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Association of Single Nucleotide Polymorphisms in Bovine Heat Shock Protein 70, Cytochrome P450, Lactate Dehydrogenase, Interleukin 8 Receptor, and Melatonin Receptor 1A with Hoof and Udder Traits in Beef Cows

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Abstract

Hoof and udder conformation are important to the longevity and productivity of beef cattle. Selection for hoof and udder quality reduces the incidence of lameness and udder disease to improve animal welfare. Genetic selection is an effective means of improving traits such as these, especially through the use of single nucleotide polymorphism(SNP) genotyping to identify indicator traits. This study used genotype data and hoof and udder scores from previous research to determine if there is significant effect between polymorphisms of heat shock protein 70 (Hsp70), cytochrome P450 (CYP450), interleukin-8 receptor (IL8), lactate dehydrogenase (LDH), and melatonin receptor 1A (MTNR1A), and hoof and udder traits in (n=76) beef cows. The SNP site A1125C of Hsp70 affected hoof angle ($P < 0.0006$) and claw set ($P = 0.0007$). The SNP C994G of Cytochrome P450 affected hoof angle ($P < 0.0024$), and claw set ($P = 0.0054$). The SNP C541A of LDH affected teat size ($P < 0.0001$), and the SNP G777C of IL8 receptor affected udder suspension ($P = 0.0355$). The MTNR1A receptor had two polymorphisms G497A, which affected teat size ($P = 0.032$) and hoof angle ($P < 0.01$), and A455G, which affected udder suspension ($P = 0.0004$) and teat size ($P = 0.02$). These results suggest that Hsp70, CYP450, IL8 receptor, LDH, and MTNR1A genes could be markers for selection of hoof and udder traits in cattle.

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Introduction

Hoof and udder scores are both critical in evaluating the productivity of cows. These scores are used to determine the longevity of cows because they govern the level of production efficiency. Selection for hoof and udder scores that improve performance can be done by visual observation, and in Arkansas the opportunity to improve cowherd performance through genetic selection is an opportunity to that the beef cattle industry has recently placed important focus (Gadberry et al., 2019).

Proper hoof structure is essential to a long productive lifespan of beef cattle, and can be evaluated by scoring hoof angle and claw set (Alexander, 2014). Animals that have abnormal growth of the hoof and claw tend to become lame and be more prone to hoof diseases that result in decreased mobility (Vermunt & Greenough, 1996).

Udder structure of beef cows is very important to longevity and calf performance. Poor teat and udder confirmation decrease profitability due to decreased calf weaning weight, increase incidence of mastitis, and decreased lifelong productivity of beef cows (Beard et al., 2019) Proper udder structure is evaluated by teat size and udder suspension. Longevity and welfare are both important to the beef cattle industry as the health of cows can impact the entire industry.

Visual selection of traits has greatly improved the beef cattle industry; however, in the dairy cattle industry, genomic selection has had excellent improvements for production (Meuwissen et al., 2013). Indicator traits of lameness can be used to improve selection and genetic gains (Ring et al., 2018). As it becomes more apparent that genetic selection can be used to improve the production and efficiency of beef cattle, it is important to start linking genes that correlate to specific traits. The analysis of single nucleotide polymorphisms in cattle is one method that can be used to correlate genes to phenotypic traits to improve genetic selection. Once genes are identified that correlate to hoof and udder scores, selection for specific genes can

be used to significantly reduce lameness and udder disease as well as well as improve production and overall efficiency of beef cattle.

Literature Review

Structural Characteristics

Hoof Structure

Cattle hooves are made up of keratin proteins that are formed when epidermal cells die and harden to create a barrier from the natural environment (Tomlinson et al., 2004). This keratinization continues throughout the animal's life and is important in maintaining hoof hardness which helps protecting cattle from hoof disorders that cause lameness (Mülling et al., 1999). The growth of this outer layer has major effect on the conformational structure of the hoof, and cattle hoof trimming is recommended to prevent overgrowth (Archer et al., 2015). Hoof trimming helps to maintain a healthy hoof angle and claw set to improve hoof quality and prevent lameness (Archer et al., 2015).

Hoof wear can be impacted by several factors including environment, nutrition, and management practices. Inadequate nutrition, lack of management, and high moisture content in the ground all lead to an increase in horn wear (Vermunt & Greenough, 1995). A study by Telezhenko et al. (2009) recorded that claw was also affected by the use of abrasive flooring such as concrete. A quality claw set is not only determined by the hardness and resistance of the hoof to wear, but also the shape and size of the claw (Vermunt & Greenough, 1995). The bones, joints, and ligaments around the hoof are the foundation of the conformational structure of the hoof and have major impact on the shape and size aspect of the quality of the hoof (Richter et al., 2011). Quality claws have low susceptibility for claw disorders and lameness and as well as low

required foot care (Politiek et al., 1986). Genetics are important to the structure of hooves as well as, they are the main contributing factor to the formation of the body structure.

Hoof Conformation

Conformation of the animal has been important in assessing desirable qualities and their effect on the value of an animal (Soule, 1902). The conformation of an animal relates to the shape or proportionate dimensions of an animal or part there of (Vermunt & Greenough, 1996). Characterizing the proper structure and conformation of the hoof as well as abnormalities that appear has been an important aspect of cattle evaluation for several decades (Phillipsson, 1989). Many studies have been done to determine appropriate measurements for the hoof to define the optimal hoof conformation. Hoof angle, which measures the slope of the hoof with respect to the floor surface, and toe length, which measures the length of the dorsal aspect of the claw from the skin-hoof junction to the apex, are the two measurements that have been proposed to be sufficient for quick and precise classification of the hoof (Vermunt & Greenough, 1995). The ability to accurately assess conformational traits is of significant benefit to selection and being able to gauge the impact of the environment or nutrition on the hoof (Laven et al., 2015). Claw measurements are partly symptoms but are also predisposing factors to lameness (Politiek et al., 1986). Ideally the claws of each foot should be symmetrical and distribute the weight of the animal evenly, however large variations in claw conformation are seen within cattle of the same breed as well as across different breed (Boelling & Pollott, 1998; Baird et al., 2009; Häggman et al., 2013). Cattle with quality hoof conformation should not have short, or straight pasterns, and the claws should not be uneven, twisted, curled, or crooked because these things can lead to an increased incidence of lameness (Barham et al., 2005). Lameness causes major losses to

production, and economically is costly due to treatments, culling, and management (Huxley et al., 2013). In dairy cattle, lameness is categorized in the top 3 reasons for culling (Kossaibati & Esselmont, 1997). Production losses result from decreased milk yield (Green et al., 2010; Hernandez et al., 2002), varied nutrition intake (Huxley et al., 2013), and decreased likelihood of ovulation (Morris et al., 2009). While the overall economic costs of lameness are difficult to quantify, it is clear that lameness negatively effects the health and production of cattle. Lameness is also a major animal welfare problem and it is important to used animal based assessment to evaluate the relationship between lameness and welfare (Sadiq et al., 2017).

Udder Structure

The udder is made up of four mammary glands, referred to as quarters, which are skin glands that have been modified to secrete milk (Richter et al., 2011). The udder is made up of the body and teat which both vary in size by the individual cow, and lactation status (Richter et al., 2011). The suspensory apparatus is important to supporting the weight of the mammary glands as they fill with milk and is the primary structure that holds the udder suspended above the ground (Richter et al., 2011). Throughout the life of a cow the udder structure may vary as older cows tend to exhibit reduced suspension and increased teat size from multiple lactations (Goonewardene et al., 2003).

Udder Conformation

Proper udder conformation is highly important to the beef cattle industry as it directly effects calf performance (Beard et al., 2019). Dairy cows with large teats, and loose, pendulous udders are shown to have an increased risk of mastitis which has major economic costs to the

dairy industry (Persson Waller et al., 2014; Romero et al., 2018). The longevity of dairy cows is also significantly affected by udder conformation as cows with well positioned teats and, moderate udder suspension are more likely to produce well and stay in herds longer (Stefani et al., 2018).

While the beef cattle industry does not place the importance of milk production at the same level of the dairy industry, it is indicated that udder conformation also influences both cow longevity and health, as well as calf performance. The size and shape of teats can limit calf growth as enlarged teats that are more difficult to suckle can decrease the growth potential of calves (Devani et al., 2019). Cows with pendulous udders were reported to negatively affect calf nursing ability (Wythe, 1970). Long teats, also known as bottle teats, have been associated with higher calf mortality due to inability to nurse, thus indicating that the size and shape of teats have an impact on calf milk consumption (Frisch, 1982). The finding that cows with small udders, or large teats with pendulous udders had slower growing calves that had lighter weaning weight also confirmed the impact of udder and teat conformation on calf performance (Goonewardene et al., 2003). Cows with tighter udder suspension and smaller teats are associated with decreased incidence of mastitis and management costs (Bradford et al., 2015). Improvement of udder quality could increase the longevity of cows and decrease economic costs of purchasing replacements (Bradford et al., 2015).

Hoof and Udder Scoring

Cattle evaluation can be effectively accomplished by visual scoring because the appearance of an animal is directly related to its reproductive efficiency (Barham et al., 2005). Several breed associations, such as Holstein Association USA, the American Angus Association

(AAA), as well as the Beef Improvement Federation (BIF) have adopted scoring systems to evaluate cattle for foot and udder quality. It is important to purebred and commercial operations to select for quality traits because selection will improve not only the characteristics of the breed, but also the profitability of the operation (Barham et al., 2005). There are scoring systems for many phenotypic aspects of cattle, but for the purpose of this study, only udder scoring and hoof scoring will be reviewed.

The Holstein Association USA, has one of the most extensive trait evaluation guidelines with 50 point scales for each trait. The foot angle is scored from 1, extremely low angle, to 50 extremely steep angle with 25 being intermediate (Holstein, 2018). Due to the importance of the udder to dairy cattle, there are several traits that are evaluated including Fore Udder Attachment (FU), Rear Udder Height (UH), Rear Udder Width (UW) Udder Cleft (UC) and Udder Depth (UD) which are also scored from 1-50 in the same format as the hoof scores (Holstein, 2018). The teats are scored by Front Teat Placement (TP) ranging from 1, extremely wide to 50 extremely close, and Teat Length (TL) ranging from 1, extremely short to 50, extremely long (Holstein, 2018). While this extensive scoring system is feasible in dairy cattle, the beef cattle scoring system of hoof and udder scores is far more simplified since beef cattle are not handled on a daily basis. The udder scoring for the Beef Improvement Federation has a 9 point scale to evaluate udder suspension (US) and teat size (TS). For udder suspension, a score of 9 indicates a very tight udder suspension while a score of 1 indicates a very pendulous udder suspension with broken floor. The range for teat size is 9, which indicates very small teats, to 1 which indicates very large, balloon shaped teats.

The American Angus Association has made the most commonly used foot scoring guidelines for beef cattle in the United States. The foot angle and claw set are evaluated on two

nine point scales with a score of 5 being ideal. For foot angle a score of 1 indicates extremely straight pasterns and very short toe, a score of 5 is ideal with an approximately 45-degree angle at the pastern joint and appropriate toe length and heel depth, and a score of 9 indicates extremely shallow heel, long toe and weak pasterns. The udder scoring guidelines from the Beef Improvement Federation and the hoof scoring guidelines from the American Angus Association were the two scoring systems used to collect the data for this thesis.

Genetic Characteristics

Genetic Selection

The goal of many beef cattle producers is to increase productivity of animals through genetic selection. The dairy industry over the past century has used genetic selection to greatly increase production traits, and over the past few decades, has begun genetic selection to increase longevity, fertility, and health (Miglior et al., 2017). For traits to be considered for selection, it is important that there is a significant economic value, that there is large genetic variation and heritability, and that the trait can be consistently recorded (Shook, 1989). As the beef cattle industry has sought to increase productivity and decrease costs through genetic selection, trait records and genetic studies to determine variation and heritability of traits has begun to increase. Selection of traits before time and resources are invested increases the quality of animals produced and decreases costs associated with production loss (Thompson et al., 2014). Genetic markers, and single nucleotide polymorphisms can be found through genetic testing and correlated to conformation traits to determine relationships and select for desirable traits (Thompson et al., 2014)

Variation and Heritability

Heritability is defined as the phenotypic variation in a population that is attributed to genetic differences. Higher heritability indicates an increased probability that genetic selection will have notable results.

Heritability estimates of teat and udder traits indicate that there is an opportunity that genetic selection for specific traits will exhibit improvement to udder scores. Teat length heritability has been reported as 0.30, 0.29, and 0.33, and teat placement heritability has been reported as 0.26, 0.29, and 0.52 by Rupp and Boichard (1999), Degroot et al. (2002), and Royal et al. (2002), respectively. Teat size heritability was reported by Kirschten et al. (2001) as 0.38, Sapp et al. (2003) as 0.21, and Bradford et al. (2015) as 0.28. The heritability for udder score related traits of beef cattle have been reported as 0.33, 0.22, and 0.31 for udder attachment and suspension by Kirschten et al. (2001), Sapp et al. (2003), and Bradford et al. (2015). Devani et al. (2019) reported that estimated heritability scores for teat and udder score were 0.32 and 0.15, respectively. The genetic correlation between teat size and suspension was 0.81 so selection for one of these traits would have positive correlation to improve the other trait.

Heritability of hoof angle in beef cattle has been reported to be 0.50, and 0.35 for the front and rear hoof angle respectively by Jeyaruban et al. (2012) and 0.20 and 0.19 by Jensen (2017). Heritability estimates of claw set in beef cattle have been reported by Jeyaruben et al. (2012) as 0.46, and 0.42 for front and rear claw set respectively, and 0.09 and 0.17 by Jensen (2017). Kasasrda et al. (2018) estimated heritability of claw traits to be 0.28. Chawala et al. (2013) supports that despite the low heritability scores, genetic selection for resistance to lameness is an option that could have substantial impact on the cattle industry.

Several studies have indicated the importance of genetic selection to improve both hoof and udder traits and due to the low to moderate heritability estimates for there is opportunity for genetic selection to improve these traits.

Single Nucleotide Polymorphisms

Single nucleotide polymorphisms (SNP) are the alteration of a single nucleotide in an animal's genetic sequence. Genotyping has become increasingly popular due to the increasing accuracy of genetic merit prediction and the availability of this form of testing (Scheifers & Weigel, 2012).

Heat Shock Protein 70

Heat shock proteins (Hsps) are molecular chaperons that are named by their weight in kilo Daltons (Marruchella et al., 2004). Heat shock proteins are activated as a response to stressors and are released both intracellularly, and extracellularly (Schmitt et al., 2007). Intracellular Hsp have a protective function while extracellular Hsp mediate immunological function (Schmitt et al., 2007). Hsps through receptor mediated interactions can deliver maturation signals and peptides to antigen presenting cells (Pockley, 2003). Hsp70 plays a significant role in both heat tolerance as well as the proinflammatory immune response (Pockley, 2003). Hsp70 has also been shown to play an important role in apoptosis or programmed cell death (Schmitt et al., 2007). Redirection of the blood flow to the periphery of the body is a common response to heat stress (Gaughan et al., 2013).

Polymorphism of the Hsp70 promoter region at base -1125 was predictive of body condition, breed lineage, LDH concentration, calving rate, hormone concentrations, and nonesterified fatty acid concentration (Rosenkrans, 2011). Other polymorphisms of Hsp70 have been predictive of somatic cell count, and milk protein concentration, and could serve as genetic

markers for production and reproductive traits (Rosenkrans, 2011). Although there have not been any significant effects reported between Hsp70 and hoof traits in cattle, due to the heat stress response of blood flow redirection to periphery, and the role it plays in apoptosis which is important in keratinization of hoof tissue there is a possible relationship to the expression of these traits.

Cytochrome P450

Cytochromes P450 (CYP450) are hemoproteins that is important to drug metabolism, and the clearing of toxins and dietary compounds (Ryder, 2009). Cytochrome P450 are significant sources of reactive oxygen species (ROS) in many tissues (Puntarulo & Cedarbaum, 1998). Reactive oxygen species contribute to oxidative stress as well as muscle function (Abdel-Rahman et al., 2016). Massive production of ROS has been suggested to induce age related muscle dysfunction and subsequent muscle atrophy (Abdel-Rahman et al., 2016). In beef cattle, polymorphisms of CYP450 coding sequence have been predictive of calving traits, milk production, milk protein concentration, calf weight, and calf height (Rosenkrans, 2011; Sales et al., 2013). Cytochrome P450 genotyping has also be associated with possible improvements to productivity of dairy heifers (Sales et al., 2016). While CYP450 has exhibited some significant effect on udder traits due to its predictability of milk production and milk protein concentration, the effect of CYP450 on hoof traits has not been studied.

Lactate Dehydrogenase

Lactate dehydrogenase (LDH) is the final enzyme in the glycolytic pathway. It is used in conversion of pyruvate to lactate in the absence of oxygen and then reverses the conversion when oxygen is present. Lactate dehydrogenase originates from somatic cells, leukocytes, and foreign microorganisms in the milk (Larsen, 2005). Isoenzyme patterns are shown to vary within

related plasmas with LDH-1 and LDH-2 being most commonly found in the liver of ruminants (Arai et al., 2003). Varying isoenzyme patterns of LDH, leading to higher concentrations in the milk, have been associated as a possible indicator of mastitis infections (Larsen, 2005; Kato et al., 1989) SNPs of LDH have also shown relations to calving weight, milk production, and calf height, and somatic cell count (Rosenkrans, 2011).

Interleukin 8 Receptor

Interleukin 8 (IL-8) is a multifunctional chemokine related to chemotaxis, enzyme release, neutrophil function, and surface adhesion molecule expression. Chemokines are classified in four main sub families: CXC, CC, CX3C, and XC, and all interact with guanine-nucleotide protein-linked receptors called chemokine receptors and IL-8 is part of the CXC subfamily (Mélik-Parsadaniantz & Rostene, 2008). Interleukin 8 has also been linked as an autocrine growth factor for carcinoma cells (Hidaka et al., 2002). Curcumin, a traditional medicine for inflammatory disease, has been found to significantly inhibit IL-8 concentration (Hidaka et al., 2002). The most common IL-8 receptor is the CXCR2. Polymorphisms of the CXCR2 gene have demonstrated a significant association to subclinical mastitis in dairy cows due to decreased neutrophil ability to suppress apoptosis and produce reactive oxygen species (Rambeaud et al., 2006). Kopecny (2013) also supports this significant effect between polymorphisms of the CXCR2 receptor and mastitis.

Melatonin

Melatonin is a hormone secreted by the pineal gland. It is the primary regulator of circadian rhythm and is important to sleep regulation (Carter & Juurlink, 2012). Melatonin is mainly metabolized in the liver by cytochrome P450, but can also be metabolized by free radicals (Singh & Jadhav, 2014). Melatonin levels are regulated by light exposure and play an important

role in long day and short day seasonally breeding mammals (Reiter et al., 2009). Melatonin receptors have two subtypes, MT1 and MT2, that are G protein-coupled transmembrane receptors. MT1 receptor activation inhibits adenylyl cyclase and reduces cAMP production to reduce protein kinase activity, while MT2 receptor activation inhibits guanylyl cyclase and reduces the formation of cGMP (Singh & Jadhav, 2014). The reduction of cAMP and cGMP by MT1 and MT2 reduces the release of hormones related to ovulation. Serotonin has been hypothesized to synthesize melatonin to act as a radical scavenger and calmodulin antagonist in ova to protect tissues from free radical damage (Singh & Jadhav, 2014). A study by Wang et al. (2017b) also recorded the importance of melatonin in bovine oocyte maturation, and oocyte complex hormone secretion. Melatonin stimulates the synthesis of glutathione which is an antioxidant that reduces electron leakage from the mitochondrial electron chain (Reiter et al., 2003).

The MTR1 receptor (MTNR1A) for melatonin has been associated to the reproductivity of water buffalo, and ability to reproduce during parts of the breeding season that are not normal as well as conception rates (Gunwant et al., 2018; Pandey et al., 2019). In bovine granulosa cells, melatonin and MTNR1A are important to regulating cellular progression, apoptosis hormone secretion, and reproduction related genes (Wang et al., 2017a).

Methodology

Design, Purpose, Data Collection

This is a quantitative study of historical data using analysis of variance between hoof and udder scores and genotype of (n=76) crossbred Angus beef cows. The purpose is to determine if

there is significant effect between single nucleotide polymorphisms (SNPs), and hoof and udder score.

The genetic polymorphisms and hoof and udder scores that were analyzed in this study were collected during previous research studies done at the University of Arkansas using crossbred Angus beef cows. The genomic data was gathered in a study completed in 2017 by collecting blood samples, and extracting DNA. Genotypes of the cows were determined using the Sequenom technology (GeneSeek, Lincoln, NE). The genetic extraction was done as explained in the methodology of Davis (2019). The hoof and udder scores of cows enrolled into this study were recorded by trained technicians in 2017 for a previous study that included the American Angus Association guidelines for hoof angle (HA) and claw set (CS), and the Beef Improvement Federation (2016) guidelines for udder suspension (US) and teat size (TS). McGuire (2019) has published a deeper explanation of the methodology used in scoring. The University of Arkansas Institutional Animal Care and Use Committee (IACUC), approved the current study (Protocol #19117).

Data Analysis

This current study analyzed SNPs of heat shock protein 70 (Hsp 70), melatonin (MTN), cytochrome p450, lactate dehydrogenase (LDH), and interleukin 8-receptor in association to the hoof and udder scores of (n=76) cows. The SNP data and hoof and udder scores were analyzed using a mixed model ANOVA with the main effects of SNPs, HA score, CS score, US score, and TS score. The differences of least square means separated the means of the scores in relation to the alleles of each SNP, and T-tests and Tukey Kramer adjustments were used to determine if the effects were significant ($P < 0.05$). The results of the T-tests and Tukey Kramer adjustments were

used to determine if there was significant effect between cattle genotype, and hoof and udder score.

Results

Heat shock protein 70

Statistical analysis showed significant effects between the coding sequence polymorphism A1125C in Hsp70 promotor region for both hoof angle and claw set. Cows that were homozygous for both the major and minor allele (AA; CC) had lower ($P=0.0006$; $P=0.0003$) hoof angle scores than heterozygous (AC) cows. Hoof angle scores were not significantly different (5.9, 5.8, and 6.5, respectively for AA, CC, and AC), with less than half a point difference in score, but they do indicate a trend for heterozygous cows to have worse hoof angle scores. A similar trend was seen in relation to claw set score as cows that were homozygous for the major allele (AA) had significant lower ($P=0.0007$) claw set scores than heterozygous cows. Homozygous cows scored close to a full point lower score than heterozygous cows, 5.9 and 6.8 respectively, which indicates that AA cows tend to have better claw set scores than heterozygous cows. The cows homozygous for the minor allele did not have significantly different scores from either the major allele homozygous cows or the heterozygous cows. (See Table 1)

Cytochrome P450

The coding sequence polymorphism C994G of cytochrome p450 indicated the major homozygous allele was significantly different ($P=0.0024$) from the minor homozygous (GG) as well as the heterozygous alleles ($P=0.002$) in relation to hoof angle. Hoof angle scores of the primary homozygous allele (CC) cows, compared to the minor homozygous, and heterozygous

cows differed by less than half a point, but this does indicate a trend for the major homozygous (CC) cows to have higher hoof angle scores that are further from an optimal hoof angle score (6.3, 5.9, 5.9; for CC, CG, and GG, respectively).

This polymorphism C994G of the coding sequence had significant effect ($P=0.0054$) on claw set. Homozygous (CC) cows had higher mean claw set scores (6.7) than the heterozygous cows (6.0), by more than half a point. (See Table 2)

Lactate dehydrogenase B

The coding sequence polymorphism C541A of lactate dehydrogenase had significant effect ($P<0.0001$) on the teat score in cows. Homozygous cows (CC) had mean teat scores of 5.8 which is more than a full point lower than the heterozygous teat scores of 6.9. Both scores could be considered acceptable, however a higher teat size score indicates smaller teats. Smaller teats would be acceptable on heifers or younger cows, but would be less desirable in cows that had calved several times. (See Table 3)

Interleukin 8 Receptor

The interleukin 8 receptor coding sequence polymorphism G777C showed a significant ($P=0.0355$) effect on the mean udder score. The homozygous (CC) cows had close to half a point lower in udder score than heterozygous (CG) cows with mean udder scores of 7.1 and 7.5, respectively. An udder suspension score in the range of 7 to 8 indicates a tight udder suspension. Tight udder attachment is essential to udder conformation, and the heterozygous cows tend to have higher scores which indicate tighter attachment of the udder, however both scores would be acceptable. (See Table 4)

Melatonin Receptor 1A

A455G

The coding sequence polymorphism A455G of melatonin receptor 1a had a significant effect ($P=0.0004$) on udder suspension scores. Cows that were homozygous (AA) had a lower mean udder suspension score of 7.1 compared the heterozygous (GA) cows which had udder scores of 7.8, more than half a point higher. Cows with udder suspension scores in the range of 7 to 8 have tight udder suspension, which is desirable because it indicates a strong udder attachment. This coding sequence polymorphism also had a significant effect ($P=0.02$) on the teat score with the homozygous AA cows having a lower mean teat score (5.8) than the heterozygous cows (6.5) by more than half a point. While both of these teat size scores fall within an acceptable range, a moderate teat size score (5) is the most desirable. (See Table 5)

G497A

The coding sequence polymorphism G497A of melatonin receptor 1a had significant effect ($P<0.03$) on teat size scores. The major homozygous allele (GG) had a higher mean teat scores of 6.0 by more than half a point than the minor homozygous allele(AA) cows which had mean teat scores of 5.3. This shows a trend for the minor homozygous allele cows to have a more desirable teat size score than the major homozygous allele cows.

Interestingly, this coding sequence polymorphism G497A also significantly affected hoof angle scores. The minor homozygous (AA) cows had lower hoof angle scores compared to the major homozygous (GG) cows ($P=0.0043$) and the heterozygous cows ($P=0.01$). The difference in hoof angle score was less than half a point but does indicate a trend for the minor homozygous cows to have better hoof angle scores (5.6, 6.0, 6.1; for AA, GA, and GG, respectively). (See Table 6)

Discussion

Heat shock protein 70

Prior studies of bovine heat shock protein 70 have been associated with productivity and health. SNPs in the coding sequence of Hsp70 have been associated with an ill effect on susceptibility of mastitis (Huang et al., 2015). Polymorphisms of Hsp70 at A1125C and G1128T have been indicated to have significant effect on fertility and lower calving percentages (Rosenkrans et al., 2010). One SNP of Hsp70 at C895D has also been indicated to have significant effect on Julian Calving date (Finney, 2018). These previous studies have all indicated an effect on reproduction and udder health, however there have not been any studies that indicate the significant effect of Hsp70 at A1125C on hoof angle and claw set, as was found in this study.

Cytochrome P450

According to Sales et al. (2012), a cytosine to guanine transversion at base 994 (C994G) had significant effect on calving rate, milk yield, and calving date. A more recent study of the transversion C994G on Hsp 70 indicated effect on average daily gain in dairy heifers (Sales et al., 2016). One study of inflammatory response in hoof dermal tissues found down regulation of subfamilies of cytochrome P450, and the potential of CYP450 to be used in development of anti-inflammatory drugs (Tian et al., 2019). Although the importance of CYP450 to drug metabolism and productivity has been found, the effect of CYP450 on hoof angle and claw set found in this study has not been researched.

Lactate Dehydrogenase B

Single nucleotide polymorphisms of LDH-B have been related to hormonal and enzymatic activity, growth and carcass traits in heifers (Turner, 2013). Polymorphisms of LDH-B have also been shown to affect calving rate at G-348A and non-essential fatty acids at C541A (Alaamri, 2011). The LDH-B has been negatively related to milk protein content (Wang et al., 2019), and enzyme concentrations of LDH are presently used for automatic detection of mastitis (Khatun et al., 2019). Compared to previous studies and the implication of LDH with productivity traits and udder health, this current study, which identified significant effect at C541A on teat size scores, furthers the previous research done on LDH.

Interleukin-8 Receptor

Polymorphisms of the Interleukin-8 receptor also known as CXCR2 have been significantly related to production traits in cattle. The SNP at C777G has been previously associated with birth weight, calf weaning weight, cow body condition score at weaning, and cow weight at weaning (Deaton, 2017). Associations between polymorphisms in IL8 Receptor A and neutrophil traits and mastitis have been described (Verbeke et al., 2012). The relation of SNPs in IL8 Receptors to production and udder health has been shown in previous studies and the finding that the C777G polymorphism has significant effect on udder suspension score supports the importance of IL8 to cow productivity.

Melatonin Receptor 1A

The SNPs of bovine MTNR1A have been associated with meat quality traits (Yang et al., 2015). Melatonin receptor 1A has also been suggested to play an important role in granulosa cell function by regulating cellular progression, hormone secretion, apoptosis, and reproduction-related genes (Wang et al., 2017b). The MTNR1A SNP site A583G has been indicated to effect both hair coat and calving rate (Meyer, 2016). Several studies have found that polymorphisms of

MTNR1A had no effect on traits such as sperm quality and testicular size, hair condition score, prolactin concentrations, or cow body weight (Davis, 2019; Kianpoor et al., 2018). While there is a variation in the effect of MTNR1A on different traits, the effect of the A455G and G497A polymorphism of MTNR1A on udder suspension scores, teat size scores, and hoof angle scores found in this study indicate that there is a link to productivity.

Tables

Table 1

Effect of A1125C coding sequence polymorphism of Hsp70 promoter region on mean cow hoof angle score, and mean cow claw set score

A1125C Genotype		
	Hoof Angle	Claw Set
Homozygous Cows (AA)	5.9 ^a	5.9 ^a
Homozygous Cows (CC)	5.8 ^a	6.3 ^b
Heterozygous Cows (AC)	6.5 ^b	6.8 ^b
P-Value	0.0006 ^{AA} 0.0003 ^{CC}	0.0007 ^{AC}

[^{ab} Least-square means without a common superscript differ (P<0.05);
Superscript on P-Value indicates allele associated with the value]

Table 2

Effect of C994G coding sequence polymorphism of cytochrome P450 on mean cow hoof angle score, and mean cow claw set score

C994G Genotype		
	Hoof Angle	Claw Set
Homozygous Cows (CC)	6.3 ^a	6.7 ^a
Homozygous Cows (GG)	5.9 ^b	6.1 ^b
Heterozygous Cows (CG)	5.9 ^b	6.0 ^b
P-Value	0.0024 ^{GG} 0.002 ^{CG}	0.0054 ^{GG}

[^{ab} Least-square means without a common superscript differ (P<0.05);
Superscript on P-Value indicates allele associated with the value]

Table 3

Effect of C541G coding sequence polymorphism of Lactate Dehydrogenase on mean cow teat size score

C541A Genotype	
	Teat Size
Homozygous Cows (CC)	5.8*
Heterozygous Cows (CA)	6.9
P-Value <0.0001	

[* means P<0.05]

Table 4

Effect of G777C coding sequence polymorphism of Interleukin 8 Receptor on mean cow udder suspension score

G777C Genotype	
	Udder Suspension
Homozygous Cows (CC)	7.1*
Heterozygous Cows (CG)	7.5
P-Value 0.0355	

[* means P<0.05]

Table 5

Effect of G497A coding sequence polymorphism of Melatonin Receptor 1A on mean cow teat size score, and mean cow hoof angle score

G497A Genotype		
	Teat Size	Hoof Angle
Homozygous Cows (GG)	6.0 ^a	6.1 ^a
Homozygous Cows (AA)	5.3 ^b	5.6 ^b
Heterozygous Cows (GA)	5.7 ^{ab}	6.0 ^a
P-Value 0.032		0.0043 ^{GG}
		0.01 ^{GA}

[^{ab} Least-square means without a common superscript differ (P<0.05);
Superscript on P-Value indicates allele associated with the value]

Table 6

Effect of A455G coding sequence polymorphism of Melatonin Receptor 1A on mean cow udder suspension score, and mean cow teat size score

A455G Genotype		
	Udder Suspension	Teat Size
Homozygous Cows (AA)	7.1*	5.8*
Heterozygous Cows (AG)	7.8	6.5
P-Value	0.0004	0.02

[* means P<0.05]

Conclusions

Based on the results of this study there are several single nucleotide polymorphisms that have significant effect on hoof and udder scores in cows, so there is possibility of genetic selection to improve hoof and udder scores in cows.

The A1125C polymorphism of Hsp 70 indicated a trend for homozygous cows to have lower hoof angle and claw set scores which would be more desirable for productivity. The homozygous (AA) cows had close to a full point better score than the heterozygous (AC) cows. The polymorphism C994G of CP450 indicates a trend for the homozygous allele cows to have higher hoof angle and claw set scores, which would be less desirable than the heterozygous (CG) cows. The LDH-B polymorphism C541A and IL8 receptor polymorphism G777C indicated significant effect on teat size scores and udder suspension scores respectively. The cows that were homozygous had lower scores than the heterozygous cows. A moderate teat size is more desirable so the cows with the homozygous genotype for LDH-B would be better for productivity. The MTNR1A polymorphism A455G indicated trends for both teat size and udder suspension as cattle that were not homozygous had more than half a point increase in both scores away from the optimum score. Melatonin receptor G497A indicated a trend in relation to teat

size score as cows that were minor homozygous(AA) had better scores than the major homozygous(GG) and heterozygous genotypes. Melatonin receptor G497A also indicated that cows with the minor(AA) homozygous allele had better hoof angle scores marginally. Based on this it can be predicted that alleles for heat shock protein 70, cytochrome p450, lactate dehydrogenase, interleukin 8 receptors, and melatonin can be selected for to improve the hoof and udder quality of beef cattle. Selection to improve these traits would improve overall productivity and health in beef cattle.

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