8-2018

Investigation of Bacterial Taxa as Biomarkers of Fescue Toxicosis and Heat Stress in Grazing Beef Cows

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Investigation of Bacterial Taxa as Biomarkers of Fescue Toxicosis and Heat Stress in Grazing Beef Cows

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Animal Science

by

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Bachelor of Science in Journalism and Electronic Media, 2014

August 2018
University of Arkansas

Thesis is approved for the recommendation to the Graduate Council.

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ABSTRACT

Fescue toxicosis in grazing beef cattle is caused by consumption of Ergot alkaloids associated with tall fescue (toxic fescue) and is responsible for substantial economic loss to the U.S. beef industry. Cattle consuming toxic fescue suffer adverse physiological responses, such as: hyperthermia, increased respiration rate (RR), poor reproduction and growth performance. Other adverse responses to fescue toxins include the retention of a winter hair coat and vasoconstriction in the extremities, which can lead to tissue necrosis. Identifying cattle with reduced susceptibility to fescue toxins would allow for efficient use of fescue pastures; thus, the objectives of this thesis was to: 1) document breed differences in heat and fescue stress phenotypes; and 2) identify fecal bacteria taxa associated with performance on toxic fescue. One hundred crossbred beef cow sired by Charolais or Hereford, and of parities 1, 2, or 3, were allocated to graze toxic tall fescue paddocks (Toxic; n=54) or novel endophyte infected fescue paddocks (Novel; n=46) for 5 months (March to August 2016). Heat and fescue stress phenotypes and fecal samples were collected pre-treatment (March) and post-treatment (August). Cows on toxic fescue had higher ($P < 0.0001$) rectal temperatures (RT), greater systolic blood pressures, and slower hair coat reduction rates (HRR) than cows on novel fescue. Hereford-sired cows had higher ($P < 0.0002$) RT, higher ($P < 0.0001$) HCS, and slower ($P < 0.0001$) HRR than Charolais-sired cows. There were tendencies for sire breed × fescue-type interactions for ADG ($P = 0.06$) and heat accumulation measured by trapezoidal area under of the curve ($P=0.07$). These results indicated Charolais-sired cows had superior tolerance to fescue toxins. Increased abundance of bacterial taxa from *Ruminococcaceae, Lachnospiracceae,* and *Bacteroides* in post-treatment fecal samples were associated with fescue toxin exposure. OTUs from *Rikenellaceae, Clostridiales, Odoribacter*, and *Lachnospiraceae* in pre-treatment samples were correlated with of hair reduction rate and growth performance while

grazing toxic fescue. Breed difference exist in bacterial taxa and may serve as indicators of toxin exposure and performance potential on tall fescue.

ACKNOWLEGEMENTS

I offer a heartfelt thanks to the diving coaches who invested in my growth as an athlete, student, and individual. Thank you to Coach Scott Reich, for encouraging me to face fears and persist in adversity. Thank you to Ted Hautau, for his genuine faith and enthusiasm, which have been a continuing encouragement in recent years. Finally, I owe Coach Dave Parrington, heartfelt thanks for taking a risk on a transfer student and reminding me that the best way forward is to "just keep pluggin' away." The encouragement from these individuals has followed me beyond the pool and carried me through innumerable challenges.

Special acknowledgments are owed to my fellow lab workers, graduate students, and researchers. I am indebted to my mothers in the lab: Laura Meyer, Julie Atchley, Dayanara Ward, and Palika Dias-Morse. Their patient instruction and joyful attitudes were invaluable to my education. Thank you to the Batesville Livestock and Forestry Station farm crew, whose continual support made our research possible. Lastly, I extend thanks to my fellow graduate students, who will be the source of my cherished memories from the University of Arkansas. I am proud to be your classmate and friend.

My family has been a consistent comfort and support through all my endeavors. I am thankful to my mother, who is the source of countless blessings. Thank you to my dad, whose support made my work possible. Both of my parents have all my love and respect. Thank you to my siblings who are an encouragement and inspiration to me.

Thank you to Dr. James Koltes, for his tenacity and enthusiasm for research and allowing me to be part of his work. I am amazed at his dedication and grateful for his welcoming me to research as his first graduate student. The opportunities afforded to me by his mentorship have exceeded anything I could have imagined. I look forward to our continued friendship.

DEDICATION

This Master's thesis is dedicated to Mrs. Shirley Clarke Chewning; a devoted grandmother and model of faith, service, and steadfast love.

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Chapter 1

Review of Literature

Introduction

Tall fescue, *Lolium arundinaceum*, (a.k.a. tall fescue or toxic fescue) is a cool-season perennial forage that feeds about 8.5 million beef cattle in the U. S. Fescue is favored for its high nutrient content and hardiness during drought and heavy grazing. Toxins associated with tall fescue induce fescue toxicosis in livestock, which cost the U.S. beef industry between $600 million and $1 billion annually (Hoveland, 1993; Strickland et al., 2011a). Fescue's ability to withstand heat and frequent defoliation is largely attributable to a symbiotic relationship with a fungal endophyte, *Epichloë coenophiala* (previously *Neotyphodium coenophialum*) (Bacon and Siegel, 1988; Belesky and West, 2009). The fungus resides in the above-ground plant tissues and confers abiotic and biotic stress resistance (Bush and Fannin, 2009). The fungus produces several ergot alkaloids, which confer stress tolerance to the plant but are toxic to grazing livestock. Ergot alkaloid increases susceptibility to heat stress and induces other adverse physiological effects collectively refered to as fescue toxicosis (Porter and Thompson, 1992; Strickland et al., 2011). Documented indicators of fescue toxicosis include hyperthermia, increased RR, decreased serum prolactin, vasoconstriction in the extremities, and growth of a thick hair coat. Fescue toxicosis, in combination with heat stress, contributes to the suppression of growth and reproductive performance (Al-Haidary et al., 2001).

Efforts to improve productivity on fescue have largely focused on limiting toxin exposure. Current strategies require considerable input cost and result in inefficient use of existing fescue pastures (Aiken and Strickland, 2013). Mitigation strategies should be developed which incorporate genetic improvement of cattle to utilize existing fescue resources. Genetic

improvement for fescue toxin resistance would be more efficient with the development of precise, quantitative measures. Current measures which reduce fescue toxicosis lack the affordability, reliability, and resolution to aide selective breeding.

Establishing novel and inexpensive ways to measure fescue toxicosis will enable identification of resilient animals and improved utilization of fescue pastures. Recent developments in microbial DNA sequencing technologies have been utilized as biomarkers for health, growth and related traits in a variety of species and conditions (Jami et al., 2014; Zackular et al., 2014; Levy et al., 2015). Fecal microbial community content may contain reliable quantitative measures of health and productivity of cattle on toxic fescue. Additionally, insight into genetic derived variations in host-microbial cooperation could lead to production strategies to manipulate microbe communities for improved performance on toxic fescue. The objectives of these studies were to: 1) document variation among beef breeds in classical indicators of heat and fescue stress; 2) assess efficacy of bacterial clades (e.g. families, genus, or species) as indicators of fescue toxicosis; 3) report statistical associations between quantitative measures of performance on fescue and bacterial abundance.

Toxic fescue and U.S. beef production

Toxic fescue covers approximately 14.2 million acres of U.S. land and feeds over 8.5 million beef cattle (Hoveland, 1993; Hoveland, 2009). Estimates of the economic loss to the livestock due to fescue toxicosis have been as high as $2 billion each year. This value surpasses the worth of U.S. beef exports to Japan in 2017 (Allen and Segarra, 2001; USDA, 2017). Fescue is competitive in the temperate transition zone of the southeastern U.S., where drought, frost, and heavy grazing eliminate many other forages. The ‘Fescue Belt’ is the region of the U.S., where toxic fescue serves as the primary forage for grazing cattle. Fescue’s heartiness is largely

attributable to a symbiotic relationship with the fungal endophyte, *Epichloë coenophiala*. The fungus improves water retention, root growth, and nitrogen uptake (Bacon et al., 1997; Siegrist et al., 2010). However, the fungus also produces ergot alkaloids, which induce a variety of adverse physiological responses in grazing livestock, including: cattle, sheep, goats, and horses. Symptoms of ergot alkaloids exposure are collectively known as fescue toxicosis (Lyons et al., 1986). Fescue toxicosis in cattle is characterized by suppressed growth, reproductive failure, retention of a thick hair coat, severe vasoconstriction, hyperthermia, endocrine imbalance, and increased susceptibility to disease (Strickland et al., 2009). Unlike other symptoms, vasoconstriction in the extremities remains an overt concern in the winter months and can result in the loss of tails, tips of ears, and even hooves (Cunningham, 1949; Goodman, 1952). Twenty percent of the U.S. beef herd is estimated to be impacted by fescue toxicosis each year (West and Waller, 2007).

Ergot alkaloids and fescue toxicosis

The ergot alkaloids produced by fescue's fungal endophyte are assumed to be causative agents of most fescue toxicosis symptoms in livestock (Guerre, 2015). Ergot alkaloids induce physiological effects due to their ability to interact with biogenic receptors in grazing livestock. Ergot alkaloids contain an ergoline ring structure similar to norepinephrine, epinephrine, serotonin, and dopamine (Klotz et al., 2008, 2012; Bastin et al., 2014). Additionally, ergot alkaloids can act as antagonists, partial agonists, or antagonist at adrenergic receptors (Oliver et al., 1998). Physiological response to alkaloids in livestock vary depending on absorption and combination of ergot alkaloids in question. Ergot alkaloids documented to induce physiological effects in livestock belong to the amides of lysergic acid (lysergic acid, ergonovine, lysergamide), and the ergopeptines alkaloids (ergovaline and ergotamine). Biological outcomes

of primary concern include: reduced growth, vasoconstriction in the extremities, suppressed serum prolactin, and altered smooth muscle contractility. Downstream implication of these responses in cattle will be discussed below.

The toxin profiles of fescue pastures are complex, dynamic and change from year to year. Weather conditions, season, growth stage, genetics, and drought stress play major roles in shaping toxin profiles of forage (Agee and Hill, 1994a; Zhang et al., 2005; Bush and Fannin, 2009; Bengtson et al., 2012). Additionally, application of nitrogen can alter ergot alkaloid composition and induce fescue toxicosis in cattle (Arechavaleta et al., 1992). Bioavailability, absorption, and modulation of ergovaline and other ergot alkaloids in ruminants are still under investigation.

The ergo peptide, ergovaline, has been considered a benchmark for forage toxicity to livestock. Ergovaline comprises between 50-80% of ergot alkaloids abundance in plant tissues (Lyons et al., 1986). Ergovaline forages containing more than 400 ppb (detail abbreviation) ergovaline induce symptoms of toxicosis in cattle. The detection of ergovaline in tissues or fluids of grazing livestock has been unsuccessful, making it a poor benchmark for toxin exposure post-ingestion. Reliable measures of true physiological doses of ergovaline in livestock diets do not exist (Klotz and Nicol, 2016). The difficulty in purifying ergovaline has made *in vivo* studies of this compound on cattle physiology difficult (Ji et al., 2014). *In vitro* studies demonstrate that ergovaline absorption across ruminal and abomasum tissue is minimal compared to lysergic acid and lysergol (Hill et al., 2001). The primary mode of ergovaline absorption is therefore believed to be active transport in the small intestine (Klotz and Nicol, 2016). However, modulation of ergovaline in the forestomach is likely (Coufal-Majewski et al., 2017).

Ergovaline works in conjunction with other ergot alkaloids to induce varying degrees of toxicosis in livestock (Klotz et al., 2008). Ergot alkaloid such as lysergic acid, lysergol, ergonovine, ergotamine, and ergocryptine absorption are capable of absorption over most regions of the intestinal epithelia, but the absorption rates vary between ergot alkaloids and the digestive tissue (Nimmerfall and Rosenthaler, 1976; Hill, 2008). An *in vitro* study demonstrated that ergovaline declines after incubation in rumen fluid, while lysergic acid increased 12-fold over two days (Ayers et al., 2009). Ergovaline disappearance was associated with the appearance of lysergic acid. Ergovaline modulation to lysergic acid was supported was by an *in vivo* study in lambs in which only 35% of dietary ergovaline was recovered in the feces from sheep fed fescue seeds, while 148% of lysergic acid was recovered (De Lorme et al., 2007). The authors suggested ergovaline and other alkaloids are modulated into lysergic acid by digestive microbes. Ruminal microbe transformation of ergot alkaloids was demonstrated by feeding undigested or rumen digested fescue seed to rats (Westendorf et al., 1992). The incubation of the endophyte infected fescue seed in rumen fluid reduced toxicity to mice and restored growth performance (Westendorf et al., 1992). Specific rumen bacteria capable of metabolizing tryptophan, a structural component of ergot alkaloids, were identified and can be cultured (Aiken and Strickland, 2013). Once in circulation, modulation of ergot alkaloids takes place in the liver (Rosenkrans et al., 2015). The modulation of Bromocriptin, a synthetic alkaloid and dopaminergic receptor agonist, in the liver has been extensively documented (Maurer et al., 1982).

Reactions to ergot alkaloids exposure in livestock are largely dependent on ambient temperatures. Fescue toxins and high ambient temperatures work in conjunction to suppress growth, milk production, fertility, and health. Indicators of fescue toxicosis often confounded with

heat stress include: increased rectal temperatures (RT), increased respiration rates (RR), reduced average daily gain (ADG), and suppressed prolactin levels (Fuquay, 1981; Settivari et al., 2008; Strickland et al., 2009). The best symptoms for differentiating fescue stress from heat stress are hair shedding scores, and vasoconstiriction in the extremities (Klotz et al., 2016; Mayberry et al., 2017). These indicators cannot provide the reliable, quantitative information required for genetic improvement in toxin resistance.

Mitigation strategies and biomarkers

Current fescue toxicosis mitigation strategies rely on limiting cattle exposure to fescue toxins. Strategies for limiting toxin exposure include: moving animals, diluting pastures with clover and other legumes, replanting fescue with low toxin cultivars, or limiting access to fescue seed head. (Lomas et al., 1999; Drewnoski et al., 2009; Kallenbach, 2015). Novel (non-toxic) fescue cultivars have been developed by reinfection of toxic fescue seed with low-toxin producing endophytes (Bouton et al., 2002). These cultivars benefit from stress resistance conferred by the endophyte and have comparable nutrient content to toxic fescue with vastly reduced toxin load (Drewnoski et al., 2009).

Novel endophyte fescue cultivars improve animal performance, but fail to optimize use of existing pastures. Removing animals from fescue pasture requires additional labor, time, expense, and availability of alternative forage (Browning, 2004). Many producers lack access to these resources. Exchanging toxic fescue pastures for low-toxin cultivars may take several years to achieve a return on investment (Beck et al., 2008). Contamination on novel endophyte pastures with the natural symbiont endophyte remains a risk (Siegel et al., 1984).

Identifying cattle with improved performance on toxic fescue would improve use of existing fescue pastures. Genetic tolerance for heat and fescue stress have been identified among

cattle breeds and sire lines within breeds (Rolf, 2015; Smith and Cassady, 2015). Productivity of cattle types under varying environmental heat conditions are documented (Haile-Mariam et al., 2008). Genetic selection for fescue toxin resistance in mice improved litter size, and ADG in mice given diets containing endophyte-infected fescue seed (Wagner et al., 2000). Likewise, genetic variation in heat tolerance and response to fescue toxins have been documented by comparing *Bos Taurus* vs *Bos Indicus* breeds of cattle (Browning, 2004; Rolf, 2015; Smith and Cassady, 2015). To achieve efficient genetic improvement in fescue resistant, reliable and quantitative measures and biomarkers should be identified. Difficulty in developing affordable, quantitative measures of fescue stress stems from confounding heat stress and fescue toxicosis symptoms.

Physiology of fescue toxicosis

Physiological indicators of fescue toxicosis in ruminants will be reviewed in the context of animal physiology. In human medicine, ergot alkaloids are widely administered for control and interruption of pituitary hormone signaling (Muller et al., 1978). Synthetic alkaloids such as bromocriptine have found medical uses in treatment of migraines, post-partum bleeding, hyperlactemia, and Parkinson's disease for their ability to alter hormonal and bionergic signaling (Schiff, 2006). In cattle fescue, consumption suppresses serum prolactin, induce vasoconstriction in smooth muscle, and cause metabolic shifts related to reduced nutrient absorption. Ergot alkaloids' physiological actions in ruminants have consequences for male and female reproductive efficiency, growth and metabolism, lactation, hair shedding, blood flow and thermoregulation will be discussed. The limitations in utilizing these symptoms as reliable selection tools will be illustrated.

Reproduction

Reproductive failure is among the leading causes of loss on fescue (Pratt et al., 2015). Male and female fertility and reproductive quality traits including sperm quality, conception rates, and maintenance of pregnancy are adversely effected by fescue exposure. These losses may be attributable to a combination of physiological challenges associated with fescue including: alteration in blood flow, heat stress, endocrine alterations. Several of these physiological factors, detrimental to reproduction, are exacerbated by or work in combination with heat stress.

Bull reproductive performance is altered by exposure to fescue toxins. Semen concentration and conformation was negatively affected by bulls grazing toxic fescue (Pratt et al., 2015). Sperm of bulls on toxic fescue had a decreased semen freezing potential after 155 days on fescue pastures (Schuenemann et al., 2005; Looper et al., 2010). Sperm from bulls fed diets containing 40 μg/kg body weight of ergotamine tartrate were suspected to have reduced fertilization potential and slower motility (Stowe et al., 2013). Bull scrotal circumferences were negatively affected by exposures to endophyte infected tall fescue seed (Klotz, 2015). The causes of these anti-quality factors in bulls may be attributable to the interruption of prolactin signaling in the testes or reduced blood flow to the testes (Auburn University. Agricultural Experiment Station. et al., 1986; Schuenemann et al., 2005).

Conception rates of cows grazing toxic fescue are considerably suppressed (Allen and Segarra, 2001). One study found that conception rates on toxic fescue were 55% compared to 96% for cows grazed on endophyte free diets (Porter and Thompson, 1992). On average, conception rates in beef cows on toxic fescue were reported to be 60% (Burke and Rorie, 2002; Seals et al., 2005). Risk of embryotic loss on fescue is greatest between ovulation and the first week of pregnancy (Burke et al., 2001; Looper et al., 2010; Klotz, 2015). Heat and toxin stress work together to impair follicle development in cows reducing estradiol and prolactin (Burke and Rorie,

2002). Experimental challenge with ergo peptides reduced luteinizing hormone, indicating that hormones associated with reproduction and cyclicity may be suppressed on fescue. Proposed explanations for failure to conceive include: lowered serum progesterone, poor blood flow, and compounded effects of heat stress (Browning et al., 2000).

Conception and reproductive performance lack the resolution to define gradients of toxin resistance for two reasons. First, conception and maintenance of pregnancy are binary indicators of fescue stress resistance. If a cow fails to conceive on toxic fescue, her fitness is poor and the cost to feed her would exceed her ability to bring in cash by producing a calf yearly. However, culling based on conception does not capture underlying gradients of fescue resistance. Secondly, heat and fescue stress work in combination to determine fitness. Heat and fescue stress have independent and interactive effects on growth and metabolism and endocrine and biometric signaling. Therefore, reproductive success on toxic fescue may not provide information for individual robustness on fescue.

Growth and metabolism

Despite toxic fescue's high nutrient content, growth and animal performance on toxic fescue is poor compared to alternative low toxin forages, especially in the summer months (McCracken et al., 1993). Several biochemical signaling interruptions work together to suppress growth and productivity on fescue. Ergot alkaloid consumption interrupts hormonal signaling, alters digestive tract motility and blood flow, reduces feeding behavior, and negatively impacts forage digestibility. Independent and interactive effects of fescue toxins and heat stress impact growth performance. Growth measures alone lack the resolution for reliable selection on fescue.

Experimental challenge of livestock with purified ergot alkaloids induce hormonal signals associated with catabolism. However, dietary challenges with fescue seed have yielded

inconsistent results for catabolic hormones, such as thyroid hormones and insulin. Experimental challenge of cattle with eropeptide, ergotamine, increased cortisol, and triiodothyronine, and additionally after one hour ergot administration, serum insulin was decreased while serum glucagon increased (Browning Jr. and Thompson, 2002). Browning and Thompson et al. 2002 noted that breed differences exist between Brahman and Hereford steers in catabolic hormone levels in response to ergotamine challenge (Browning and Thompson, 2002). Browning also proposed that that alteration in reproductive hormonal signals may be attributable to ergotamine interaction with alpha-adrenergic receptors (Browning et al., 2001). Adrenergic signaling induces mobilization of fat stores. A decrease in serum insulin was reported in steers provided toxin-free or ergot alkaloid-containing diets (Harmon et al., 1991; Aldrich et al., 1993; Al-Haidary et al., 2001b). In other experiments, dietary ergot alkaloids did not alter catabolic hormones (Looper et al., 2010). Cows grazing on endophyte infected fescue showed suppression in anabolic proteins such as insulin-like growth factor 1(IGF-I) (Collier et al., 2008). The failure to identify alterations in catabolic hormones on fescue diets in these experiments may be due to small sample size, small biological dose or modulation of ergot alkaloids.

Heat stress in the absence of toxic fescue can induce similar catabolic signaling in livestock. Catabolic activities are well documented under heat stress conditions. When energy expenditure is needed need to cope with elevated ambient temperatures, similar metabolic shifts are observed in heat stressed cattle (Johnson et al., 2015). Extended periods above thermoneutral direct metabolic resources away from growth and muscle development to reduce heat exposures (Rhodes et al., 1991a). This alteration in metabolism by heat stress were compounded by the actions of ergot alkaloids (Spiers et al., 2005).

Reduced growth and nutrient absorption may be attributable to alterations in digestive tract blood flow. Diets containing high-endophyte concentrations suppressed blood flow to the duodenum and colon of steers and sheep compared to those provided low-endophyte diets (Rhodes et al., 1991b). *In vitro*, ergovaline had potent vasoconstrictive effects on the ruminal vein and artery (Foote et al., 2013). Constriction of blood to these tissues may reduce blood flow in the rumen. The addition of tall fescue seed to diet reduced blood flow to the absorptive surface of the rumen in both steers and sheep (Mote et al., 2017). Reduced blood vessels may reduce flow to these absorptive tissues and likely contributes to decreased nutrient uptake.

Additional digestive disruption may be due to decreased digestive track motility, or fat accumulation. In sheep, reticulorumen contraction became less frequent after challenge with ergot alkaloids (McLeay and Smith, 2006). The contractile tension in isolation of rat colon was found to be altered by ergotamine *in vitro* (Dalziel et al., 2013). In sheep, tonality of the rumen and reticulum was elevated and frequency of reticulum contractions were reduced by the administration of ergotamine (Poole et al., 2009). Consumption of toxic fescue leads to difficulty in digestion and fat necrosis in the intestine (McLeay and Smith, 2006). Fat accumulation in the abdominal cavity of cattle was a problem common among cattle grazing fescue fertilized with large amounts of nitrogen fertilizer (Stuedemann et al., 1985). The accumulation of abnormal abdominal fat has been documented to interfere with passage of digestia in cattle (Williams et al., 1969).

Alteration in grazing behavior may also contribute to poor growth on fescue. Feed intake may be reduced due to decrease in rumen motility, less time spent grazing and more time seeking cooling. Animals on toxic fescue spend more time standing, loafing or seeking cooling (Schmidt and Osborn, 1993; Browning, 2004). Cattle have been documented spending more time seeking

cooling and less time grazing. Feed intake is reduced for animals offered diets containing endophyte toxins, and the reduction is especially evident under heat stress (Aldrich et al., 1993a; Settivari et al., 2008;Eisemann et al., 2014). In the field, frequency of grazing for steers on toxic fescue was negatively correlated with ambient temperatures (Mcclanahan et al., 2008a).

The presence of fescue's endophyte and ergot alkaloids are speculated to reduce digestibility of forage. Reduced gains may be attributable to the decreased digestibly of organic compounds in the presence of ergot alkaloids. Digestibility of forage decreases with increasing amounts of toxins (Coufal-Majewski et al., 2017). Outside of the rumen, the presence of the fungi has been documented to slow decomposition of organic matter (Lemons et al., 2005) suggesting the capability of the toxins to demonstrate anti-microbial activities. Though the interaction of the fungus with rumen microbes is poorly understood, several studies indicated the presence of the endophyte itself, regardless of the presence of ergot alkaloids, in slow fescue decomposition rates (Looper et al., 2010; Procedures, 2010; Pratt et al., 2015). The presence of the endophyte itself decreases the decomposition of organic and dry matter (Siegrist et al., 2010). The digestibility of cellulose decreased for sheep in the presence of endophyte infected seed and caused a reduction in feed intake (Hannah et al., 1990)**.** In sheep, when dry matter intake was controlled with rumen cannulation feeding, the presence of the fungal endophyte reduced digestion of dry matter, neutral detergent fiber and acid detergent fiber (Fiorito et al., 1991)**.**

Growth can serve as an indicator of robustness on fescue, however several confounding factors inhibit utilizing this measure as a response to toxin robustness alone. Firstly, poor growth on fescue pastures is often confounded with heat stress condition. If cattle are selected for growth on toxic fescue in heat stress, this may not translate to a robust performance on toxic fescue in cool weather. Parallel selection based on growth or under heat and fescue stress may not translate to

robust performance under heat stress in the absence of toxic fescue. Metabolic shifts and reduction in feed intake are parallel symptoms of heat stress. Poor weight gain may be the result of a complex interaction to fescue toxins on animal physiology and intake behavior. Altered feeding behavior make determining resistance to toxin load among individuals difficult. Due to the uncertainty which surrounds biological doses of alkaloids, direct measures of growth to toxin ingestion cannot be calculated.

Suppressed prolactin

Prolactin is one of several pituitary hormones suppressed after absorption of ergot alkaloids. Suppressed serum prolactin is a trade mark of fescue toxin exposure, and is especially evident under heat stress (Hurley et al., 1980; Strickland et al., 2009; Li et al., 2017). Ergot alkaloids interact with D2 dopamine receptors of the anterior pituitary to induced vasoconstriction and suppress release of prolactin (Li et al., 2017). The suppression of serum prolactin is thought to occur by inhibiting transcription of prolactin in pituitary cells (Schillo et al., 1988; Aiken et al., 2011). The cessation of prolactin is accompanied by suppressed milk production (Lean, 2001). Lactation likely contributes to 40% of that variation in beef calf weights at 205 days of age (Lubritz et al., 1989). Milk yield may also be negatively affected by reduced nutrient intake, heat intolerance, and/or other hormonal interruptions associated with fescue (Lean, 2001). Other probable consequences of suppressed prolactin in cattle include: poor reproduction, reduced hair shedding, and abnormal hair growth.

While prolactin suppression is a reliable indicator of toxin exposure, it lacks the resolution for identifying gradients response to toxin stress. Serum prolactin measures are quantitative in nature, but single time point observations are largely binary (high or low) on fescue. Additionally, prolactin suppression in cattle depends on ambient temperature (Hurley et al., 1980). At higher

ambient temperatures, prolactin suppression become more severe under fescue challenge. Using prolactin levels as reliable and quantitative measures for selection would require costly, repeated sampling under controlled environmental conditions (Porter and Thompson, 1992).

Hair shedding

In cattle, hormonal signals keep hair growth and hair shedding behavior linked to the photoperiod. Cattle hair coats thicken in short day seasons and shed hair coats during long day seasons (Turner and Schleger, 1960). The interruption of the natural thickening and shedding of hair coats is a notable indicator of fescue stress. Cattle are expected to have slick (or shed) coats in mid-summer and grow winter hair coat as day length shortens. However, cattle exposed to toxic fescue, grow long hair coats even in long-day periods.

Mechanisms by which entophyte toxins interrupt the hair shedding cycle have been speculated, but are yet to be defined. Evidence suggests that several pituitary hormones associated with seasonal behavior are interrupted by consumption of ergot alkaloids. Thick summer hair coats on fescue were determined to be comprised of new hair grown abnormally long during long-day photo period. Prolactin signaling has been linked to seasonal control of the hair growth cycle in cattle and other ruminants (Larson et al., 1995; Mayberry et al., 2017). Fescue toxins disrupt prolactin signaling, which may be responsible for prolonged hair growth. Ergot alkaloids contain an indole ring capable of interacting with other seasonal hormone receptors such as serotonin and dopamine receptors-D2 receptors (Larson et al., 1995). Serotonin is a precursor for dopamine which is responsible for many photoperiod regulated traits, such as prolactin release, reproductive cyclicity, and hair growth (A Steak in Genomics^{TM}, 2016). Thyroid hormones are also responsible for shedding and growth of cashmere goats. In rats the daily rate of hair elongations is halted by

estrogens and stimulated by thyroid hormone thyroxine (Stowe et al., 2013). These may also contribute to the long dull coat induced by fescue toxicosis, as both prolactin and thyroid hormones can be altered by ergot alkaloid exposure (Celi et al., 2003).

Cattle hair coats (length and thicknesses) are relevant indicators of growth even in the absence of fescue exposures. Since the early descriptions of hair coat score (HCS) in cattle, hair coat thickness was associated with growth performance in cattle (Turner and Schleger, 1960). Heat tolerance has been linked to the slick hair coat mutation in the prolactin receptor gene (Littlejohn et al., 2014). The relationship between growth performance and hair shedding have been previously reported (Dikmen et al., 2014). Sex hormones such as estrogen and testosterone also influence the length, growth and thickness in several mammals, and may contribute to severity of hair coat growth and susceptibility to heat stress.

Hair scoring systems have been developed to quantify individual variation in hair shedding and growth behavior. The initial scoring system developed by Turner and Schleger (1960) was created to describe the percent of coat shed in a 7-level scoring system. This system was eventually adapted to a 5-level scoring system at Mississippi State University. This system differentiates shedding percent on a 1 to 5 scale (slick, no winter coat to a full winter coat during the summer, respectively)(Tor-Agbidye et al., 2001). These systems are useful, but subject to bias by the scorers' consistency and expectations. Turner and Schleger et al. 1960, noted that the hair coat is constantly in flux, indicating that time in point measures should be carefully considered. Because hair coats are constantly changing, some dates may have the potential to indicate breed differences more so than other dates in the year. For example, in March, most animals are expected to have a full winter coat regardless of prolactin gene. The scoring system has been utilized to quantify hair shedding behavior on toxic fescue.

Several techniques have been attempted to estimate heritability, reduce bias, and maximize individual variation. Previous studies have accounted for likelihood of judger bias by group individuals based on the high, medium or low scores. Grouping animals minimizes effects of scorer bias and can serve as a blocking factor in experiments. Gray et al. (2011) utilized the 5-level system to document individual variation based on date of first shed. Animals were grouped based on the month their hair coat first appeared to be three by judger. This technique reduced the difficulty in distinguishing between hair scores 4 and 5 (Gray et al., 2011). This scoring system was used as a new grouping variable called 'month of first shed'. Calves of cows exhibiting earlier shed had heavier calves. Barman influenced cattle showed faster hair shed than cattle of European decent. Estimates for heritability of month of first shed was proposed to be $h^2 = 0.35$ (Gray et al., 2011).

Blood flow and vascular control

Unlike other symptoms of fescue toxicosis, alterations in blood flow are not confounded with the effects of heat stress and remain a relevant concern in the winter months. Alterations in blood flow are thought be caused by interaction on ergot alkaloids with serotonin receptors in smooth muscle. These contractions and dilation in the vascular system have implications for heat dissipation, blood pressure, heart rate, and nutrient absorption (Rhodes et al., 1991; Strickland et al., 2009). Vasoconstriction in the extremities is a notable indicator of fescue toxin exposure. Vasoconstriction in response to toxin exposure can yield tenderness in the hoof region, lead to lameness, or tissue necrosis in the ears and tails (Dyer, 1993). Livestock may lose the tail switch, ends of nose or ears (Thompson, 1993). In extreme, but not uncommon circumstances, cattle will become lame and slough hooves due to poor blood flow. This condition is referred to as fescue foot (Cunningham, 1949). The loss of hooves may require culling of an individual from the

population. Decreased blood flow to the skin has been confirmed by measuring blood flow and body heat of the skin. In combination with freezing ambient temperatures, vasoconstrictions in the extremities can lead to necrosis of the tips of ears, tails, and hooves. (Cunningham, 1949). The ergot alkaloids interact with the α2-adrenergic receptor and induce alterations in blood flow dynamics (Oliver et al., 1998). The reduction of blood flow was not associated with increased blood flow to other tissues, rather total cardiac output of cattle on toxic fescue is likely to be reduced (Rhodes et al., 1991a). Changes in blood flow were documented to occur independent of the feed intake (Finch, 1985).

The vasoconstrictive effects of ergot alkaloids have been documented in several tissues *in vitro*. Ergovaline is a potent vasoconstriction agent and likely acts on 5-hydroxytryptamine receptors **(**5-HT2) serotonin receptors (Klotz et al., 2007). Ergotamine, another ego peptide, is commonly used in humans for the treatment of migraines, however side effects of intake beyond the recommended dose are vasoconstriction in the extremities. The potent vasoconstrictive abilities of ergovaline have been demonstrated *in vitro* in bovine lateral saphenous veins (Eisemann et al., 2014). *In vivo,* constriction of the tail vein has been measured by Doppler ultra sound after challenge with ergot alkaloids. Recent studies demonstrate the toxins may have lingering effects on the vascular system. It may take several weeks after being removed from toxic pasture for normal vasodilation to return. Cattle need 4-5 weeks to recover proper vascular control after grazing on toxic fescue. (Klotz et al., 2016). Exposure to ergot alkaloids are also responsible for thickening of smooth muscle vascular tissues (Eisemann et al., 2014). The thickening of muscular vascular tissue along with prolonged constrictive effects of ergot alkaloids, such as ergovaline and ergotamine, are likely the source of downstream consequences in heat dissipation.

Doppler ultrasound blood flow measurements have been suggested as a means of quantifying vasoconstriction in the caudal tail vein, but this technology is too costly for use in the field, and only addresses one of the many physiological effects of fescue toxicosis. Alteration in heart rates and blood pressure have also been measured after toxin exposures (Koontz et al., 2012). Differences in these measures are often undetectable under thermoneutral conditions. However, under heat stress, fescue impacted both of these measurements (Strickland et al., 1996).

Thermoregulation and heat stress

Heat dissipation is controlled in the anterior hypothalamus. Increasing body temperatures should suppress sympathetic activity, in skin blood vessels, allowing for heat dissipation by radiation and convection (Renaudeau et al., 2012). Hyperthermia in fescue stressed cattle is a primary health and productivity concern. The primary ways in which toxin stress exacerbates heat stress are though the alterations in blood flow to skin and skeletal muscles, metabolic alterations, and the retention of the winter hair coat. Increased rectal temperature is one possible indicator of heat stress, which can occur at lower ambient temperatures in animals grazing toxic fescue than those grazing non-toxic forage. Under lipopolysaccharide (LPS) challenge, steers fed toxic fescue seed had higher rates of elevated body temperature compared to steers on a toxin-free diet. (Al-Haidary et al., 2001).

Several factors may contribute to hyperthermia in cattle after absorption of fescue toxins. First, reduced blood flow to the skin and skeletal muscle is a primary inhibitor of heat dissipation. The increase in core body temperature and stability of skin temperature illustrates the inefficiency of heat dissipation. Heat transfer from the body primarily occurs via convection or evaporation from the skin. Only 15% of body heat will be dissipated by the ? tract in cattle under heat stressed conditions (Dikmen et al., 2012). High-endophyte diets challenged by weather and steers

demonstrate the blood flow in the skin covering the ribs (Rhodes et al., 1991b). After challenge with ergopeptines, core body temperature in sheep spiked while the skin temperatures decrease (Rhodes et al., 1991). As previously reviewed, interruption of hormonal signaling results in unseasonal hair growth on toxic fescue. The retention of long hair inhibits heat transfer from the skin and sweating (Mcclanahan et al., 2008b). Increases in core body temperature are correlated with high respiration rates, and skin temperature (Eisemann et al., 2014). These have been confirmed in the context of fescue toxin stress. Injection of ergotamine increased respiration rate in heifers and steers (Browning Jr. and Thompson, 2002). Increase in core body temperature is associated with increased sweating and panting (Finch, 1986). Both physiological responses require energy for growth and robustness.

Selection for lower rectal temperatures may be possible; however it may not be beneficial. The heritability for rectal temperature in dairy cows was estimated to be 0.17 +/- 0.13 (Dikmen et al., 2012). However, higher rectal temperatures were negatively correlated with important economic traits, such as milk production, which would make selection for this trait detrimental (Westendorf et al., 1992). Reliable measurements for rectal temperature in beef cow would be influenced by environmental instability. For instance, bringing in grazing beef cows from the field increases body temperature beyond what is typical in the field. Standing long periods before sampling, and or usage of pond and shade may skew interpretation of rectal temperatures for selection.

The microbiome as a phenotype and health indication

Microbial communities have gained unprecedented attention for their role in host health with the development of new DNA sequencing technologies. The microbial communities inhabiting the digest tract of mammals have been deemed a necessary organ that contributes to

health and development (Malmuthuge et al., 2015). The composition of the gut microbiota has been associated with a variety of health and robustness measures in humans and livestock species. Analysis of the microbial communities in cattle grazing toxic fescue holds promise for three primary reasons. First, gut microbe-host genetic interaction may hold novel information regarding control of microbial communities that can lead to selective breeding practices. Secondly, bacterial composition could serve as an indicator of future performance on fescue. Lastly, the growing attention of probiotic development in livestock may lead to potential candidates for probiotic mitigation of fescue stress.

First, microbes may help us understand host genetic control and cooperate with gut microbe communities. Host genetic control of microbial population may illuminate breed differences in microbe-host relationships (Levy et al., 2015; Roehe et al., 2016). The microbiome may be heritable in multiple species, including cattle. The heritability of microorganisms through mothers is well documented in many eukaryotic organisms including nematodes, insects, mice, and even humans. Maternal transition of bacteria in cattle may play a major role in immune function and digestive health throughout life and may also impact performance on toxic fescue. Ley (2005) postulates that inheritance of the gut microbiota in mice can occur from the immediate family (Ley et al., 2005).

Secondly, study of the microbial communities may serve as biomarkers for fescue tolerance or future performance on fescue pastures. Gut community composition has already been used as indicators of mammalian health status including inflammatory bowel disease, Crohn's, diabetes, and obesity (Turnbaugh et al., 2006; Frank et al., 2007; Willing and Van Kessel, 2010). Current understanding of microbial communities indicate that host organisms maintain a natural balance of microbial organisms that dictate heath and performance. Bacterial modulation of toxins in the

gastro intestinal tract has implication for severity of host response to toxin exposure (Smith, 1992; Zhang et al., 2013) The microbiome of livestock has also been associated with other growth and stress traits in livestock (McCormack et al., 2017). Identifying dysbiosis, in these microbial communities inhabiting livestock, could serve as health predictors.

Lastly, a growing body of research suggests that microbial communities can be improve and or modulated with pro- or pre-biotic treatments (Fuller, 1989; Saarela et al., 2000). The use of probiotics in the livestock industry is an area of growing research, as the market seeks to reduce reliance on anti-microbial growth promotors. The bacterial composition of the rumen is a highly diverse niche important for deriving nutrients from forages, and detoxification of harmful compounds. Modulation of ergot alkaloids by gastrointestinal microbes has been documented (Westendorf et al., 1992; Harlow et al., 2017). The bacteria, in the gut of ruminants, is capable of detoxifying many plant compounds and holds promise for fescue robustness information (Smith, 1992; Wallace, 2008). Probiotics may be used to modulate fescue toxicosis (Yeoman and White, 2014; Uyeno et al., 2015). I*n vitro* studies demonstrate that the rumen and abdomen show minimal capability of absorbing ergovaline (Chen et al., 2011; McCann et al., 2014).

The objectives of this thesis were to: 1) quantify breed differences in documented measures of heat and fescue stress in two common beef breeds; and 2) assess efficacy of gut microbial communities as novel measures of heat and fescue stress.

Chapter 2

Analysis of heat and fescue stress phenotypes in crossbred beef cows

Abstract

Cattle grazing toxic fescue suffer adverse physiological effects, costing the US beef industry an estimated $1 billion annually. Symptoms of fescue toxicosis include retention of a thick hair coat, vasoconstriction in the extremities, hyperthermia, increased respiration rate (RR), decreased feed intake, suppressed serum prolactin, and subsequent suppression of growth and reproductive performance. Several of these symptoms are exacerbated by heat stress. Current measures of fescue toxicosis, including: hair coat score (HCS), rectal temperature (RT), and fescue foot (hoof loss), lack the resolution needed to capture genetic variation of fescue toxin resistance. Thus, the objectives of this study were to: 1) evaluate breed differences among Charolais-sired cows and Hereford-sired cows for recognized indicators of heat stress and fescue toxicosis; and 2) assess the efficacy of hair reduction rate (HRR) and serial vaginal temperatures as novel quantitative measures of fescue tolerance. A total of 100 crossbred fall-calving Charolais- or Hereford-sired cows of parities 1-3 were allocated to graze either toxic fescue paddocks (Toxic: n=54) or novel endophyte (low-toxin) tall fescue paddocks (Novel: n=46) for 5 months. Documented measures of heat and fescue stress were recorded in March (low heat stress) and at the end of the trial, in August (high heat stress). Hair coat scores (HCS), body weight (BW), and body condition scores (BCS) were recorded monthly. Toxic fescue increased RT ($P < 0.0002$), systolic blood pressure ($P = 0.04$), HCS ($P < 0.001$), slowed HRR ($P < 0.0001$), and decreased BW gain ($P < 0.0001$). Charolais-sired cows sired cows exhibited lower ($P = 0.0017$) HCS, faster ($P = 0.0004$) HRR, and lower ($P < 0.0001$) RT than Hereford-sired cows. There was a tendency ($P = 0.07$) for a breed × fescue-type interaction for daily heat accumulation

measured by trapezoidal area under the temperature curve (AUC). There was a tendency (P = 0.06) for a breed × fescue-type interactions in ADG, where Charolais-sired cows gained more weight on toxic fescue than Hereford-sired cows. Impacts of toxic fescue or novel fescue (low-toxin) pastures on fescue toxicosis symptoms are documented and breed differences may exist in related phenotypes. This study documents the impact of fescue toxicosis in cattle exposed to toxic vs. novel fescue and indicates breed differences may exist in related phenotypes.

Introduction

Toxic fescue [*Lolium arundinaceum* (Schreb.) Darbysh.] feeds over 8.5 million cattle in the US, and is a staple forage in the southeastern transition zone, known as the fescue belt (Hoveland, 1993). Toxic fescue's heartiness is largely attributable to a fungal endophyte (*Epichloë coenophiala)* that confers resistance to drought and heavy grazing (Hill et al., 1991). The fungus produces ergot alkaloids, which interact with biogenic amine receptors in herbivores (Klotz et al., 2012; Foote et al., 2013). Ingestion and absorption of ergot alkaloids induce a variety of adverse physiological effects, collectively referred to as fescue toxicosis (Strickland et al., 2009). Recognized indicators of fescue toxicosis include retention of a thick hair coat, suppressed serum prolactin, vasoconstriction in the extremities, and reduced feed intake and nutrient absorption (Strickland et al., 2009; Strickland et al., 2011). These symptoms work in combination with heat stress to suppress growth, conception rates, milk yield, and inhibit thermoregulation (Aldrich et al., 1993b; Al-Haidary et al., 2001a).

Attempts to mitigate fescue losses have centered around limiting exposure to toxic pastures by replanting with low toxin cultivars, pasture dilution, or removal of cattle from toxic pastures (Drewnoski et al., 2009; Jackson et al., 2015). Replanting fescue is costly and time consuming; thus selective breeding of cattle for robust performance on toxic fescue would

improve utilization of existing pastures in beef cattle operations. Genetic variation in thermoregulation and robustness traits on fescue have been documented (Cole et al., 1989; Browning, 2000; Smith and Cassady, 2015; Lees et al., 2018); however, current measures of fescue toxicosis symptoms lack the resolution to capture underlying genetic variation in heat and fescue toxicosis tolerance. Several documented indicators of fescue toxicosis are either confounded with heat stress (i.e., rectal temperature (RT), respiration rate (RR), ADG), subjective in nature (i.e., hair coat score (HCS) and body condition score (BCS)), currently lack reliable estimates of genetic variation (development of fescue foot, suppressed serum prolactin, or pregnancy status) (Larson et al., 1996; Foote et al., 2012; Stowe et al., 2013; Eisemann et al., 2014; Shoup et al., 2016), or not practically implemented by producers (vasoconstriction measures by Doppler ultrasound)(Aiken and Flythe, 2014). Individual variation exists in the severity of fescue toxicosis indicators, both on toxic and non-toxic forage (i.e. hair coat scores and body temperatures). Quantifying the severity of fescue toxicosis by phenotyping all symptoms simultaneously is impractical in commercial settings. Investigation of new indicator traits for fescue toxicosis would allow for practical standards of toxic fescue tolerance in cattle. Documentation of genetic variation in multiple fescue toxicosis traits will provide context for identification of higher resolution indicators for fescue stress resistance in cattle. Comparison of these fescue toxicosis indicator traits within and across breeds will determine if sufficient genetic variation exists to allow selection for tolerance or robust phenotypic performance on toxic fescue pastures.

The objectives of this study were to: 1) document breed differences between Charolais- and Hereford-sired crossbred, cows for documented indicators of heat stress and fescue

toxicosis; and 2) assess the efficacy of hair reduction rate (HRR) and serial vaginal temperatures as indicators of fescue tolerance.

Material and Methods

Cows on this study were housed at University of Arkansas Division of Agriculture Livestock and Forestry Research Station in Batesville, AR and handled in accordance with the regulations of the University of Arkansas Institute for Animal Care and Use Committee (IACUC) under protocol number 16037.

Animals and Experimental Design

A total of 100 crossbred fall-calving cows were included in a five-month (March to August) grazing trial in 2016. Cows were distributed by breed (n= 61 and 39 for Charolais-sired and Hereford-sired cows respectively), and parity (1st, 2nd, or 3rd) to graze either toxic fescue or novel (low-toxin) fescue pastures for 127 days (March 20 to August 5, 2016). Cows were bred by AI and subsequent natural service in December 2015. A total of 81 cows were confirmed pregnant and open cows remained on the grazing trial. Cows grazed on non-toxic pastures for at least four months prior to the initiation of the trial. The pasture management system included weekly rotations at a stocking rate of (0.5 acre/cow). Animals on the toxic fescue-type grazed rotationally on one of two pasture pairs. Each pasture was divided into two 12 to 13-acre toxic paddock (Toxic, n=54). One paddock in each pasture contained a pond, resulting in pond access every other week for each cow. Likewise, novel fescue-type cows grazed rotationally on one of two novel (low-toxin) fescue pasture pairs (four paddocks). Pastures were tested monthly for ergovaline (University of Missouri Veterinary Diagnostic Lab). One novel pasture was contaminated with toxic fescue (> 300 ppb ergovaline), which resulted in exposure of 20 individuals to toxic fescue two weeks each month. These individuals were analyzed with the

Novel fescue condition, because statistical differences were not detected for most phenotypes. Two major sampling and phenotyping events were performed on days 0 (March; pre-treatment and lower ambient temperatures) and 127 (August; post treatment and elevated ambient temperatures). Measurements collected pre- and post-treatment included: RT, RR, BW, BCS, and HCS; whereas blood pressure and blood serum for minerals were collected only at post-treatment phenotyping. A serial vaginal temperature collection was conducted between days 120 and 127 of the trial, and HCS, BW, and BCS were recorded at least once a month on days – 20, 0, 29, 55, 84, 112, 121, and 127. Serial vaginal temperatures are not reported for the 20 cows on the contaminated pasture treatment.

Phenotyping Technique

Blood pressure was collected using an Omoron® digital wrist blood pressure monitor and wrist cuff (Omron Healthcare Co., Ltd: Muko, Kyoto). Blood pressure was measured with the wrist cuff secured around the coccygeal vessels. Serum of mineral analysis were collected from the jugular vein and analyzed University of Arkansas veterinary diagnostic lab. Body weights from day 0 and day 127 were used to calculate ADG and percent BW increase over the trial (BIF, 2018). Serial vaginal temperatures were collected using iButton DS1922L thermosensors (Digi-Key, Thief River Falls, MN) embedded in progesterone-free controlled internal drug release devices (CIDRs) (Zoetis Parsippany-Troy, NJ). The temperature collection protocol was programed using the OneWireViewer program (Maxim Integrated San Jose, CA). Thermosensors were labeled with an animal identification code, synchronized to computer clock, and programed to collect temperatures every five minutes at a resolution of ± 0.5 ^{o}C. Sensors were secured in CIDR by slicing the silicone casing at the center groove of the CIDR and embedding the sensor in the CIDR. The sensor was secured with electrical tape and groves were

sealed with a clear, waterproof silicone sealant (LOCTITE 2.7-oz Specialty Adhesive). CIDRS were placed intravaginal on day 121 and removed on day 127.

Temperature recorded from 5 days were analyzed to remove the effects handling stress. Summary statistics of serial temperatures were analyzed, including: average, variance, maximum, minimum , and daily area under the temperature curve (AUC) for vaginal temperatures. Daily vaginal temperature AUC estimates were calculated using a trapezoidal method of area under the curve, where the rectangular base represented the change in time in hours and the height was the difference in maximum and minimum vaginal temperatures (^{o}C); and the resulting statistic is reported in ^{o}C × h and represents an individual's accumulated "heat load" over time. Five daily AUC ^{o}C × h measures were average for an individual's average daily ^{o}C × h AUC measurement. Hair coat scores (HCS) were designated as described by Grey et al., 2011. The scores were adjusted using a sliding window average to capture underling trends in hair growth. A sliding average is calculated such that each observation is equal to the average of the original observation, the original observation preceding, and the original observation following. The rate of hair reduction (HRR) over the trial was estimated using the hair scores (after sliding average) on days 0 and 121.

$$\text{Hair Reduction Rate} = \left(\frac{ajusted\ HCS\ on\ Day\ 112 - ajusted\ HCS\ on\ Day\ 0}{112\ days}\right)$$

Statistical Analysis

All models were fit using PROC GLIMMIX or PROC MIXED for repeated measures in SAS (version 9.4; SAS Inst., Inc., Carry, NC). The threshold for significance was considered ($P < 0.05$) and tenancies were considered ($P < 0.1$). Models for single record quantitative variables were analyzed in PROC GLIMIX using a base model of fescue-type + sire breed + parity + pregnant + fescue-type × sire breed. Fescue-type × sire breed × pond interactions were

considered when appropriate. Repeated measures for hourly vaginal temperature were analyzed using PROC MIXED of SAS using the model fescue-type + sire breed + parity + pregnant + hour + date + fescue-type × sire breed + fescue-type × sire breed × hour. For the serial vaginal temperature analysis, the 20 cows on novels pastured exposed to fescue toxins were excluded from the analysis; resulting in three treatment conditions (toxic with a pond during temperature collection, novel with a pond during temperature collection, or toxic with no pond during temperatures collection). Individual cow was treated as the experimental unit in each model. HCS was modeled in PROC GLIMMIX with a multinomial distribution the model HCS = fescue-type + sire breed. HSCs were also analyzed as a repeated measure to evaluated breed difference over time using the model HCS = fescue-type + sire breed + parity + pregnant + date + fescue-type ×sire breed + fescue-type × sire breed × date.

Results

Exposure to toxic fescue induced symptoms of fescue toxicosis (**Table 1**). Rectal temperatures were approximately 0.42 °C higher ($P = 0.0002$) on toxic fescue than on novel (low-toxin) fescue. Cows on toxic fescue had greater ($P < 0.0001$) HCS, less ($P < 0.0001$) BW gain, and slower ($P < 0.0004$) HRR. Cows on novel fescue gained approximately 0.53 kg/day more ($P < 0.0001$) than cows on toxic fescue. Fescue-type did not influence vaginal temperature mean, range, or maximum. However, cows on novel fescue had greater ($P =0.003$) vaginal temperature variance. Cows on toxic fescue tended to have lower ($P = 0.09$) serum potassium and lower ($P=0.10$) BCS than those on novel fescue. Fescue-type × sire breed interactions were not significant for any of the phenotypes recorded, however there were tendencies ($P > 0.05$) for interactive effects of Fescue-type × sire breed for hair coat scores recorded at the end of the trial

(August), average daily gain over the trail period (ADG, kg/d), and the daily accumulation of degree area under the curve degree × hours (daily AUC, ^{o}C×h).

Main effects of sire breed are reported in **Table 2**. At pre-treatment sampling, Hereford- and Charolais-sired cows had similar (P = 0.22) RT and similar (P = 0.70) RR; however, at post-treatment, Hereford-sired cows had RT approximately 0.58 ^{o}C warmer (P < 0.0001) than Charolais-sired cows. Moreover, Hereford-sired cows had greater mean vaginal temperatures (P = 0.002), larger ranges of vaginal temperatures (P = 0.0006), and greater vaginal temperature variance (P = 0.003) than Charolais-sired cows. Hereford-sired cows tended to have lower (P = 0.09) concentrations of serum potassium than Charolais-sired cows. Hereford-sired cows had higher HCS at summer solstice (P < 0.0001) and at trial's end (P = 0.05). Charolais-sired cows had a faster (P < 0.0004) HRR over the trial, and increased BW more (P < 0.0001) than Hereford-sired cows.

There were tendencies for fescue-type × sire breed interactions for HCS in August, ADG and average daily AUC (**Table 3**). Hereford-sired cows had higher (P < 0.05) hair coat scores at the end of the trial than Charolais-sired cows, regardless or fescue-type. However, Hereford-sired HCS were significantly different across fescue-types, while Charolais-sired cows were not different across fescue type (**Figure 1A**). Differences in ADG were not different by sire breed on novel fescue. However, on toxic fescue, Charolais-sired cows gained significantly more (P <0.05) than Hereford-sired cows (**Figure 1B**). On toxic fescue, daily AUC (^{o}C × h) were not different by sire breed (P < 0.05). However, on novel fescue, Hereford-sired cows had significantly more (P < 0.05) heat accumulation in degree × hours (^{o}C × h) than Charolais-sired cows (**Figure 1C**).

Cows were rotated weekly; resulting in pond access for half of the cows the last week of the trial. Several fescue-type × sire breed × pond interactions were observed for phenotypic indicators of heat stress and fescue toxicosis (**Table 4**). A three-way interaction for RR was observed (**Figure 2**). Charolais and Hereford sired cows had similar respiration rates across treatment when no pond was available. However, when a pond was available, breed differences were statistically significant across treatments. On novel (low-toxin) pasture with pond access, Charolais-sired cows had lower ($P < 0.05$) RR than Herefords. On toxic pastures with pond access, respiration rates were greater ($P < 0.05$) than on toxic pastures with no pond access for both breeds. Similarly, interactions were observed in mineral and electrolytes associated with heat and sweating and feed intake including: P, Ca, Mg, Fe, and, Cu (**Table 4**). For cows with pond availability in the final week of the trial, RT was approximately 0.86 °C higher ($P < 0.0001$) than cows with no pond access (**Table 5**). Cows with pond access had greater ($P \leq 0.05$) serum K and greater ($P < 0.02$) serum Zn than those with no pond access. Cattle with pond access also had smaller ($P < 0.02$) ranges of vaginal temperature, smaller ($P = 0.01$) vaginal temperature variance, and higher ($P = 0.02$) vaginal temperature maximums than cattle without a pond.

At summer solstice, HCS was significantly different depending on fescue-type ($P = 0.009$) and no evidence of a difference was observed by sire breed ($P = 0.12$) (**Figure 3**). About 65.0% of novel individuals exhibited a slick hair coat (HCS =1), while only 7.0% individuals on toxic pastures were considered slick. HCS at trial's end tended to depend ($P = 0.10$) of fescue-type, but dependent ($P = 0.031$) on sire breed (**Figure 4**). At the end of the trial, 63.0% of Charolais-sired cows on toxic pasture were considered slicked while only 26.0% of Hereford-sired cows exhibited slick hair coats. Based on the repeated measures analysis of HCS, a fescue-

type × breed × date interaction was observed. Least square means for the fescue-type × sire breed × date for HCS are provided in **Figure 5**. The average HCS across time points for cows on novel pasture was 2.7 units, while the average hair score for toxic pasture was 3.5 ($P < 0.0001$). Hereford-sired cows were approximately 0.5 units higher in hair score ($P < 0.0001$) than Charolais-sired cows across time points. Sire breed differences were not detectable, except at the last three sampling dates on toxic fescue ($P < 0.05$) (**Table 6**).

Sliding window averages in HCS resulted in 13 unique rates of hair reduction values over the course of the trial. Cows on toxic fescue had reduced shedding of 0.01 HCS units × day^{-1} compared to cows on novel (low-toxin) fescue (**Table 1**). Charolais-sired cows had reduced shedding of 0.005 HCS units × day^{-1} faster ($P < 0.02$) than Hereford-sired cows (**Table 2**). Individual variation in HRR by fescue-type and sire breed is represented in **Figure 6**. Hair reduction rate had a linear association with ADG for cows on both novel ($r = -0.32$, $P = 0.03$) and toxic fescue ($r = -0.65$, $P < 0.0001$) types (**Figure 7**). The negative correlation between HRR and ADG indicated that the faster the reduction rate in HCS, the higher the ADG over the trial (**Figure 7**).

Repeated measures analysis of serial vaginal temperature had a significant ($P < 0.0001$) three-way interaction between fescue-type × breed × hour. Least square means (LSM) for breed differences over time on three pond-fescue-type combination are reported in Figures **8A**, **8B**, and **8C**. On a novel pasture with pond access, Hereford-sired cows maintained vaginal temperatures 0.39 ^{o}C warmer ($P < 0.0001$) than Charolais-sired cows regardless of the time of day. On average, Hereford-sired cows reached peak vaginal temperature between 1700 and 1900h, while Charolais-sired cows reached peak vaginal temperature between 1800 and 2000h. On average, Hereford-sired cows reached their minimum vaginal temperature between 500 and 700h, while

Charolais-sired cows their minimum vaginal temperature between 700 and 900h. On toxic pasture without pond access, Hereford-sired cows maintained a vaginal temperature 0.30 ± 0.018 ^{o}C warmer than Charolais-sired cows, regardless of the time of day. Both breeds minimum vaginal temperature occurred between 800 and 1900h and had peak temperatures between 600 and 2000h. On toxic pastures with pond access, Charolais-sired cows stayed 0.07 ± 0.018 ^{o}C cooler than Hereford-sired cows throughout the day. Breed differences in vaginal temperature did not exist between 400 and 1600h. From 1700 to 300h, Charolais-sired cows exhibited higher body temperatures than Hereford-sired cows. Temperatures measures of vaginal temperature probes were statistically significant covariates in modeling average daily gain. (**Table 9**). Vaginal temperatures measures were added to the base model as covariates. The p-values indicated that each variable significantly accounted for variation in ADG and outperformed traditional measures such as RT and RR. **Table 7** provides Pearson correlations among indicators of heat stress and fescue toxicosis.

Discussion

Breed differences existed in these phenotypes such that Hereford-sired cows appeared to suffer from toxin exposure more so than Charolais. Regardless of fescue-type, Hereford-sired cows had higher rectal temperatures and higher average vaginal temperature recordings. Hereford-sired cows had higher hair coat scores at the end of the trial, and at summer solstice than Charolais-sired cows. Hereford-sired cows also exhibited less relative growth, as measures by percent body mass increase, and poorer overall growth as measured by average daily gain. Vaginal temperatures from Hereford-sired cows also had higher maximums, larger ranges, and greater variance in body temperatures than Charolais-sired cows. Tendencies for fescue-type x sire breed interactions in ADG indicates that Charolais may maintain better gain on Toxic fescue

pastures. Variation exist in the rate at which cattle shed their hair throughout the summer. The rate of hair shed is dependent on breed, and fescue-type.

Pond availability during the last week of the trial altered phenotypes related to heat stress and fescue toxicosis measured at trial's end (August). Significant three way interactions were observed for several serum minerals related to heat stress (i.e. phosphorous, calcium)(Bouchama et al., 1991; Shieh et al., 1995). Lowered serum copper levels in Herefords-sired cows on toxic pasture with pond access may be associated with reduced feed intake (Stewart et al., 2010). Pond availability increased reparation rates, especially for Hereford-sired cows. While Hereford-sired cows had higher body temperatures than Charolais-sired cows as demonstrated by rectal temperature and average vaginal temperature, this relationship was reversed as measured by vaginal temperatures probes on toxic fescue with pond access. The marked reduction in vaginal temperature in Herford-sired cows during the evening hours is speculated to be due to more average pond usage than that of Charolais-sired cows. As respiration rates were higher in cattle with pond access, the marked increase in respiration rates in Hereford-sired cows on toxic fescue with a pond supports this speculation. Hereford likely maintain higher reparation rates and rectal temperatures when the opportunity to cool is not available. When pond cooling is available, Herefords-sired cows are more likely to engage in cooling behavior. The marked increase in rectal temperatures with pond access should be considered in management prior to timed AI, especially on toxic fescue. Time spent in the pond may be negatively correlated with time spent grazing.

The ability to detect sire breed and fescue-type differences in HCS is sensitive to the date of scoring. Single date observation for hair coat score are not only subject to judger error, but are also variable based on photoperiod and associated hormonal signaling. Because HRR is a

summary of several HCS observation over time, it minimizes impact of judger bias, while providing higher resolution for capturing individual variation. HRR rate correlated with ADG on novel fescue and was the variable most highly correlated with ADG on toxic fescue. Correlations of HRR with ADG, RT, RR, Fe, Cu, ACU, vaginal temperature mean on toxic fescue, indicated that the faster a cow decreases hair coat over the summer, the better growth, higher the feed intake, and the lower the body temperature is likely to be.

Limitations exist in the interpretation of serial vaginal temperatures in the context of the pond. While temperature probes were waterproof sealed, it is unknown how accurate vaginal temperatures probes reflect vaginal temperature while cows stand in ponds. As Herefords are typically smaller framed than Charolais, it is possible that there was more water exposure to vaginal probes in Hereford-sired cows than in Charolais-sired cows. While serial vaginal temperature measurements and HRR provided higher resolution, hair reduction rate calculation requires repeated collection of HCS thought out the summer to maximize information gleaned and temperature probe are expensive and likely not useful on most cattle operations

Chapter 3

Fecal bacterial taxa associated with fescue toxin exposure and robustness

Abstract

Toxins associated with tall fescue grass cause fescue toxicosis in beef cattle, which cost the U.S. beef industry approximately $1 billion dollars annually. Symptoms of fescue toxicosis include elevated body temperatures, growth of a thick hair coat, vasoconstriction in the extremities, and increased susceptibility to heat stress. The objectives of this study are to identify bacterial clades indicative of toxic fescue exposure and resistance. The V4 region of 16s rRNA genes from fecal samples of 99 fall-calving crossbred cows grazing toxic fescue (Toxic, n=54) or low toxin novel fescue pastures (Novel, n=45) collected at the beginning (March) or end (August) of a five-month grazing trial were examined. Operational taxonomic units (OTUs) were constructed at 97% similarity using the Mothur MiSeq SOP. Toxic fescue increased abundances of OTUs from *Bacteroides* and *Coriobacteriaceae* and decreased abundance of *Mogibacterium* and unidentified bacteria. Several OTUs from *Lachnospiraceae* and *Ruminococcaceae* were negatively correlated with body weight (BW) gain, and positively correlated with rectal temperatures (RT) and the rate of hair shedding. Abundance of a bacterial OTU from *Odoribacter,* in pre-treatment samples, was negatively correlated with BW gain (r= -0.44, P = 0.001) and the rate of hair shedding (r =0.40, P = 0.003). Bacterial OTUs may indicate the robustness toxic fescue. This study identified several potential targets for product development.

Introduction

Toxic fescue [*Lolium arundinaceum* (Schreb.) Darbysh.] is a stress-resistant pasture grass grazed by approximately 8.5 million cattle in the southeastern United States (Hoveland, 1993). Despite fescue's quality nutritive content, it is known to result in poor performance of grazing

beef cattle (Burns, 2009). Toxic fescue lives symbiotically with a fungal endophyte (*Epichloë coenophiala*), which improves plant performance but produces ergot alkaloids (Arechavaleta et al., 1992). Ingestion and absorption of ergot alkaloids (i.e. ergovaline) by cattle induces symptoms including: hyperthermia, increased respiration rate, hormonal imbalance, maintenance of a thick hair coat, and vasoconstriction in the extremities; collectively known as fescue toxicosis. These symptoms exacerbate the effects of heat stress on milking ability, growth, reproduction, nutrition absorption, and overall health and productivity (Browning, 2004; Roberts et al., 2005; Johnson et al., 2015). Even though ergovaline is used as the benchmark for forage toxicity, its modulation and absorption in ruminants is poorly understood (Agee and Hill, 1994). Current evidence suggests that ergovaline absorption is minimal over the foregut epithelium of ruminants and is likely absorbed in the small intestine or transformed to other alkaloids (Hill et al., 2001; Ayers et al., 2009; Foote et al., 2013). Replanting toxic fescue pastures with novel endophyte fescue can improve animal performance, but is impractical for many producers (Beck et al., 2008; Drewnoski et al., 2009). Identifying variation in fescue stress resistance among livestock would allow for more efficient use of fescue pastures. Current measures, such as hair coat scores (HCS), rectal temperature (RT), and body weight (BW) lack the resolution and reliability needed to understand individual response to fescue toxins. Establishing new measures of toxin exposure and severity of response to fescue toxins would improve selection.

Bacterial communities are sensitive to their host environment and capable of modulating toxins in ruminants (Mao et al., 2012; Weimer, 2015; Leng, 2017). Community measures of microbiota in the hind gut niches have been associated with health and productivity in a variety of animal species (Turnbaugh et al., 2006; Mao et al., 2012; McCormack et al., 2014). These communities have been associated with the ability to glean nutrients from diet and ward-off

detrimental infection. Random forest allows for the identification of specific hindgut bacterial clades capable of predicting biological outcomes (Calle et al., 2011; Bento et al., 2013; Zackular et al., 2014). Thus, the objectives of this study were to: (i) characterize the hindgut microbial communities of crossbred beef cows grazing on toxic or novel fescue; and (ii) identify specific bacterial taxa predictive of fescue toxin exposure and performance on fescue grass.

Materials and methods

All animal handling and experiments were performed in accordance with approved guidelines and regulations of the University of Arkansas Institute for Animal Care and Use Committee (IACUC) under protocol number 16037. The study was conducted at the University of Arkansas Division of Agriculture Livestock and Forestry Research Station in Batesville, AR.

Study population and experimental design

The study population consisted of 100 fall-calving crossbred beef cows distributed to graze either toxic fescue pasture (Toxic; n=54) or low-toxin novel endophyte fescue (Novel; n=46). Pastures consisted of four 12 to 13-ha^2 paddock in each pair. Allocation and pasture management was performed as described in Chapter 2. Twenty cows on novel fescue pastures were exposed to pasture contaminated with toxic fescue. This group was considered an alternating treatment (T+N, n=21). Only the 54 animals on toxic fescue were used for identification of bacterial OTUs indicative of robustness on toxic fescue.

Sample collection and phenotyping

Fecal samples, heat stress indicators, and fescue toxicosis measures were collected pre-toxin exposures in cool weather (March) and post-toxin exposures in hot weather conditions (August). Description of phenotypic collection was described previously (Chewning et al., 2018; Chapter 2). Briefly, body weight (BW), hair coat score (HCS) (1=slick summer coat to 5=full,

thick winter coat) (A Steak in Genomics™, 2016), and body condition scores (BCS) were monitored monthly. Hair reduction rate (HRR) over the trial was used as a quantitative summary of hair shedding behavior on tall fescue (Chewning et al., 2018; Chapter 2). Phenotypes collected at the beginning (March) and the end (August) of the grazing trial included: respiration rate (RR), rectal temperature (RT) and body weight (BW) as described previously (Chewning et al., 2018; Chapter 2). Fecal samples were collected with a nitrile glove by fecal grab technique, placed in sterile Whirl-pak® bags (Nasco, Ft. Atkinson, WI), transported to the laboratory on ice and subsequently stored at -20 ^{o}C until DNA isolation.

DNA isolation and sequencing

Bacterial DNA sequence reads were merged, filtered, annotated, and quantified using the Miseq Mothur SOP (https://www.mothur.org/wiki/MiSeq_SOP) as described by the Schloss lab group (Kozich et al., 2013). Sequences were screened for maximum length of 275 bps, and sequences containing ambiguous bases were removed. Sequence noise was reduced using a pre-cluster technique (Huse et al., 2010), where sequences were grouped by similarity and ranked by abundance of reads. Chimeras were flagged and removed using the UCHIME v4.2.40 algorithm (Edgar et al., 2011). Operational taxonomic units (OTUs) were defined at 97% similarity of the V4 region, and classified to the genus level using the Bayesian method against the RDP database. All samples were standardized to the lowest read number found in a single sample by subsampling. Thus, 2,609 reads per sample were used to calculate alpha and beta diversity estimates. Jaccard and Bray-Curtis distance matrices were calculated to examine changes in fecal microbiome community membership and structure over time and between treatments.

Statistical analysis

Treatment difference in bacterial abundances were quantified by models constructed PROC GLIMMIX of SAS (version 9.4: SAS Institute, Cary, NC.) with fixed effects of fescue-type + pond + breed + parity + pregnant + fescue-type × breed. Treatment differences in diversity measures over time were quantified using the model above with the addition date and date × fescue-type interactions were considered when appropriate. Person correlations were constructed in PROC CORR of SAS (version 9.4: SAS Institute, Cary, NC.).

Random forest analysis

Classification and regression random forest was used to identify variables predictive of toxin exposure and quantitative phenotypes associated with fescue toxicosis, respectively. The Random Forest algorithm was executed in R version 3.2.4 using random Forest 4.6-12 (Bento et al., 2013). Two random forest models were used to predict toxin exposure using fecal samples collected at post-treatment (August). Twenty-five individuals were randomly selected from the Toxic and Novel treatment groups. Variables included in the first classification random forest included the 2,000 most abundant OTUs, whereas the second model included the 2,000 most abundant OTUs and three quantitative measures previously associated with toxic fescue (rectal temperature (RT), percent weight gain (%BW), hair reduction rate (HRR)) (Chewning et.al. 2018). The algorism ranked variables based on mean decrease accuracy in treatment prediction with 10,000 trees. Any variables with a negative mean decrease accuracy (detrimental to out-of-bag error rate) were removed, and remaining variables were submitted for re-ranking by random forest. Variable elimination and re-ranking were repeated until all variables improved the out of bag error rate (OOB).

Regression random forests were performed using the 54 cows from toxic treatment to identify the most predicative variables for quantitative measures of heat and fescue stress on toxic fescue. RT, % BW gain, and HRR were modeled for the cows in the Toxic group using the relative abundance of the 2,000 most abundant OTUs alone or with experimental fixed effects (fescue-type, pond, sire breed, pregnant, parity), and quantitative variables associated with heat and fescue stress (% BW gain, HRR, RR, RT, serum phosphorous [P], magnesium [Mg], and sodium [Na]) (Chewning, Ch 2). Variable were ranked by percent increase in mean square error (%IncMSE) based on 10,000 trees. Variables with negative increase in means square error (%IncMSE) were removed and remaining variables were submitted for re-ranking. This variable removal and re-ranking was repeated until each variable had a positive%IncMSE.

Results

Sequences and quality

All samples were subsampled to 2609 sequence to match lowest number of reads. One sample only contained 7 reads and was excluded from analysis, thus 18,536 unique OTUs were identified.

Alpha and beta diversity over time

Shannon diversity of gut microbiomes was greater (P = 0.0395) in the Toxic than the Novel treatment, but the T+N treatment was not different from Toxic (P = 0.4755) or Novel (P = 0.6462) (**Figure9a**). Treatment tended (P =0 .083) to affect the number of OTU in fecal samples. At the post treatment time point, cows grazed on Toxic pasture had more (p= 0.0026) OTUs in August than the Novel treatment condition; however, Toxic animals did not differ (p= 0.9892) from the Alternating treatment (T+N) for observed OTUs (**Figure 9b**). Number of observed OTUs in the T+N condition were greater (P = 0.0392) than that of the Novel fescue condition.

There was a significant sire breed x time point effect ($P < 0.0001$) such that sire breed differences were detected pre-treatment, but not post treatment (**Figure 10**) in which Herefords had more diverse communities before the trial. Beta diversity of fecal samples at the post-treatment time point was not notably divergent based on treatment (**Figure 12** & **Table 10**). Beta diversity overtime, as measured by Bray-Curtis, showed significant community structure separation over time, but not by treatment **(Figure 13 & Table 11**). Despite the distinct clustering over time, behavior in community shifts appeared similar regardless of treatment.

Bacterial taxa indicative of toxin exposure

Classification random forest identified 115 variables that improved prediction of toxin exposure (Toxic v.s. Novel treatment) in fecal samples collected post-treatment (August). The 25 OTUs with the highest mean decrease accuracy for treatment prediction are reported in **Table 12a**. The top performers, in treatment prediction identified by classification random forest with the inclusion of indicators of heat and fescue stress (RT, weight gain, and Hair reduction rate), are reported in **Table 12b**. Traditional measures of RT and weight gain had large MDAs; confirming their efficacy in treatment prediction. When bacterial taxa and phenotypic indicators were included, RT was the top predictor of toxin exposure, followed by 8 bacterial OTUs and BW gain. Despite inclusion or exclusion of phenotypic indicators, several OTUs from *Bacteriodes*, *Clostridales*, *Ruminococcaceae* and *Flavobateriaces* were in the top 25 predictors for toxin exposure. *Coriobacteriaceae* and *Mogibacterium* were helpful in the absence of RT, growth or hair shedding information. Several OTUs were differentially abundant by treatment (**Figure 14**). OTUs from *Bacteroides*, *and Coriobacteriaceae* increased in cows grazing on toxic fescue. Alternatively, an OTU from *Mogibacterium* and OTU 395 (unidentified) were statistically less abundant in cows on toxic fescue.

Post-treatment taxa associated with performance on fescue

Regression random forest identified several OTUs indicative of quantitative indicators of fescue stress on toxic fescue. **Table 13** summarizes the OTUs of post-treatment fecal samples most predictive of phenotypic indicators. **Table 14** contextualizes performance of bacterial abundance information against documented measures of heat and fescue stress. In the absence of associated phenotypic indicators and fixed effects, the top indicators of weight gain among post treatment samples was an OTU176 from *Streptococcus*. *Blautia* was the best predictor for hair shedding behavior, and the 3rd best preforming bacteria in the prediction of August RTs. *Ruminococcacea, Clostridiales, Lachnospiracia* were among the top 25 predictors for all three phenotypes. Hair reduction rate was the best phenotypic indicator of weight gain among cows on toxic treatment, however this variable was outperformed in the prediction of weight gain by 4 OTUs. Serum P, RR, an unclassified bacterium, and access to a pond were the best indicators of RT in August. The OTU691 from *Blautia* remained the best predictor of HRR, followed by BW gain and OTU from *Lanospiracceae*. OTU176 from *Streptococcus* was the predictor most indicative of BW gain, regardless of inclusion of fixed effects.

Table 15 reports Person correlations and associated P-values among OTUs and RT, BW gain and HRR, respectively. Abundance of two OTUs from *Lachnospiraceae* were negatively correlated with growth performance over the trial. Cows with the greatest abundance OTU 710 *Lachnospiracea* in August had the poorest BW gain over the trial. *Lachnospiracea* was positively correlated with RT. Cows with the highest enrichment in several OTU's from this family had the lowest gain on toxic fescue. Similarly, an OTU from *Ruminococcus* and an unidentified bacterium of OTU651 had significant negative correlation with gain. Breed differences were observed in several taxa indicative of performance (Figure 9). Hereford-sired

cows had fecal community's enrichment for several taxa negatively associated with favorable performance on fescue.

Pre-treatment taxa associated with performance on fescue

Fecal taxa identified in pre-treatment samples were also indicative of cow performance on toxic fescue over the trial. **Table 16** reports that best performing OTUs in the prediction of heat stress and fescue toxicosis by regression ransom forest for weight gain, rectal temperature and hair reduction rate. An OTUs from *Odoribacter* was the second best bacterial predictor of hair reduction on fescue and the top predictor of weight gain on toxic fescue (Table 16). *Lachnospiracceae, Clostridiales, Ruminococcus,* and *Coriobacteriaceae* were in the top three predicators for all three outcomes. Pearson's correlations among OTUs in pretreatment samples and indicators of fescue toxicosis and heat stress are reported in **Table 17**.

Discussion

The composition of the hind gut microbiome of pregnant cows grazing on toxic fescue contribute to the understanding of fescue pasture on the composition of the hind gut community. Shifts in the microbiome over time were similar regardless of treatment type. The shifts in microbial community overtime may be attributable to shifts in pasture nutrient availability, the effects of pregnancy hormones, and/or ambient environmental temperatures (McCracken et al., 1993). Minor differences were found in the community structure of fecal bacterial communities based on Bray-Curtis distance matrixes; yet obvious clustering by toxic and novel fescue were not observed. This indicates that the community structure measures may not have the resolution to capture the underlying diversity contributing to health and resilience phenotypes on fescue. Some differences in community diversity were observed between treatments; notably, diversity was greater for the toxic treatment conditions. There are two probable explanations for this

difference. As documented in the corresponding study, pond usage increased when cows grazed toxic fescue, and the models confirmed that pond access was also associated with an increase of fecal microbiota diversity. It is possible that pond usage introduced more OTUs into the fecal microbiome, without shifting overall community structure. These findings are consistent with those of Li et al. 2012, who demonstrated that community structure measures such as Shannon and Bray-Curtis, may lack the resolution to assess performance and robustness in livestock.

Classification and regression random forest machine learning methodology identified taxa most indicative of toxic fescue exposures from Percent weight gain, HRR and RT. These were confirmed as top indicators of toxic fescue exposure. Bacterial OTUs from *Ruminococcaceae*, *Lachnospiraceae*, and others, may be greatly altered by toxic fescue exposure. *Odoribacter* **(**of *Bacteroidetes*) was a dominant taxa inhabiting the cecum and fecal matter of pre-weaned dairy calves when fed untreated waste milk (Deng et al., 2017). This taxa was depleted in vitamin D receptor knock out mice (Jin et al., 2015). In the treatment of constipation in mice, the reduction of *Odoribacter* and several *Bacteriodetes* were associated with constipation relief (Wang et al., 2017). *Odoribacter* is butyrate-producing genus that was found to be inversely correlated with systolic blood pressures in early pregnancy women (Gomez-Arango et al., 2016). This relationship was attributed to the lowering of serum plasminogen activator inhibitor-1 when *Odoribacter* is enriched. *Odoribacter,* along with *Coriobacteriaceae* and *Alistipes* were significantly enriched in mice stressed with grid floor housing. *Odoribacter* and *Alistipes* are both common members of a healthy human gut microbiome (Nagai et al., 2010).

Abundance of *Bacteroides* from pretreatment fecal samples were positively correlated with growth performance on toxic fescue. *Bacteroides* were enriched in vitamin D receptor knock-out mice, while beneficial lactobacillus were depleted (Jin et al., 2015). In mice,

Lachnospiraceae abundance has been correlated with anxiety behavior in mice under grid floor stress (Bangsgaard Bendtsen et al., 2012). Treatment with minocycline, which alleviates stress-related inflammation and reduces depressive-like behavior, also induced enrichment *Lachnospiraceae* along with *Ruminococcaceae* (Wong et al., 2016). When both chronic stress and minocycline (inflammatory stress response reducer) were administered *Lachnospiracea* was enriched along with *Blautia* and *Akkermansia*.

Enrichment of OTU522 *Akkermansia* in pre-treatment samples were negatively associated with weight gain over the trial. OTU522 was also a top predictor of toxin exposure in post-treatment samples and hair shedding behavior over the trial. These results suggest that enrichment in *Akkermansia* may be indicative of chronic stress response on toxic fescue or predict future response to chronic toxin stress on fescue pastures. Enrichment of Akkermansia in human gut is inversely related to body weight(Santacruz et al., 2010). *Akkermansia* is a mucin degrading bacterium. A decrease in this bacteria is associated with obesity, however *Akkermansia* is a normal portion of human gut microbiota (Everard et al., 2013).

Roseburia is also a butyrate-producing Fermicute that was highly predictive of post-treatment rectal temperature. This taxa was increased by severe tryptophan restriction in obesity prone mice (Zapata et al., 2018). *Roseburia* are depleted in children suffering from phenylketonuria (Verduci et al., 2018). Despite *Roseburia* being a top predictor for RT, the linear relationship was not significant. Enrichment of *Blautia* in post-treatment was a top indicator for both RT and rate of HRR. Enrichment of *Blautia* in post treatment fecal samples was associated with slower HRR and elevated RT on toxic fescue. *Blautia* has been identified in the feces of dairy cows and was found to be elevated under induced ruminant acidosis conditions (Mao et al., 2012).

Likewise, *Lachnospiraceae* and *Blautia* where increased in the feces of steers under ruminal acidosis (Mao et al., 2012). The bacteria from *Blautia*, and *Lachnospiraceae* were shown to persist in the gut even under diet transitions from roughage to concentrate (Mao et al., 2012). Enrichment of *Lachnospiraceae* was found in the rumens of low feed efficient beef cattle (Li and Guan, 2017).

Li and Guan et al. 2017 used a hierarchical cluster analysis which suggested *Lachnospiraceae* and *Ruminococcaceae* shared a functional cluster in the rumen. The *Lachnospiraceae* and *Ruminococcaceae* families are abundant in both the rumen and hind gut of ruminants and capable of producing short chain fatty acids from plant materials (Biddle et al., 2013; Paz et al., 2016). These two families were both identified as core microbes of the rumen and hindgut of other species (Henderson et al., 2015; Li and Guan, 2017). Although considered part of the healthy microbial community in humans, these families are known to be enriched on high-fiber diets in dairy cows (Thoetkiattikul et al., 2013). These taxa are enriched during dietary challenges in cattle, and are reduced by the administration of anti-microbial growth promoters (Thomas et al., 2017). *Roseburia*, *Blautia*, and *Lachnospiraceae* were all enriched in the stool of HIV positive individuals in Columbia (San-Juan-Vergara et al., 2018). When random forest accounted for rectal temperature and weight gain, several *Ruminiococcaceae* OTUs were predicative of toxin exposures.

Lachnospiracea was a top predictor for toxic fescue exposure in post-treatment fecal samples and a predictor for weight gain. OTU 710 *Lachnospiracea* was negatively correlated ($r = -0.44$, $P = 0.0008$) with percent weight gain over the trial. Those with the lowest abundance of this OTU exhibited the poorest growth. The average percent in body weight gain over the trial on toxic pastures was 16.59% with a standard deviation of 7.2%. Two pregnant cows performed

exceptionally poorly in growth with 1.5 and 1.7% increase in BW over the trial. These poor performing individuals were 2 of only 5 individuals with 0% abundance of the predicative OTU 431 in post treatment samples. The other 3 individuals with no detected 431 *Coriobacteriaceae* were Charolais-sired cows within 1 standard deviation of the mean growth performance. This suggest that *Coriobacteriaceae* may be indicative of adaption to toxic fescue toxins. *Coriobacteriaceae* was found to be enriched in mice suffering from environmental stress (Bangsgaard Bendtsen et al., 2012). Abundance of this bacteria family was identified as malleable based on rumen transplantation (Zhou et al., 2018). *Coriobacteriaceae* was also associated with offspring whose mother were fed polyunsaturated fatty acids (PUFA) deficient diets (Robertson et al., 2017). These results indicated that abundance of *Coriobacteriaceae* may be indicative of the ability to handle toxic fescue stress and have the potential to be modulated via probiotic manipulation.

Application of previous association of bacterial family is the primary challenge to overcome in applying the findings of this study. Although significant association between some bacterial taxa and fescue resilience phenotypes exist, it is unknown if the presence of these groups are causes of robustness or artifacts of underlying ability to handle fescue exposure. The results of this study indicate that fecal bacteria have enough compositional variation to explain economically important phenotypes, but cause and effect studies should be designed for the identification of biomarkers and probiotics.

Table 1. Fescue-type effects for fescue toxicosis trait phenotypes.

	Fescue Type							
	Toxic	Novel	Diff[6]	SE	Breed (B)	Fescue (F)	Pond (P)	B × F
n	54	46						
August Phenotypes								
Rectal temperature, °C	40.61	40.19	0.42	0.11	<0.0001	0.0002	<0.0001	0.62
Systolic blood pressure, mmHg	129.76	114.18	15.58	7.53	0.36	0.04	0.89	0.56
Diastolic blood pressure, mmHg	77.13	74.05	3.08	4.69	0.23	0.51	0.27	0.92
Serum K, mg/L	28.42	27.83	0.58	1.22	0.09	0.64	0.05	0.59
Serum Zn, mg/L	0.10	0.10	0.00	0.003	0.75	0.43	0.02	0.73
Body condition score[5]	6.06	5.95	0.11	0.15	0.10	0.49	NA	0.40
Hair coat score[3]	2.40	1.61	0.79	0.22	<.0001	0.001	NA	0.07
Other Phenotypes								
Hair reduction rate, score/d	-0.019	-0.028	0.001	0.002	0.0004	<0.0001	NA	0.83
Hair coat score[3,4]	3.37	1.98	1.39	0.20	0.05	<0.0001	NA	0.78
Body mass increase, %1	14.24	21.49	-7.26	1.28	<.0001	<0.0001	NA	0.11
ADG, kg/d	1.13	1.66	-0.53	0.08	<.0001	<0.0001	NA	0.06
Vaginal temperature, °C[2]								
mean	39.14	39.18	-0.04	0.08	0.002	0.63	0.29	0.43
range	2.08	2.19	-0.12	0.08	0.0006	0.16	0.001	0.53
maximum	40.27	40.19	0.08	0.10	<0.0001	0.39	0.021	0.80
variance	0.19	0.23	-0.04	0.02	0.003	0.01	0.008	0.20

[1][(BWd0 – BWd147) / BWd0) × 100.
[2]Temperatures recorded across the last 7 d of the trial using ibutton thermosensors (Digi-Key, Thief River Falls, MN) in progesterone-free controlled internal drug release devices (CIDRs) (Zoetis Parsippany-Troy, NJ).
[3]1 = slick summer coat (100% shed) to 5 = full, thick winter coat (0% shed) (Gray et al., 2011).
[4]Recorded 1 d before the summer solstice.
[5]1 = emaciated to 9 = extremely obese (Richards et al., 1986).
[6] column 1 – column 2
Only variable that were statistically significant are reported in the table

Table 2. Sire breed effects for fescue toxicosis trait phenotypes.

	Sire Breed							
	Hereford	Charolais	Diff[6]	SE	Breed (B)	Fescue (F)	Pond (P)	B × F
n	39	61						
August Phenotypes								
Rectal temperature, °C	40.69	40.11	0.58	0.12	<.0001	0.0002	<.0001	0.62
Systolic blood pressure, mmHg	125.65	118.29	7.36	7.97	0.36	0.04	0.89	0.56
Diastolic blood pressure, mmHg	78.58	72.60	5.98	4.96	0.23	0.51	0.27	0.92
Serum K, mg/L	27.02	29.23	-2.21	1.30	0.09	0.64	0.05	0.59
Serum Zn, mg/L	0.10	0.102	-0.0009	0.003	0.75	0.43	0.02	0.73
Body condition score[5]	5.87	6.14	-0.27	0.16	0.10	0.49	NA	0.40
Hair coat score[3]	2.62	1.39	1.23	0.23	<.0001	0.0005	NA	0.07
Other Phenotypes								
Hair reduction rate, score/d	-0.020	-0.027	0.007	0.002	0.0004	<.0001	NA	0.83
Hair coat score[3,4]	2.90	2.46	0.44	0.22	0.05	<.0001	NA	0.78
Body mass increase, %[1]	14.83	20.90	-6.07	1.36	<.0001	<.0001	NA	0.11
Vaginal temperature, °C[2]								
mean	39.30	39.02	0.28	0.09	0.002	0.63	0.29	0.43
range	2.29	1.99	0.30	0.09	0.0006	0.16	0.00	0.53
maximum	40.44	40.02	0.41	0.10	<.0001	0.39	0.02	0.80
variance	0.23	0.18	0.05	0.02	0.003	0.01	0.01	0.20

[1][(BWd0 – BWd147) / BWd0) × 100.
[2]Temperatures recorded across the last 7 d of the trial using ibutton thermosensors (Digi-Key, Thief River Falls, MN) in progesterone-free controlled internal drug release devices (CIDRs) (Zoetis Parsippany-Troy, NJ).
[3]1 = slick summer coat (100% shed) to 5 = full, thick winter coat (0% shed) (Gray et al., 2011).
[4]Recorded 1 d before the summer solstice.
[5]1 = emaciated to 9 = extremely obese (Richards et al., 1986).
[6]column 1 – column 2
Only variable that were statistically significant are reported in the table.

Table 3. Fescue-type × sire breed interactive means for fescue toxicosis trait phenotypes.

	Hereford		Charolais		P-value				
	Toxic	Novel	Toxic	Novel	Breed (B)	Fescue (F)	Pond (P)	B × F	B x F x P
n	19	20	35	26					
August Phenotypes									
Hair coat score[3]	3.21	2.03	1.58	1.20	<0.0001	0.0005	NA*	0.07	NA*
Other Phenotypes									
ADG, kg/d	0.85	1.54	1.41	1.78	<0.0001	<.00001	NA	0.06	NA
Vaginal temperature, °C[2]									
Mean daily AUC, °C×h[6]	25.70	29.70	23.84	23.37	0.002	0.15	0.007	0.07	0.56

[1][(BWd0 – BWd147) / BWd0) × 100.
[2]Temperatures recorded across the last 7 d of the trial using ibutton thermosensors (Digi-Key, Thief River Falls, MN) in progesterone-free controlled internal drug release devices (CIDRs) (Zoetis Parsippany-Troy, NJ).
[3]1 = slick summer coat (100% shed) to 5 = full, thick winter coat (0% shed) (Gray et al., 2011).
[4]Recorded 1 d before the summer solstice.
[5]1 = emaciated to 9 = extremely obese (Richards et al., 1986).4,
[6]daily accumulation of degree area under the curve degree × hours (daily AUC, °C×h)

*Pond and breed x fescue type x pond interactions were not molded for hair coat score or average daily gain over the trial. These effects were only tested if the pond was likely to alter values at the final phenotypic collection.

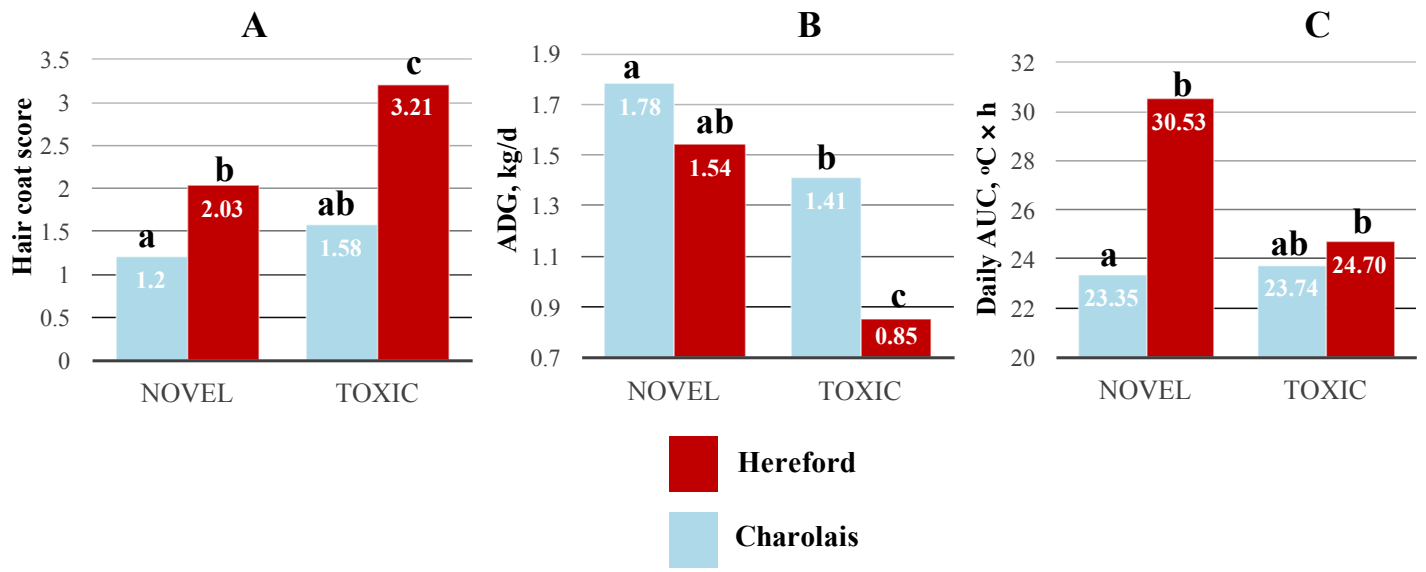

Figure 1. Fescue-type × sire breed tendencies for August phenotypes of HCS (A), ADG (B), and Daily AUC (C). Subscripts denote statistical differences across breed and treatment at a significance level of $P \leq 0.05$. **(A)** Interactive means for hair coat score collected in August (under high heat stress). Herefords had greater hair coat scores (HCS) regardless of treatment. Exposure to toxic fescue did not cause significant increase in hair coat score for Charolais-sired cows, but Hereford-sired cows were different across treatment. **(B)** Average daily gain (ADG) for Charolais- and Herford-sired cows was similar on novel (low-toxin) fescue type. However, Charolais-sired individuals gained significantly more than Hereford-sired cows on toxic pasture. **(C)** Heat accumulation as measured by trapazoidal area under the curve (AUC) during the last week of the trail indicated that Hereford sired-cows had accumulated significantly more degree× hours ($^{o}C \times h$) than Charolais-sired cows on novel (low-toxin) fescue. However, sire breed differences were not observed on toxic fescue. Serial vaginal temperatures were recorded across the last 7 d of the trial using ibutton thermosensors (Digi-Key, Thief River Falls, MN) in progesterone-free controlled internal drug release devices (CIDRs) (Zoetis Parsippany-Troy, NJ). The internal 5 days where averaged for daily AUC heat accumulation

Table 4. Fescue-type × sire breed × pond interactive means separated by (A) with a pond and (B). without a pond

A. With a pond

	Hereford		Charolais		P-value				
	Toxic	Novel	Toxic	Novel	Breed (B)	Fescue (F)	Pond	B × F	B x F x P
August Phenotypes									
Respiration rate, breaths/min	102.84	79.44	89.39	58.81	0.0001	<0.0001	<.0001	0.73	0.03
Serum P, mg/L	4.58	6.55	5.88	6.98	0.0002	<0.0001	<.0001	0.11	0.09
Serum Ca, mg/L	7.43	7.32	7.76	7.41	0.32	0.86	0.82	0.32	0.03
Serum Mg, mg/L	2.18	2.21	2.33	2.26	0.03	0.001	0.17	0.60	0.001
Serum Fe, mg/L	0.099	0.075	0.106	0.082	0.44	0.67	0.12	0.38	0.003
Serum Cu, mg/L	0.092	0.085	0.090	0.086	0.10	0.66	0.16	0.59	0.03

B. Without a pond

	Hereford		Charolais		P-value				
	Toxic	Novel	Toxic	Novel	Breed (B)	Fescue (F)	Pond	B × F	B x F x P
August Phenotypes									
Respiration rate, breaths/min	74.37	57.13	57.13	52.30	0.0001	<.0001	<.0001	0.73	0.03
Serum P, mg/L	6.56	7.60	7.01	7.92	0.0002	<.0001	<.0001	0.11	0.09
Serum Ca, mg/L	7.40	7.64	7.40	7.56	0.32	0.86	0.82	0.32	0.03
Serum Mg, mg/L	2.11	2.38	2.16	2.46	0.03	0.001	0.17	0.60	0.001
Serum Fe, mg/L	0.061	0.097	0.075	0.090	0.44	0.67	0.12	0.38	0.003
Serum Cu, mg/L	0.078	0.085	0.089	0.089	0.10	0.66	0.16	0.59	0.03

*Blood samples for serum mineral analysis were collected from the jugular vein on the last day of the trial, transported on ice, and analyzed at the veterinary diagnostic laboratory (University of Arkansas, Fayetteville, AR). Only variable that were statistically significant are reported in the table.

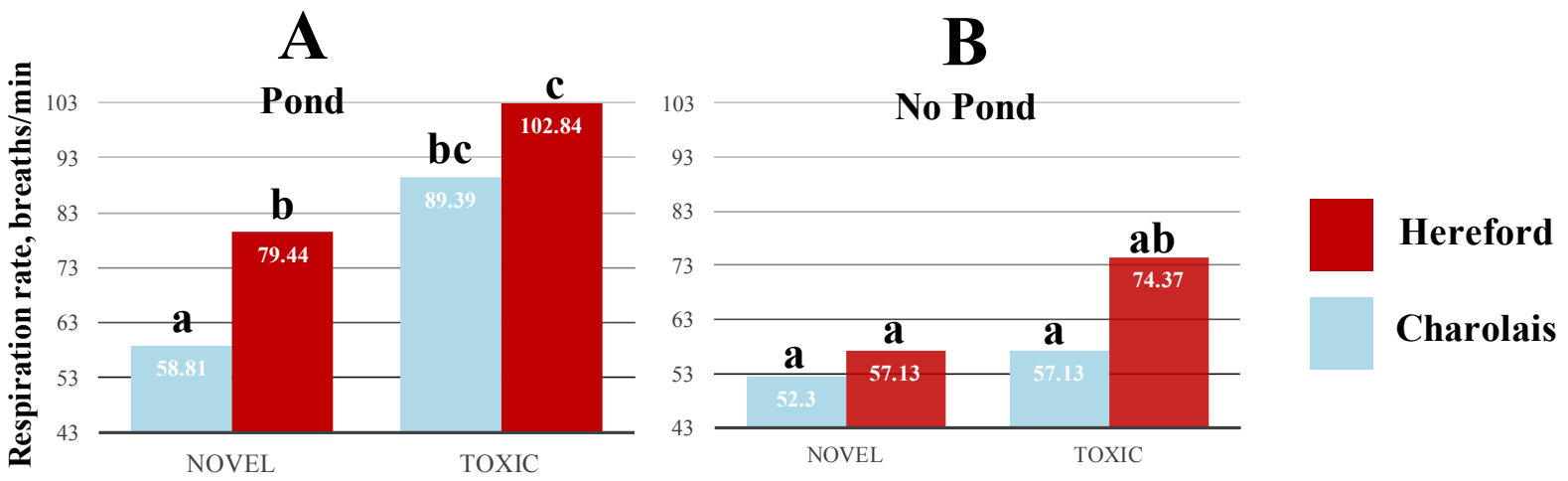

Figure 2. Fescue-type × sire breed × pond interactive means for respiration rate separated by (A) with a pond and (B) without a pond. Subscripts denote statistical difference across breed and treatment at a significance level of $P \leq 0.05$. The three-way interaction depicts a two-way interaction among fescue-type (Novel: low-toxin or Toxic) and sire breed (Charolais or Hereford), however this interaction is dependent on pond availability. **(A)** When pond access is available, reparation rates are highest for cattle on toxic fescue, but there are not breed differences in this condition. **(B)** With no pond availability, Hereford-sired cows had greater respiration rates than Charolais-sired cows on novel fescue. When no ponds were available, breed differences were not detectable on novel (low-toxin) or toxic fescue. Herefords had numerically higher respiration rates on in all pond-fescue combinations.

Table 5. Pond effects on fescue toxicosis phenotypes.

	Pond							
	Yes	No	Diff	SE	Breed (B)	Fescue (F)	Pond (P)	B × F
n	39	61						
August Phenotypes								
Rectal temperature, °C	40.83	39.97	0.86	0.11	<0.0001	0.0002	<.0001	0.62
Systolic blood pressure, mmHg	121.44	122.50	-1.06	7.80	0.36	0.04	0.89	0.56
Diastolic blood pressure, mmHg	72.87	78.31	-5.45	4.96	0.23	0.51	0.27	0.92
Serum K, mg/L	29.35	26.89	2.46	1.26	0.09	0.64	0.05	0.59
Serum Zn, mg/L	0.105	0.098	0.01	0.003	0.75	0.43	0.02	0.73
Vaginal temperature, °C[2]								
mean	39.12	39.21	-0.09	0.09	0.002	0.63	0.29	0.43
range	1.99	2.28	-0.29	0.09	0.001	0.16	0.001	0.53
maximum	40.11	40.35	-0.24	0.10	<.0001	0.39	0.02	0.80
variance	0.19	0.23	-0.04	0.02	0.003	0.01	0.01	0.20

[1][(BWd0 – BWd147) / BWd0) × 100.
[2]Temperatures recorded across the last 7 d of the trial using ibutton thermosensors (Digi-Key, Thief River Falls, MN) in progesterone-free controlled internal drug release devices (CIDRs) (Zoetis Parsippany-Troy, NJ).
[3]1 = slick summer coat (100% shed) to 5 = full, thick winter coat (0% shed) (Gray et al., 2011).
[4]Recorded 1 d before the summer solstice.
[5]1 = emaciated to 9 = extremely obese (Richards et al., 1986).4,
[6]daily accumulation of degree area under the curve degree × hours (daily AUC, ^{o}C×h)

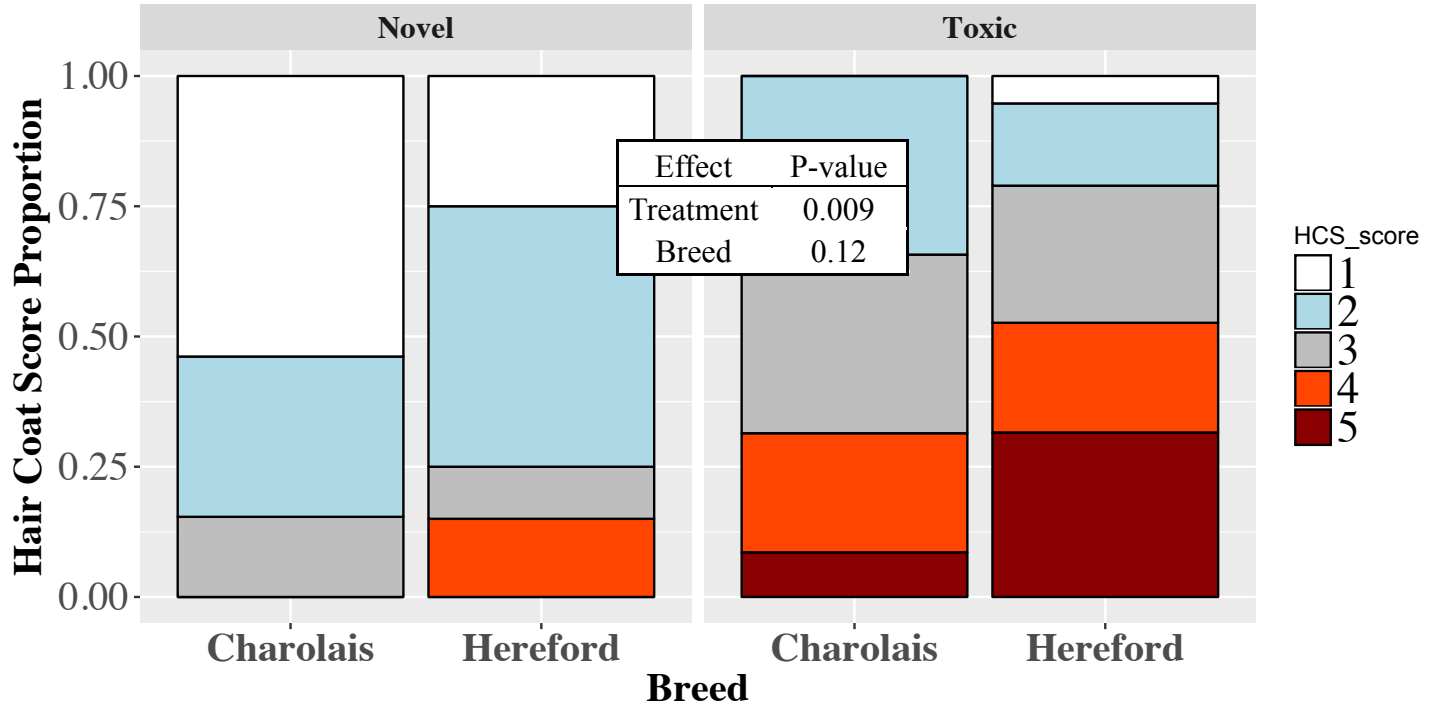

Figure 3. HCS at summer solstice by fescue-type and sire breed. Sire breed (Charolais or Hereford) and Fescue-type (toxic or novel/low-toxin) differences for cows grazing on toxic fescue was analyzed using a multinomial distribution in PROC GLIMIX of SAS (version 9.4; SAS Inst., Inc., Carry, NC). Cow hair coat scores (HCS) were judged using the system used by Grey et al. 2011 (1 = slick summer coat (100% shed) to 5 = full, thick winter coat (0% shed). The proportion of cow hair coat scores (HCS) at summer solstice were independent of sire-breed effects but dependent on fescue-type. At summer solstice, few cows on toxic fescue exhibited slick (HCS =1) coats, while several cows exhibited full (HCS=5) winter coats. On novel (low-toxin) fescue, all cows were scored lower than a HCS 5, and bout 65.0% exhibited complete hair shed.

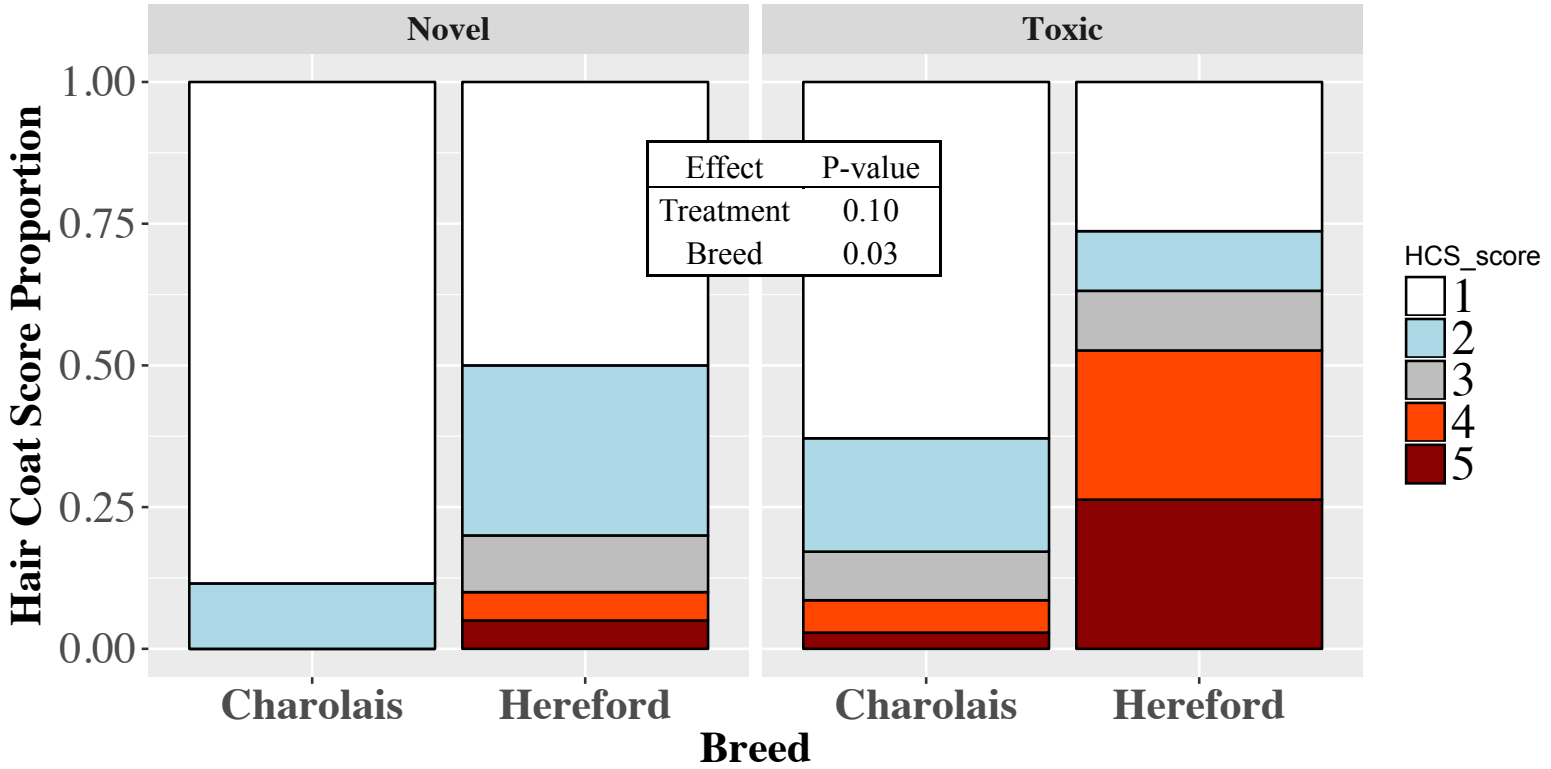

Figure 4. HCS at trial's end by fescue-type and sire breed. Sire breed (Charolais or Hereford) and Fescue-type (toxic or novel/low-toxin) differences for cows grazing on toxic fescue were analyzed using a multinomial distribution in PROC GLIMIX of SAS (version 9.4; SAS Inst., Inc., Carry, NC). Cow hair coat scores (HCS) scores were judged using the system used by Grey et al. 2011 (1 = slick summer coat (100% shed) to 5 = full, thick winter coat (0% shed). The proportion of cow hair coat scores (HCS) at trial's end (August) were independent of fescue-type effects but dependent on sire-breed. By August, about 80% of Hereford-sired cows on novel (low-toxin) fescue exhibited HCS of 1 or 2 (completely shed or mostly shed), However only about 30% of Hereford-sired cows on toxic fescue had HCS of 1 or 2. Charolais on novel (low-toxin) pastures were represented entirely of HCS 1 or 2, but, on toxic fescue, spanned all five hair score levels.

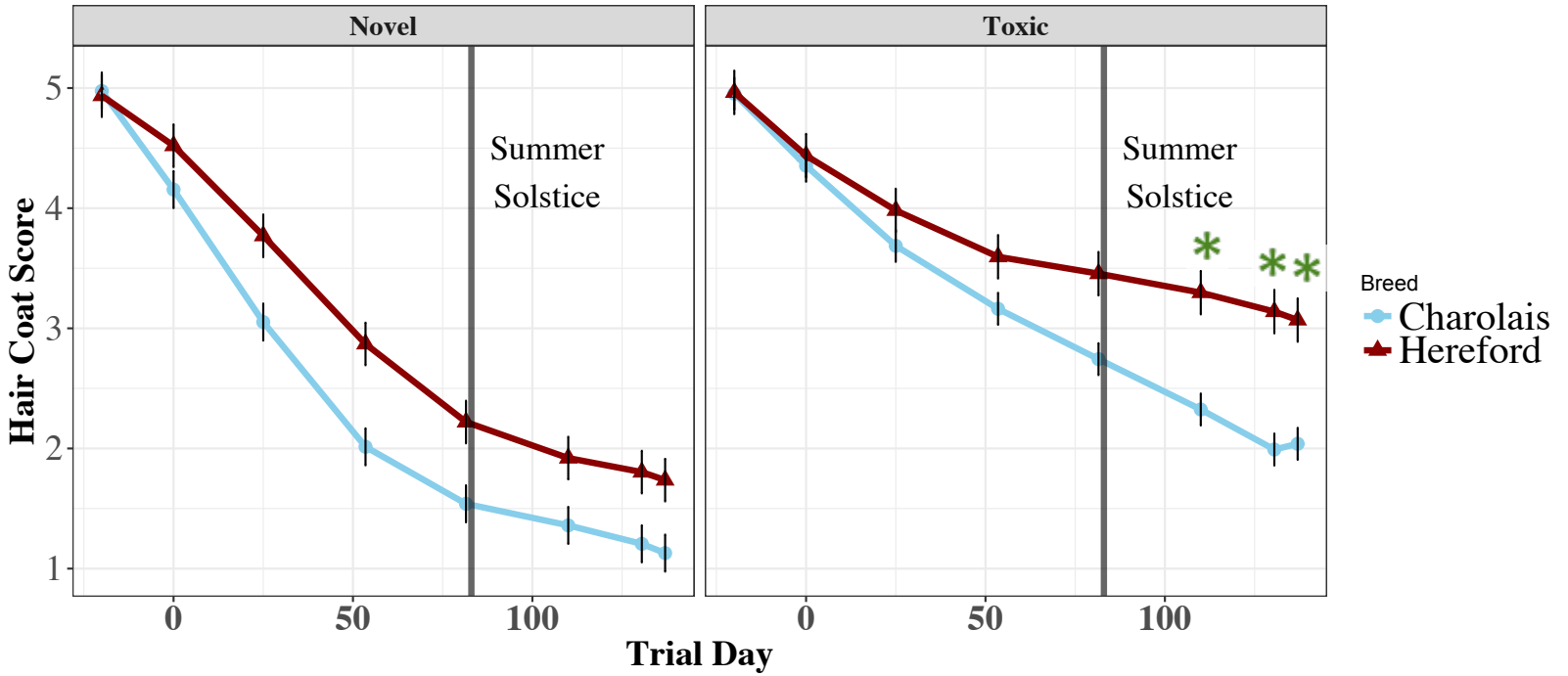

Figure 5. Least square means plot for repeated measure of HCS. Sire breed (Charolais or Hereford) and Fescue-type (toxic or novel/low-toxin) differences were analyzed as a repeated measured in PROC MIXED of SAS (version 9.4; SAS Inst., Inc., Carry, NC). Cow hair coat scores (HCS) scores were judged about once each month using the system described by Grey et al. 2011 (1 = slick summer coat (100% shed) to 5 = full, thick winter coat (0% shed). A significant three-way interaction. Stars indicated statistical difference at $P \leq 0.05$. A fescue-type × sire breed × date interaction was observed in repeated measure of hair coat scores (HCS) over time. Breed differences were not detectable ($P < 0.05$) until the last three sampling dates on toxic fescue. These results suggest that HCS taken later in the summer may more accurately capture genetic variation in performance on toxic fescue.

Table 6. Least square means for repeated measure of HCS.

Trial Day	Sire breed		SE	Adjusted P-value
	Charolais	Hereford		
n	58	43		
Toxic				
-20	4.95	4.96	0.22	1.00
0	4.35	4.44	0.22	1.00
29	3.69	3.98	0.22	1.00
55	3.16	3.60	0.22	0.99
84	2.74	3.46	0.22	0.28
112	2.32	3.30	0.22	0.01
121	1.99	3.14	0.22	0.00
127	2.04	3.07	0.22	0.00
n	49	43		
Novel				
-20	4.98	4.94	0.23	1.00
0	4.15	4.52	0.23	1.00
29	3.05	3.77	0.23	0.35
55	2.01	2.87	0.23	0.07
84	1.54	2.22	0.23	0.48
112	1.36	1.92	0.23	0.86
121	1.21	1.80	0.23	0.76
127	1.13	1.74	0.23	0.73

1 = slick summer coat (100% shed) to 5 = full, thick winter coat (0% shed) (Gray et al., 2011).

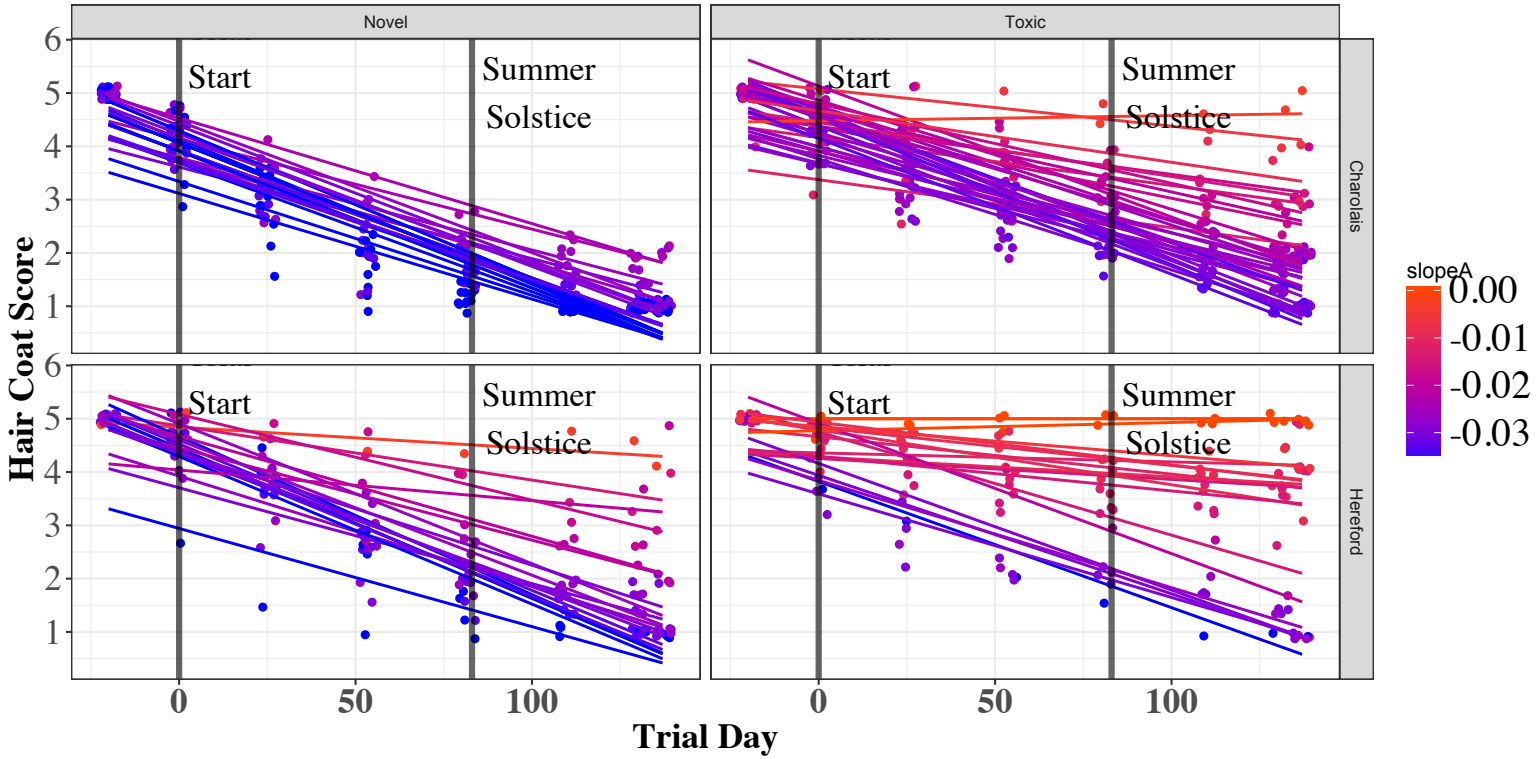

Figure 6: Individual variation in hair reduction rate by fescue-type and sire breed. Cow hair coat scores (HCS) scores were judged monthly using the system described by Grey et al. 2011 (1 = slick summer coat (100% shed) to 5 = full, thick winter coat (0% shed). Hair reduction rate (HRR) was calculated by performing a sliding window average of hair coat score (HCS). Adjusted hair coat scores on day 0 and Day 112 were used to calculate a rate of hair shed over time. Each line represents the rise/run (change in hair coat score/ change in time in days). The left and right panels depict rate of hair reduction for novel (low-toxin) cows and toxic fescue grazed cows respectively. The top and bottom panels represent rates of hair reduction for Charolais- and Hereford-sired cows respectively. Steeper slopes indicate more efficiency in hair reduction over time. Hereford-sired cows exhibited less efficiency in hair shed, especially on toxic fescue. Individual variation in rates of hair exist on toxic fescue for both sire breeds.

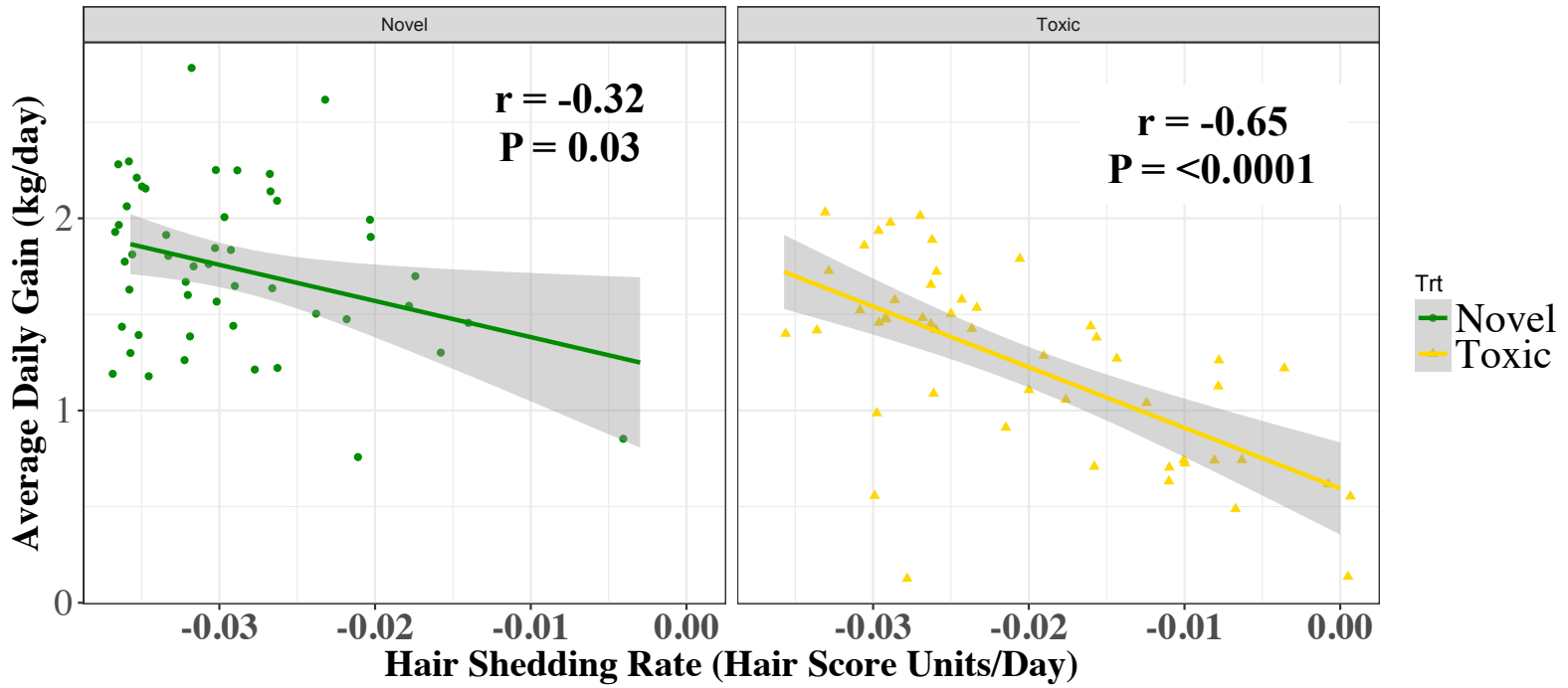

Figure 7: Pearson's correlation of hair reduction rate and ADG on toxic and novel fescue. Cow hair coat scores (HCS) scores were judged monthly using the system described by Grey et al. 2011 (1 = slick summer coat (100% shed) to 5 = full, thick winter coat (0% shed). Hair reduction rate (HRR) was calculated by performing a sliding window average of hair coat score (HCS). Adjusted hair coat scores on day 0 and Day 112 were used to calculate a rate of hair shed over time. Correlations were calculated in PROC CORR for SAS (version 9.4; SAS Inst., Inc., Carry, NC). Cow HRR were significantly correlated with average daily gain on both toxic and novel (low toxin fescue). Cows with faster rates of hair reduction over the trial also had greater weight gain.

Table 7. Pearson's correlation among heat stress and fescue toxicosis trait phenotypes contrasted by fescue-type[1].

Variable	RR	RT	HCS	ADG	P	K	Ca	Mg	Na	Fe	Zn	Cu	AUC	vmean	vmin	vrange	vmax	vvar	HHR
RR	--	*** 0.61	*** 0.50	† -0.25	*** -0.63	0.08	-0.08	-0.07	0.04	0.07	0.19	0	0.04	0.18	0.18	0.06	0.18	0.02	* 0.30
RT	*** 0.49	--	*** 0.44	** -0.40	*** -0.66	0.03	-0.21	* -0.31	† 0.26	0.14	-0.04	-0.01	0.2	* 0.29	0.2	0.18	* 0.30	0.06	* 0.32
HCS	-0.01	0.08	--	*** -0.60	-0.16	-0.04	† -0.23	-0.1	0.04	-0.21	-0.05	* -0.32	0.22	** 0.39	* 0.30	* 0.28	*** 0.45	*0.27	*** 0.90
ADG	* -0.36	** -0.46	* -0.36	--	0.13	0.15	* 0.32	* 0.37	-0.04	** 0.40	0.19	* 0.33	* -0.36	*** -0.54	* -0.39	* -0.34	*** -0.56	*0.27	*** -0.65
P	-0.17	*** -0.53	-0.17	* 0.36	--	0.03	0.2	* 0.28	-0.1	-0.2	0.01	-0.22	0.15	0.09	0	0.13	0.11	0.14	0.06
K	-0.01	0.17	* -0.34	0.09	0.14	--	* 0.34	† 0.24	*** -0.60	** 0.42	0.23	* 0.34	* -0.33	-0.12	0.08	† -0.27	-0.18	*-0.31	-0.02
Ca	-0.25	-0.21	0	0.14	-0.08	-0.01	--	** 0.45	0.05	** 0.43	* 0.32	0.13	-0.2	-0.21	-0.11	-0.15	-0.21	-0.22	-0.2
Mg	* -0.39	*** -0.62	0	* 0.33	** 0.46	-0.05	0.1	--	-0.01	† 0.27	* 0.29	0	* -0.30	-0.11	0.07	* -0.38	† -0.27	*-0.34	-0.15
Na	0.09	0.17	-0.21	-0.17	*-0.33	-0.22	0.3	-0.35	--	-0.15	-0.04	-0.08	t0.27	0.05	-0.12	0.21	0.09	0.11	0.05
Fe	† -0.27	-0.11	-0.22	0.16	0.09	0.19	*** 0.56	0.11	0.18	--	* 0.29	* 0.32	* -0.27	** -0.42	* -0.31	-0.19	* -0.38	*-0.34	* -0.31
Zn	0.22	0.17	† -0.25	0.05	-0.2	-0.04	0.21	-0.05	0.09	0.23	--	0.09	-0.01	t-0.28	* -0.31	** -0.003	-0.22	-0.1	-0.01
Cu	-0.13	* -0.32	0.11	-0.07	0.08	0.07	0.09	0.18	0.13	-0.16	† -0.26	--	* -0.33	*** -0.48	* -0.33	* -0.33	*** -0.51	**-0.40	** -0.39
AUC	0.22	* 0.31	* 0.38	-0.14	0	-0.15	0.03	* -0.33	-0.13	-0.21	-0.16	† -0.26	--	*** 0.50	-0.09	*** 0.81	*** 0.62	***0.75	* 0.32
vmean	0.25	0.19	*** 0.55	** -0.50	-0.18	-0.19	-0.04	-0.26	-0.04	† -0.28	-0.24	-0.03	*** 0.56	--	*** 0.82	* 0.30	*** 0.85	**0.39	*** 0.51
vmin	0.1	-0.05	* 0.32	** -0.47	-0.21	-0.1	-0.07	-0.01	0.06	-0.15	-0.14	0.2	-0.22	*** 0.69	--	-0.19	*** 0.56	-0.05	** 0.38
vrange	0.18	0.23	0.24	0.09	0.12	-0.05	-0.03	* -0.30	-0.09	-0.25	-0.26	† -0.27	*** 0.83	* 0.37	† -0.30	--	*** 0.70	*** 0.77	* 0.40
vmax	0.24	0.18	** 0.45	-0.23	-0.03	-0.12	-0.08	t-0.3	-0.04	* -0.34	* -0.34	-0.13	*** 0.66	*** .82	* 0.39	*** 0.76	--	*** 0.62	*** 0.6
vvar	† 0.30	† 0.31	* 0.32	-0.14	0.02	-0.17	-0.18	t-0.26	-0.07	* -0.33	-0.23	-0.23	*** 0.77	** 0.45	-0.15	*** 0.85	*** 0.71	--	* 0.34
HRR	0.04	-0.12	*** 0.83	* -0.32	0.02	-0.23	0.037	0.11	* -0.28	* -0.29	** -0.37	0.13	* 0.31	*** 0.54	* 0.35	† 0.28	*** 0.52	** 0.34	--

Table 7 (Cont.)

[1]Correlations among variables for cows on toxic fescue are reported above the diagonal below for novel (low-toxin) fescue

$^{***}P \leq 0.001$; $^{**}P \leq 0.01$; $^{*}P \leq 0.05$; $^{\dagger}P \leq 0.10$

RR = respiration rate; RT = rectal temperature; ADG= average daily gain (kg/d); P = serum phosphorus; K = serum potassium; Ca = serum calcium: Mg = serum magnesium; AUC = daily cumulative degree*hrs; vmean = average vaginal temperature; vmin = minimum vaginal temperature; vrange = maximum – minimum vaginal temperature; vmax = maximum vaginal temperature; vvar = vaginal temperature variance; HRR = hair reduction rate (hair score* units/day).

* 1 = slick summer coat (100% shed) to 5 = full, thick winter coat (0% shed) (Gray et al., 2011).

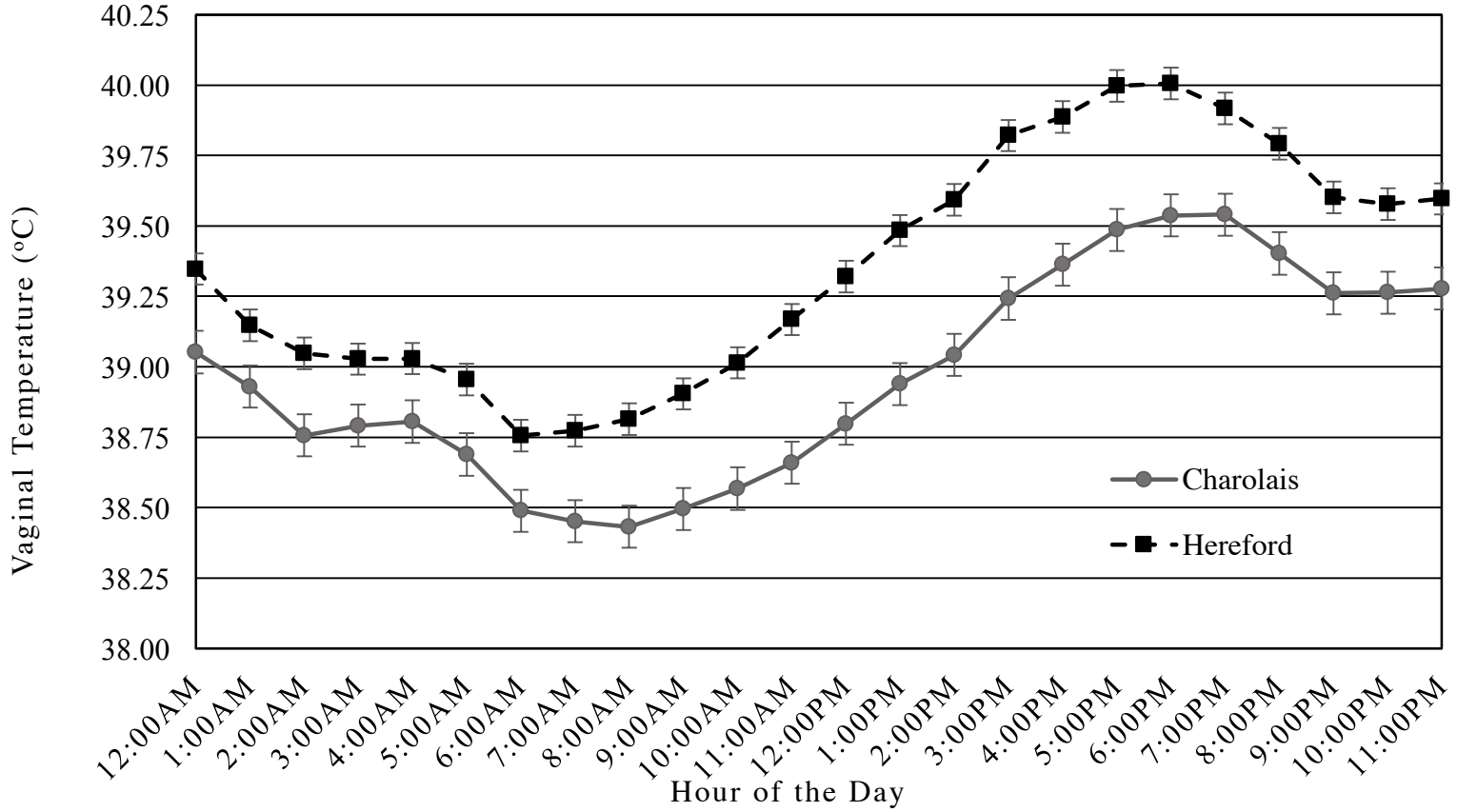

Figure 8a. Least square means for repeated measures of vaginal temperature by sire breed on novel pasture with a pond. Significance at $P \leq 0.05$ is indicated where error bars do not overlap. The last week of the trial, after cows had grazed either Toxic or novel fescue for 5 months**,** cow vaginal temperatures were recorded using ibutton thermosensors (Digi-Key, Thief River Falls, MN) in progesterone-free controlled internal drug release devices (CIDRs) (Zoetis Parsippany-Troy, NJ). Temperatures were captured every 5 minutes at a sensitivity of 0.5 degrees ^{o}C. Five days of temperatures were analyzed to exclude handling bias. A significant fescue-type × sire breed × time interaction was observed. On novel (low-toxin) pasture with a pond, Hereford-sired cows remained about 0.4 ^{o}C warmer than Charolais-sired cows regardless of the time of day.

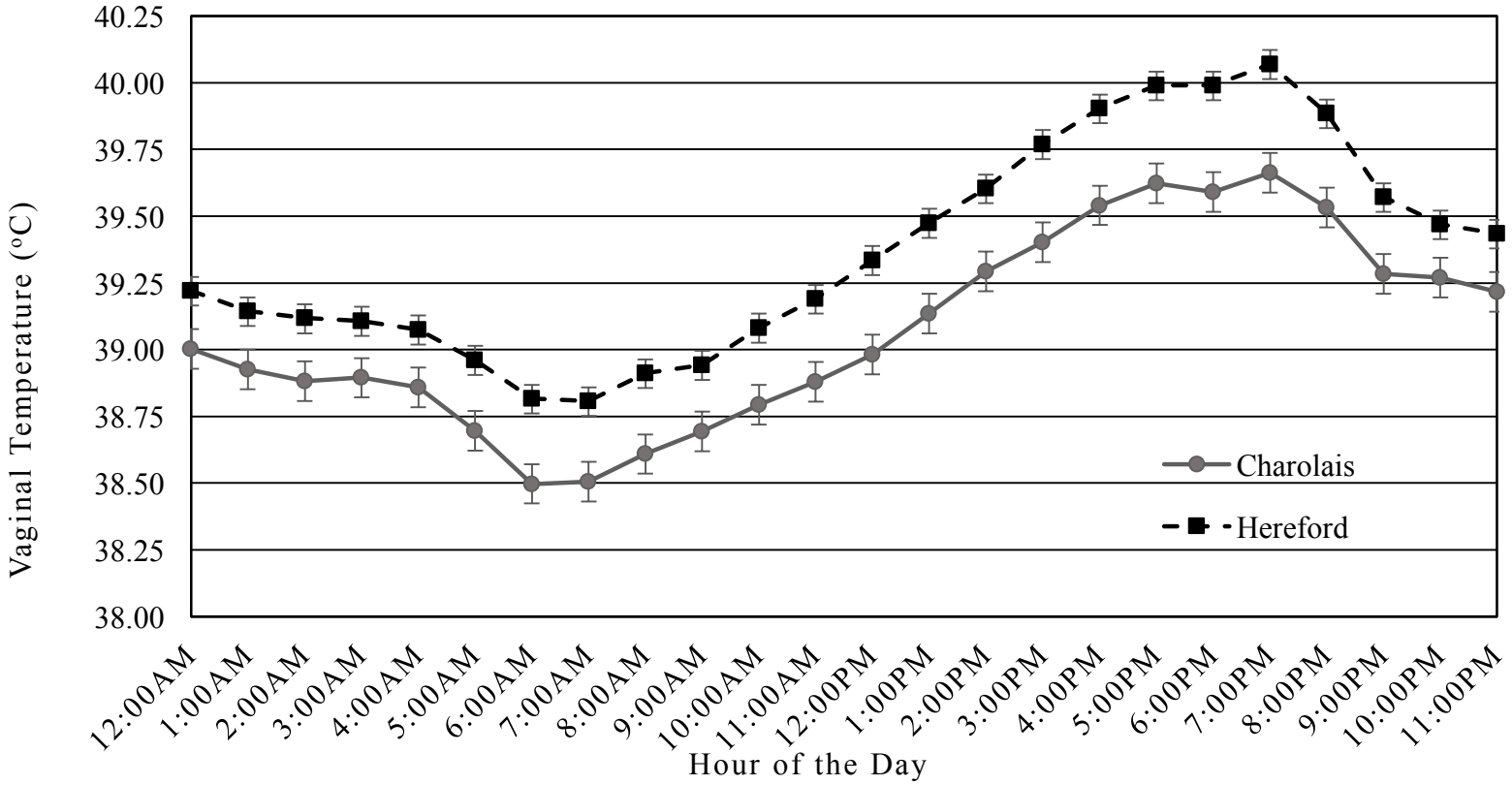

Figure 8b. Least square means for repeated measures of vaginal temperature by sire breed on toxic pasture without a pond. Significance at $P \leq 0.05$ is indicated where error bars do not overlap. The last week of the trial, after cows had grazed either Toxic or novel fescue for 5 months**,** cow vaginal temperatures were recorded using ibutton thermosensors (Digi-Key, Thief River Falls, MN) in progesterone-free controlled internal drug release devices (CIDRs) (Zoetis Parsippany-Troy, NJ). Temperatures were captured every 5 minutes at a sensitivity of 0.5 degrees ^{o}C. Five days of temperatures were analyzed to exclude handling bias. A significant fescue-type × sire breed × time interaction was observed. On toxic pasture with no pond, Hereford-sired cows remained about 0.3 ^{o}C warmer than Charolais-sired cows regardless of the time of day. Significance at $P \leq 0.05$ is indicated where error bars do not overlap.

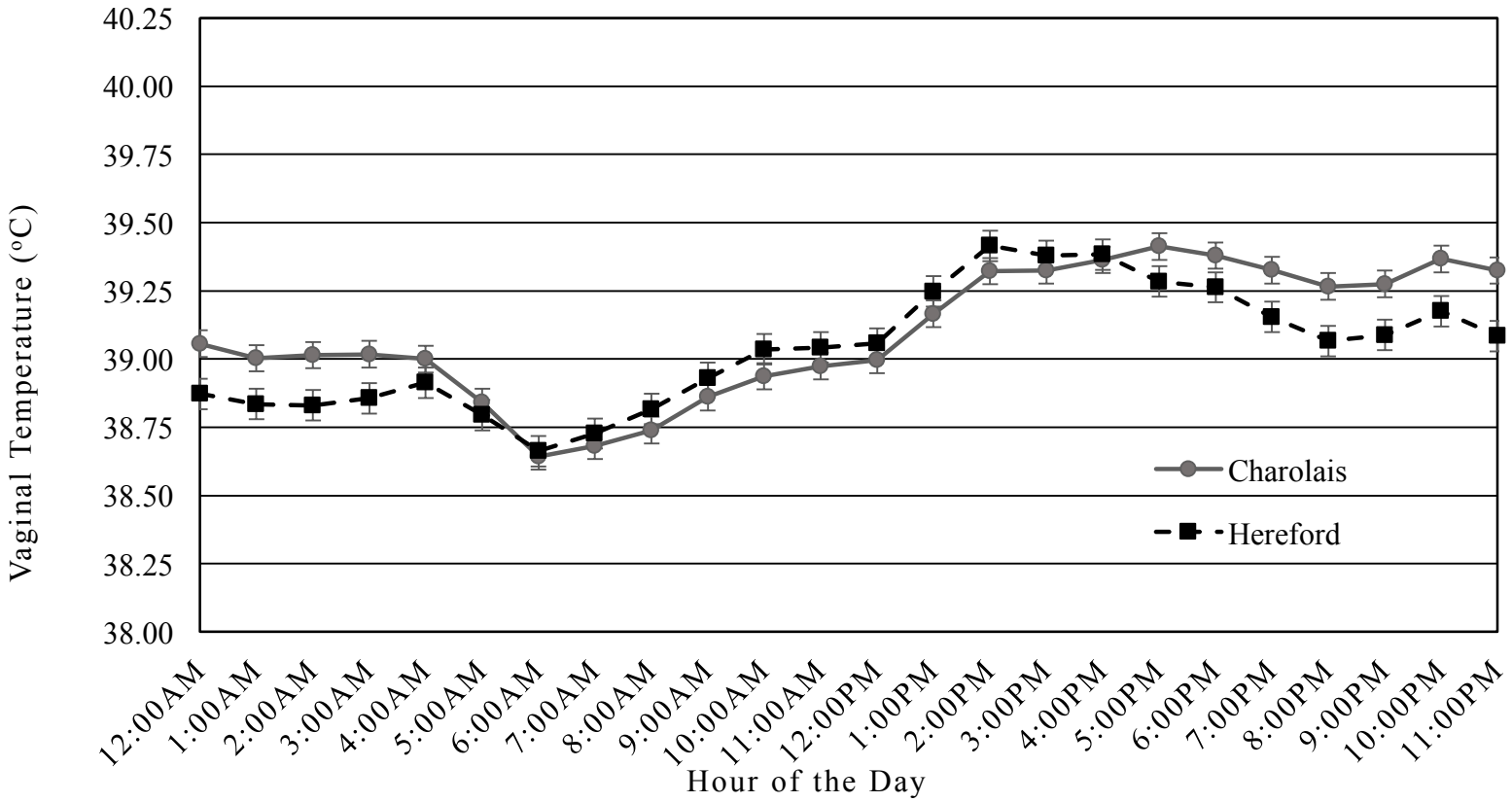

Figure 8c. Least square means for repeated measures of vaginal temperature by sire breed on toxic pasture with a pond. Significance at $P \leq 0.05$ is indicated where error bars do not overlap. The last week of the trial, after cows had grazed either Toxic or novel fescue for 5 months**,** cow vaginal temperatures were recorded using ibutton thermosensors (Digi-Key, Thief River Falls, MN) in progesterone-free controlled internal drug release devices (CIDRs) (Zoetis Parsippany-Troy, NJ). Temperatures were captured every 5 minutes at a sensitivity of 0.5 degrees ^{o}C. Five days of temperatures were analyzed to exclude handling bias. On toxic pasture with a pond, breed differences were not detectible between 400 and 1600h. Charolais-sired cows exhibited higher body temperatures than Hereford-sired cows between 1700 and 300h. These results indicated that Hereford-sired cows may have utilized pond cooling on toxic fescue more than Charolais. Significance at $P \leq 0.05$ is indicated where error bars do not overlap.

Table 8. Vaginal temperatures as covariates of ADG.

Dependent Variable	Heat stress measures added to base model[1]	P-value
Average Daily Gain		
	Vaginal temperature range[3]	0.5825
	August respiration rate[2]	0.1071
	August rectal temperature[2]	0.003
	Vaginal temperature variance[3]	0.122
	Vaginal temperature maximum[3]	0.0007
	Vaginal temperature minimum[3]	0.0001
	Daily area under the curve[3,4]	0.0663
	Mean vaginal temperature[3]	<.0001

[1]ADG = fescue-type + sire breed + parity + pregnant + hour + date + fescue-type × sire breed
[2] Recorded the last day of the trial
[3]Temperatures recorded across the last 7 d of the trial using ibutton thermosensors (Digi-Key, Thief River Falls, MN) in progesterone-free controlled internal drug release devices (CIDRs) (Zoetis Parsippany-Troy, NJ).
[4]daily accumulation of degree area under the curve degree × hours (daily AUC, ^{o}C×h)

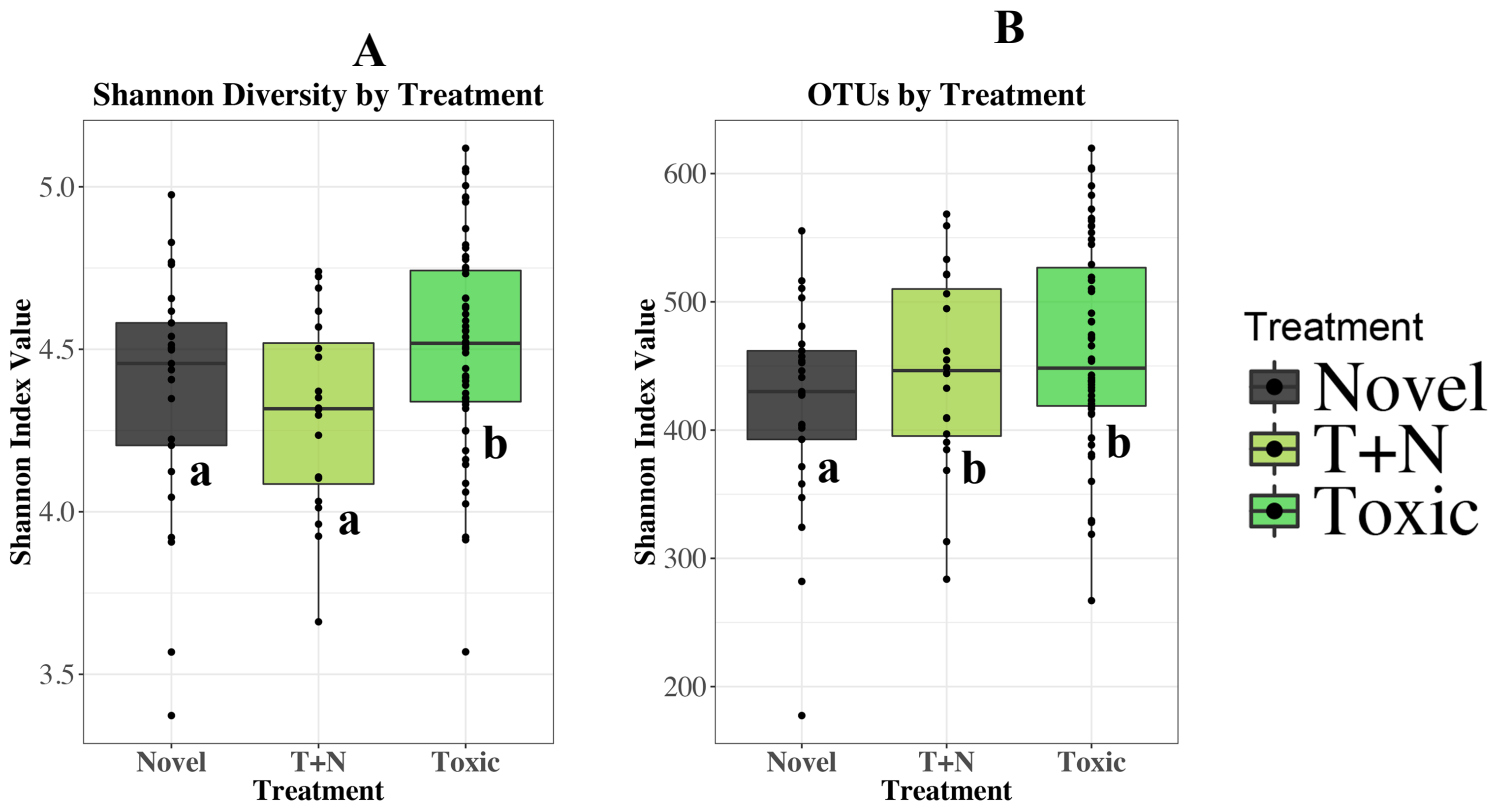

Figure 9. Post-treatment alpha diversity by treatment. Subscripts indicated significance at a level of $P \leq 0.05$. **(A)** Fecal sample diversity as measured by Shannon diversity measures index, indicated that the cattle grazed on toxic fescue for 5 months had more diversity in their fecal samples. **(B)** Similarly, fecal samples collected after 5 months on toxic fescue increased the number of observed OTUs in feces over cattle grazed on novel (low-toxin fescue). However, the number of OTUs in the toxic condition, was not different from the alternating treatment condition.

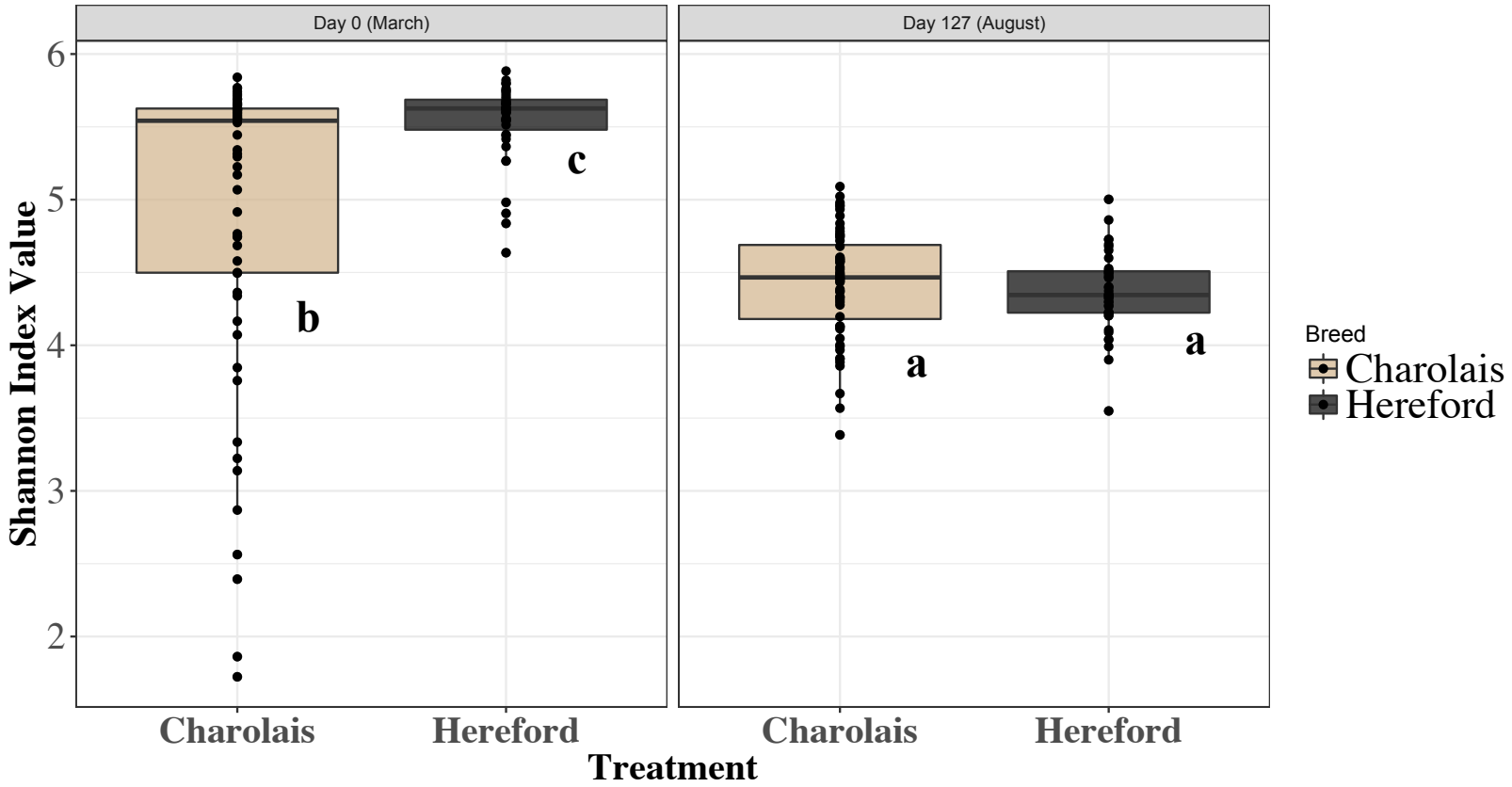

Figure 10. Sire breed × time point effect for fecal Shannon diversity. Subscripts indicated significance at a level of $P \leq 0.05$. A significant sire breed × time point interaction was observed for changes in fecal community diversity over time as measures by the Shannon index values. Shannon index values were greater for both sire breeds in March (pre-treatment) than in August (post treatment). Hereford-sired cows had greater Shannon index values than Charolais, prior to toxin exposure, however, after five months of grazing, sire breed differences were not detectable.

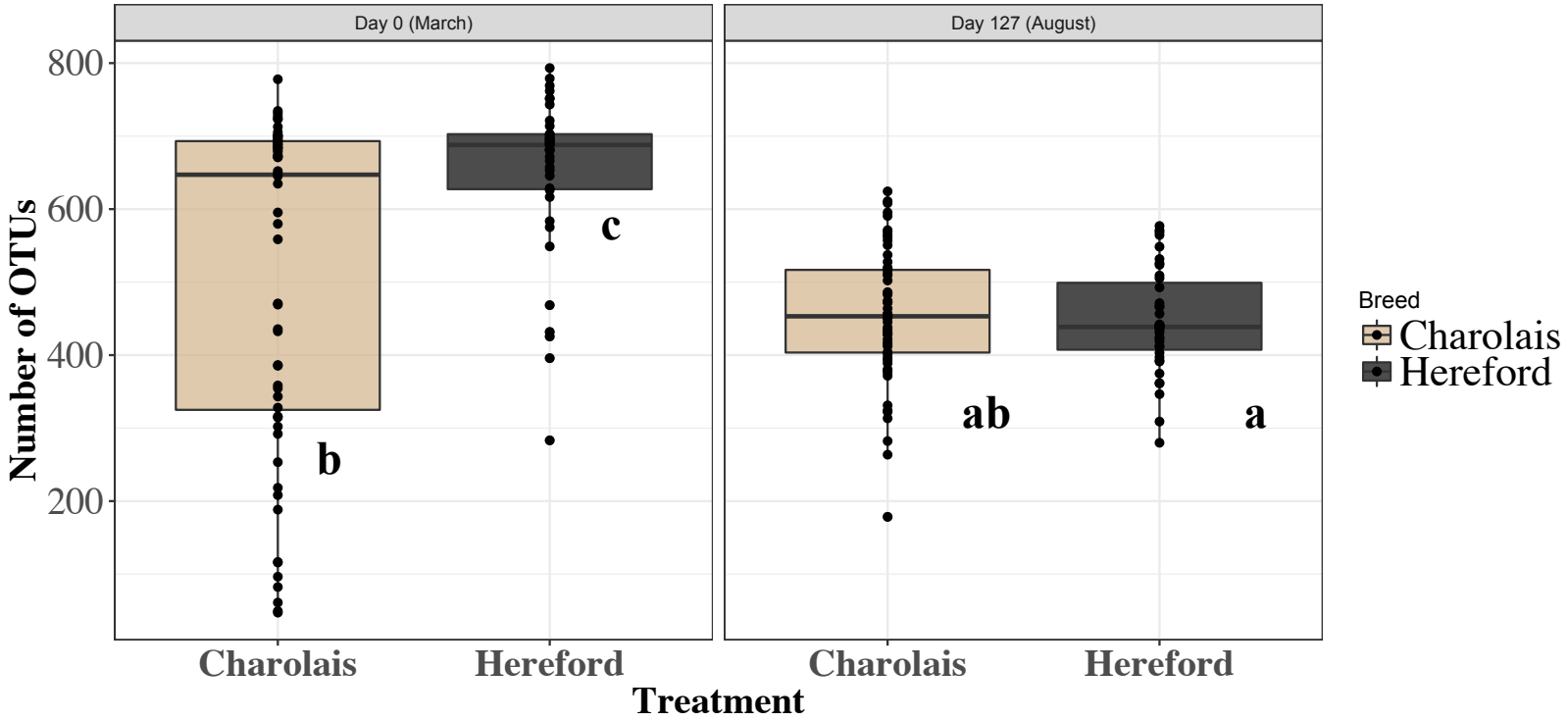

Figure 11. Sire breed × time point effect for fecal OTUs. Subscripts indicated significance at a level of $P \leq 0.05$. A significant sire breed × time point interaction was observed for changes in fecal community diversity over time as measured by the number of OTUs. Hereford-sired cows had greater number of OTUs prior to toxin exposure (Day 0) than Charolais-sired cows. After toxin exposures, Hereford-sired cows had significantly reduced number fecal OTUs, while Charolais-sired cows did significantly reduce the number of OTUs between pre- and post-treatment tripoints. Sire breed effects were not statistically significant at the post-treatment time point.

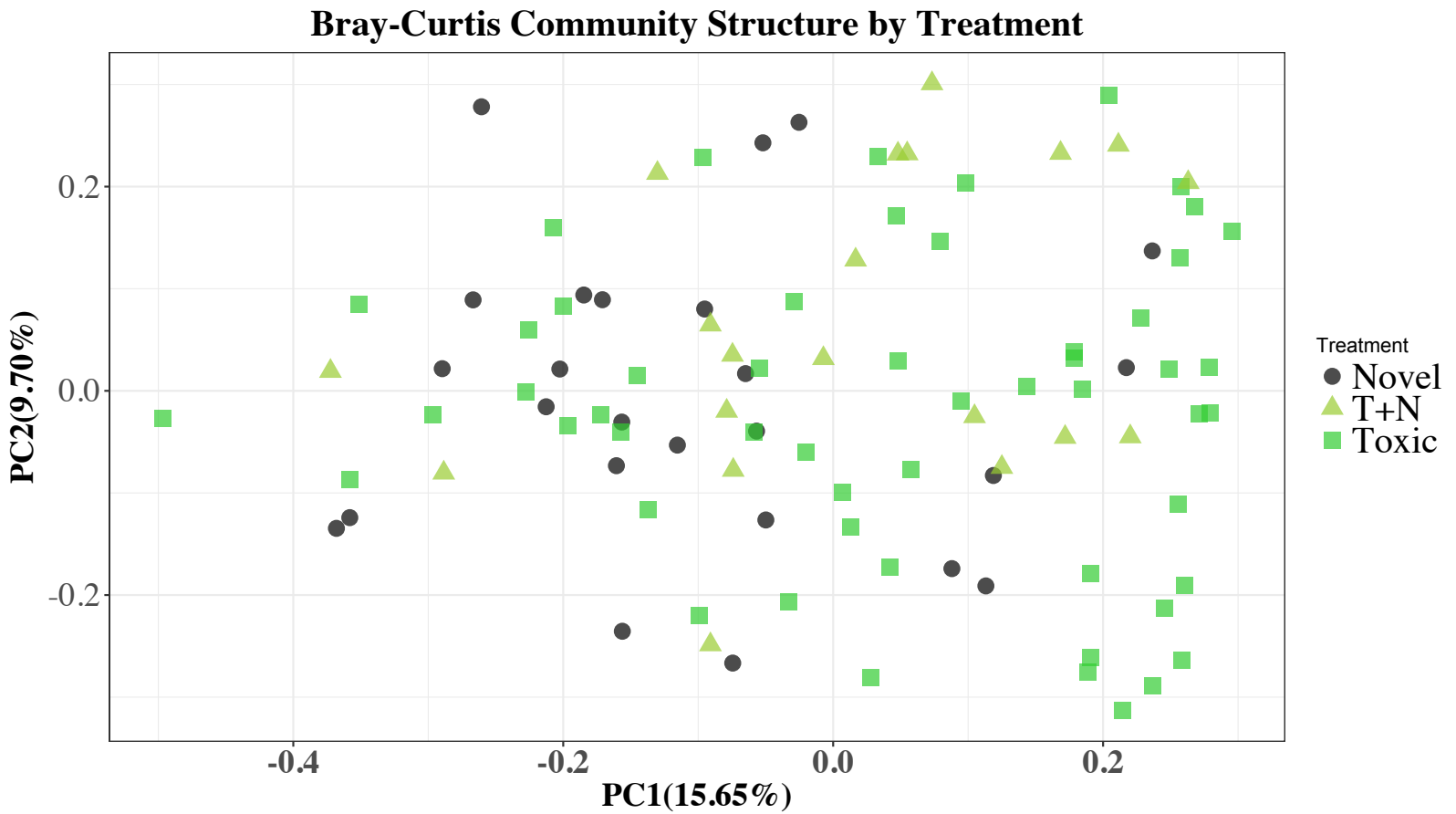

Figure 12. Bray-Curtis PCoA of post-treatment fecal samples. Fecal samples collected from cows after grazing toxic fescue, novel fescue, or a combination of both forages for five months were analyzed for community stricter at the post treatment time point. Community clustering by treatment condition was not obvious. This indicates that toxin exposure in the diet did not shift community structure to cause significant clustering as calculated by the Bray-Curtis index.

Table 9. Bray-Curtis R and p-values in post-treatment samples

Comparison	R-value	P-value
Novel-Toxic-Alt.	0.0690	0.037*
Novel-Toxic	0.0913	0.037*
Novel-Alt.	0.0958	0.006*
Toxic-Alt.	0.0690	0.291

*indicates likelihood that R-value for Bray-Curtis community structure comparison was generated at random in by Monte-Carlo simulations in Mothur.

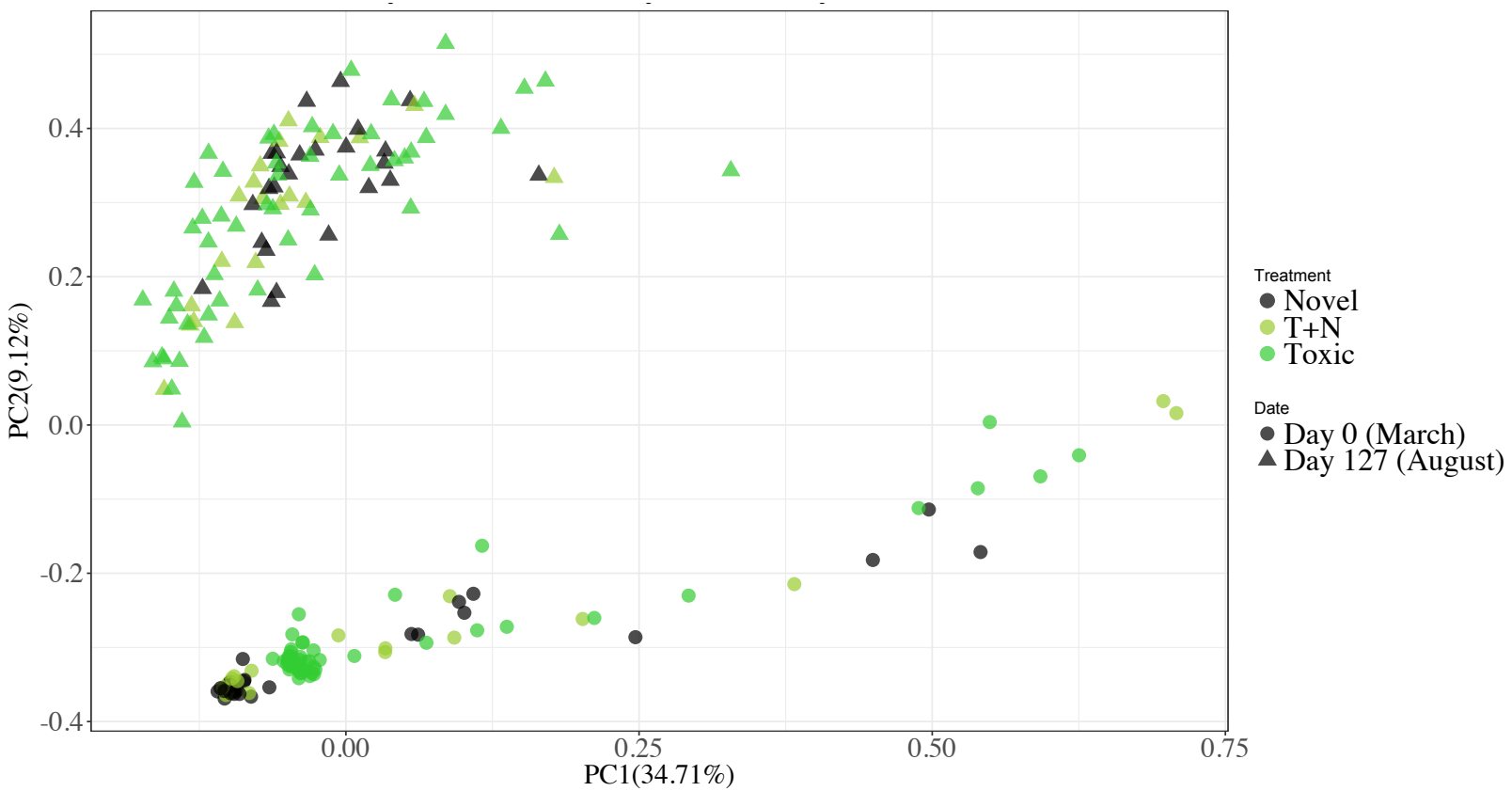

Figure 13. Bray-Curtis PCoA of treatments across time points. PCoA plot of cow fecal Bray-Curtis community. Significant clustering of fecal community structure was observed by pre- and post-treatment time points. The effect of grazing toxic fescue, novel fescue, or a combination of both on Bray-Curtis community structures was not statistically significant. Two clusters of community are defined by fecal collection time point.

Table 10. Bray-Curtis community structure R and P-values by time point

Comparison	R-value	P-value
Toxic	0.81	<0.001*
March- August		
Novel or Alt.		
March- August	0.86	<0.001*

*****indicates likelihood that R-value for Bray-Curtis community structure comparison was generated at random in by Monte-Carlo simulations in Mothur.

Table 11. Best post-treatment predictors of toxin exposure

A. OTUs without Traditional Phenotypes ‡		B. OTUs with Traditional Phenotypes †	
Variable	MDA*	Variable	MDA*
Otu00221_Bacteroides	22	Rectal_Temperature[2]	28
Otu00390_Coriobacteriaceae_UNCL	21	Otu00220_Flavobacteriaceae_UNCL	19
Otu00472_Bacteroides	20	Otu00389_Ruminococcaceae_UNCL	18
Otu00366_Mogibacterium	18	Otu00471_Rhizobiales_UNCL	18
Otu00672_Bacteria_UNCL	18	Otu00671_Ruminococcaceae_UNCL	16
Otu00395_Bacteria_UNCL	15	Otu00365_Bacteroidetes_UNCL	15
Otu00380_Clostridiales_UNCL	14	Otu00379_Sphingomonas	14
Otu00544_Lysobacter	13	Otu00394_Clostridiales_UNCL	12
Otu01757_Arthrobacter	13	Otu01756_Ruminococcaceae_UNCL	11
Otu00639_Clostridia_UNCL	12	Percent_Weight_Gain[1]	10
Otu00644_Lachnospiraceae_UNCL	12	Otu00493_Ruminococcaceae_UNCL	10
Otu00097_Clostridiales_UNCL	11	Otu00543_Akkermansia	10
Otu00335_Actinobacteria_UNCL	11	Otu00638_Arcanobacterium	10
Otu00494_Ruminococcaceae_UNCL	11	Otu01221_Bacteroidales_UNCL	10
Otu01265_Clostridiales_UNCL	11	Otu01561_Clostridiales_UNCL	10
Otu01562_Bacteria_UNCL	11	Otu01728_Clostridium_IV	10
Otu01588_Bacteria_UNCL	11	Otu00096_Acinetobacter	9
Otu00417_Erysipelotrichaceae_UNCL	10	Otu00134_Alcaligenaceae_UNCL	9
Otu01138_Clostridiales_UNCL	10	Otu00283_Bacteroidetes_UNCL	9
Otu01222_Bacteria_UNCL	10	Otu00334_Erysipelothrix	9
Otu01533_Flavobacteriaceae_UNCL	10	Otu00576_Clostridiales_UNCL	9
Otu01629_Geminicoccus_UNCL	10	Otu00643_Firmicutes_UNCL	9
Otu01729_Lachnospiraceae_UNCL	10	Otu00749_Bacteria_UNCL	9
Otu00172_Comamonadaceae_UNCL	9	Otu01587_Ruminococcaceae_UNCL	9

*****Mean decrease accuracy if variable excluded from random forest
[1][(BWd0 – BWd147) / BWd0) × 100.
[2]Recorded that last week of the trial at (post-treatment)
‡Random forest constructed from 2,00 most abundant OTUs only
† Random forest constructed from 2,000 most abundant OTUs, experimental fixed effects and (Treatment, Breed, Parity, Pregnacy) and quantitative measures of fescue stress (rectal temperature, weight gain, and hair reduction rate)

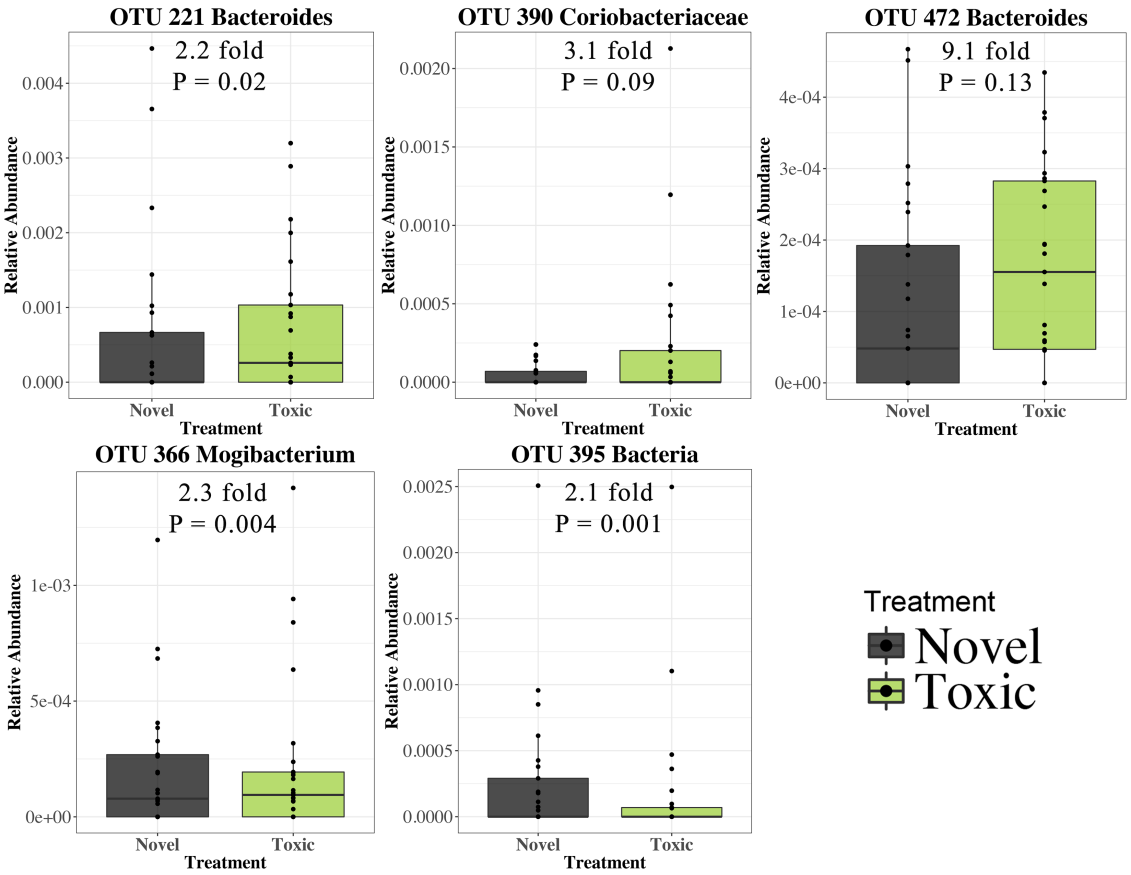

Figure 14. **Taxa differentially abundant by treatment**. Twenty-five random animals from the toxic treatment and 25 five grazed on novel fescue for 5 months were randomly selected and submitted to Random forest for identification taxa indicative of toxin exposure. Operational taxonomic unites (OTUs) identified by random forest to have high mean decrease accuracy (MDA) in treatment prediction were differentially abundant by treatment.

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Table 12. Best post-treatment OTU predictors for quantitative measures of (A) weight gain, (B) rectal temperature, and (C) hair reduction rate

Weight Gain[1], lbs (Toxic, n=54)		Rectal Temperature[2], °C (Toxic, n=54)		Hair Reduction[3,4] (Toxic, n=54)	
	MSE*	Variable	MSE*	Variable	MSE*
Otu00176_Streptococcus	29	Otu00184_Intestinimonas	24	Otu00691_Blautia	27
Otu00277_Ruminococcaceae_UNCL	20	Otu00863_Bacteria_UNCL	23	Otu01395_Lachnospiraceae_UNCL	24
Otu00651_Bacteria_UNCL	20	Otu00095_Ruminococcaceae_UNCL	19	Otu00651_Bacteria_UNCL	18
Otu00789_Lachnospiraceae_UNCL	19	Otu01389_Blautia	15	Otu01729_Bacteria_UNCL	16
Otu00431_Coriobacteriaceae_UNCL	16	Otu00132_Roseburia	14	Otu00564_Firmicutes_UNCL	14
Otu00564_Firmicutes_UNCL	16	Otu00057_Bacteroides	13	Otu00139_Bacteroidales_UNCL	13
Otu01647_Lachnospiraceae_UNCL	13	Otu00231_Ruminococcaceae_UNCL	12	Otu01185_Bacteria_UNCL	13
Otu00710_Lachnospiraceae_UNCL	12	Otu00403_Lachnospiraceae_UNCL	12	Otu01986_Lachnospiraceae_UNCL	13
Otu00752_Firmicutes_UNCL	12	Otu00838_Ruminococcaceae_UNCL	12	Otu00232_Olsenella	12
Otu00040_Cryomorphaceae_UNCL	11	Otu00583_Clostridiales_UNCL	11	Otu00422_Prevotellaceae_UNCL	12
Otu01696_Clostridiales_UNCL	11	Otu00044_Clostridiales_UNCL	10	Otu00080_Clostridium_XlVb	11
Otu00267_Lachnospiraceae_UNCL	10	Otu00080_Clostridium_XlVb	10	Otu00092_Ruminococcaceae_UNCL	11
Otu00357_Microbacterium	10	Otu00082_Ruminococcaceae_UNCL	10	Otu00433_Devosia	10
Otu00015_Acinetobacter	9	Otu00842_Bacteroidales_UNCL	10	Otu00520_Erysipelotrichaceae_UNCL	10
Otu00160_Oscillibacter	9	Otu01764_Erysipelotrichaceae_UNCL	10	Otu01072_Ruminococcaceae_UNCL	10
Otu00350_Lachnospiraceae_UNCL	9	Otu00260_Ruminococcaceae_UNCL	9	Otu01846_Steroidobacter	10
Otu00397_Clostridiales_UNCL	9	Otu01125_Paenibacillus	9	Otu00291_Lachnospiraceae_UNCL	9
Otu00498_Clostridiales_UNCL	9	Otu00187_Bacteria_UNCL	8	Otu00293_Lachnospiraceae_UNCL	9
Otu01523_Porphyromonadaceae_UNCL	9	Otu00232_Olsenella	8	Otu00492_Ruminococcaceae_UNCL	9
Otu01908_Ruminococcaceae_UNCL	9	Otu00338_Bacteroidetes_UNCL	8	Otu00548_Clostridiales_UNCL	9
Otu00007_Comamonas	8	Otu00390_Lachnospiraceae_UNCL	8	Otu00619_Subdivision5_UNCL	9
Otu00027_Sphingomonas	8	Otu00415_Clostridiales_UNCL	8	Otu00337_Clostridiales_UNCL	8
Otu00049_Bacteroides	8	Otu00566_Paenibacillus	8	Otu00344_Clostridiales_UNCL	8
Otu00067_Bacillales_UNCL	8	Otu00940_Clostridiales_UNCL	8	Otu00609_Sedimentibacter	8

[1][(BWd0 – BWd147) / BWd0) × 100.

[2]Recorded that last week of the trial at (post-treatment)

[3]1 = slick summer coat (100% shed) to 5 = full, thick winter coat (0% shed) (Gray et al., 2011)

[4] Hair reduction rate is a rate of HCS reduction (change in HCS from day 0 to 112)/ 112 days after a sliding window HCS adjustment

*Percent increase mean square error if variable excluded from random forest.

Table 13. Best post-treatment OTU predictors including covariates and fixed effects for (A) weight gain, (B) rectal temperature, and (C) hair reduction rate.

Weight Gain[1] (Toxic, n=54)		Rectal Temperature[2] (Toxic, n=54)		Hair Reduction[3,4] (Toxic, n=54)	
Variable	MSE*	Variable	MSE*	Variable	MSE*
Otu00176_Streptococcus	25	Serum Phosphorous	38	Otu00691_Blautia	26
Otu00651_Bacteria_UNCL	19	Respiration Rate	24	Percent Weight Gain	25
Otu00277_Ruminococcaceae_UNCL	18	Otu00863_Bacteria_UNCL	24	Otu01395_Lachnospiraceae_UNCL	25
Otu00789_Lachnospiraceae_UNCL	18	Pond (yes or no for last week of trial)	22	Otu01729_Bacteria_UNCL	18
Hair Reduction Rate	16	Otu00132_Roseburia	19	Otu00651_Bacteria_UNCL	16
Otu00564_Firmicutes_UNCL	14	Otu00403_Lachnospiraceae_UNCL	17	Otu00139_Bacteroidales_UNCL	13
Otu01871_Clostridiales_UNCL	12	Percent Weight Gain	16	Otu01185_Bacteria_UNCL	13
Otu01647_Lachnospiraceae_UNCL	11	Otu00073_Ruminococcaceae_UNCL	14	Otu00232_Olsenella	12
Otu01696_Clostridiales_UNCL	11	Otu00082_Ruminococcaceae_UNCL	13	Otu00564_Firmicutes_UNCL	12
Respiration Rate	10	Otu00231_Ruminococcaceae_UNCL	13	Otu01072_Ruminococcaceae_UNCL	12
Otu01271_Microbacteriaceae_UNCL	10	Otu00080_Clostridium_XlVb	12	Otu01986_Lachnospiraceae_UNCL	12
Otu00040_Cryomorphaceae_UNCL	9	Otu00068_Bacteroidetes_UNCL	10	Otu01846_Steroidobacter	11
Otu00350_Lachnospiraceae_UNCL	9	Otu00583_Clostridiales_UNCL	10	Otu00433_Devosia	10
Otu00420_Lachnospiraceae_UNCL	9	Hair Reduction Rate	9	Otu00080_Clostridium_XlVb	9
Otu00710_Lachnospiraceae_UNCL	9	Otu00011_Bacteroides	9	Otu00092_Ruminococcaceae_UNCL	9
Otu00752_Firmicutes_UNCL	9	Otu00014_Bacteroidetes_UNCL	9	Otu00422_Prevotellaceae_UNCL	9
Otu00783_Clostridiales_UNCL	9	Otu00031_Bacteroidetes_UNCL	9	Otu00522_Akkermansia	9
Otu01455_Bacteria_UNCL	9	Otu00065_Firmicutes_UNCL	9	Otu00619_Subdivision5_UNCL	9
Otu00476_Erysipelotrichaceae_UNCL	8	Otu00181_Bacteroidetes_UNCL	9	Otu00684_Firmicutes_UNCL	9
Otu00498_Clostridiales_UNCL	8	Otu00281_Oscillibacter	9	Otu01264_Bacteria_UNCL	9
Otu00500_Roseomonas	8	Otu00305_Clostridiales_UNCL	9	Otu00191_Bacteroidetes_UNCL	8
Otu00787_Lachnospiraceae_UNCL	8	Otu00320_Corynebacterium	9	Otu00293_Lachnospiraceae_UNCL	8
Otu01056_Ruminococcaceae_UNCL	8	Otu00338_Bacteroidetes_UNCL	9	Otu00520_Erysipelotrichaceae_UNCL	8
Otu01786_Firmicutes_UNCL	8	Otu00940_Clostridiales_UNCL	9	Otu00548_Clostridiales_UNCL	8

[1][(BWd0 – BWd147) / BWd0) × 100.

[2]Recorded that last week of the trial at (post-treatment)

[3]1 = slick summer coat (100% shed) to 5 = full, thick winter coat (0% shed) (Gray et al., 2011)

[4] Hair reduction rate is a rate of HCS reduction (change in HCS from day 0 to 112)/ 112 days after a sliding window HCS adjustment

*Percent increase mean square error if variable excluded from random forest

Table 14. Pearson's correlations and p-values of post-treatment OTUs and stress indicators.

Herefords n=19	RT	HRR	%Gain	Otu 176	Otu 651	Otu 277	Otu 789	Otu 403	Otu 73	Otu 691	Otu 1395	Otu 1729
	Charolais n=35											
RT	1.00	0.10 0.83	-0.30 0.51	0.35 0.44	0.07 0.88	-0.26 0.58	-0.60 0.16	-0.64 0.12	0.33 0.47	-0.54 0.21	-0.40 0.37	-0.05 0.91
HRR	0.36 0.13	1.00	**-0.72** **0.07**	-0.24 0.61	0.65 0.12	0.32 0.48	0.60 0.16	0.21 0.65	0.55 0.20	0.06 0.90	**0.79** **0.03**	**0.77** **0.04**
%Gain	-0.24 0.31	**-0.63** **0.004**	1.00	-0.11 0.81	-0.08 0.87	-0.50 0.25	-0.10 0.84	0.28 0.54	-0.21 0.66	0.47 0.28	-0.38 0.40	**-0.80** **0.03**
Otu 176	0.38 0.11	-0.06 0.79	0.05 0.83	1.00	-0.02 0.97	-0.35 0.44	-0.18 0.69	0.01 0.98	-0.23 0.62	-0.28 0.55	-0.11 0.81	-0.36 0.43
Otu 651	0.18 0.47	**0.56** **0.01**	**-0.50** **0.03**	-0.11 0.67	1.00	-0.06 0.90	**0.68** **0.09**	0.61 0.15	**0.74** **0.06**	0.60 0.16	**0.70** **0.08**	0.19 0.69
Otu 277	0.08 0.76	0.41 0.09	-0.57 0.01	-0.25 0.29	**0.63** **0.004**	1.00	0.13 0.79	-0.10 0.83	0.34 0.46	0.16 0.74	0.18 0.70	**0.74** **0.06**
Otu 789	-0.29 0.24	0.31 0.20	**-0.43** **0.06**	-0.27 0.26	**0.70** **0.001**	**0.65** **0.003**	1.00	**0.89** **0.01**	0.26 0.57	0.65 0.11	**0.95** **0.001**	0.36 0.43
Otu 403	**0.56** **0.01**	0.23 0.35	-0.20 0.42	0.14 0.56	**0.48** **0.04**	**0.42** **0.07**	0.16 0.52	1.00	0.16 0.74	**0.80** **0.03**	**0.72** **0.07**	-0.01 0.98
Otu 73	0.13 0.59	0.11 0.65	0.09 0.72	-0.26 0.29	-0.10 0.70	0.13 0.58	-0.05 0.84	0.25 0.31	1.00	0.48 0.28	0.29 0.52	0.45 0.32
Otu 691	**-0.60** **0.007**	**-0.67** **0.002**	0.30 0.22	-0.13 0.61	**-0.44** **0.06**	-0.35 0.14	0.02 0.95	-0.26 0.29	-0.06 0.79	1.00	0.42 0.35	-0.02 0.97
Otu 1395	0.02 0.94	0.37 0.12	**-0.46** **0.05**	-0.02 0.92	**0.89** **<.0001**	**0.56** **0.01**	0.64 **0.003**	0.38 0.11	-0.20 0.42	-0.34 0.15	1.00	0.50 0.25
Otu 1729	-0.10 0.69	0.14 0.56	-0.20 0.41	-0.25 0.30	0.20 0.41	**0.65** **0.003**	0.20 0.40	0.17 0.48	0.28 0.24	-0.18 0.46	0.27 0.26	1.00

Table 14 (Cont.)

1 Pearson's correlations were calculated in PROC CORR for SAS (version 9.4; SAS Inst., Inc., Carry, NC). Correlations among variables for Charolais-sired-cows on toxic fescue are reported above the diagonal. Correlations for variable for Hereford-sired cows on toxic fescue are reported below the diagonal.

Abbreviation	Definition
RT	Rectal Temperature[2]
HRR	Hair Shedding Rate[3,4]
%gain	Percent Weight Gain[1]
Otu 176	Otu 176 *Streptococcus*
Otu 651	Otu 651 Bacteria
Otu 277	Otu 277 *Ruminococcaceae*
Otu 789	Otu 789 *Lachnospiraceae*
Otu 403	Otu 403 *Lachnospiraceae*
Otu 73	Otu 73 *Ruminococcaceae*
Otu 691	Otu 691 *Blautia*
Otu 1395	Otu 1395 *Lachnospiraceae*
Otu1729	Otu 1729 *Bacteria*

[1][(BWd0 – BWd147) / BWd0) × 100.
[2]Recorded that last week of the trial at (post-treatment)
[3]1 = slick summer coat (100% shed) to 5 = full, thick winter coat (0% shed) (Gray et al., 2011)
[4] Hair reduction rate was calculated as a rise (change in HCS from day 0 to 112)/ 112 days after a sliding window HCS adjustment

Table 15. Best pre-treatment OTU for prediction for quantitative measures of (A) weight gain, (B) rectal temperature, and (C) hair reduction rate.

Weight Gain Predictors (Toxic, n=54)		Rectal Temperature Predictors (Toxic, n=54)		Hair Reduction Predictors (Toxic, n=54)	
	MSE*	Variable	MSE*	Variable	MSE*
Otu00845_Odoribacter	27	Otu00930_Clostridiales_UNCL	17	Otu00390_Lachnospiraceae_UNCL	25
Otu01255_Bacteroides	19	Otu00105_Rhizobiales_UNCL	14	Otu00845_Odoribacter	24
Otu00011_Bacteroides	18	Otu00164_Bacteroidales_UNCL	14	Otu00388_Clostridiales_UNCL	19
Otu01374_Ruminococcaceae_UNCL	13	Otu00241_Clostridiales_UNCL	14	Otu00405_Rikenellaceae_UNCL	18
Otu00204_Firmicutes_UNCL	12	Otu01311_Lachnospiraceae_UNCL	13	Otu00927_Bacteria_UNCL	18
Otu00399_Clostridium_IV	12	Otu00323_Bacteria_UNCL	12	Otu01156_Lachnospiraceae_UNCL	14
Otu00302_Clostridiales_UNCL	11	Otu00585_Treponema	12	Otu01075_Bacteria_UNCL	12
Otu00510_Clostridiales_UNCL	11	Otu00754_Clostridiales_UNCL	11	Otu01879_Bacteroidetes_UNCL	12
Otu00522_Akkermansia	11	Otu00790_Firmicutes_UNCL	11	Otu01078_Bacteria_UNCL	11
Otu00754_Clostridiales_UNCL	11	Otu00881_Bacteria_UNCL	11	Otu01296_Firmicutes_UNCL	10
Otu00139_Bacteroidales_UNCL	10	Otu01025_Bacteria_UNCL	10	Otu00229_Clostridium_sensu_stricto	9
Otu00359_Ruminococcaceae_UNCL	10	Otu00161_Bacteria_UNCL	9	Otu00348_Clostridiales_UNCL	9
Otu00549_Porphyromonadaceae_UNCL	10	Otu00487_Clostridiales_UNCL	9	Otu00384_Clostridiales_UNCL	9
Otu01003_Firmicutes_UNCL	10	Otu01573_Bacteroidetes_UNCL	9	Otu01335_Clostridiales_UNCL	9
Otu01248_Prevotella	10	Otu00272_Ruminococcaceae_UNCL	8	Otu00289_Coriobacteriaceae_UNCL	8
Otu01632_Ruminococcaceae_UNCL	10	Otu00447_Porphyromonadaceae_UNCL	8	Otu00520_Erysipelotrichaceae_UNCL	8
Otu01924_Lachnospiraceae_UNCL	10	Otu00450_Coriobacteriaceae_UNCL	8	Otu00632_Succiniclasticum	8
Otu00289_Coriobacteriaceae_UNCL	9	Otu00994_Ruminococcaceae_UNCL	8	Otu01633_Clostridiales_UNCL	8
Otu00481_Clostridiales_UNCL	9	Otu01016_Ruminococcaceae_UNCL	8	Otu00706_Ruminococcaceae_UNCL	7
Otu00561_Bacteria_UNCL	9	Otu01052_Bacteroidetes_UNCL	8	Otu00881_Bacteria_UNCL	7
Otu00585_Treponema	9	Otu01203_Faecalibacterium	8	Otu01006_Bacteria_UNCL	7
Otu01418_Clostridiales_UNCL	9	Otu01627_Coriobacteriaceae_UNCL	8	Otu01191_Lachnospiraceae_UNCL	7
Otu00182_Paraprevotella	8	Otu01715_Lachnospiraceae_UNCL	8	Otu01222_Bacteria_UNCL	7
Otu00232_Olsenella	8	Otu00018_Paraprevotella	7	Otu01728_Coriobacteriaceae_UNCL	7

[1][(BWd0 – BWd147) / BWd0) × 100.

[2]Recorded that last week of the trial at (post-treatment)

[3]1 = slick summer coat (100% shed) to 5 = full, thick winter coat (0% shed) (Gray et al., 2011)

[4] Hair reduction rate was calculated as a rise (change in HCS from day 0 to 112)/ 112 days after a sliding window HCS adjustment

*Percent increase mean square error if variable excluded from random forest

Table 16. Pearson's correlation and p-values of pre-treatment OTUs and stress indicators.

Herefords n=19 \ Charolais n=35	RT	HRR	%Gain	Otu 845	Otu 1255	Otu 1374	Otu 204	Otu 399	Otu 930	Otu 105	Otu 164	Otu 241	Otu 1311	Otu 390	Otu 388	Otu 405	Otu 927
RT	1.00	**0.37** **0.03**	**-0.39** **0.02**	0.18 0.29	-0.02 0.92	-0.27 0.12	-0.12 0.50	-0.16 0.36	0.27 0.12	0.33 0.05	**-0.38** **0.03**	**-0.31** **0.07**	-0.20 0.25	-0.12 0.49	-0.03 0.85	-0.14 0.41	0.16 0.35
HRR	0.36 0.13	1.00	**-0.34** **0.05**	**0.51** **0.002**	-0.14 0.41	-0.17 0.32	-0.13 0.46	-0.12 0.49	-0.11 0.54	0.02 0.90	-0.07 0.68	-0.10 0.56	0.10 0.55	**0.39** **0.02**	**0.33** **0.06**	**0.33** **0.06**	**0.29** **0.09**
%Gain	-0.24 0.31	**-0.63** **0.004**	1.00	**-0.33** **0.05**	**0.40** **0.02**	-0.16 0.37	0.23 0.18	-0.21 0.22	-0.13 0.46	**-0.41** **0.02**	0.22 0.21	0.18 0.30	-0.01 0.95	-0.12 0.48	-0.14 0.41	0.00 0.99	-0.25 0.15
Otu 845	-0.10 0.70	0.14 0.55	**-0.40** **0.09**	1.00	0.15 0.38	-0.16 0.37	0.10 0.58	0.20 0.26	-0.17 0.33	0.18 0.30	-0.15 0.39	-0.12 0.48	-0.10 0.55	0.35 0.04	0.14 0.41	0.31 0.07	**0.40** **0.02**
Otu 1255	-0.22 0.37	-0.23 0.35	0.34 0.16	-0.04 0.86	1.00	0.13 0.45	0.14 0.42	0.16 0.36	-0.07 0.69	-0.12 0.48	-0.13 0.44	-0.08 0.66	-0.15 0.38	0.09 0.63	-0.08 0.65	**0.33** **0.05**	0.14 0.43
Otu 1374	0.01 0.97	-0.14 0.57	-0.09 0.72	-0.20 0.42	-0.31 0.20	1.00	0.07 0.69	0.17 0.33	-0.12 0.49	-0.04 0.81	0.11 0.51	0.00 0.98	-0.12 0.51	-0.02 0.92	0.11 0.53	0.21 0.23	-0.14 0.42
Otu 204	0.004 0.99	-0.23 0.34	0.11 0.66	0.18 0.47	0.03 0.89	0.13 0.58	1.00	**0.47** **0.004**	0.18 0.31	0.14 0.43	**0.30** **0.08**	0.14 0.42	-0.08 0.64	0.07 0.68	0.19 0.27	**0.33** **0.05**	0.18 0.31
Otu 399	0.23 0.35	0.14 0.58	-0.01 0.98	0.11 0.67	-0.03 0.89	0.29 0.23	0.38 0.11	1.00	0.19 0.27	0.24 0.17	0.09 0.62	0.07 0.70	0.06 0.73	**0.47** **0.005**	0.05 0.77	**0.33** **0.05**	**0.40** **0.02**
Otu 930	0.01 0.98	0.30 0.21	0.01 0.96	-0.16 0.52	**0.40** **0.09**	-0.09 0.73	-0.30 0.22	0.39 0.10	1.00	0.14 0.42	-0.12 0.48	0.00 0.99	-0.06 0.71	0.02 0.93	**0.53** **0.001**	0.03 0.85	0.10 0.55
Otu 105	**0.45** **0.06**	-0.31 0.19	0.21 0.38	-0.05 0.83	-0.17 0.48	0.23 0.35	0.12 0.62	**0.44** **0.06**	-0.20 0.42	1.00	0.03 0.85	0.01 0.94	-0.01 0.94	0.04 0.84	-0.10 0.58	-0.05 0.76	0.06 0.71
Otu 164	**-0.41** **0.08**	0.01 0.97	0.04 0.88	-0.05 0.84	-0.11 0.67	0.21 0.39	-0.09 0.72	-0.08 0.75	-0.05 0.83	-0.08 0.74	1.00	**0.54** **0.001**	-0.11 0.54	0.02 0.91	-0.07 0.68	-0.04 0.83	0.21 0.23
Otu 241	**-0.48** **0.04**	**-0.47** **0.04**	0.26 0.27	-0.36 0.13	0.25 0.31	0.14 0.57	0.29 0.22	-0.22 0.36	-0.29 0.23	0.04 0.87	0.24 0.33	1.00	-0.06 0.72	0.16 0.35	-0.03 0.88	-0.04 0.82	-0.15 0.40
Otu 1311	-0.33 0.17	-0.04 0.87	**-0.39** **0.09**	**0.66** **0.002**	0.08 0.75	-0.33 0.17	-0.10 0.67	-0.23 0.34	-0.12 0.62	-0.19 0.43	-0.15 0.55	0.004 0.99	1.00	-0.08 0.64	0.27 0.12	-0.07 0.68	-0.09 0.60
Otu 390	0.26 0.28	**0.52** **0.02**	-0.12 0.61	0.22 0.37	0.23 0.34	-0.37 0.12	0.00 1.00	0.27 0.26	**0.42** **0.07**	-0.02 0.93	-0.46 0.05	-0.29 0.22	0.04 0.88	1.00	0.11 0.55	**0.45** **0.01**	**0.29** **0.10**
Otu 388	0.18 0.45	**0.43** **0.06**	**-0.41** **0.08**	-0.09 0.71	-0.08 0.76	-0.12 0.61	-0.16 0.51	**-0.39** **0.09**	-0.19 0.44	-0.22 0.37	0.19 0.44	0.01 0.95	- 0.001 1.00	0.01 0.96	1.00	**0.30** **0.08**	-0.01 0.94
Otu 405	-0.13 0.61	**0.39** **0.10**	-0.26 0.28	0.04 0.87	-0.13 0.61	0.10 0.68	-0.15 0.53	0.02 0.92	-0.13 0.59	0.01 0.98	**0.41** **0.08**	0.09 0.73	0.12 0.62	0.13 0.60	**0.61** **0.01**	1.00	0.14 0.41
Otu 927	0.06 0.82	0.37 0.12	0.13 0.59	-0.02 0.93	0.13 0.61	-0.23 0.35	-0.15 0.53	0.05 0.84	**0.46** **0.05**	-0.27 0.27	-0.07 0.78	-0.27 0.26	-0.12 0.63	**0.52** **0.02**	-0.23 0.35	-0.12 0.64	1.00

Table 16 (Cont.)

[1]Pearson's correlations were calculated in PROC CORR for SAS (version 9.4; SAS Inst., Inc., Carry, NC). Correlations among variables for Charolais-sired-cows on toxic fescue are reported above the diagonal. Correlations for variable for Hereford-sired cows on toxic fescue are reported below the diagonal.

Abbreviation	Definition
RT	Rectal Temperature[2]
HRR	Hair Shedding Rate[3,4]
%Gain	Percent Weight Gain[1]
Otu 845	Otu 845 *Odoribacter*
Otu 1255	Otu 1255 *Bacteroides*
Otu 1374	Otu 1374 *Ruminococcaceae*
Otu 204	Otu 204 *Firmicutes*
Otu 399	Otu 399 *Clostridium IV*
Otu 930	Otu 930 *Clostridiales*
Otu 105	Otu 105 *Rhizobiales*
Otu 164	Otu 164 *Bacteroidales*
Otu 241	Otu 241 *Clostridiales*
Otu 1311	Otu 1311 *Lachnospiraceae*
Otu 390	Otu 390 *Lachnospiraceae*
Otu 388	Otu 388 *Clostridiales*
Otu 405	Otu 405 *Rikenellaceae*
Otu 927	Otu 927 Bacteria

[1][(BWd0 – BWd147) / BWd0) × 100.
[2]Recorded that last week of the trial (post-treatment)
[3]1 = slick summer coat (100% shed) to 5 = full, thick winter coat (0% shed) (Gray et al., 2011)
[4] Hair reduction rate was calculated as a rise (change in HCS from day 0 to 112)/ 112 days after a sliding window HCS adjustment

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UNIVERSITY OF ARKANSAS

Office of Research Compliance

To: James Koltes
FR: Craig Coon
Date: September 27th, 2016
Subject: IACUC Approval
Expiration Date: January 14th, 2019

The Institutional Animal Care and Use Committee (IACUC) has APPROVED your personnel additions of Tom Hess and Sarah Chewnig to protocol # 16037 *Investigation of genetic factors involved in tolerance to heat and fescue based ergot alkaloid stress in cattle*.

In granting its approval, the IACUC has approved only the information provided. Should there be any further changes to the protocol during the research, please notify the IACUC in writing (via the Modification form) prior to initiating the changes. If the study period is expected to extend beyond January 14th, 2019 you must submit a newly drafted protocol prior to that date to avoid any interruption. By policy the IACUC cannot approve a study for more than 3 years at a time.

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

CNC/aem