during gestation ($P = 0.03$; Table 6). There was no pre-LPS effect of time or a treatment x time interaction on hematocrit percentage ($P > 0.05$; Table 6). There was a post-LPS effect of treatment on hematocrit percentage with calves born to dams consuming E+ during gestation increased relative to calves born to dams consuming NE+ during gestation ($P < 0.01$; Table 6). There was an expected effect of time on hematocrit percentage with hematocrit percentage increased within 1 h and remained increased ($P = 0.03$; Table 6). There was no post-LPS treatment x time interaction on hematocrit percentage ($P > 0.05$; Table 6).

DISCUSSION

This study assessed the influence of consumption of toxic fescue by pregnant cattle on postnatal innate immune response to an endotoxin challenge. A major mechanism of the innate immune response is inflammation, which is characterized by leukocytes, plasma proteins, and fluid leaving the peripheral blood and accumulating at the site of infection (Abbas et al., 2018; Medzhitov and Janeway, 1997). Therefore, the decreased amounts of leukocytes found in the blood of calves born to dams consuming both E+ and NE+ during gestation can be considered a typical response to infection.

In the present study, calves born to dams consuming E+ during gestation had decreased lymphocyte counts and increased eosinophil counts relative to the calves born to dams consuming NE+ during gestation in the post-LPS period. Similar results have been found in mice exposed to immobilization stress and who's dams were exposed to 2 hours of stress daily during the last week of gestation exhibiting decreased lymphocyte counts and increased eosinophil counts and neutrophils (Llorente et al., 2002). The present study did not find any differences between calves born to dams consuming E+ and NE+ during gestation on neutrophils, but calves born to dams consuming E+ during gestation did have numerically increased neutrophils relative to calves born to dams consuming NE+ during gestation. Similar to the current study, pigs born to dams exposed to social stress during late gestation and then underwent the stress of weaning, exhibited decreased lymphocyte and WBC counts (Couret et al., 2009). While the current study did not exhibit significantly decreased WBC counts, calves born to dams consuming E+ during gestation exhibited numerically decreased WBC counts relative to calves born to dams consuming NE+ during gestation.

In the present study, there were no differences in glucose concentrations between treatments, however, glucose concentrations did decrease in response to LPS at 3 h and lymphocyte counts decreased at 1 h followed by an increase in lymphocyte counts at 3 h. It can be proposed that the timing of the decrease in glucose could be associated with the increase in lymphocytes at that same time because glucose has been shown to be the preferred metabolic fuel during inflammation for lymphocytes (Ingvartsen and Moyes, 2013). In mice, there was an increasing risk of infection due to the inhibition of glucose uptake (Lang and Dobrescu, 1991; Barghouthi et al., 1995). Maintaining immune cellular function is dependent on the efficiency of glucose uptake; therefore, low glucose availability could limit immune function (Ingvartsen and

Moyes, 2013) Since calves born to dams consuming E+ during gestation exhibited decreased lymphocytes relative to calves born to dams consuming NE+ during gestation during the post-LPS period, there could be a difference in glucose utilization supporting the immune system between calves born to dams consuming E+ relative to NE+ during gestation.

In the present study, calves born to dams consuming E+ during gestation had increased eosinophil counts compared to calves born to dams consuming NE+ during gestation. Eosinophils are historically known for their role in allergic and parasitic responses, and calves born to dams consuming E+ during gestation could potentially be having a hyperactive immune response involving eosinophils similar to mice with asthma allergies. Mice with asthma allergies have exhibited an increased concentration of eosinophils within bronchoalveolar lavage fluid, potentially serving as mediators in airway inflammation (Akuthota et al., 2008; Isobe et al., 2012). The difference in eosinophils between calves born to dams consuming E+ and NE+ during gestation from the current study could possibly be used to predict susceptibly to bovine respiratory disease (BRD). In a receiving trial, cattle with low circulating eosinophil counts were more susceptible to BRD (Richeson et al., 2013). In another receiving trial, cattle that showed clinical signs of BRD also showed decreased eosinophil counts compared to cattle that developed subclinical BRD within the first 28 days upon arrival (Cuevas-Gomez et al., 2020). Although the studies mentioned were not measuring eosinophil counts in response to LPS, the stress of transportation and relocation could provoke a similar stress response as the post-LPS period in the current study. It could be possible that the increased eosinophils in calves born to dams consuming E+ during gestation allow the animal to be better equipped against BRD.

Although steers in the present study had *ad libitum* access to water prior to and throughout challenge, E+ steers exhibited an increased hematocrit percentage relative to NE+

both pre- and post-LPS. Hematocrit percentage can also be referred to as the percentage of red blood cell (RBC) in the blood. Typically, an increased RBC count is the result of dehydration.

In the present study, there was a post-LPS treatment x time interaction on monocyte counts with calves born to dams consuming E+ during gestation exhibiting decreased monocyte counts relative to calves born to dams consuming NE+ during gestation at 0 h and then remained similar between treatment groups. During the pre-LPS period, calves born to dams consuming E+ during gestation did not have significantly lower monocyte counts, but it was numerically lower relative to calves born to dams consuming NE+ during gestation. This could explain why calves born to dams consuming E+ during gestation had a decreased monocyte counts at 0 h during the post-LPS period relative to calves born to dams consuming NE+ during gestation. This contrasts with previous literature where bull calves born to dams exposed to transportation stress had increased monocyte counts prior to the administration of LPS relative to bull calves born to dams not exposed to transportation stress (Littlejohn et al., 2019).

In the present study, there was a post-LPS tendency for a treatment x time interaction on basophil counts with calves born to dams consuming E+ during gestation being increased relative to calves born to dams consuming NE+ during gestation at 4 h and 6 h. Basophils can serve as antigen presenting cells and promote cytokine associated inflammation, as well as the release of histamine in allergic responses (Siracusa et al., 2013). Basophils can also be activated by IL-3 and can promote the release of IL-4 and IL-6 (Siracusa et al., 2013). Basophil-deficient mice that underwent a cecal ligation and puncture model of sepsis, exhibited decreased tumor necrosis factor (Piliponsky et al., 2019). Overall, the basophil-deficient mice exhibited increased morbidity and mortality, suggesting that basophils can enhance the innate immune response (Piliponsky et al., 2019). Because certain leukocytes can stimulate the production of specific

cytokines, the differing concentrations of leukocytes in the current study could impact the circulating cytokine concentrations in calves born to dams consuming E+ and NE+ during gestation. Ongoing analysis will evaluate the cytokine profiles.

In the present study, there was no pre- or post-LPS effect of treatment on cortisol. These results coincide with no differences of cortisol in pigs at pre- and post-weaning that were born to dams exposed to social stress during late gestation and pigs born to dams who were not stressed (Couret et al., 2009). However, these results do contrast with increased cortisol concentrations following the administration of LPS in bull calves born to dams exposed to transportation stress relative to bull calves born to dams not exposed to transportation stress (Littlejohn et al., 2019). There was no post-LPS effect of treatment on time to return to baseline cortisol concentrations; however, calves born to dams consuming E+ during gestation took a numerically longer amount of time to return to baseline cortisol and had numerically decreased concentrations both pre- and post-LPS relative to calves born to dams consuming NE+ during gestation. The hastened time to return to baseline for calves born to dams consuming NE+ during gestation contrasts with pigs of no prenatal treatment returning to baseline concentrations at 12 h following the injection of 0.5 µg/kg of LPS intraperitoneally versus 8 h in the current study (Webel et al., 1997). Cortisol is known to have negative effects on the immune system, but necessary to prevent a hyper-inflammatory state caused by increasing pro-inflammatory cytokines in response to a pathogen (Lefcourt and Elsasser, 1995); therefore, the numerical difference in time to return to baseline in calves born to dams consuming E+ and NE+ during gestation could impact the cytokine concentrations during later time points post-LPS.

CONCLUSION

To our knowledge, this study is among the first to evaluate the influence of pregnant dams grazing toxic fescue on the innate immune response of offspring to an endotoxin challenge. Calves born to dams consuming E+ during gestation had increased hematocrit percentage pre- and post-LPS and eosinophils post-LPS relative to calves born to dams consuming NE+ during gestation, potentially affecting their ability to fight respiratory infections. Calves born to dams consuming E+ during gestation had decreased lymphocytes post-LPS relative to calves born to dams consuming NE+ during gestation, potentially having an altered utilization of glucose in support of lymphocytes. These preliminary data suggest altered innate immune response in offspring born to dams consuming E+ relative to NE+ fescue during gestation, and the difference could affect cattle health and growth rates in stocker and finishing operations. This warrants future evaluations with increased animal numbers.

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TABLES AND FIGURES

Figure 1. Overview of complete project.

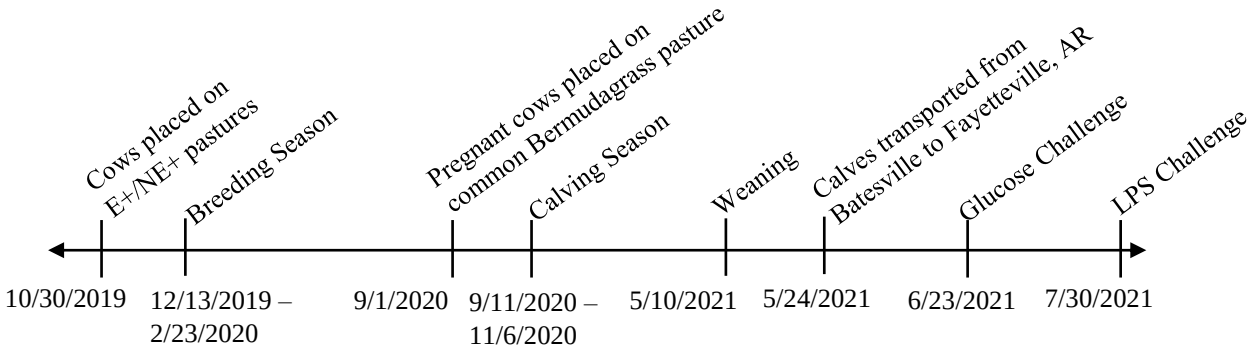


Figure 2. Sampling timeline for blood and data collection.

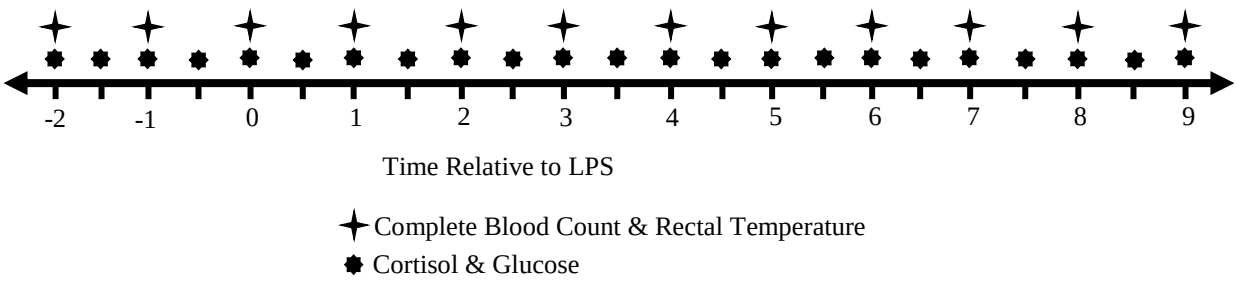
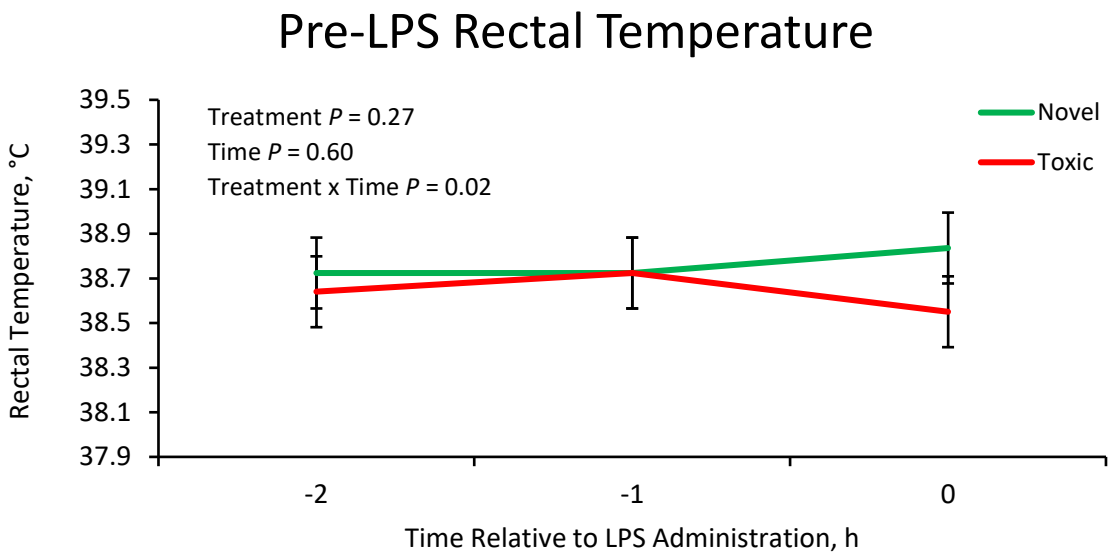


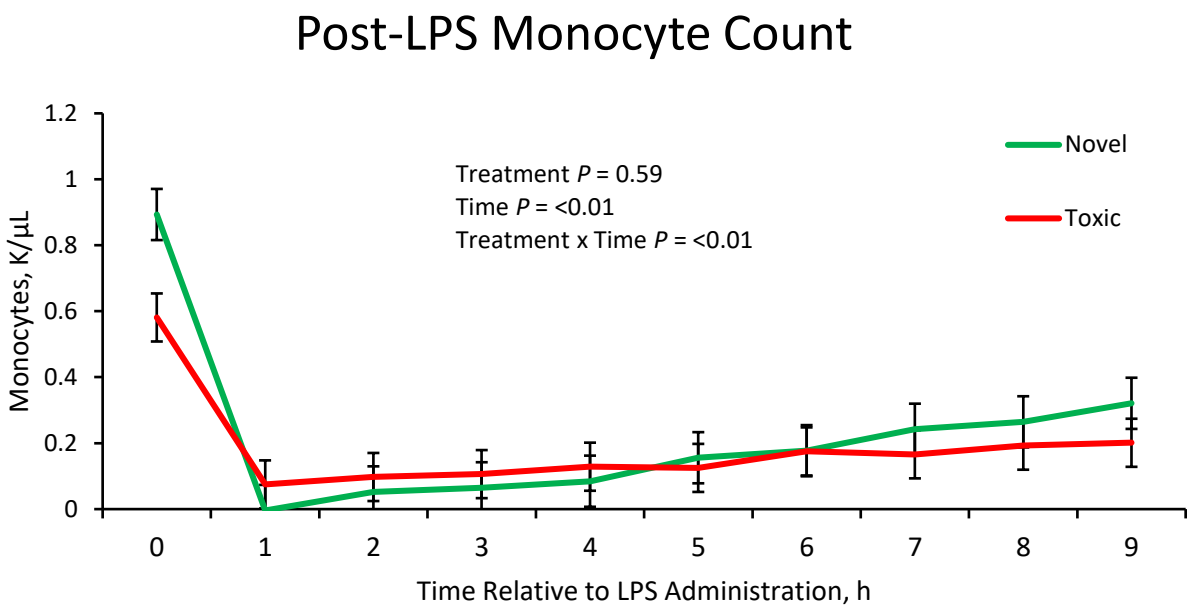
Figure 3. Treatment x time interaction graph of calf rectal temperature during the 2-h period prior to (Pre-LPS) the administration of lipopolysaccharide (LPS) to steer calves born to dams consuming toxic (E+) or novel (NE+) fescue during gestation[a,b].



[a]Data compare calves born to dams consuming E+ or NE+ fescue during gestation and are presented as least squares means ± SE.

[b]Data were analyzed using the MIXED procedures of SAS specific for repeated measures.

Figure 4. Treatment x time interaction graph of the circulating concentrations of monocytes during the 9-h period following (Post-LPS) the administration of lipopolysaccharide (LPS) to steer calves born to dams consuming toxic (E+) or novel (NE+) fescue during gestation[a,b].



[a]Data compare calves born to dams consuming E+ or NE+ fescue during gestation and are presented as least squares means ± SE.

[b]Data were analyzed using the MIXED procedures of SAS specific for repeated measures.

Table 2. Summary of circulating concentrations of cortisol during the 2-h period prior to (Pre-LPS) and the 9-h period following (Post-LPS) the administration of lipopolysaccharide (LPS) to steer calves born to dams consuming toxic (E+) or novel (NE+) fescue during gestation[a,b].

	Treatment (Trt) Means		*P*-value		
Variable	NE+	E+	Trt	Time	Trt x Time
Pre-LPS					
Cortisol, ng/mL	1.56 ± 0.17	1.28 ± 0.17	0.23	0.11	0.29
Post-LPS					
Cortisol, ng/mL	14.43 ± 1.60	13.44 ± 1.48	0.55	<0.01	0.56
Cortisol AUC[c], (ng/mL)/h	119.65 ± 11.52	134.74 ± 11.52	0.38	--	--
Maximum cortisol response concentration, ng/mL	33.21 ± 2.06	30.37 ± 2.06	0.42	--	--
Time at maximum cortisol response concentration, h	2.75 ± 0.33	2.81 ± 0.33	0.90	--	--
Time at return to baseline cortisol, h	8.31 ± 0.48	9.19 ± 0.48	0.22	--	--

[a]Data compare calves born to dams consuming E+ or NE+ fescue during gestation and are presented as least squares means ± SE.

[b]Data were analyzed using the MIXED procedures of SAS specific for repeated measures.

[c]AUC (area under the curve)

Table 3. Summary of circulating concentrations of glucose during the 2-h period prior to (Pre-LPS) and the 9-h period following (Post-LPS) the administration of lipopolysaccharide (LPS) to steer calves born to dams consuming toxic (E+) or novel (NE+) fescue during gestation[a,b]

	Treatment (Trt) Means		*P*-value		
Variable	NE+	E+	Trt	Time	Trt x Time
Pre-LPS					
Glucose, mg/dL	71.31 ± 2.86	71.57 ± 2.86	0.95	0.59	0.47
Post-LPS					
Glucose, mg/dL	61.96 ± 1.77	59.68 ± 1.77	0.36	<0.01	0.85
Glucose AUC[c], (mg/dL)/h	559.64 ± 27.55	535.54 ± 27.55	0.55	--	--
Maximum glucose response concentration, mg/dL	88.66 ± 3.98	94.06 ± 3.98	0.36	--	--
Time at maximum glucose response concentration, h	0.93 ± 0.55	1.02 ± 0.53	0.91	--	--
Time at return to baseline glucose, h	1.69 ± 0.38	1.63 ± 0.38	0.91	--	--

[a]Data compare calves born to dams consuming E+ or NE+ fescue during gestation and are presented as least squares means ± SE.

[b]Data were analyzed using the MIXED procedures of SAS specific for repeated measures.

[c]AUC (area under the curve)

Table 4. Summary of rectal temperature during the 2-h period prior to (Pre-LPS) and the 9-h period following (Post-LPS) the administration of lipopolysaccharide (LPS) to steer calves born to dams consuming toxic (E+) or novel (NE+) fescue during gestation[a,b]

	Treatment (Trt) Means		*P*-value		
Variable	NE+	E+	Trt	Time	Trt x Time
Pre-LPS					
Rectal temperature, °C	38.46 ± 0.14	38.33 ± 0.14	0.27	0.60	0.02
Post-LPS					
Rectal temperature, °C	38.86 ± 0.10	38.84 ± 0.10	0.86	<0.01	0.23
Maximum rectal temperature response, °C	39.62 ± 0.30	39.67 ± 0.28	0.83	--	--
Time at maximum rectal temperature response, °C	3.65 ± 0.28	3.71 ± 0.26	0.85	--	--

[a]Data compare calves born to dams consuming E+ or NE+ fescue during gestation and are presented as least squares means ± SE.

[b]Data were analyzed using the MIXED procedures of SAS specific for repeated measures.

Table 5. Summary of complete blood count during the 2-h period prior to (Pre-LPS) and the 9-h period following (Post-LPS) the administration of lipopolysaccharide (LPS) to steer calves born to dams consuming toxic (E+) or novel (NE+) fescue during gestation[a,b]

	Trt Means		*P*-value		
Variable	Novel	Toxic	Trt	Time	Trt x Time
Pre-LPS					
White Blood Cell, K/μL	8.91 ± 0.71	8.71 ± 0.64	0.81	0.55	0.49
Neutrophils, K/μL	2.71 ± 0.52	3.45 ± 0.49	0.14	0.24	0.86
Lymphocytes, K/μL	5.31 ± 0.33	4.70 ± 0.33	0.21	0.48	0.44
Monocytes, K/μL	0.93 ± 0.14	0.61 ± .012	0.19	0.11	0.29
Eosinophils, K/μL	0.06 ± 0.01	0.05 ± 0.01	0.70	0.30	0.65
Basophils, K/μL	0.019 ± 0.005	0.011 ± 0.004	0.30	0.89	0.70
Lymphocytes:Neutrophils	2.10 ± 0.39	1.48 ± 0.35	0.20	0.10	0.49
Hematocrit %	24.00 ± 0.38	25.30 ± 0.38	0.03	0.09	0.86
Post-LPS					
White Blood Cell, K/μL	3.45 ± 0.44	3.10 ± 0.39	0.56	<0.01	0.28
Neutrophils, K/μL	1.17 ± 0.20	1.25 ± 0.20	0.78	<0.01	0.96
Lymphocytes, K/μL	2.07 ± 0.11	1.65 ± 0.11	0.01	<0.01	0.09
Monocytes, K/μL	0.23 ± 0.07	0.18 ± 0.06	0.59	<0.01	<0.01
Eosinophils, K/μL	0.03 ± 0.01	0.05 ± 0.01	0.05	<0.01	0.15
Basophils, K/μL	0.010 ± 0.003	0.012 ± 0.003	0.67	0.81	0.09
Lymphocytes:Neutrophils	2.86 ± 0.63	3.23 ± 0.70	0.68	<0.01	0.99
Hematocrit %	24.59 ± 0.34	26.63 ± 0.34	<0.01	0.03	0.87

[a]Data compare calves born to dams consuming E+ or NE+ fescue during gestation and are presented as least squares means ± SE.

[b]Data were analyzed using the MIXED procedures of SAS specific for repeated measures.