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Associations of single nucleotide polymorphisms in the bovine prolactin gene with phenotypic traits in beef cattle

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ABSTRACT

Fall-calving Angus-based cows (n = 170 unique cows; 400 calving records during three years) were used to determine prolactin (*Prl*) genotype and haplotype effects on phenotypic traits. Genomic DNA, from buffy coat, was genotyped at three *Prl* SNP sites (C1286T, A1134T, and G8398A). Traits of interest were: pre-breeding body condition score (BCS) and weight, Julian calving date, calf birth weight, cow weight and BCS at weaning, calf weaning weight, adjusted 205-day weight and cow efficiency. Hair coat scores were determined each year in May, June, and July. Heterozygous cows at SNP C1286T had a lower ($P < 0.05$) calving rate when compared to homozygous cows. Calf birth weight was affected ($P < 0.05$) by genotypes at A1134T. Genotype at G8398A did not affect ($P > 0.10$) phenotypic traits. Six haplotypes were identified: CAG (n = 107), TAA (n = 173), CTG (n = 50), TTA (n = 32), TAG (n = 50), and TTG (n = 37); n represents total number of records for that haplotype during 3-year study. Calving percentage for CAG cows was greater ($P < 0.05$) than TTA, TAG, and TTG cows (96 vs. 83%; respectively, CAG vs. mean of TTA, TAG, and TTG). Haplotype CAG cows had earlier hair coat shedding. In addition, CAG cows had a larger calving rate ($P < 0.05$) and greater ($P < 0.05$) cow efficiency (45 ± 0.9%) than TTG cows. Cows with *Prl* haplotype CTG or TAG cow efficiency decreased from ≤ 3 to 4–10 and were missing in ≥ 11 years' group, suggesting those cows may have sustainability issues. Our results suggest that mutations associated with the bovine *Prl* gene may be useful as early selection tools for replacement cattle.

1. Introduction

Mutationsin the bovine prolactin (*Prl*) gene have been identified and associated with biological function. Single nucleotide polymorphisms (SNP) in the upstream elements have been associated with cattle productivity traits; specifically, mutations at bp 1286 (cytosine to thymine transversion) and 1167 (adenine to guanine transversion) influenced calving percentages and weaning weights of spring-calving cows (Looper et al., 2010). In addition, a transversion of guanine to adenine at coding sequence bp 8398 resulted in homozygous guanine cows' milk having more fat content, and heterozygous (GA) cows with increased milk yield (Brym et al., 2005).

Cattle productivity can be affected by multiple factors including genetic, environment (e.g. heat stress) and their interactions. Wild-type endophyte-infected (*Neotyphoduym coenophialum*) tall fescue (E+; [*Lolium arundinaceum* (Schreb.) S. J. Darbyshire]) plants produce ergot alkaloids that result in decreased cattle performance throughout much of the world (Strickland et al., 2011). Ergot alkaloid poisoning has been studied extensively, and prolactin is a reliable biomarker for fescue toxicosis (Rosenkrans et al., 2012). Prolactin also may be an indicator of nutritional status and serve as a communication link between the reproductive and neural systems (Flores et al., 2008). Cattle suffering from fescue toxicosis routinely experience hyperthermia which is exacerbated by retention of winter hair coat (Aiken et al., 2011). The detrimental effects of ergot alkaloids can be ameliorated through animal breeding and genetic selection, which results in improved reproductive efficiency and overall cattle productivity (Brown et al., 1993, 1997, 2000). However, traditional cattle selection methods are slow due to lengthy generation intervals. Modern genetic selection tools including genomic-enhanced expected progeny differences, next generation sequencing, and candidate gene analyses can hasten genetic progress. Our objective was to evaluate the associations between bovine *Prl* SNP and phenotypic traits in fall-calving beef cows.

Abbreviations: BCS, body condition score; *Prl*, prolactin; SNP, single nucleotide polymorphism

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2. Materials and methods

2.1. Animals and management

Data were collected from crossbred Angus-based cows (n = 170 unique cows with 400 calving records; 5.4 ± 3 years of age) from three consecutive breeding seasons (2012, 2013, and 2014). During the three breeding seasons 15 bulls were used, of which 9 bulls served as sires in all three breeding seasons. All animals were managed in accordance to the University of Arkansas Institutional Animal Care and Use Committee (protocol #13062). Cattle grazed mixed grass forages consisting of common bermuda grass (*Cynodondactylon* (L.) Pers.) and E + tall fescue with ad libitum access to trace mineral supplement and water. Cow pre-breeding weight and body condition score (BCS; 1 = emaciated, 9 = obese; Wagner et al., 1988) were recorded in November of each year. Cows were on a fall-calving schedule (August–December), and calf weights were documented at birth. At weaning (223 ± 39 days of age), cow BCS and weight, and calf weights were noted. As recommended by the Beef Improvement Federation (2010) birth weight was adjusted for dam age, and weaning weights were adjusted for cow age, sex of calf, and calf age at weaning. Cow efficiency was calculated by dividing each calf's 205-day adjusted weaning weight by dam weight at weaning and expressed as a percent.

Hair coat scores were determined each year in May, June, and July by a trained technician. Coat score was scored on a scale from 1 to 5 where 1 = slick summer coat, 100% shed and 5 = full winter coat, 0% shed (Turner and Schlegar, 1960).

2.2. DNA isolation and genotyping

Blood was collected via jugular venipuncture into EDTA treated tubes, placed in ice, and transported to the lab where tubes were centrifuged (2500 × g for 25 min). Buffy coats were removed and stored at − 20 °C until DNA extraction. Genomic DNA (gDNA) was extracted (DNeasy blood and tissue kit; QIA-GEN, Valencia, CA) from buffy coats, and quantified (Qubit® fluorometer; Invitrogen, Carlsbad, CA). Genomic DNA (600 ng) was pipetted into 96-well plates, dried (~50 °C), sealed, and sequenced (GeneSeek; Neogen Genomics, Lincoln, NE). Genotypes were determined at three *Prl* SNP (C1286T, A1134T, and G8398A; Table 1) using Sequenom's MassARRAY® system. Haplotypes were deduced based on each cow's genotype at the three *Prl* SNP sites. At each SNP (C1286T, A1134T, and G8398A), cows that carried one copy of the minor allele (heterozygous) and cows that carried two copies of the minor allele (homozygous minor allele) were coded as containing the minor allele; homozygous primary allele cows were coded with the primary allele. For example, cows that were homozygous primary allele at all three SNP had a haplotype of CAG; whereas, a cow that was heterozygous at all three SNP would have a haplotype of TTA.

2.3. Statistical analyses

Cows were categorized by age; ≤ 3 years, 4–10 years, and ≥ 11 years. Data were analyzed using a reduced mixed model ANOVA (SAS Inst. Inc., Cary, NC) with cow as the experimental unit and main effects of year, sire, genotype or haplotype, and cow age group. Repeated measures analyses were conducted using maximum likelihood method (Jennrich and Schluchter, 1986). Dependent variables were pre-breeding body condition score (BCS), pre-breeding cow weight, Julian calving date, calf birth weight, adjusted birth weight, calf weaning weight, 205-day adjusted weaning weight, cow BCS at weaning, cow weight at weaning, and cow efficiency. Haplotype and each SNP were analyzed in separate models. When F-tests for main affects were significant ($P < 0.05$), multiple t-tests and Tukey's adjustment were performed to separate means.

3. Results

3.1. Base position 1286

A transition from cytosine to thymine was detected at base 1286 (https://www.ncbi.nlm.nih.gov/nuccore/X16641). One-hundred eleven cows were either heterozygous or homozygous with the minor allele (Table 2). Heterozygous cows had lower ($P < 0.05$) calving rates than homozygous (CC or TT) cows (Table 3). Cows that were homozygous primary allele (CC) had lower ($P < 0.05$) BCS at weaning when compared with heterozygous cows.

3.2. Base position 1134

A transversion from adenine to thymine was detected at base 1134 (https://www.ncbi.nlm.nih.gov/nuccore/X16641). Heterozygous cows (n = 41; Table 2) tended ($P = 0.10$) to have lower calving rates than AA cows (Table 3). Homozygous adenine cows calved later ($P < 0.001$) in the calving season, but had heavier ($P < 0.05$) calf birth weights. Heterozygous cows had calves with adjusted 205-day weights that were lighter ($P < 0.05$) than calves from AA cows (Table 3).

3.3. Base position 8398

A transition from guanine to adenine was identified at base 8398 (https://www.ncbi.nlm.nih.gov/nuccore/AF426315.1). Eighty-six cows were either heterozygous or homozygous with the minor allele; minor allele frequency = 30.6% (Table 2). Phenotypic traits were not affected ($P > 0.06$) by G8398A genotypes (Table 3).

3.4. Prolactin haplotype effects

Three SNP (C1286T, A1134T, and G8398A) genotypes were used to assign *Prl* haplotypes to each cow. Prebreeding weight of TTA cows was heavier ($P < 0.05$) than cows with haplotypes CAG, TAA, and CTG

Table 1
Mutations associated with bovine prolactin gene.

Polymorphism[a]	Sequence	Accession Number[b]	Reference
C1286T	AGTGAACATGACTGT **[C/T]** TAGAATTTTGTTTTA	X16641	Looper et al., 2010
A1134T	TCATCTCATTCAGGA **[A/T]** ATCTCTAAAAGGCAA	X16641	
G8398A	CCTAGTCACCGAGGT **[G/A]** CGGGGTATGAAAGGA	AF426315.1	Brym et al., 2005

[a] Single nucleotide polymorphism (SNP) occurred at the number indicated. First letter indicates the primary allele and the letter following the digits is the minor allele.
[b] Based on the National Center for Biotechnology Information number.

Table 2
Distribution of SNP in the bovine prolactin gene.

Polymorphism[a]	Genotype Distribution[b]			MAF[c]
	Homo	Hetero	homo	
C1286T	59	86	25	40.0
A1134T	129	41	0	21.1
G8398A	84	68	18	30.6

[a] Single nucleotide polymorphism (SNP) 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 homozygous for the primary allele (Homo), heterozygous (hetero), and homozygous for the minor allele (homo).
[c] Minor allele frequency (MAF) expressed as percent.