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Genetic Polymorphisms of the Glucocorticoid Receptor and Interleulin-8 Receptor Genes and their Relationship to Production Traits and Hair Coat Scores in Crossbred Cattle

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Genetic polymorphisms of the glucocorticoid receptor and interleukin-8 receptor genes and their relationship to production traits and hair coat scores in crossbred cattle

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Abstract

Little is understood about how the diversity of genes, specifically the glucocorticoid receptor (GR) and interleukin-8 receptor (CXCR2), are related to reproductive health and how this affects physical traits in cattle. Glucocorticoid receptors have been positively associated with higher milk yields, lactose content, feed intake, and feed conversion rates. Interleukin-8 genes are part of the innate immune response and help with many aspects of female reproductive health, such as protecting the embryo from the maternal immune system during pregnancy. The objective of this research was to identify polymorphisms in the GR and CXCR2 genes and to associate genotypes between the abovementioned polymorphisms and production traits in crossbred cattle. The hypothesis was that polymorphisms will exist for GR and CXCR2 genes and will be linked to production traits. Blood samples were collected from 94 crossbred cattle over a period of 3 years (2012, 2013, 2014) and the DNA was extracted, amplified, and sent to GeneSeek in Lincoln, Nebraska, to be analyzed and genotyped for single nucleotide polymorphisms (SNP). Phenotypic data, including cow pre-breeding body condition score (BCS) and weight, Julian calving date, calf birth weight, cow weaning BCS and weight, calf weaning weight, calf adjusted 205-day weight, cow efficiency, and hair coat scores (HCS) were collected from the 94 crossbred cattle and analyzed alongside the genotypic results. Significant relationships were determined using *t*-tests. Single nucleotide polymorphisms were found for the GR and CXCR2 genes and the polymorphisms were significantly related to production traits in cattle. Scientists and breeders could manipulate these genes to produce cattle that are more efficient and possess more desirable production traits.

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Meet the Student-Author

Avery Deaton

I grew up in Maumelle, Arkansas and graduated from Arkansas Baptist High School in 2014. I will have graduated magna cum laude from the University of Arkansas in May 2017 with a major in Animal Science and a Pre-Professional Science concentration. During my undergraduate career, I was an active member of the Pre-Vet club, Block and Bridle, and several honors societies. I was an intern at Shackleford Road Veterinary Clinic in Little Rock, Arkansas during the summer of 2016 and gained a lot of veterinary experience. I applied to veterinary school the summer of 2016 and have been accepted at several universities. I will attend the veterinary school of my choosing in August of 2017 and pursue a career in large and small animal medicine. I want to thank my mentor Charles Rosenkrans for all his help while writing my thesis. His continued guidance throughout the whole process made my research possible. I would also like to thank my committee members, Jeremy Powell and Lauren Thomas, for their support of my thesis.

Introduction

Glucocorticoids

Steroid hormones can be classified as anabolic, which result in increased lean body mass, and catabolic, which facilitate metabolism. Glucocorticoids, specifically cortisol and corticosterone, are the primary catabolic steroid hormones which assist with homeostasis in the body by mobilization of glucose and increase glucose synthesis through gluconeogenesis in the liver. In response to stress, the hypothalamic-pituitary-adrenal (HPA) axis is activated and glucocorticoids are synthesized and released by the adrenal cortex which triggers the fight-or-flight response. Specifically, when an animal becomes stressed, the hypothalamus releases corticotropin-releasing hormone which triggers the anterior pituitary to secrete adrenocorticotropin hormone (ACTH) into the bloodstream. Circulating ACTH acts on the adrenal cortex, which then releases glucocorticoids, which circulate throughout the body. Intracellular glucocorticoid receptors (GR) are located in the cytoplasm and nucleus which are members of the nuclear receptor subfamily of ligand-dependent transcription factors. The GR remains inactive until glucocorticoids bind to it. The GR then transports the glucocorticoid to the nucleus through the use of nuclear pores. Once bound to the DNA, the GR acts as a transcription factor and can either induce or repress certain target genes (Oakley and Cidlowski, 2013).

In an experiment with Brown Swiss cows, a polymorphism of the glucocorticoid receptor DNA-binding factor 1 (GRLF1) was identified and positively associated with milk yields and lactose percentages. Two single nucleotide polymorphisms (SNP) had previously been found that were associated with feed intake and feed conversion rates in cattle (Cecchinato et al., 2014). In a study done on meat quality traits in male Nellore cattle, GR polymorphisms were found to be associated with various traits, including: glucocorticoid sensitivity, bone mineral density, body mass index, abdominal obesity, cholesterol, and lower concentrations of plasma cortisol (Poleti et al., 2014). This supported the prediction that the GR is related to energy production in cattle (Cecchinato et al., 2014).

Interleukin 8

Chemokines are a family of small (8-10 kDa) chemotactic cytokines that help coordinate the movement of cells. They are grouped into four families based on their amino acid sequences: α, β, γ, and δ (Tizard, 2013). The β, or CXC, chemokines are further classified into two subgroups, ELR- and ELR+. These subgroups are based on the presence of the amino acid sequence glutamic acid-leucine-arginine, or ELR (Umasuthan et al., 2014). Interleukin-8 (IL-8) is a β chemokine that attracts and activates neutrophils for inflammatory and immune responses. It is produced by macrophages, and is often denoted as CXCL8 due to its structure and function as a ligand (Tiz-

ard, 2013). It is ELR+ and typically found in or associated with liver, acute lung injury, and atherosclerotic lesions (Olson and Ley, 2002). The most common receptor for IL-8 is CXCR2, which binds all ELR+ CXC chemokines (Olson and Ley, 2002). Recruitment of myeloid cells is facilitated by CXCR2 for inflammation sites in the liver, lungs, and atherosclerotic lesions (Olson and Ley, 2002). Different genotypes for CXCR2 have been linked to impaired neutrophil migration and increased occurrences of mastitis (Tizard, 2013). The CXCR family of genes including CXCR1 and CXCR2 have affinities for IL-8 that may result in altered animal form and (or) function.

Very little is known about the impacts of the polymorphisms of the GR and CXCR family of genes. If identified, scientists and breeders could begin to manipulate those genes for a desired phenotype. Cattle could be produced that are more efficient and possess more desirable production traits. The objective of this study was to identify polymorphisms in the GR and CXCR2 genes, and associate a specific phenotype between these polymorphisms and production traits in crossbred cattle.

Materials and Methods

This study used samples from 94 crossbred Angus cows grazing mixed grass pastures over a period of 3 consecutive years (2012, 2013, and 2014). All cows were on a fall calving schedule with a 100% calving rate during those three years. Blood samples were taken from the jugular vein and immediately put in ice. The samples were centrifuged and then buffy coat was extracted. Buffy coats were stored at -20 °C until DNA analysis.

Genomic DNA was extracted from the buffy coats and plated into 96-well plates. These plates were then shipped to GeneSeek in Lincoln, Nebraska to be sequenced and for SNP analysis. Genotyping for GR and CXCR2 SNP was performed using the Sequenom technique, as explained in GeneSeek Brochure: Agrigenomic Solutions for Breeding and Improvement (http://genomics.neogen.com/pdf/catalogs/geneseekbrochure.pdf).

Hair coat scores (HCS) were recorded for all 94 cows during each of the 3 years (2012, 2013, and 2014). Trained personnel determined HCS for each cow monthly and used a scale ranging from 1 to 5 (Table 1) (Gray et al., 2011).

Dependent variables collected from the 94 crossbred cows included cow pre-breeding body condition score (BCS) and weight, Julian calving date, calf birth weight, cow weaning BCS and weight, calf weaning weight, calf adjusted 205-day weight, cow efficiency, and HCS. The experimental unit was the cow. Significance of the genotypes and their relation to the mentioned phenotypic traits was determined using *t*-tests where significance was determined at $P < 0.05$.

Results and Discussion

The SNPs T105G and C777G were identified for the GR and CXCR2 genes, respectively (Table 2). Seventy-eight cows were homozygous dominant for the GR gene. Sixteen cows were heterozygous for the polymorphism and

Table 1. Description of hair coat scores (HCS) used in this experiment.

HCS	Description
5	Full winter coat, 0% shed
4	Initial shedding
3	Half way shed
2	Almost shed
1	Slick summer coat, 100% shed

This table was derived from Gray et al., 2011.

Table 2. Distribution of single nucleotide polymorphism (SNP) in the bovine genes GR (T105G) and CXCR2 (C777G).

	Genotype distribution[b]			MAF[c]
SNP[a]	Homo	hetero	homo	%
T105G	78	16	0	8.5
C777G	79	12	2	8.6

[a] Single nucleotide polymorphism occurred at the number indicated. First letter indicates the primary allele and the letter following the digits is the minor allele.

[b] Number of cows that were homozygous for the primary allele (Homo), heterozygous (hetero), and homozygous for the minor allele (homo).

[c] Minor allele frequency (MAF) expressed as percent.

zero cows were homozygous recessive. The minor allele frequency was 8.5% in the population. Of the 94 cows, 79 were homozygous dominant for the CXCR2 gene. Twelve cows were heterozygous and two were homozygous for the recessive allele resulting in an 8.6% minor allele frequency.

T105G Polymorphism

Cow pre-breeding weight was affected ($P = 0.02$) by genotype (Table 3). The heterozygous cows averaged 500 kg while the homozygous cows averaged 527 kg. The significant difference in weight could be due to the fact that the GR is related to feed intake, feed conversion, and energy production. Cow weight at weaning and calf weight at weaning tended ($P = 0.08$, $P = 0.11$) to be affected by genotype. All other dependent variables were not affected by the genotype.

C777G Polymorphism

Cow pre-breeding body condition score and pre-breeding weight tended ($P = 0.06$, $P = 0.08$) to be affected by CXCR2 genotype. Calf birth weight was affected ($P = 0.0003$) by genotype. Homozygous calves averaged 35 kg and heterozygous calves averaged 29 kg. Calf weaning weight also was affected ($P = 0.05$) by genotype with homozygous calves averaging 199 kg and heterozygous calves at 179 kg. Cow body condition score at weaning and weight at weaning were affected ($P < 0.05$) by genotype. Homozygous cows averaged a 5.0 BCS and 500 kg; heterozygous cows averaged a 4.7 BCS and 456 kg. The differences between genotypes could be attributed to the effect that CXCR2 has on immune responses in the body. Calf adjusted 205-day weight tended ($P = 0.10$) to be affected by genotype.

Cow Age and Year

Cow pre-breeding weight, calf birth weight, cow weaning BCS, cow weaning weight, calf weaning weight, and cow efficiency were all affected ($P < 0.05$) by cow age group (Table 4). These results are to be expected due to the correlation between cow age and productivity. The 4-10 age group provided the most significant results out of the three categories due to their age and productivity traits. Cow pre-breeding BCS, cow pre-breeding weight, Julian calving date, cow weaning weight, calf adjusted 205-day weight, and cow efficiency were affected ($P < 0.05$) by year (Table 4). Year had a significant effect on these traits due to the weather differences exhibited in 2012, 2013, and 2014. Traits were negatively affected during the drought of 2012 and can be seen recovering in the following years of 2013 and 2014.

Hair Coat Score

Cow HCS was affected by year and month ($P < 0.0001$). Over the course of 12 months, HCS tended to be affected ($P = 0.07$) by the genotype C777G. By look-

Table 3. Main effects of GR (T105G) and CXCR2 (C777G) single nucleotide polymorphism (SNP) on bovine productivity traits.

	T105G				C777G			
Productivity Traits[b]	**TT**	**TG**	**SEM**	***P*-value**	**CC**	**CG**	**SEM**	***P*-value**
No.[c]	234	48	-	-	237	42	-	-
Pre-Breeding								
BCS	5.5	5.4	0.12	0.26	5.6	5.1	0.15	0.06
Weight, kg	527	500	8.31	0.02	528	493	12.5	0.08
Calving								
Julian	268	271	2.32	0.42	267	270	2.94	0.78
Birth Weight, kg	34	33	0.88	0.51	35	29	1.01	0.0003
Weaning								
BCS	5.0	4.9	0.08	0.61	5.0	4.7	0.10	0.04
Cow Weight, kg	497	476	8.94	0.08	500	456	13.5	0.05
Calf Weight, kg	198	189	4.45	0.11	199	179	6.35	0.05
Adj. 205 Wt., kg	206	206	4.20	0.94	208	192	6.04	0.10
Cow Efficiency	43	45	1.26	0.26	43	43	1.26	0.63

[a] Single nucleotide polymorphism occurred at the number indicated. First letter indicates the primary allele and the letter following the digits is the minor allele; SEM represents the mean standard error of least square means.

[b] BCS = Body Condition Score; Cow efficiency calculated by dividing the Adj. 205-day weight by cow weight at weaning.

[c] No. = total number of records over 3 consecutive years (2012, 2013, and 2014).

ing at a period of 4 months from May to August, HCS was affected ($P = 0.008$) by the genotype C777G (Fig. 1). Cows with the homozygous gene exhibited HCS of 2.2 while the heterozygous cows exhibited HCS of 2.5. Hair coat score could possibly be linked to immune responses caused by CXCR2.

Conclusions

The polymorphism T105G for the glucocorticoid receptor gene was found to be associated with production traits in crossbred cows. This SNP was related to cow pre-breeding weight and tended to affect cow weight at wean-

Table 4. Main effects of cow age group and year on bovine productivity traits.

	Cow Age Group[a]					Year				
Productivity Traits[b]	**≤ 3**	**4 - 10**	**≥ 11**	**SEM**	***P*-value**	**2012**	**2013**	**2014**	**SEM**	***P*-value**
No.[c]	53	207	22	-	-	94	94	94	-	-
Pre-Breeding										
BCS	5.4^{x}	5.6^{x}	5.0^{x}	0.18	0.07	5.1^{y}	5.4^{xy}	5.6^{x}	0.14	0.002
Weight, kg	476^{y}	546^{x}	510^{xy}	14.5	0.0001	501^{y}	489^{y}	542^{x}	10.8	0.0001
Calving										
Julian Date	271^{x}	270^{x}	267^{x}	3.58	0.71	264^{y}	268^{xy}	275^{x}	3.11	0.02
Birth Weight, kg	30^{y}	34^{x}	31^{xy}	1.18	0.005	31^{x}	32^{x}	32^{x}	0.95	0.41
Weaning										
BCS	5.0^{z}	5.1^{x}	4.5^{y}	0.12	0.007	4.9^{x}	4.9^{x}	4.7^{x}	0.10	0.30
Cow Weight., kg	453^{y}	505^{x}	477^{xy}	16.4	0.002	462^{xy}	488^{x}	484^{x}	37.0	0.008
Calf Weight, kg	166^{xy}	216^{x}	186^{x}	8.10	0.0001	193^{x}	178^{x}	196^{x}	6.88	0.06
Adj. 205 Wt., kg	207^{x}	206^{x}	187^{x}	7.60	0.34	199^{x}	186^{xy}	214^{x}	6.12	0.0003
Cow Efficiency	48^{x}	41^{y}	40^{yz}	1.55	0.0004	45^{x}	39^{xy}	45^{x}	1.41	0.0008

[a] Cow age groups = ≤3 years of age, young; 4–10 years of age, adult; ≥11 years of age, mature adult.
[b] BCS = body condition score; cow efficiency calculated by dividing the adj. 205-day weight by cow weight at weaning.
[c] No. = total number of animals over three consecutive years (2012, 2013, and 2014).
[xyz] shows similarities and differences between categories in cow age group and year.

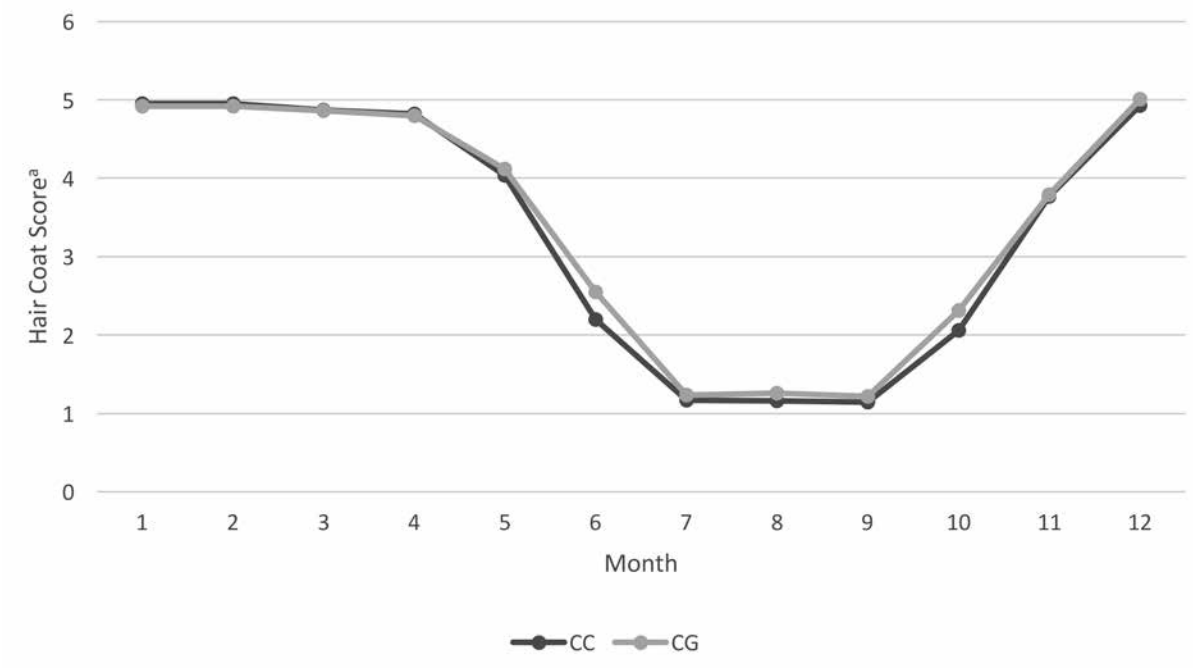

Fig. 1. Genotypic effects of CXCR2 on average hair coat score over three consecutive years (2012–2014). Based on a scale of 1–5; 5 = 0% shed, 1 = 100% shed.

ing and calf weaning weight. The interleukin-8 receptor gene, CXCR2, polymorphism C777G was shown to be associated with calf birth weight, calf weaning weight, cow BCS at weaning, and cow weight at weaning. Specifically for a period over 4 months from May to August, polymorphism C777G also affected HCS in cows. This study also shows that cow age and year have effects on production traits and HCS in cattle. Future research should be done in order to determine if these mutations are related to other traits in cattle and if they occur in great enough frequency to be useful for further genetic modifications.

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