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Steer stress response as affected by genotype and transportation

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ABSTRACT

Bovine cytochrome P450 3A28 is responsible for metabolizing ergot alkaloids that cattle ingest when feeding on endophyte-infested tall fescue grass. The objective of this research was to determine associations among genotype, transportation, and stress responses. Angus crossbred steers (n = 47) were genotyped (CC, CG, or GG) for a single-nucleotide polymorphism (C994G) in cytochrome P450 3A28. Genotypes were determined by polymerase chain reaction (PCR) amplification followed by restriction enzyme (Alu1) digestion. Steers were backgrounded on a mixed-cultivar tall fescue pasture. Following the stocker phase, steers were transported to the feedlot for finishing. Stress responses were determined 27 h prior to, and 6 and 20 h after transport. Plasma concentrations of prolactin and cortisol, and white blood cell expression of prolactin, cytochrome P450, tumor necrosis factor-alpha, and short form prolactin receptor were our indicators of stress. Both time and genotypic effects were determined. Time ($P < 0.05$) relative to transportation was associated with expression of all four genes tested. In addition, plasma concentrations of cortisol and prolactin, as well as their ratio were affected ($P < 0.05$) by time. In contrast, neither genotype nor the interaction between genotype and time affected ($P > 0.1$) our stress indicators. In previous studies, C994G genotype has been associated with cattle productivity; however, those effects were not observed in this study.

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# Charles Rosenkrans, Jr. is the research mentor and professor in the Department of Animal Science.

MEET THE STUDENT-AUTHOR

Megan Wary

I grew up in Rogers, Arkansas and attended Rogers High School, graduating in 2010. I chose to stay close to home to pursue a degree in Biochemistry at the University of Arkansas with a pre-pharmacy emphasis. After my freshman year, I decided to also declare minors in Mathematics and Animal Science because I have always had a passion for animals and enjoy challenging myself. During my time here at the University of Arkansas, I have been a student ambassador, active in my sorority Kappa Kappa Gamma, and have worked part-time as a pharmacy technician. After graduation in the spring, I will move to Denver, Colorado to pursue a Pharm. D. at the University of Colorado School of Pharmacy.

I enjoyed doing my Honors thesis project in the Animal Science department, where I was able to have hands-on experience in the field and the laboratory. I would like to thank Dr. Charles Rosenkrans for agreeing to be my mentor and for diligently teaching me all I needed to know. He was supportive and directive in the writing of my thesis and preparation for my defense. Special thanks should be given to Dr. Ali Moubarak who provided expert understanding of ergot alkaloids, and to Marites Sales and Jonathan Anthony who taught me technique in the laboratory.

INTRODUCTION

Many cattle producers in the Midwestern and Southern United States use tall fescue, *Lolium arundinaceum*, which is a cool season perennial grass that is typically infected with a fungus, *Neotyphodium coenophialum* (Browning, 2003). When cattle ingest the fungal-infected fescue, they consume ergot alkaloids which results in a condition termed fescue toxicosis. Fescue toxicosis is characterized by poor growth, decreased weight gain, suppressed appetite, reduced conception and calving rates, poor peripheral circulation, uneven hair coats, heat stress, decreased prolactin and milk production (Browning, 2003; Lyons et al., 1986; Paterson et al., 1995; Settivari et al., 2008). Fescue toxicosis is estimated to cost the U.S. beef industry approximately $500 million to $1 billion annually in lost revenue because of reduced growth and reproductive rates in cattle (Browning, 2003).

One enzyme family involved with detoxification of alkaloid toxins is cytochrome P450 (CYP450) heme-thiolatemonooxygenases. Those proteins are particularly prevalent in the smooth endoplasmic reticulum of liver cells (Hardin et al., 2012). One of the expressed bovine cytochrome P450 genes is CYP450 3A28. Cattle have a single nucleotide polymorphism (SNP) in the genetic region that codes for CYP450 3A28 and that SNP is related to cattle productivity (Sales et al., 2012).

This study aimed to determine if SNP C994G genotypes and(or) transportation affected prolactin and cortisol concentrations in plasma, and white blood cell expression of CYP3A28, PRL, sPRLR, and TFN-α. Understanding the underlying cellular and metabolic pathways following exposure to ergot alkaloids will allow for pharmacological developments to diminish the symptoms associated with fescue toxicosis.

MATERIALS AND METHODS

Animals and Sample Collection. Forty-seven Gelbvieh × Angus steers were weaned and developed on a pasture of mixed (toxic and non-toxic) tall fescue grass. After 154 d of grazing, steers were transported from Booneville, Ark. to Stillwater, Okla. (approximately 250 miles, and 5 h duration) where they were finished in a feedlot. Blood samples from the 47 steers were collected by venipuncture at 27 h prior to transport (T – 27), 6 h after arrival (T + 6), and 20 h after arrival (T + 20). Blood samples were immediately put on ice, and centrifuged to isolate the buffy coat and plasma.

DNA Preparation and Amplification. Using the blood samples collected, each steer was genotyped at the CYP450 3A28 coding sequence SNP C994G. Genomic DNA was purified from the buffy coat of each steer, and the DNA region that contains SNP C994G was amplified by poly-

merase chain reaction (PCR). Components used for PCR were 43 μL supermix, 1.3 μL reverse primer, 1.3 μL forward primer, and 5 μL template DNA (diluted to 20 ng/mL). Initial denaturing was done at 94 °C for 2 min, followed by denaturing at 94 °C for 30 sec. Annealing occurred at 55 °C for 1 min, with extension subsequent at 68 °C for 1 min. Thirty-five cycles were completed before the final extension at 68 °C for 10 min. The PCR primers for the CYP450 3A28 coding sequence (Forward: 5'-CAACAACATGAATCAGCCAGA-3'; Reverse: 5'-CCTACATTCCTGTGTGTGCAA-3') amplified a 565-base pair DNA fragment.

Genotype Assignment. Amplification products were genotyped by restriction fragment length polymorphism (RFLP). The RFLP reaction consisted of restriction enzyme AluI (5 μL), Alu I buffer (2.5 μL), and amplified DNA product (3 μL) incubated at 37 °C for one hour. Genotype was assigned after digestion products were separated by gel electrophoresis (2% agarose). The polymorphism is a transversion in the SNP; consisting of either a cytosine or a guanine in the CYP450 3A28 region with three possible genotypes, homozygous cytosine (CC) or guanine (GG), or heterozygous (CG).

Stress Indicators. From blood samples that were taken at T – 27, T + 6, and T + 20, plasma concentrations (ng/mL) were determined for cortisol and prolactin using validated radioimmunoassay. White blood cell gene expression was assessed by purifying total RNA from steer buffy coats using RiboPure Blood kit (Life Technologies Corp., Carlsbad, Calif.). Prolactin, short form prolactin receptors (sPRLR), CYP450 3A28, and tumor necrosis factor alpha (TNFα) gene expression was determined by quantitative PCR (StepOnePlus Real-Time PCR System; Life Technologies Corp., Carlsbad, Calif.). To avoid bias, real-time PCR results were normalized to one or more internal control genes, also known as 'housekeeping genes.' Those are genes whose expression are critical for basic cell function and should be expressed during normal and pathological conditions (Nicot et al., 2005). Cyclophilin is a common housekeeping gene, and was used as the reference gene in this study.

Statistical Analyses. Mixed model analysis of variance was used to determine effects of C994G genotype (CC, CG, GG), time (T – 27, T + 6, T + 20), and their interaction on stress indicators. When F-tests were significant ($P < 0.05$), then least squares means were separated using the Tukey-Kramer method. Expression values for transcription levels were computed by a comparative threshold method. Comparative threshold (Ct) is the number of cycles of PCR before the reaction meets threshold. The Ct values for the gene of interest were normalized twice, first to the control gene Ct, cyclophilin in this case, and second to the biological control, which for this experiment was CC genotype at time T – 27, this method is known as δδ-Ct method (Dharmaraj, 2014).

RESULTS AND DISCUSSION

Of the 47 Angus crossbred steers used in this study, 5 were homozygous cytosine (CC) genotype, 31 were homozygous guanine (GG), and 11 were heterozygous (CG) at the SNP in the CYP450 3A28 coding sequence. Allele frequency for the herd was 22% cytosine, and 78% guanine. Genotype at C994G did not affect ($P > 0.1$) any of the traits evaluated in this study (Table 1). The interaction of main effects was not significant for traits evaluated in this study.

Table 1. Steer stress response as affected by CYP450 3A28 genotype.

Item†	CC	GC	GG	SE	*P*-value
WBC Gene Expression					
TNFα	1.07	1.09	1.07	0.07	0.98
sPRLR	1.05	1.06	1.09	0.08	0.93
Prolactin	1.12	1.13	1.23	0.05	0.25
CYP450 3A28	0.97	0.99	1.07	0.05	0.34
Hormones					
Cortisol, ng/mL	70.1	65.5	66.4	4.7	0.83
Prolactin, ng/mL	177.6	241.1	241.1	41.7	0.61
Prolactin:cortisol	2.7	3.9	3.5	0.54	0.31

† Genes were: tumor necrosis factor alpha (TNFα), short form prolactin receptors (sPRLR), prolactin, and cytochrome P540 3A28 (CYP450 3A28). Cortisol and prolactin are plasma concentrations.

Main affect of transportation affected all stress indicators in this study (Table 2). White blood cells (WBC) are not typically known for expression of prolactin or CYP450 3A28; however, this study demonstrates that those two genes are differentially expressed in WBC. Prolactin increased ($P < 0.05$) in relation to transportation; whereas, CYP450 3A28 expression decreased ($P < 0.05$) shortly after transportation but returned to pre-travel expression by 20 h post-travel. Both sPRLR and TNFα expression profiles in WBC were highest ($P < 0.05$) at 20 h post-transportation. Circulating concentrations of cortisol were lowest ($P < 0.05$) at 6 h post-travel. In contrast, prolactin concentrations increased ($P < 0.05$) in relation to transportation and were highest at 20 h after arrival at feedlot. The prolactin:cortisol followed the prolactin profile and was different ($P < 0.05$) at each time point.

A reliable, quantifiable result of fescue toxicosis is decreased serum prolactin concentrations and this can be attributed to ergot alkaloid interaction with dopamine receptors that mediate prolactin production (Paterson et al., 1995). Ergot alkaloids interacting with other neurotransmitter receptors that are responsible for controlling blood flow, especially epinephrine and norepinephrine, are thought to cause vasoconstriction and increased respiration, which are often associated with fescue toxicosis (Paterson et al., 1995).

One enzyme family involved with detoxification of alkaloid toxins is CYP450 heme-thiolatemonooxygenases, which are particularly prevalent in the smooth endoplasmic reticulum of liver cells (Hardin et al., 2012). Previously, our research team has demonstrated that cattle have altered production traits that were associated with genotype of SNP C994G (Sales et al., 2012). In C994G, the SNP results in a point mutation (C994G) with amino acid replacement of leucine with valine at amino acid location 331 for steers of the homozygous guanine genotype.

A few other genes of interest that may be involved in animal response to fescue toxicosis include cytokine tumor necrosis factor-α (TNFα), and short form prolactin receptors (sPRLR). Prolactin receptors are cytokine receptors that interact with the prolactin molecule. Cytokines are small chemical messengers that mediate critical brain endocrine immune responses to infection and are produced by white blood cells (Feuerstein et al., 1993). White blood cells express TNFα for involvement in inflammation, and also for ischemia (restriction of blood supply) and trauma in addition to immune function (Feuerstein et al., 1993). In conclusion, our results confirm that WBC express genes that are essential for immune function, and demonstrate that WBC also express genes related to animal toxicology response.

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Table 2. Steer stress response as affected by transportation stress.

	Time Relative to Transportation				
Item†	T – 27	T + 6	T + 20	SE	*P*-value
WBC Gene Expression					
TNFα	1.02^{b}	1.07^{b}	1.15^{a}	0.04	0.001
sPRLR	1.03^{b}	1.06^{ab}	1.11^{a}	0.05	0.02
Prolactin	1.11^{b}	1.17^{ab}	1.21^{a}	0.03	0.001
CYP450 3A28	1.05^{a}	0.96^{b}	1.03^{a}	0.03	0.005
Hormones					
Cortisol, ng/mL	68.8^{a}	57.5^{b}	75.6^{a}	3.4	0.001
Prolactin, ng/mL	106.7^{b}	166.0^{b}	387.1^{a}	37.7	0.001
Prolactin:cortisol	1.6^{c}	3.3^{b}	5.2^{a}	0.52	0.001

[abc] Least squares means within the same row with different superscripts are different ($P < 0.05$).
† Genes were: tumor necrosis factor alpha (TNFα), short form prolactin receptors (sPRLR), prolactin, and cytochrome P540 3A28 (CYP450 3A28). Cortisol and prolactin are plasma concentrations.

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