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Relationships Among Beef Cow Productivity Traits and Single Nucleotide Polymorphisms in the Bovine Heat Shock Protein 70 Gene

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Abstract

When eukaryotes are exposed to stressors such as heat, toxins, and low oxygen levels, heat shock proteins (HSPs) are synthesized to maintain normal cellular function within the body. Single nucleotide polymorphisms in the heat shock protein 70 (Hsp70) gene have been associated with calving percentage, and Julian calving date in spring-calving crossbred Brahman cows (Rosenkrans, et al., 2010). Our objective was to determine associations between previously identified polymorphisms in the promoter region and coding sequence of the bovine Hsp70 gene and beef cow efficiency. We evaluated productivity traits, including Julian calving date and calving rates, of fall-calving cows at each single nucleotide polymorphism (SNP) site. Genomic DNA was extracted from blood samples collected from crossbred cows (n=109) and amplified by polymerase chain reaction (PCR) using specific forward and reverse primers. Upon amplification, samples were purified, quantified, and sequenced in a commercial laboratory. Sequences were analyzed to determine SNP and genotypes were assigned to each of them. Production data of the cows from 2012-2014 were analyzed at each SNP site. Cows with CD genotype at C895D calved about one week later ($P = 0.0001$) and tended ($P < 0.06$) to have calves with lighter birth weights compared to cows with CC genotype. Genotype at G2033C affected ($P < 0.02$) weaning weight and cow efficiency. Genotype of a haplotype composite (No SNP, Deletion, and Yes SNP) affected ($P < 0.02$) Julian date and cow efficiency. Polymorphisms associated with the bovine Hsp70 gene were related to important cattle productivity characteristics. Selecting replacement cattle with known Hsp70 genotypes may result in herds that are more thermotolerant and sustainable.

Keywords: Cattle, Hsp70, Polymorphism, Productivity

Introduction

Heat stress, which is defined as "any combination of temperature, humidity, radiation, and wind producing conditions higher than the animal's thermal neutral zone", has dangerous effects on the health of cattle and can lead to illness and in some cases death (Alberta Agriculture and Forestry, Livestock and Crops Division, Animal Health and Assurance Branch, & Animal Welfare Section, 2018). Environmental stressors, for example heat stress, can reduce livestock productivity by billions of dollars annually by affecting reproductive function, physiological parameters, and performance when an animal is not within its thermoneutral zone (Alfonzo et al., 2016; St-Pierre et al., 2003). To decrease the impact of this stress, breeds with a higher tolerance to stress are selected over those that are not, and there is evidence to support that breed is related to differences in heat tolerance and fertility (Turner, 1982). Due to evidence supporting a genetic linkage to reproductive fitness, it is valuable to expand our knowledge of the stress response axis of cattle and its relationship to genetic mechanisms.

A special class of proteins, called heat shock proteins (HSP), are synthesized in response to elevated temperatures, and some are present in all organisms at normal temperatures as well (Lindquist and Craig, 1988). Because of their vital role in normal cell function, determining if polymorphisms in the gene that codes for heat shock protein 70 (Hsp70) is related to beef cow efficiency could provide a better understanding of how the physiological stress mechanism works. Previous studies have found that polymorphisms in the Hsp70 gene are related to milk characteristics, as well as calving percentage; however, the present study reviews productivity traits from calves born in the fall, as opposed to the spring (Lamb, et al. 2007; Rosenkrans, et al. 2010). Although Hsp70 has been documented previously relating to cow productivity, it is

uncertain the effect on cow profitability of fall-calving cows, which is examined in the present study. If polymorphisms in the gene are related to cow profitability, then breeding decision tools can be developed for cattle producers to aid in increasing livestock thermotolerance. In certain regions of the United States where the warm season lasts longer, cattle producers face challenges with heat stress in their herds. Therefore, there is a need for developing breeding plans to combat this issue.

Literature Review

Evidence suggests that single nucleotide polymorphisms (SNP) have an association with profitability traits in cattle. For example, in a study focused on SNPs in the bovine cytochrome p450 region and its effects on cow productivity, four SNPs for cytochrome p450 were related to cow productivity. A lower lifetime calving rate, and significant differences in birth weight and weaning size were evaluated. Their results suggested that SNP in cytochrome P450 gene were associated with cattle productivity (Sales et al., 2013). Nutrition studies have also discovered a correlation with SNP and productivity. When considering nutritional factors, we can also see genetic alterations coupled with toxic tall fescue to result in overall lower productivity in cows (Sales et al., 2012). Collectively, those studies suggest that cattle managed under stressful conditions such as heat would likely have altered HSP expression.

Stress, in this case an increased body temperature, can physiologically affect cattle by raising cortisol levels. Cortisol, which is the main stress steroid in cattle, can indicate both acute and chronic stress. When experiencing stress, a cell will stop or slow down some of its functions, which includes DNA, RNA, and protein synthesis (Bhat et al., 2016). In response to

this stress, HSP are synthesized and released into the body and are critical in maintaining normal cellular function in the body. The three most prevalent members of the HSP family are Hsp60, Hsp70, and Hsp90 (Lindquist and Craig, 1988). Because of these induced responses to stress, understanding the relationship between cortisol and HSP could help in predicting animal stress response. The HSP family has several functions, one of which is to "regulate protein folding, transport, translocation and assembly" as well as refolding proteins (Wang et al., 2014). Along with folding heat-denatured proteins, they also block caspase dependent apoptosis, which permits repair and prevents cellular death (Calderwood et al., 2007). Heat shock proteins also provide support for steroid nuclear receptors and aide in steroid response (Kovacs et al., 2005). Nuclear receptors are needed for normal function of cortisol, estrogen, progesterone, and testosterone. Those steroids affect stress response and reproductive success in mammals. Therefore, improving steroid function could improve cattle efficiency and the HSP family is integral in this process of steroid response.

Heat Shock Factor 1

Heat shock factor 1 (HSF1) is an "upstream transcriptional regulator of HSPs" and is important in inducing the response to heat (Wang et al., 2014). Because it regulates the expression of the HSPs, a SNP in HSF1 could affect the synthesis of the heat shock proteins. Genetic mutations that keep HSF1 from doing its job properly could theoretically be correlated with beef cow efficiency because it would negatively affect steroid function. Certain traits that are favorable in beef cattle could be affected because of the decreased physiological function that would have adverse effects on the reproductive system causing a loss in productivity, for example calf birth weight. Both invertebrate and vertebrate HSP synthesis and release in

response to stress is primarily controlled by HSF1 (Anckar and Sistonen, 2011). More than thirty SNP have been identified in the HSF1 gene and were related to human cell function in vitro (Bridges et al., 2015). Also, in dairy cattle, two SNP were identified and were related to milk production and heat tolerance (Li et al., 2011). Unfortunately, there are not many studies that investigate the relationship between polymorphisms in HSF1 and production values specifically in beef cattle.

Heat Shock Protein 60

Members of the HSP60 group are large oligomeric complexes that are essential for growth, and are induced by forms of cellular stress that cause protein denaturation (Frydman and Hartl, 1994). Although gene expression of HSP60 in cattle has been reported as higher in the summer months than in winter months, genetic variants in the bovine HSP60 have not been related to cattle productivity (Bhat et al., 2016). In the human reproductive tract, concentrations of the protein were related to genotype at Hsp60 SNP sites and have been related to fertility success (Lev-Sagie et al., 2009). Because of this evidence, this particular heat shock protein might be of interest moving forward.

Heat Shock Protein 70

The most abundant member of the heat shock protein family, Hsp70, is essential for cell survival at high temperatures and in normal cellular physiology (Lindquist and Craig, 1988). Functions of HSP70 include acting as molecular chaperones when an organism is exposed to stressful pathological or environmental conditions, like viral infection, fever, hypoxia, etc. (Ohtsuka and Hata, 2000). A molecular chaperone will protect cells against exposure to lethal heat shock, which can denature proteins, by binding to the denaturing protein and stabilizing it,

which preserves its activity. In a study conducted on Tharparkar cattle to discover possible differences in thermotolerance, it was discovered that the allelic variants of HSP70 gene were associated to heat tolerability (Bhat et al., 2016). Results from a study conducted on crossbred Brahman-influenced cows, where a deletion of cytosine was detected at base 895, discovered that cows that were homozygous for the deletion had a significantly lesser calving percentage when compared with heterozygous or homozygous cytosine cows (Rosenkrans et al., 2010). These studies support that the gene is important in thermotolerance in cattle of different breeds, and suggest that cattle managed under stressful conditions such as heat due to climate change would likely have altered HSP expression. Although HSP70 is heavily induced by stress, "non-stressed" cows were found to have circulating concentrations of plasma HSP70, which might indicate that it is produced in preparation of combating increased stress (Kristensen et al., 2004).

Heat Shock Protein 90

Highly conserved members of the heat shock protein 90 family are important in carrying out biological functions in the cytosol and endoplasmic reticulum of eukaryotes (Frydman and Hartl, 1994). Polymorphisms in Hsp90 of sheep has been related to heat stress response (Marcos-Carcavilla et al., 2010). Since cellular HSP90 is required for steroid biological response, it could be useful to study polymorphisms in the bovine Hsp90 gene since steroid function is critical for cattle reproduction, and response to stress.

Materials and Methods

Animals and Sample Collection

Blood samples were collected from crossbred cows (n=109). Genomic DNA extraction was performed by using QIAgen extraction kit (QIA-gen, Valencia, CA) and stored at -70° for amplification. After purification, a Hoefer DYNA Quant 200 Florometer (Amersham Biosciences Corp; Piscataway, NJ) was utilized to quantify DNA. Primers were designed based on the bovine Hsp70 gene sequence (Gen Bank accession number M98823), using primer 3 software (Rozen and Skaletsky, 2000).

PCR and AgaroseD Gel Electrophoresis

Each reaction for amplification by PCR was prepared by pipetting 95 µl of PCR mix and 5 µl of genomic DNA into each PCR sample tube. The final mix was spun down and halved so that the final volume was 50 µl in each tube before inserting into the thermocycler. Each PCR started with 2-min heating at 94°C, followed by 35 S cycling at 94°C, then I-min at 55°C, and 1-min at 68°C. Then final elongation occurred for 10 min at 68°C. An agarose gel stained with ethidium bromide was used to verify amplicons through gel electrophoresis. Amplification products were then purified using QIAgen MinElute PCR purification kit (QIAgen).

Quantifying DNA and Analysis of Sequences

DNA samples were quantified using NanoDrop, then prepared for sequencing at Eurofins. Once samples and data were returned, MEGA7 and Clustal Omega software was used to evaluate for SNP genotypes.

Results

The SNPs detected were C895D [indel; cytosine was deleted and was not replaced with a base(D)], G1851A (transition; guanine was replaced with adenine), and G2033C (transversion; guanine was replaced with cytosine; Table 1). The minor allele frequency was 5.5, 4.6, and 10.1%, respectively for C895D, G1851A, and G2033C (Table 2).

Base position 895

The deletion of cytosine was detected at base 895, part of the promoter region. Ninety-seven cows were homozygous CC and twelve were heterozygous CD (Table 3). No cows were homozygous for the minor allele. The heterozygous CD cows had a significantly later (P = 0.0001) Julian calving date when compared to the homozygous cytosine cows. The difference in calving percentage rate, birth weight, cow weight, 205-day calf weight, and cow efficiency were insignificant between the two.

Base position 1851

Transition of guanine to adenine detected at base 1851 was part of the coding region. Ninety-nine cows were homozygous GG and 10 cows were heterozygous GA (Table 4). No cows were homozygous for the minor allele. Genotype at G1851A was not ($P > 0.24$) associated with any of the production data.

Base position 2033

Transversion of guanine to cytosine detected at base 2033 was part of the coding region. Eighty-seven cows were homozygous GG and twenty-two were heterozygous GC (Table

5). No cows were homozygous for the minor allele (Table 2). The heterozygous GC cows had a lower ($P < 0.05$) cow weight at weaning compared to the homozygous GG cows (GG was 503 kg and GC was 472 kg). Cows that were heterozygous had a higher ($P < 0.05$) efficiency compared to the homozygous guanine cattle.

HSPSNP

Development of a composite haplotype based on genotype at the three SNP found cow productivity differences that were very similar to C895D (Table 6). The deletion group had a later ($P = 0.0001$) Julian calving date when compared to cows without a mutation ("No") and cows with a mutation other than the deletion ("Yes"). Cow efficiency for "Yes" cows was greater ($P < 0.02$) compared to "No" cows. The difference in calving percentage rate, birth weight, cow weight, 205-day calf weight, and cow efficiency were not affected by haplotype.

Table 1. Mutations associated with the Hsp70 gene of cattle

Polymorphism[a]	Sequence	Reference
C895D	GCCAGGAAACCAGAGACAGA[C/D]CCTACGCAGGAGTAGGTGGT	Rosenkrans et al., 2010
G1851A	GAAGAGCGCCGTGGAGGATG[G/A]CTTGGAAGTAAACAGAAACGGG	
G2033C	CTGGCGGCTTTGGGGCTCAGG[G/C]CCCTAAAGGGGGCTCTGGGTGG	Brown et al., 2010

[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

Table 2. Distribution of SNP in the bovine Hsp70 gene

Polymorphism[a]	Genotype distribution[b]			MAF[c]
	Homo	hetero	homo	
C895D	97	12	0	5.5%
G1851A	99	10	0	4.6%
G2033C	87	22	0	10.1%

Table 3. Effects of heat shock protein 70 genotype at single nucleotide polymorphism site C895D on cattle productivity traits.

	C895D Genotype[a]			
Trait	CC	CD	SEM[b]	*P*-value[c]
Number of cows	97	12	-	-
Calving				
Rate, %	92.9	85.7	-	0.18
Julian, day	262	269	1.0	0.0001
Adj. birth weight[d], kg	36	34	1.1	0.0591
Weaning				
Cow weight, kg	498	495	9.7	.8364
Adj. 205-d calf weight[d], kg	198	202	5.8	.5365
Cow efficiency[e]	42	44	1.4	.1926

[a]Single nucleotide polymorphism occurred at 895 bases 5' of the putative gene start site based on Gen- Bank accession number M98823. Amplicon length was 539 bases, 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 C895D genotype.
[d]Birth weight and 205-d weaning weights were adjusted as recommended by the Beef Federation (2010).
[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.

Table 4. Effects of heat shock protein 70 genotype at single nucleotide polymorphism site G1851A on cattle productivity traits.

	G1851A Genotype[a]			
Trait	GG	GA	SEM[b]	*P*-value[c]
Number of cows	99	10	-	-
Calving				
Rate, %	91.7	95.7	-	0.5
Julian, day	265	263	2.7	0.4930
Adj. birth weight[d], kg	36	35	1.3	0.2415
Weaning				
Cow weight, kg	497	503	10.6	0.6717
Adj. 205-d calf weight[d], kg	198	201	5.2	0.3557
Cow efficiency[e]	42	44	1.4	0.3554

[a]Single nucleotide polymorphism occurred at 1851 bases 3' of the putative gene start site based on Gen- Bank accession number U09861. Amplicon length was 523 bases, 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 C895D genotype.
[d]Birth weight and 205-d weaning weights were adjusted as recommended by the Beef Federation (2010).
[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.

Table 5. Effects of heat shock protein 70 genotype at single nucleotide polymorphism site G2033C on cattle productivity traits.

	G2033C Genotype[a]			
Trait	GG	GC	SEM[b]	*P*-value[c]
Number of cows	87	22	-	-
Calving				
Rate, %	92.6	90.2	-	0.57
Julian, day	265	267	2.7	0.5546
Adj. birth weight[d], kg	36	36	1.2	0.6834
Weaning				
Cow weight, kg	503	472	9.5	.0092
Adj. 205-d calf weight[d], kg	198	200	5.7	0.7489
Cow efficiency[e]	42	45	1.3	.0147

[a]Single nucleotide polymorphism occurred at 2033 bases 3' of the putative gene start site based on Gen- Bank accession number U09861. Amplicon length was 523 bases, 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 C895D genotype.
[d]Birth weight and 205-d weaning weights were adjusted as recommended by the Beef Federation (2010).
[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.

Table 6. Effects of heat shock protein 70 genotype at single nucleotide polymorphism site HSPSNP on cattle productivity traits.

	HSPSNP Haplotype[a]				
Trait	No	Deletion	Yes	SEM[b]	*P*-value[c]
Number of cows	72	12	25	-	-
Calving					
Rate, %	93.3	85.7	91.7	-	0.38
Julian, day	262[b]	269[a]	260[b]	1.6	0.0001
Adj. birth weight[d], kg	36.0	34.4	36.7	1.1	0.13
Weaning					
Cow weight, kg	503	495	480	10.3	0.12
Adj. 205-d calf weight[d], kg	198	202	199	6.1	0.79
Cow efficiency[e]	41.8[b]	44.4[ab]	44.9[a]	1.4	0.016

[a]Haplotype based on genotype at C895D, G1851A, and G2033C.
[b]Mean standard error of the least squares means.
[c]F-test probability of main effects for HSPSNP haplotype.
[d]Birth weight and 205-d weaning weights were adjusted as recommended by the Beef Federation (2010).
[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.

Discussion

As previously discovered, single nucleotide polymorphisms related to Hsp70 gene were associated with profitability traits in beef cattle. One SNP located in the promoter region (site C895D) was related to both the Julian calving date, and cow efficiency. Julian date was also later for the deletion group in HSPSNP and cow efficiency suffered as well for the cows without a mutation versus those with a deletion or a mutation other than a deletion. One SNP, located in the coding region, (site G2033C) was associated to cow weight and cow efficiency. These results confirm the effect of polymorphism in the Hsp70 on cattle reproductive traits; however, there was also a large amount of insignificant data. There could be several factors that caused this, including not having a large enough sample size, or the time of year the calves were born. Compared to research already present, we have identified polymorphisms and gained a better understanding on how stress response is driven by genetics. We have also seen that since calving percentage is affected by the time of year, heat stress response may differ from spring to fall.

Recommendations for future research would be to investigate other heat shock protein families, specifically Hsp60, Hsp90 and HSF-1. More knowledge about multiple family's involvement in stress response can help us better understand how they physiologically work together in the body and can lead to improved breeding plans. It would be valuable to emphasize studies on HSF-1 in bovine genomic DNA because of the lack of information currently on this topic in relation to beef cattle. Since HSF-1 is an upstream element, it could be useful to understand more about its relationship to beef cattle stress response, and how its polymorphisms can affect productivity traits.

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