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Relationships Between Cattle Genetic Polymorphisms and Profitability Traits in Tall Fescue Forage Systems

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Introduction

Ergot alkaloid toxins produced by *Neotyphodium coenophialum* in association with tall fescue (*Festuca arundinacea*) have been shown to affect numerous physiological systems of livestock resulting in decreased profitability of those enterprises. One management technique that has been promoted to reduce the production losses in cattle is crossbreeding (Brown et al., 1997). In addition to crossbreeding *Bos indicus* (Brahman) and *Bos taurus* (Angus, Hereford, Senepol, etc.) cattle, one also can rotate cattle off of toxic fescue prior to the breeding season (Brown et al., 2000). While it was apparent that heterosis improved cattle productivity, specific genetic effects related to an animal's tolerance to ergot alkaloids is not known.

Heat shock proteins (HSPs), also known as stress proteins, are present in all cells of the body. The HSPs increase when an animal is subjected to stressors such as heat, cold, oxygen deprivation, and other conditions (for review see Lindquist and Craig, 1988). Although HSPs are present in high concentrations during cellular stress, they also are present at normal temperatures and play vital roles in normal cell function via protein chaperones, immune cell regulation, and steroid receptor function (Bresnick et al., 1989; Morishima et al., 2000; Smith et al., 1995).

Prolactin is a protein hormone secreted by the lactotropic cells of the anterior pituitary. The primary role of prolactin in mammals is the development of the mammary gland and lactation in the mature mammary gland during the last stage of pregnancy and following parturition (McCann, 1988; Bawden and Nicholas, 1999). Consumption of endophyte-infected tall fescue results in reduced feed intake, weight gains, blood flow to the periphery, pregnancy rates, as well as lower concentrations of serum prolactin and milk production (Nihsen et al., 2004; Browning, 2000; Samford-Grigsby et al., 1997).

In this report, the results of studies are presented that evaluated the relationships between cattle productivity and genomic polymorphisms in cattle. Specifically, the enhancer region of the prolactin gene and coding sequences of the HSP-70 gene in *Bos indicus* and *Bos taurus* cattle were genotyped and related to cattle productivity while grazing toxic tall fescue.

Materials and Methods

Animals

The cows were part of a long-term breeding program at the USDA-ARS Dale Bumpers Small Farms Research Center near Booneville, Arkansas. Blood samples were collected and the plasma was harvested. Buffy coats were then stored at -80°C to await genomic analysis. Genetic data was successfully collected on 157 cows. The breed composition of the cows and the number of each breed used were as follows: *Bos taurus* (Angus; n = 42), *Bos indicus*

(Brahman; n = 41), and *Bos taurus*/*Bos indicus* crosses (n = 74). The crossbred cows were distributed as follows: 38 Angus sired Brahman dams, 36 Brahman sired Angus dams.

Prolactin Genotyping

The prolactin enhancer sequence was predicted to be approximately 1.5 kb to 300 bp before the coding region, and sequences between positions 985 and 1124 were essential to enhancer activity (National Center for Biotechnology Information (NCBI) nucleotide sequence X16641; Wolf et al., 1990, 1992). Primers were designed to amplify a 501 bp fragment from positions 892 to 1392. Primer +PRL 892 (AAGTCCCCATAAGCACACTTGG) and primer –PRL 1392 (CTAACTTTAGGGAGTTCATACTG) were synthesized and supplied by Sigma–Genosys (Saint Louis, MO). The thermocycler conditions for PCR were 96°C 30 seconds denaturation, 50°C 30 seconds primer annealing, and 68°C 1 minute elongation for 36 cycles. The first 13 PCR products were gel purified using the Qiagen MiniElute gel purification kit according to the manufacturer's instructions (Qiagen Inc., Valencia, CA) and sequenced by the DNA Resource Center, University of Arkansas, using a Beckman CEQ 8000. The sequence was analyzed using DNAStar software. A single nucleotide polymorphism (SNP) was identified at base position 1286 (a cytosine to a thymine) and represents a distinct restriction enzyme site *Xba* I (T^CTAGA). The alleles that did not contain the SNP were 'cut' and coded as CC; whereas, those alleles that had the SNP were 'uncut' and labeled as TT.

Heat Shock Protein 70 Genotyping

Based on NCBI sequence accession number U09861 of *Bos taurus* HSP-70, three primers were designed for PCR amplification and sequencing. The primers were synthesized by Sigma-Genosys (Saint Louis, MO). Primers HSP1778F (CGCTGGAGTCGTACGCCTTC) and HSP2326R (CTTGGAAGTAAACAGAAACGGG) were used for amplification of a 548 base pair fragment from positions 1778 to 2326. After amplification, HSP1803F (GAAGAGCGCCGTGGAGGATG) and HSP2326R were used to sequence a 523 base pair fragment within the amplified region from positions 1803 to 2326. Specific DNA amplification occurred after 35 cycles of denaturation at 94°C for 2 minutes and then cycled at 94°C for 30 seconds, 55°C for one minute and 68°C for one minute.

Statistics

Distribution of cow genotype and breed were tested using Chi-square. Analysis of variance was used to determine genotype effects on the mean lifetime calving rate, milk quality and quantity (as reported by Brown et al., 1993), and calf weaning weight and height. Main effects included: genotype, forage type (tall fescue vs. bermudagrass), genotype x forage interaction. Age and sex of calf, month of sampling, and lactation number were used as covariates where appropriate. When F-tests were significant for main effects means were separated using multiple t-tests.

Results

Prolactin

The distribution of Xba SNP was affected ($P < 0.01$) by breed of cow (Table 1). Brahman cows had a greater proportion of TT genotype; whereas, Angus cows had a greater proportion of CC genotype. All breed groups had at least one cow in all three genotype

possibilities. Table 2 presents the interactive means of forage type and prolactin genotype on cattle traits. Cows that were homozygous thymine and grazing tall fescue had lower ($P < 0.1$) lifetime calving rates when compared to cows grazing bermudagrass or other genotypes grazing tall fescue. Fat percent in milk was affected by an interaction ($P < 0.05$) of forage type and prolactin genotype. Homozygous cytosine cows grazing tall fescue had ($P < 0.05$) the least amount of butterfat in their milk when compared to all other groups, and tended ($P < 0.1$) to wean calves that were shorter than other genotypes.

Heat Shock Protein 70

The bovine HSP-70 gene was amplified from base 1778 to base 2326. By comparing the region of interest from our samples to the NCBI published sequence (accession number U09861), eight SNPs were identified with four of the eight resulting in an altered peptide sequence. The SNPs were identified at the following base positions on the HSP-70 gene: 1851, 1899, 1902, 1917, 1926, 2033, 2087, and 2098.

The frequency and breed composition of each of the eight SNPs are summarized in Table 3. The base change, location, and effect on amino acid profile are summarized in Table 4. The presence of SNP 2033 was not affected ($P > 0.5$) by breed. Brahman ancestry was related ($P < 0.11$) to the occurrence of SNPs at positions 1902, 1917, 1926, 2087, and 2098. Whereas, the presence of SNP 1851 was associated ($P < 0.11$) with Angus lineage.

Associations of HSP-70 SNPs and cattle traits are presented in Tables 5 and 6. Cows with genotypes inconsistent with NCBI sequence at bases 1902, 1917, and 1926 had milk with a greater ($P < 0.05$) percentage of butterfat and protein than other cows. Those same cows tended ($P < 0.1$) to have calves that were taller than calves from non-SNP cows (Table 5). Heterozygous (GC) cows at base 2033 had fewer ($P < 0.05$) somatic cells in their milk when compared with homozygous guanine cows. In addition, heterozygous cows grazing tall fescue had the lightest ($P < 0.1$) calves at weaning (Table 6).

Discussion

Fescue toxicosis has been shown to have varying effects on different breeds of cattle. In a study conducted by Criss (1986), Brahman and Angus cows were fed endophyte-infected tall fescue during the first 3 months of lactation; milk production by Brahman exceeded Angus. Brown et al. (1993, 1997) found that Angus cows were more susceptible to the ergot alkaloids when compared to Brahman. The percentage of milk fat was 0.68% greater in Brahman than Angus. They also found that crossbred cows (both Angus-Brahman and Brahman-Angus) were more resistant to the negative effects of fescue toxicosis than their purebred counterparts. Our study was aimed at identifying polymorphisms in the enhancer region of the prolactin gene in *Bos indicus* and *Bos taurus* that may account for the higher toxin tolerance of the Brahman. Our results suggest that prolactin promoter polymorphisms were associated with calving rate; however, that association was biased against Brahman on tall fescue.

The HSP-70 SNPs at positions 1902, 1917, 1926, and 2098 appeared to be linked, if a cow had one of the SNPs they had all four, and these SNPs appear to be driven by Brahman lineage. Somatic cell counts were associated with the SNP at position 2033 which was the most prevalent in the HSP-70 segment and was equally distributed over breed groups. In humans, a similar guanine to cytosine transition at position 2074, which is close to the G to C transition at 2033 in cattle, was associated with the risk of Parkinson's disease (Wu et al., 2004).

Both the prolactin and HSP-70 sequences posted at NCBI were derived from Angus samples (*Bos taurus*); therefore, the SNPs associated with our *Bos indicus* samples may represent subtle species differences in genetic coding and possibly give rise to future advances in the understanding of the ability of one breed to perform better in stress situations than another breed within a genus.

Implications

These results suggest that single nucleotide polymorphisms associated with the prolactin and(or) heat shock protein 70 genes may server as genetic markers for production and reproductive traits related to cattle profitability. Genomic DNA evaluations will allow producers to evaluate the genetic potential of animals and, in the future, will increase the accuracies of selecting breeding stock that are less susceptible to ergot alkaloids.

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Table 1. Allele frequencies of *Xba* I restriction site by breed composition.

Breed[1]	CC[2]	CT	TT
AA	8	13	2
AB	1	19	1
BA	3	6	3
BB	1	8	8

[1] Breed designations are AA = purebred Angus; AB = sire was Angus, dam was Brahman; BA = sire was Brahman, dam was Angus; BB = purebred Brahman.
[2] Allele CC represents the samples that were homozygous for the restriction site allele (TCTAGA), WX represents the heterozygous cows, and TT allele represents the samples that were homozygous for the SNP (TTTAGA).

Table 2. Relationship between prolactin promoter restriction enzyme, *Xba*, digestion and milk composition, reproduction, and calf traits.

Item	Forage						SEM	Effects[1]
	Bermudagrass			Tall Fescue				
	Genotype							
	CC	CT	TT	CC	CT	TT		
Calvings, %	88	83	88	90	84	58	7	f*g
Prolactin, ng/ml	91	119	103	13	17	25	33	F
Milk								
Volume, kg/d	5.5	5.7	5.2	3.8	4.2	3.3	0.46	F
Butterfat, %	4.6	4.3	3.8	2.7	3.6	3.8	0.35	F, F*G
Protein, %	3.1	3.2	3.3	3.5	3.4	3.6	0.1	F
SCC, n	437	277	311	140	346	253	163	-
Calves								
Birth wt, kg	35.2	35.0	35.7	37.0	35.5	34.3	1.21	-
Weaning wt, kg	240	256	259	227	230	226	9.5	F, S, A
Weaning ht, cm	114	117	118	113	114	116	1.5	g, S, A

[1]Effects: for the statistical model main effects were forage (F, f), genotype (G, g), age at weaning (A, a), sex of the calf (S, s), and month (M, m) in the case of multiple dates of collection. Uppercase letters indicate that the main effect was significant at a probability of less than 0.05; whereas, lowercase letters indicates a significance below 0.1. Interactions between main effects are indicated with an asterisk using the same uppercase and lowercase designations.

Table 3. Effects of breed composition[1] on SNP occurrence in HSP-70 gene.

SNP	Sequence Position	Frequency[2]	Breed AA	AB	BA	BB
1	1851	0.045	2	0	5	0
2	1899	0.006	0	0	1	0
3	1902	0.038	0	1	1	4
4	1917	0.038	0	1	1	4
5	1926	0.038	0	1	1	4
6	2033	0.140	8	5	3	6
7	2087	0.064	0	2	2	6
8	2098	0.038	0	1	1	4

[1] The number of animals with the detected SNP by breed; AA-purebred Angus; AB-Angus sire; BA-Angus dam; BB-purebred Brahman
[2] Percentage of cows with that SNP in our population of 157 cows

Table 4. Relationship of HSP-70 SNP to potential codon position and translational products.

SNP	Base Change[1]	Codon Position[2]	Amino Acid Change[3]
1 (1851)	G to A	3	Ala (no change)
2 (1899)	G to A	3	Leu (no change)
3 (1902)	C to T	3	Asp (no change)
4 (1917)	G to T	3	Ala (no change)
5 (1926)	C to G	3	Asp to Glu
6 (2033)	G to C	2	Gly to Ala
7 (2087)	C to G	-	Post-translational
8 (2098)	T to A	-	Post-translational

[1] G-Guanine; A-Adenine; C-Cytosine; T-Thymine
[2] 1-first base in codon; 2-second base in codon; 3-third base in codon
[3] Ala-alanine; Asp-aspartic acid; Glu-glutamic acid; Gly-glycine; Leu-leucine

Table 5. Relationship between HSP-70 polymorphisms (c1902t, g1917t, and c1926g) and milk composition, reproduction, and calf traits.

	Forage					
	Bermudagrass		Tall Fescue			
	Genotype					
Item	**CGC**	**TTG**	**CGC**	**TTG**	**SEM**	**Effects[1]**
Calvings, %	84	83	80	89	9	-
Prolactin, ng/ml	87	83	23	11	28	F
Milk						
Volume, kg/d	5.7	6.2	4.0	5.1	0.67	f
Butterfat, %	3.9	5.4	3.2	4.1	0.54	G, F,
Protein, %	3.2	3.8	3.4	3.5	0.12	G, f*g
SCC, n	301	637	215	63	180	-
Calves						
Birth wt, kg	35.2	34.4	35.7	36.3	1.7	F*G*S
Weaning wt, kg	257	257	229	228	5	A
Weaning ht, cm	116.5	118.3	114.2	120.9	2.1	g, A

[1]Effects: for the statistical model main effects were forage (F, f), genotype (G, g), age at weaning (A, a), sex of the calf (S, s), and month (M, m) in the case of multiple dates of collection. Uppercase letters indicate that the main effect was significant at a probability of less than 0.05; whereas, lowercase letters indicates a significance below 0.1. Interactions between main effects are indicated with an asterisk using the same uppercase and lowercase designations.

Table 6. Relationship between HSP-70 polymorphisms (g2033c) and milk composition, reproduction, and calf traits.

Item	Forage				SEM	Effects[1]
	Bermudagrass		Tall Fescue			
	Genotype					
	GC	GG	GC	GG		
Calvings, %	85	83	72	82	5.1	-
Prolactin, ng/ml	60	91	16	23	17	F
Milk						
Volume, kg/d	5.9	5.7	3.7	4.2	0.38	F
Butterfat, %	4.1	3.9	2.9	3.3	0.24	F
Protein, %	3.2	3.3	3.5	3.4	0.07	F
SCC, n	175	350	47	259	103	G
Calves						
Birth wt, kg	34.6	35.3	36.4	35.6	0.9	s
Weaning wt, kg	253	258	220	231	7.5	F, S, f*g*s, A
Weaning ht, cm	115.6	116.7	114	114.6	1.1	f, S, A

[1]Effects: for the statistical model main effects were forage (F, f), genotype (G, g), age at weaning (A, a), sex of the calf (S, s), and month (M, m) in the case of multiple dates of collection. Uppercase letters indicate that the main effect was significant at a probability of less than 0.05; whereas, lowercase letters indicates a significance below 0.1. Interactions between main effects are indicated with an asterisk using the same uppercase and lowercase designations.