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Heat Shock Protein 90 Mutation is Associated with Beef Cattle Traits

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Original Research Article

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Abstract: Our objective was to determine if polymorphisms associated with bovine Hsp90 gene have potential as a selection tool for productivity traits in cattle. Genomic DNA was extracted from buffy coat samples of crossbred Angus (n = 26) cows. Data were analyzed with cow as the experimental unit, genotype as the main effect, and dependent variables (calving rate, Julian calving date, calf birth weight, calf weight and cow weight at weaning, and calculated cow efficiency). When F-tests were significant ($P < 0.05$) least-squares means were separated using multiple T-tests. Polymorphism A97G is a transition from adenine to guanine at base 97 of the 283 base amplicon. Of the 26 cows, 19 were homozygous for adenine, 7 were heterozygous, and no homozygous guanine, which resulted in a minor allele frequency of 13.5%. Calving rate and cow weaning weight were not ($P > 0.9$) associated with genotype at A97G. However, 205-day adjusted calf weight was associated ($P = 0.0002$) with A97G genotype (188 vs. 208.1 ± 7.1 kg; respectively AA and AG). That heavier calf weaning weight improved ($P=0.08$) cow efficiency for AG cows (Table 2). Heterozygous cows shed their winter hair coats earlier than AA cows. June hair coat score for AG cows (1.6 ± 0.17) was lower ($P < 0.03$) than June hair coat scores for AA cows (2.2 ± 0.11). Single nucleotide polymorphism A97G was associated with productivity traits in beef cattle. Our results suggest that additional research with this mutation is warranted to determine its value as a genetic tool for selecting animals that are less susceptible to heat stress and related problems.
Keywords: weaning weights, HSP90, calving date.

INTRODUCTION

It has been estimated that lost productivity due to heat stress cost the cattle industry billions of dollars annually [1]. Wild-type endophyte-infected tall fescue contains ergot alkaloids that can exacerbate the effects of heat stress on animal productivity [2]. The interactive effects of heat stress and ergot alkaloids results in noticeable reductions in fertility of both females and males [3, 4]. Selecting cattle with tolerance of heat stress and (or) ergot poisoning would be an approach to reduce that lost productivity.

Heat shock proteins (HSP) are a highly conserved class of cellular proteins that protect cells when stressed [5, 6]. Under normal stress-free conditions heat shock proteins account for 1-2 % of total cellular protein content; however, hyperthermy can increase HSP concentrations to 4-6 % of all cellular proteins [6]. The gene for heat shock protein 90 (Hsp90) codes for a protein that is approximately 90 kd. Protein HSP90 acts as a molecular chaperone that stabilizes proteins when stressed, it is involved with steroid signal transduction, and with intracellular transport [7, 8]. Our objective was to determine if polymorphisms associated with bovine Hsp90 gene have potential as a selection tool for productivity traits in cattle.

MATERIALS AND METHODS

Animals and sample collection

Crossbred Angus (26) cows were managed at the University of Arkansas Savoy Cow-Calf Unit where they grazed mixed grass pastures of endophyte-infected tall fescue and bermudagrass, and had free access to both trace mineral supplement and water (University of Arkansas IACUC approved protocol #13062). Blood (~ 10 mL) was collected from each animal via the jugular vein using vacuum tubes containing ethylenediaminetetraacetic acid (Vacutainer, Becton-Dickinson, and Rutherford, NJ). Blood samples were placed in ice, transported to laboratory, and centrifuged at 2,500xg for 25 min at 5°C. Plasma was decanted, and buffy coats were collected and stored at -80°C.

DNA isolation, genotyping, and analysis

Genomic DNA was isolated from buffy coats using the DNeasy® Blood and Tissue Kit protocol (QIAGEN, Valenica, CA). Polymerase chain reaction (PCR) was performed using a C1000 Touch ™ Thermal Cycler (Bio-Rad, Hercules, CA). Specific primers were designed based on *Bos taurus* Hsp90 gene sequence

(NCBI accession number NM_001079637.1) and commercially synthesized by (Invitrogen, Carlsbad, CA). Forward primer was (bHSP90AB1_283F; 5′-GTGACGATCTCCAACAGGC -3′) and reverse primer was (bHSP90AB1_283R; 5′-CCTCAAGCGAGAAGCCAGA -3′)].

The PCR protocol consisted of an initial denaturation for 2 min at 94°C, followed by 35 cycles at 94°C for 30s, 1 min at 55°C, and 1 min at 68°C. The process was completed with a final step of 10 min at 68°C and was cooled to 8°C. Each amplification reaction included 2.5 µL of genomic DNA (~20 ng/µL), 1.25 µL of both forward and reverse primers (10 µM), and 45 µL of Platinum PCR Supermix (Invitrogen, Carlsbad, CA). Amplification products (10 µL) and 100 bp DNA ladder (GenScript, Piscataway, NJ) were loaded into individual wells of 1.2% agarose gels, separated using electrophoresis (TBE buffer; 130 volts for 30 min), stained with ethidium bromide, and visualized using a UVP Epi Chemi II Darkroom (Upland, CA). Expected amplicon size was compared against the DNA ladder. After PCR product was confirmed as the correct amplicon size, samples were purified using GenScript QuickClean II PCR Extraction Kit (Piscataway, NJ). Following the purification process, concentrations of purified product were quantified using a Qubit® Fluorometer (Invitrogen, Carlsbad, CA). Amplicons for each animal were sequenced in both forward and reverse directions (Eurofins SimpleSeq Louisville, KY). Sequences were compared using the web-based program Clustal Omega (http://www.ebi.ac.uk/Tools/msa/clustalo/), both heterozygous and homozygous genotypes were visually verified using sequence chromatograms [9].

STATISTICAL ANALYSIS

Mixed model procedures were used to analyze data for main effects of genotype on dependent variables (Julian date of calving, calf birth and weaning weights, cow weight at weaning, and cow efficiency was calculated). Cow was the experimental unit, and when main affect F-tests were significant ($P < 0.05$), means were separated using multiple T-tests.

RESULTS AND DISCUSSION

Six SNP sites within the Hsp90 amplicon were identified (Table 1). However, after data were analyzed on this small set of cows, only SNP A97G had significant effects on the traits of interest. Polymorphism A97G is a transition from adenine to guanine at base 97 of the 283 base amplicon (Fig-1). In this population of 26 cows, 19 were homozygous for adenine, 7 were heterozygous, and no homozygous guanine. Minor allele frequency was 13.5%.

Table-1: Mutations associated with the HSP90 gene of cattle

Polymorphism[a]	Sequence
A97G	TGAAAGCCCAG[A/G]CGCTTCGGGACAA
A104G	AAGCCCAGACGCTTC[A/G]GGACAACTC
T144C	TGGCCAAAAAGCA[T/C]CTGGAGATCAA
A201G	GAAGGCAGAGGC[A/G]GACAAAAACGA
C219T	ACGACAAGGC[C/T]GTCAAGGACCTGGT
A247G	TGGTGCTGCTGTTC[A/G]AAACTGCACTG

[a]Single nucleotide polymorphism occurred at the base number indicated. First letter indicates the primary allele and letter following digits is the minor allele. Red letters represent SNP location relative to NCBI accession entry: NM_001079637.1.

GTGACGATCTCCAACAGGCTTGTGTCGTCACCCTGCTGCATCGTGACCAGCACCTACGGCTGGACCGCCA
ACATGGAGCGCATCATGAAAGCCCAG**[A/G]**CGCTTCGGGACAACTCGACCATGGGCTACATGATGGCCAAA
AAGCATCTGGAGATCAACCCTGACCACCCCATCGTGGAGACCCTGCGGCAGAAGGCAGAGGCGGACAAAA
ACGACAAGGCCGTCAAGGACCTGGTGGTGCTGCTGTTCGAAACTGCACTGCTCTCC**TCTGGCTTCTCGCT
TGAGG**

Fig-1: Amplicon (283 bases) of bovine heat shock protein 90 (NCBI Reference Sequence: NM_001079637.1). Forward and reverse primer sequences are bold and in green, and single nucleotide polymorphism of interest (A97G) is bold in red

Calving rate and cow weaning weight were not ($P > 0.9$) associated with genotype at A97G (Table 2). However, 205-day adjusted calf weight was associated ($P = 0.0002$) with A97G genotype (188 vs. 208.1 ± 7.1 kg; respectively AA and AG). That heavier calf weaning weight improved ($P = 0.08$) cow efficiency for AG cows (Table 2). Heterozygous cows shed their winter hair coats earlier than AA cows [June hair coat score for AG cows (1.6 ± 0.17) was lower ($P < 0.03$) than June hair coat scores for AA cows (2.2 ± 0.11; Fig-2)].

Increased synthesis of certain proteins after cells were exposed to stressors such as heat shock was first discovered in drosophila cells, and subsequently in a wide range of organisms [10]. Heat shock proteins

serve as molecular chaperones that are required to maintain normal protein structure both in homeostatic conditions as well as when cells are exposed to stressors [11]. Expression products of HSP27, HSP70 and HSP90 are increased in cells under stressful conditions or exposed to death stimuli, which makes them very efficient at preventing apoptosis and thus blocking cellular death processes [12-15]. Our results in this study are consistent with our previous work, in which we demonstrated that polymorphisms associated with Hsp70 were related to calving rates, milk quantity and quality, and horn fly infestations of beef cattle [16-18]. Future studies will focus on linking polymorphisms with signal transduction pathways, physiology, and animal traits.

Table-2: Effects of heat shock protein 90 genotype at single nucleotide polymorphism site A97G on cattle productivity traits

	A97G Genotype[a]			
Trait	AA	AG	SEM[b]	P-value[c]
Number of cows	19	7	-	-
Calving rate, %	89.5	89.5	-	1.0
Julian, day	269.4	273.4	7.3	0.65
Adj. birth wt[d], kg	33.8	35.8	2.0	0.40
Cow weight at weaning, kg	521.7	521.8	24.5	0.99
Adj. 205-d calf wt[d], kg	188.0	208.1	7.1	0.0002
Cow efficiency[e],%	36.8	41.5	2.6	0.08

[a]Single nucleotide polymorphism occurred at the 97th base of the 283 base amplicon. First letter indicates the primary allele and the letter following the digits is the minor allele.
[b]Mean standard error of the least squares means.
[c]F-test probability of main effects for A97G genotype.
[d]Birth weight and 205-d weaning weights were adjusted as recommended by the Beef Improvement Federation (https://beefimprovement.org/library-2/bif-guidelines).
[e]Cow efficiency was calculated by dividing each calf's 205-day adjusted weaning weight by dam weight at weaning and expressed as a percent.

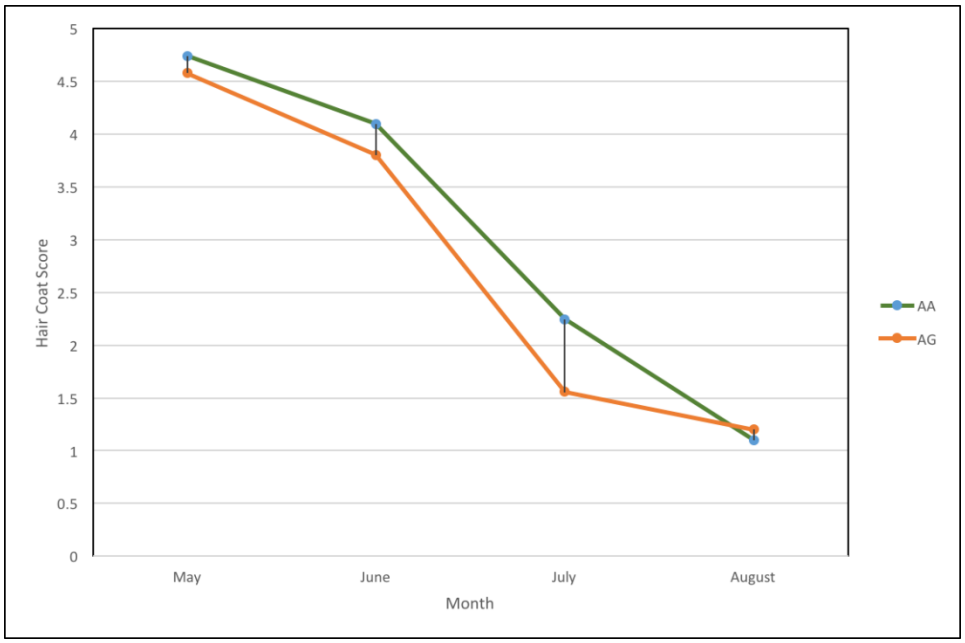

Fig-2: Least-squares means for hair coat scores by month and A97G genotype

CONCLUSION

Cows carrying the minor allele at bovine Hsp90 polymorphism A97G had heavier 205-day adjusted calf weights, greater cow efficiency, and earlier hair coat shedding. Those factors directly impact beef cattle productivity, which suggests that Hsp90 polymorphism A97G may be a useful genetic marker for cattle selection. This brief report sets the stage for mechanistic studies, and breeding studies with larger more diverse cattle populations.

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