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A Quantitative and Molecular Evaluation of Bovine Respiratory Disease, Growth Traits, and Carcass Traits in Crossbred Steers

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A QUANTITATIVE AND MOLECULAR EVALUATION OF BOVINE RESPIRATORY
DISEASE, GROWTH TRAITS, AND CARCASS TRAITS IN CROSSBRED STEERS

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
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in

The School of Animal Sciences

by

Samantha Lidia Miller
B.S., Texas State University, 2012
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ABSTRACT

The first objective of the two studies presented herein evaluated on farm sources of variation and correlated effects contributing to BRD incidence in a population of crossbred steers sent to the feedlot from 2010-2013. Analyses revealed that incidence of BRD was lowly positively correlated to birth weight (BW) and lowly negatively correlated to weaning weight (WW), hip height (HH), sire breed, site, and birth year. These results indicate that the traits analyzed herein are not precursors for BRD in the current population. However, since little is still known about the correlation between genetic predisposition to BRD and animal performance, further studies should be conducted in the future.

The second study presented herein evaluated single nucleotide polymorphisms (SNP) located on previously described QTL regions of Bos Taurus Autosome 6 and Bos Taurus Autosome 20 for potential associations with growth traits, carcass traits, and incidence of BRD in crossbred steers. Growth traits analyzed included birth weight (BW), weaning weight (WW), and hip height (HH). Carcass traits evaluated included hot carcass weight (HCW), yield grade (YG), marbling score (MS), ribeye area (REA), and back fat thickness (BF). Along with growth and carcass traits, incidence of BRD was also evaluated in the current population. Genotyping analyses identified fourteen unique SNP located on BTA 6 and eleven unique SNP located on BTA 20 that were significantly ($P < 0.05$) associated with the aforementioned traits. However, no markers on either BTA 6 or BTA 20 were identified as significantly associated with BF. These results indicate that there may be a higher genetic predisposition to BRD than previously thought. However, before their incorporation into MAS programs, additional SNP located on BTA 6 and BTA 20 should be genotyped, along with other QTL regions spanning the genome. Additionally, a larger population of crossbred steers should be utilized to further validate the results herein.

CHAPTER I INTRODUCTION

Bovine Respiratory Disease (BRD) is the most common disease affecting feedlot cattle today (Brooks et al., 2011). Commonly referred to as shipping fever, BRD is most prevalent in calves being transported to feedlots after being weaned (Bowland et al., 2000). Previous reports have indicated that lighter-weight calves were at greater risk for BRD than heavier calves (Taylor et al., 2010). However, additional studies have reported that growth traits including birth weight and weaning weight had no significant effect on BRD incidence (Schneider et al., 2010). Overall, little has been reported about genetic predisposition to BRD susceptibility (Berry, 2014). The first part of the research presented herein evaluated on farm sources of variation and correlated effects contributing to BRD incidence in crossbred steers over a four-year period. Growth traits analyzed included BW, WW and HH.

Identifying SNP on specific QTL regions associated with growth traits, carcass traits and disease resistance has become of great importance in the beef industry (Dekkers, 2004). Previous reports have indicated that SNPs have become popular due to the need for high densities of genetic markers that are utilized in studies of multifactorial diseases, such as BRD (Vignal et al., 2002). The second part of the research presented herein evaluated SNP located on previously described QTL regions of BTA 6 and BTA 20 for potential associations with growth traits, carcass traits, and incidence of BRD in a population of crossbred steers. The QTL regions selected on BTA 6 and BTA 20 were previously described as being associated with BRD susceptibility (Li et al., 2004; Casas et al., 2010). Traits analyzed included the previously mentioned growth traits BW, WW, and HH along with carcass traits including HCW, YG, MS, REA, and BF.

CHAPTER II

REVIEW OF LITERATURE

Bovine Respiratory Disease Background

Bovine Respiratory Disease (BRD) is the most common disease affecting feedlot cattle in the United States (Brooks et al., 2011). Bovine respiratory disease contributes to approximately 75% of morbidity and 50%-75% of mortality in feedlots annually (Brooks et al., 2011).

Economic losses incurred by animals contracting BRD due to reduced feed efficiency, veterinary treatment and death, are estimated to cost 640 million dollars annually in the United States (Bowland et al., 2000). Although cattle of all ages can be affected by BRD, most are susceptible during time of high stress as young calves (Schneider et al., 2010). These times of high stress include the production periods of weaning, castration, and entrance into the feedlot.

Transportation is the most accepted non-infectious risk factor for BRD and losses occur as a result of pneumonic pasteurellosis, or “shipping fever” (Bowland et. al., 2000). Previous reports involving morbidity levels of traveling calves indicated that calves transported 12 hours had higher morbidity levels than those transported 24 hours (Taylor et al., 2010). No significant difference was detected between cattle transported 24 hours and control calves that were fasted but not transported. Final results indicated that sorting, loading, and early transit are likely the most stressful components of transportation (Taylor et al., 2010). Previous studies also reported that mortality due to BRD was twice as high in calves experiencing the combination of social reorganization and transport (Hodgson et al., 2005). Patterns of respiratory disease observed during the first 12 weeks on feed among feedlot cattle is consistent with the majority of the disease cases occurring in the early part of the feeding period. Moreover, longer transport distances for arriving calves was associated with an increased morbidity incidence as well (Sanderson et al., 2008).

Bovine respiratory disease in feedlot cattle is typically characterized by severe bronchopneumonia and/or pleuropneumonia (Hodgson et.al. 2005). Fatal bovine respiratory infections are frequently characterized by a primary viral infection followed by a secondary bacterial infection. Fatal respiratory infections occur when a primary viral infection compromises host defenses and enhances the severity of a secondary bacterial infection (Hodgson et.al. 2005). Common viral agents that are associated in BRD include infectious bovine rhinotracheitis, bovine viral diarrhea virus, bovine respiratory syncytial virus, and parainfluenza type 3. In addition, bacterial strains associated with BRD include *Mannheimia hemolytica*, *Pasteurella multocida*, *Hemophilus somnus*, and mycoplasma (Schneider et al., 2010). Viral agents have the ability to cause direct damage to the respiratory clearance mechanisms and lung parenchyma. This damage facilitates translocation of bacteria from the upper respiratory tract, establishing an infection in compromised lung (Schneider et al., 2010). Inflammation caused by BRD decreases dry matter intake, average daily gain, and gain to feed ratio in feedlot calves. Overall, this decreases growth rate and increases days on feed, which results in economic losses during the feeding period (Gifford et al., 2012).

Bovine Respiratory Disease Prevention

Prevention of BRD begins with management procedures to minimize stress on the animal (Leach et.al, 2012). Vaccination has been effective in controlling BRD in cattle populations, however, vaccinations alone cannot always induce long lasting immunity and young calves can be repeatedly infected (Leach et.al, 2012). Although vaccines reduce the incidence of BRD, they don't eliminate the threat completely, and therefore, health management protocols cannot be based solely on vaccinations. Protocols must also consist of records systems, proper husbandry and nutrition, along with pen maintenance (Lechtenberg et al., 1998). This report also

emphasized that careful observation, effective therapy and hospital management should be a feedlot operator or producers' main practices in reducing the incidence of BRD (Lechtenberg et al., 1998). It was previously reported that BRD vaccination upon arrival at the feedlot was equivocal at best (Perino and Hunsaker, 1997), and if the feedlots administer a modified live virus vaccine as prevention of BRD, this may result in reduced gain performance (Richeson et al., 2008). Administration of live virus vaccines has become a common practice for BRD prevention in feedlots to vaccinate against viral agents, such as: *P. haemolytica*, *H. somnus* and *P. multocida*, and has been proven ineffective (Bowland et al., 2000).

Treatments of Bovine Respiratory Disease

When administering treatment for BRD, it was reported that antimicrobial tulathromycin as first line treatment was a more successful antibiotic treatment for BRD compared to florfenicol or tilimicosin (Nartrup et al., 2013). Treatments utilizing tulathromycin resulted in more first treatment successes and fewer deaths compared to the other anti-microbials utilized in the study (Nartrup et al., 2013). The susceptibility of ceftiofur, penicillin, danofloxacin, enrofloxacin, florfenicol, tetracycline, tilimicosin and tulathromycin was previously evaluated in animals over a ten year period. It was concluded that ceftiofur remained very active against BRD pathogens over the 10 years of the program (Portis et. al., 2012). Previously, the effects of incorporating ceftiofur along with flunixin, ketoprofen and carprofen into a treatment regimen for BRD had been observed (Lockwood et al., 2003). The three non-steroidal anti-inflammatory drugs utilized did significantly reduce the fever of the treated groups compared to the ones only treated with ceftiofur. However, there was no significant decrease in depression, illness scores, dyspnea or coughing (Lockwood et al., 2003).

A previous study conducted by Bateman and associates (1990) reported the efficacy of three antimicrobials in the treatment of BRD. The antimicrobials utilized in the trial included oxytetracycline, penicillin and trimethoprim-sulfadoxine. Results indicated no significant differences among the oxytetracycline, penicillin or trimethoprim-sulfadoxine treatments for BRD (Bateman et al., 1990). Furthermore, an additional study evaluated the efficacy and safety of a florfenicol plus flunixin meglumine formulation in BRD affected calves less than six weeks of age (Thiry et al., 2014). Compared to florfenicol, the florfenicol-flunixin formulation was found to be more effective, and alleviated the clinical signs of BRD more rapidly. This indicated that this specific formulation is both efficacious and safe in the treatment of BRD (Thiry et al., 2014).

Bovine Respiratory Disease Effects on Performance Characteristics

Previous studies reported that BRD incidence had no significant effect on weaning weight and that phenotypic correlations between BRD incidence and birth weight and weaning weight were low (Schneider et al., 2010). Results of a previous study also reported that clinical BRD resulted in decreased body weight and average daily gain at the end of a 63-day preconditioning period (Holland et al., 2010). However, after segregating the animals and following previous BRD treatment protocol, a compensatory response in average daily gain (ADG) and gain to feed ratio (G: F) was observed in treated animals. Moreover, heifers at harvest had similar birth weight, carcass weights, and carcass characteristics regardless of the number of BRD treatments (Holland et al., 2010). These results specified that animals requiring multiple treatments for BRD appear to maintain the ability to produce carcasses of similar value to healthy animals given the additional days and intake energy required. However, marbling

score was negatively impacted and showed a decrease as the number of BRD treatments increased in the same population (Holland et al., 2010).

A previous study reported the effects of BRD on an animal's performance, and focused on traits including average daily gain (ADG), daily dry matter intake (DDMI) and dry matter intake to gain ratio (DM:G). Results indicated no significant differences in ADG and DM:G when comparing healthy animals to ones diagnosed with BRD (Jim et.al., 1993). Utilizing lung lesions, it was concluded that steers with lesions had lower daily gain and lighter hot carcass weight compared to the steers with no lung lesions at time of slaughter (Gardner et. al., 1999). Moreover, it was reported that heifers treated for BRD had a decreased overall average daily gain, final body weight, and hot carcass weight (Montgomery et al., 2009).

Heritability/Incidence

Heritability is the fraction of the observed variance which is caused by differences between the genes or the genotypes of the individuals (Lush, 1949). A heritability estimate is a numerical value that is assigned to a trait of either low, moderate, or high heritability. This gives a producer a more accurate method to improve economically important traits (Muggli-Cockett et al., 1992). Heritability estimates for resistance to BRD have previously been reported in pre-weaned calves at $h^2 = 0.10$, and at $h^2 = 0.00$ to 0.26 (Muggli-Cockett et al., 1992; Snowden et al., 2005). In feedlot cattle it has been estimated that heritability of BRD resistance is between 0.04 and 0.08 , with an estimate of 0.18 (Snowden et al., 2005). The Germplasm Evaluation Project at the U.S. Meat Animal Research Center estimated that the heritability of bovine respiratory disease is 0.1 , with the genetic variation of bovine respiratory disease being considered low (Casas et al., 2010).

Genetic Markers

Microsatellite markers are defined as simple sequence repeats that are found throughout the genome. Microsatellites consist of motifs which are made up of 1-6 base pairs tandemly repeated several times (Yang et al., 2013). Since microsatellites have a high number of alleles present at a single locus, this allows them to have high heterozygosity values. These high values can dramatically reduce the number of reference families necessary for building a genomic map (Vignal et al., 2002). The first DNA-based molecular marker for constructing genetic linkage maps was restriction fragment length polymorphisms (RFLP) (Yang et al., 2013). These markers are characterized as changes in length of defined DNA fragments produced by digestion of the DNA sample with restriction endonucleases (Beckmann et al., 1986). Restriction fragment length polymorphisms are used to identify polymorphisms among different individuals (Yang et al., 2013). The molecular basis for RFLP, like SNP markers, is that nucleotide substitutions, insertions, deletions, duplications, and inversions within the genome can remove or create new restriction sites (Yang et al., 2013).

Single nucleotide polymorphisms (SNPs) have gained high popularity due to their abundance in the genome and genetically stable nature (Yang et al., 2013). An SNP is a single nucleotide base pair change in a DNA sequence (Vignal et al., 2002). These changes in the DNA sequence have the potential to alter phenotypic outcomes. Previous reports have indicated that SNPs have become more popular due to the recent need for high densities of genetic markers. High density markers are used in studies of multifactorial diseases, and in polymorphism detection and genotyping techniques (Vignal et al., 2002).

Heterosis/ Crossbreeding/Terminal Sire/Paternal Breeds

Offspring resulting from crossbreeding two different breeds or species are referred to as hybrids (ZoBell et al., 2011). The display of superior qualities demonstrated by these hybrids is referred to as heterosis or hybrid vigor (ZoBell et al., 2011). Heterosis is the difference between the mean of reciprocal F1 crosses and the mean of two parental breeds (Cundiff, 2004). Heterosis in beef cattle can result in offspring with enhanced traits in reproduction, survivability, fertility, growth, meat quality and disease resistance. Heterosis through crossbreeding increases performance with little additional cost to the producer (ZoBell et al., 2011).

Two types of crossbreeding systems include terminal cross breeding or rotational cross breeding. In a terminal cross breeding system, all calves born are going to be sold and replacement cows are acquired from other sources. These systems stress bull selection in order to achieve better growth and carcass qualities (ZoBell et al., 2011). Breeds used for terminal crosses are known for their growth rates, muscle lean-ness and large mature size compared to maternal breeds (Cundiff, 2004). Rotational cross breeding systems select bulls for growth, carcass, and maternal traits, while replacement cows come from within the herd (ZoBell et al., 2011). Continental breeds show significantly greater growth rates and heavier body weights at weaning, yearling, and mature ages compared to British breeds. Previous studies, however, reported that British breeds are comparable to Continental breeds in growth rate. Females sired by Continental breeds have a tendency to be older at puberty and have a greater propensity to gain fat (Cundiff, 2004).

Quantitative Trait Loci Background

Quantitative trait loci (QTL) are chromosomal regions of the genome that may harbor genes affecting quantitative trait variation (Snelling et al., 2010). The goal of genome research in

cattle is to map and characterize loci that control phenotypic traits (Andersson, 2011). Molecular marker information is used to map major QTLs on chromosomes (Zeng, 1994). Mapping with QTLs focuses more on chromosomal segments, while fine mapping requires more recombination to separate genes from closely linked markers (Complex Trait Consortium, 2003). Quantitative trait loci mapping links two types of information—phenotypic data and molecular marker inheritance in an attempt to explain the genetic basis of variation in complex traits (Falconer and Mackay, 1996). Quantitative trait loci mapping identifies the action, interaction, number, and precise location of these gene regions. The total number of QTL that control a given trait is not absolute and depends on a randomly chosen threshold (Andersson, 2011).

It has been reported that the most conclusive evidence to identify a QTL is in the ability to replace one allele with another and test for function (Glazier et al., 2002). A QTL mapping approach uses statistical analyses of genome-wide molecular markers to identify chromosomal regions contributing to phenotypic variation (Stinchcombe et al., 2007). Strategies for locating trait loci includes association tests using candidate genes and genome scans based on linkage mapping (Andersson, 2011). Although QTLs can be costly and challenging, they still remain the most comprehensive method of identifying genomic regions and genes contributing to variation for multi-genic traits (Stinchcombe, 2007).

Quantitative Trait Loci Associated with Bovine Respiratory Disease

It has been previously reported that the segregation of an allele(s) at the putative QTL may have been influenced by indirect selection for adaptation related to pathogenic diseases (Casas et al., 2010). A pathogenic disease occurs when an infectious agent causes illness in a host (Alberts et al., 2002). Bovine chromosome 20 has been shown to harbor genes associated with disease resistance (Casas et al., 2010). Two genes, ANKRA2 and RP105, located on

chromosome 20 were identified as being involved with immune response in cattle (Casas et al., 2010). Previous QTL studies have supported the claim that chromosome 20 harbors genes associated with the immune system (Casas et al., 2010). Results of a previous study reported that the centromeric region of bovine chromosome 20 harbors a putative QTL related to BRD incidence (Casas et al., 2008).

Previously reported loci associated with Bovine Viral Diarrhea Persistent Infection were shared with loci linked to BRD (Zanella et al., 2011). This result was in agreement with a second study that identified a QTL on BTA 2 and BTA 26 that was linked to BRD infection in cattle after an initial genome-wide linkage analysis (Neiberger et al., 2010). Quantitative trait loci regions coding for one trait, may harbor linked markers for selection that affect another trait, such as disease resistance in animals. These QTL regions may also overlap with regions that harbor markers that affect traits such as carcass quality (Garcia et al., 2010).

Carcass Traits

The quality grade of a carcass is determined by the amount of marbling on the cut surface of the rib-eye between the 12th and 13th rib (Drake, 2004). The amount of closely trimmed retail cuts of meat that a carcass can produce is measured by yield grade. Yield grades range from 1 to 5, 1 being the greatest amount of yield and 5 being the lowest. Yield grade is calculated by using the amount of external fat cover, the percentage of fat in the kidney, pelvic and heart areas, the rib eye size and carcass weight. Along with yield grade, carcass weight, also referred to as hot carcass weight, is measured. This incorporates the weight of the carcass just before it enters the chilling room during the finishing process. Then, the rib eye area is measured as the surface area on the cut surface of the rib eye muscle between the 12th and 13th ribs. Back fat thickness is also measured between the 12th and 13th rib and consists of the amount of fat opposite the rib eye at the cut surface (Drake, 2004).

Carcass Traits and Bovine Respiratory Disease

It has been reported that incidence of BRD has negative effects on performance and carcass traits (Schneider et al., 2009). Selection for BRD resistance may have little effect on hot carcass weight (HCW), longissimus muscle area (LMA), and fat due to the low genetic correlation estimates. Favorable genetic correlations exist for acclimation ADG, overall ADG, final body weight, and marbling score with either health measure (Schneider et al., 2010).

Previous reports indicated that when comparing a healthy pen and an unhealthy pen, there was no significant differences observed for carcass weight, average fat, grade fat, rib eye area, marbling score, and cutability estimate (Jim et al., 1993). Alternatively, it has been reported that steers with clinical signs of BRD and lung lesions at time of slaughter had less internal fat, and lower marbling scores compared to the steers with no clinical sign of BRD and no lung lesions at time of slaughter (Gardner, et.al., 1999). An additional study, in agreement with Gardner and associates (1999), observed decreased performance traits in heifers and that heifers with apparent BRD had decreased fat thickness and marbling score (Montgomery et al., 2009).

Single Nucleotide Polymorphisms Associated with Carcass Traits

Previous reports identified significant SNP associated with hot carcass weight located on BTA 6 (Lu et al., 2013). Additionally, SNP significantly associated with rib eye area and marbling score were identified located on BTA 6 (Casas et al., 2000; Lee et al., 2012). Moreover, significant markers associated with back fat thickness on BTA 6 were previously identified (Li et al., 2004). Previous reports also identified SNP that were significantly associated with yield grade located on BTA 20 (Saatchi et al., 2014).

Previously, it was reported that at the genome wide level, eight SNP were significantly associated with hot carcass weight, seven of which were located on BTA 6. At a stringent

significance level, 48 SNP were located on BTA 6 and 22 of those SNP were associated with hot carcass weight (Duc Lu et al., 2013). Moreover, 12 of the 53 SNP associated with percentage of rib bone were located on BTA 20 (Duc Lu et al., 2013). Previous studies reported that the CAST SNP C allele (a G to C substitution) was associated with increased LM tenderness, however it tended to reduce LM area and lean yield and significantly increase fat yield. This suggests that the CAST SNP may also affect other important carcass traits, such as fat yield, but it warrants further investigation (Schenkel et al., 2006). Associations of molecular polymorphisms within exon 2 of the leptin gene with fat yield, lean yield, grade fat and LM tenderness were reported in beef cattle (Schenkel et al., 2005). Reports indicated that there is an association between the SNP E2FB located in leptin exon 2 and carcass lean meat yield and fatness. The second SNP located in leptin exon 2, E2JW, is also associated with carcass lean meat yield and fatness, with no association with inter-muscular fat (Schenkel et al. 2005).

Previously identified SNPs located on the DNMT1, DNMT3A, DNMT3B and CAPN1 genes were found significantly associated with carcass traits (Liu et al., 2015). The carcass traits analyzed included dressing percent, carcass weight, chilled carcass weight, rib eye width, rib eye length, rib eye area, flank thickness, chuck short rib thickness, chuck short rib score, back fat thickness, chuck flat weight, fat color score, lean meat color score, and marbling score. A recent study reported that the septin 7, CDC10 gene is located on a genomic region of a QTL associated with growth-related traits (Tong et al., 2015). This report previously identified SNP located on the CDC10 gene associated with growth traits in three different breeds of beef cattle. The CDC10 gene is more likely to be expressed in the skeletal muscle in steers with higher growth performance compared to those with lower growth performance (Tong et al., 2015).

Single Nucleotide Polymorphisms Associated with Bovine Respiratory Disease

A previous study utilizing two populations, one with *Mycobacterium avium* subsp. paratuberculosis (MAP) infected individuals and one with BRD affected individuals, evaluated single nucleotide polymorphisms on the *ANKRA2* and *CD180* genes (Casas et al., 2011). The BRD-affected population had five SNPs on the *ANKRA2* gene that were significantly ($P < 0.05$) associated, and two SNPs were significantly ($P < 0.01$) associated with incidence of BRD. Using significant markers, haplotypes showed a positive association with incidence of BRD. Subsequently, markers in the *ANKRA2* and *CD180* genes are associated with the ability of the animal to cope with pathogens (Casas et al., 2011).

Along with *ANKRA2* and *CD180* genes, the *RP105* gene on chromosome 20 had 2 SNPs associated with BRD (Casas et al., 2010). Results indicate that markers *ANKRA2* and *RP105* may be complementary in the expression of the immune system. The U.S. Meat Animal Research Center (MARC) reported 30 SNPs on 15 chromosomes that were associated with BRD. When significance was relaxed, however, 550 SNPs were found to be associated with BRD (Casas et al., 2010)..

Marker Assisted Selection

Once chromosomes have been localized on a trait locus, information can be applied in breeding programs by using marker assisted selection (MAS) (Andersson, 2011). Previous reports have defined marker-assisted selection as allowing for the accurate selection of specific DNA variations that have been associated with a measurable difference or effect on complex traits (Van Eenennaam, 2006). Marker assisted selection has enabled opportunities to enhance genetic improvement programs in livestock by direct selection of genes or genomic regions that affect economic traits (Dekkers, 2004). The presence or absence of the numerous other genes and

the production environment will determine whether an animal actually displays the desired phenotype. The traits that benefit most from MAS are ones that are lowly heritable (Van Eenennaam, 2006). Knowing the alleles at particular genetic loci will identify individuals that carry beneficial alleles and help avoid making improvements in one trait while negatively affecting another. Thus, this allows for direct selection of genetically superior animals at several loci simultaneously (Williams, 2005). Marker assisted selection should be a tool used to assist with traditional selection techniques and not a tool to replace them all together (Van Eenennaam, 2006).

Whole Genome Selection

Whole genomic selection (WGS) is a form of marker assisted selection in which genetic markers, covering the whole genome, are used so that all quantitative trait loci are in linkage disequilibrium with at least one marker (Goddard et al., 2007). Additionally, WGS has become more popular due to large number of single nucleotide polymorphisms discovered by genome sequencing (Goddard et al., 2007). Whole genome selection uses genotypes of thousands of single nucleotide polymorphism markers to predict breeding values (Thallman, 2009). A previous study reported that the ideal method to estimate breeding value from genomic data was to calculate the mean of the breeding value given the genotype of the animal at each QTL (Goddard et al., 2007). Overall, implementation of whole genomic selection can have major implications for genetic improvement, including improving economically important traits (Goddard et al., 2007).

Many traits affecting profitability and sustainability of meat, milk, and fiber production are polygenic, with no single gene having an overwhelming influence on observed variation. No knowledge of the specific genes controlling these traits has been needed to make substantial

improvement through selection (Snelling et al., 2013). Significant gains have been made through phenotypic selection enhanced by pedigree relationships and continually improving statistical methodology. Previous reports have indicated that genomic selection has the potential to increase selection accuracy and accelerate genetic improvement by emphasizing the SNP most strongly correlated to phenotype (Snelling et al., 2013). Dense SNP genotype associations with only the use of a phenotype provide a one-dimensional approach to identifying genes affecting specific traits. However, associations with multiple traits allows for better identification of defining networks of genes interacting to affect correlated traits. This may help provide greater insight into the genes and genomic mechanisms affecting polygenic traits and facilitate genomic selection for economically important traits (Snelling et al., 2013).

Researchers can more accurately predict the genetic potential of animals from their genotypes with the use of genomic selection (Snelling et al., 2012). Simple DNA tests may eventually replace low-accuracy predictions for expensive or lowly heritable measures. However, associations between individual SNP and similar phenotypes are inconsistent across data sets, and genomic predictions do not appear to be globally applicable to cattle of different breeds (Snelling et al., 2012). These discrepancies may be a result of different QTL segregating in the sampled populations, differences in linkage disequilibrium patterns and spurious correlations with phenotype (Snelling et al., 2012). Example analyses are provided to demonstrate how integrating information about gene function and regulation with statistical associations from whole genome SNP genotyping assays enhance knowledge of genomic mechanisms that affect traits. This results in more reliable DNA tests to guide heifer selection decisions (Snelling et al., 2012).

Selective Genotyping

Selective genotyping is a term used to describe the determination of linkage between marker loci and quantitative trait loci affecting a particular trait carried out by genotyping individuals from the high and low phenotypic tails of the entire sample population (Darvasi et al., 1991). Selective genotyping is often used to locate QTL because only animals with extreme phenotypes are genotyped (Henshall et al., 1998). It has been previously observed that with this approach, for a given number of animals genotyped, there is a greater chance of locating QTL (Henshall et al., 1998). Previous studies reported that selective genotyping can decrease the number of individuals genotyped for a given power at the expense of an increase in the number of individual's phenotyped (Darvasi, 1991). However, the limitation of selective genotyping is that when analyzing multiple traits, by selecting the extremes of each trait, one would select most of the population. Therefore, no reduction in genotyping can be obtained (Darvasi, 1992). Unless selective genotyping and correlation are taken into account, parameter estimates in the correlated trait will be biased due to it only considering population outliers (Henshall et al., 1998). When experimenting with both selective genotyping and QTL detection, the proportion of the population selected for genotyping with the additional markers will be determined by the effect of selective genotyping on QTL mapping accuracy (Darvasi, 1996).

CHAPTER III

EVALUATION OF SOURCES OF VARIATION AND CORRELATED EFFECTS CONTRIBUTING TO BRD INCIDENCE IN CROSSBRED STEERS

Introduction

Bovine Respiratory Disease (BRD) is the most common disease affecting feedlot cattle and contributes to approximately 75% of morbidity and 50%-75% of mortality in feedlots annually (Brooks et al., 2011). Economic losses incurred by animals contracting BRD due to reduced feed efficiency, veterinary treatment and death, are estimated to cost the U.S. beef industry 640 million dollars annually (Bowland et al., 2000). Although cattle of all ages can be affected by bovine respiratory disease, most are susceptible during time of high stress as young calves (Schneider et al., 2010). Commonly referred to as shipping fever, BRD is most prevalent in calves being transported to feedlots after being weaned (Bowland et. al., 2000). The severity of bovine respiratory infections has been linked to a variety of factors, including environmental and nutritional changes, transportation, and weaning factors. Of these factors, transportation is the most accepted non-infectious risk factor for BRD (Bowland et. al., 2000). Fatal respiratory infections occur when a primary viral infection compromises host defenses and enhances the severity of a secondary bacterial infection (Hodgson et.al. 2005).

Inflammation caused by BRD decreases dry matter intake, average daily gain, and gain to feed ratio in feedlot calves (Gifford et. al., 2012). Overall, this decreases an animal's growth rate and increases the number of days on feed, which results in economic losses during the feeding period (Gifford et. al., 2012). Results of previous studies reported that clinical BRD resulted in decreased body weight and average daily gain at the end of a 63-day preconditioning period (Holland et al., 2010). Preconditioning has previously been shown to significantly reduce morbidity and mortality as well as improve weight gain and feed efficiency (Lalman et. al.,

2001). The objective of the current study was to evaluate on farm sources of variation and correlated effects contributing to BRD incidence in a population of crossbred steers sent to the feedlot.

Materials and Methods

Experimental Animals

All animals were treated and maintained in accordance with the principles and guidelines outlined in the Guide for the Care and Use of Agricultural Animals in Research and Teaching. The animals utilized in the current study were comprised of 323 crossbred steers born at the Louisiana State University Agricultural Center Central Research Station in Baton Rouge, La and Hill Farm Research Station in Homer, LA from 2010 to 2013. Spring born calves were managed at their respective locations until weaning, or approximately six to seven months of age. Calves were sired by Charolais, Braunvieh, Simmental, Angus or Braford bulls. The dam breeds at the LSU Agricultural Center utilized for this study have been previously described during the characterization of the Germplasm Evaluation VIII studies (Wheeler et al., 2011). The dams utilized by the Hill Farm Research Station in Homer, LA were from an Angus based crossbred commercial herd.

Steers were given a preconditioning ration at weaning and were fed 2.27kg per head each morning for 45 days. Along with feed, calves had ad libitum to pasture grazing and free choice Bermuda grass hay. At weaning, the calves were vaccinated with Bova Shield Gold FP5 and Clostra Shield 7. Calves were then boosted with Bova Shield Gold FP5 and Clostra Shield 7 approximately 30 days later. Steers that met pre-defined shipping criteria were vaccinated and shipped to commercial feedlots in Texas and Oklahoma for the finishing period. During the finishing period, animals were recorded as 0 if the animal was never affected with BRD and 1 if

the animal was affected by BRD at some point in the finishing period. In this study, a total of 302 animals over the four-year period were recorded as unaffected by BRD, while 21 animals were affected by BRD (Table 3.1).

Statistical Analysis

The Mixed Model procedure of SAS (version 9.4, SAS Institute, Cary, NC) was utilized to identify potential sources of variation causing BRD. The dependent variable in the model was incidence of BRD, while the independent variables included sire breed, site, and birth year. Sire breed (year) was fit into the model as a random nested variable to account for confounding effects of sire breeds among the four years. The Pearson Correlation Coefficient was utilized to evaluate correlated effects of on farm variables and traits with incidence of BRD. The variables utilized for the Pearson's Correlation Coefficient included BW, WW, HH, sire breed, site, birth year and incidence of BRD. Significance was set at $P < 0.05$.

Results

Results from the Pearson Correlation Coefficient analyses (Table 3.2) revealed that incidence of BRD was lowly positively correlated to BW and lowly negatively correlated to WW, HH, sire breed, and birth year. Birth weight was lowly positively correlated to BRD, WW, and HH, and lowly negatively correlated to sire breed and birth year. Weaning weight was lowly positively correlated to BW and highly positively correlated to HH, while lowly negatively correlated to BRD, sire breed, and birth year. Hip height was lowly positively correlated to BW and highly positively correlated to HH, while lowly negatively correlated to BRD, sire breed, and birth year. Sire breed was lowly negatively correlated to BRD, BW, WW, HH, and birth year. Birth year was lowly negatively correlated to BRD, BW, WW, HH, and sire breed. Overall,

no significant sources of variation from the experimental design were identified as significant contributors to BRD.

Table 3.1 Total number of animals for each sire breed and total number affected by BRD for each sire breed.

Sire Breed	Total Number of Animals	Total Number Affected by BRD
Angus	55	4
Braford	29	2
Braunvieh	46	0
Charolais	133	12
Simmental	60	3

Table 3.2. Pearson Correlation Coefficients for BRD, birth weight, weaning weight, hip height, sire breed and birth year.

	BRD	BW	WW	HH	Sire Breed	Birth Year
BRD	1.00000	0.03724	-0.04853	-0.03644	-0.01105	-0.16250
BW (kg)	0.03724	1.00000	0.07825	0.13971	-0.13570	-0.15200
WW (kg)	-0.04853	0.07825	1.00000	0.75095	-0.06709	-0.06151
HH (cm)	-0.03644	0.13971	0.75095	1.00000	-0.13600	-0.07930
Sire Breed	-0.01105	-0.13570	-0.06709	-0.13600	1.00000	-0.29107
Birth Year	-0.16250	-0.15200	-0.06151	-0.07930	-0.29107	1.00000

Discussion

The current study revealed that incidence of BRD was lowly positively correlated to birth weight and lowly negatively correlated to weaning weight, hip height, sire breed and birth year. The current study was in agreement with previous reports that indicated weaning weight had no significant effect on BRD incidence and that correlations between BRD incidence and birth weight and weaning weight were low (Schneider et al., 2010). Moreover, it was also reported that genetic correlation estimates for BRD incidence with birth weight and weaning weight were low in Angus and Simmental calves (Schneider et al., 2010). The study herein reported that hip

height was not a significant source of variation contributing to BRD incidence and this result is novel to the current study.

Previously, it was reported that lighter-weight calves were at greater risk for BRD than heavier calves (Taylor et al., 2010). However, a previous study contradicted these results and reported no difference in arrival weight among animals that did not suffer from BRD compared to animals that contracted BRD (Thompson et al., 2006). The current study identified no significant sources of variation between the heavier and lighter weight calves in association with incidence of BRD. Moreover, the current study reported that incidence of BRD was lowly or negatively correlated with growth performance. A previous study reported that Charolais sired calves were less likely to receive BRD treatments compared to other breeds (Haggland et al., 2006). This result is not in agreement with the current study that found no breed effects in Angus, Simmental, Braford, Braunvieh or Charolais sired calves for incidence of BRD. Previous reports identified Braunvieh as having the highest BRD frequency out of 9 breeds, including Angus, Charolais and Simmental (Muggli-Cockett et al., 1992). This result is not in agreement with the current study that found no significant sources of variation amongst any of the breeds, including Braunvieh, for incidence of BRD. Moreover, of all the Braunvieh sired calves in the current study, none were recorded as affected by BRD. It was previously reported that birth year effects were important for incidence of BRD in both pre-weaning and post-weaning periods (Muggli-Cockett et al., 1992). However, in the current study, birth year was not a significant source of variation contributing to incidence of BRD.

Previously, it was reported that conventionally sold calves not only had higher morbidity than preconditioned calves but mortality was also notably higher in the conventionally sold calves (Roerber et al., 2001). Moreover, a second study reported that vaccinated and

preconditioned treatment groups had lower incidence of morbidity compared with calves sold through conventional auction with no preconditioning (Macartney et al., 2003). The benefit to preconditioning was substantial in that study, with conventionally sold calves being 4.5 times more likely to be treated for BRD than preconditioned calves (Macartney et al., 2003). Although preconditioning programs were not the focus of the current study, all animals utilized herein were preconditioned before being shipped to the feedlot, and it's important to mention previous reports and how they affect incidence of BRD.

Summary

The current study reports that incidence of BRD was lowly positively correlated to birth weight and lowly negatively correlated to weaning weight, hip height, sire breed and birth year. These results indicate that the traits analyzed are not precursors for BRD in the current population. Thus, suggesting, that both genetic factors and management practices may have a more substantial impact on incidence of BRD than growth traits alone.

The research herein validated previous studies that reported certain growth traits are not indicators for BRD incidence, and that an animal's size cannot always account for its ability to stay healthy. Management, and the utilization of a proper preconditioning program, are key components to good herd health that can save a producer money by having less sick animals. Future studies should focus on preconditioning programs and their advantages in disease prevention, along with proper handling of animals and utilization of proven effective vaccinations. Since little is still known about the relationship between genetic predisposition to BRD and animal performance, this should be a priority for future studies as well. The growth traits analyzed in the current study should be validated and additional growth traits, such as average daily gain should be analyzed as possible precursors of BRD. Also, a larger, more diverse population of animals should be utilized to further validate the results herein.

CHAPTER IV

AN SNP ASSOCIATION STUDY EVALUATING ASSOCIATIONS BETWEEN GROWTH TRAITS, CARCASS TRAITS AND INCIDENCE OF BRD IN CROSSBRED STEEERS

Introduction

The objective of genomic research in cattle is to map and characterize trait loci that control phenotypic traits (Andersson, 2011). Identifying SNP on specific QTL regions associated with growth traits, carcass traits and disease resistance has become of great importance in the beef industry (Dekkers, 2004). Previous reports have identified QTL regions associated with BRD susceptibility located on BTA 6 and BTA 20 (Li et al., 2004; Casas et al., 2010). Moreover, the U.S. Meat and Animal Research Center reported thirty SNPs on fifteen chromosomes that were associated with BRD, including the previously described regions located on BTA 6 and BTA 20 (Casas et al., 2010). It was also previously reported that BTA 6 and BTA 20 have been shown to harbor the majority of the significant SNP associated with growth (Snelling et al., 2010).

A previous study reported that incidence of BRD has negative effects on performance and carcass traits such as average daily gain and hot carcass weight (Schneider et al., 2009). Additionally, it was reported that selection for BRD resistance may have little effect on hot carcass weight, longissimus muscle area (LMA), and fat due to the low genetic correlation estimates. However, results indicated genetic factors existed for birth weight and marbling score with both BRD affected and unaffected animals (Schneider et al., 2010). An additional study reported that steers with clinical signs of BRD had less internal fat, and lower marbling scores compared to steers with no clinical sign of BRD at time of slaughter (Gardner et al., 1999). The objective of the current study was to evaluate single nucleotide polymorphisms (SNP) located on

previously described QTL regions of BTA 6 and BTA 20 for potential associations with growth traits, carcass traits, and incidence of BRD in crossbred steers sent to the feedlot.

Materials and Methods

Experimental Animals

All animals were treated and maintained in accordance with the principles and guidelines outlined in the Guide for the Care and Use of Agricultural Animals in Research and Teaching. The animals utilized in the current study was comprised of 323 crossbred steers born at the Louisiana State University Agricultural Centers Central Research Station in Baton Rouge, LA and LSU Hill Farm Research Station in Homer, LA from 2010 to 2013. Spring born calves were managed until weaning, or approximately six to seven months of age. Calves were sired by Charolais, Braunvieh, Simmental, Angus or Braford bulls. The dam breeds at the LSU Agricultural Center utilized for this study have been previously described during the characterization of the Germplasm Evaluation VIII studies (Wheeler et al., 2011). The dams utilized by the LSU Ag Hill Farm Research Station in Homer, LA were from an Angus based crossbred commercial herd.

Steers that met pre-defined shipping criteria were vaccinated and shipped to commercial feedlots in Texas and Oklahoma. Once an animal reached an acceptable pen weight by the feed yard, it was harvested. During the finishing period, animals were recorded as 0 if the animal was never affected with BRD and 1 if the animal was affected by BRD at some point during the finishing period. A total of 302 animals over the four-year period were recorded as unaffected by BRD, while 21 animals were affected with BRD. When the finishing period was completed, animals were sent to a commercial packing plant where carcass quality and composition traits were recorded. These trait measurements included HCW, MS, REA, YG, and BF.

DNA Extraction and Genotyping

Ear notches were collected from all calves at birth for future DNA extraction. Extraction of DNA was conducted using a saturated salt procedure previously described by Miller et al (1998) (Appendix A). DNA stock solutions were diluted to 25 ng/μl concentrations for future genotyping reactions. Fifty eight SNP were selected from a previously described QTL region associated with incidence of BRD spanning between 40-80 Mbp on BTA 6 (Li et al., 2004). Twenty four SNP were selected from a previously described QTL region associated with incidence of BRD spanning 0-30 Mbp on BTA 20 (Casas et al., 2011). Single nucleotide polymorphisms were selected using the SNP database (http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs=rs42524450). Single nucleotide polymorphisms, allele substitutions, and upstream and downstream genomic sequences are reported in tables 4.1 and 4.2. Single nucleotide polymorphism genotyping was performed by Neogen, Inc. (Lincoln, Nebraska) via the Sequenom mass genotyping platform.

Table 4.1 Single nucleotide polymorphisms ID, allele substitutions, and upstream and downstream genomic sequences utilized for amplification and visualization of genotypes for BTA 6.

SNP ID	Allele Substitution	Forward Sequence	Reverse Sequence
rs29025265	C/T	CAGTTAGAGTTCAAAGGGACTTTTG	GTCAAACCTGAGTACAAAATCTTTTC
rs41626155	C/T	TCCTGCCCTGCCTTCTTTAACTTCT	TCCCCAATCTCTGGTTGCCATTCAT
rs41653357	A/C	TGGAGAATCCTTTAGACAATAGGAG	TTGGTGGGCTATAGTCCATGGGGTT
rs42402825	A/G	GAGAATCCAAAGACAATACCAAAAT	AAGTCTATTGAAAGCCCACTCCTTG
rs42403565	C/T	ATTTCTATTACCCTATGTGTCAGAT	TCTGATTCACTCTTCTGCCTCCTCT
rs42571566	A/G	GCCGTCTATGGGGTCGCACAGAGTC	GACACGACTGAAGCAACTTAGCAGC
rs42579150	C/T	ATATGCCAATGATCTTAAATTTACT	GGTAAATATTTGAACATTTTTCTGC
rs42579164	A/C	CTCTATTTTTTACAACATGGATGGAC	TAGAGATGATTATACTAAGTGAAGA
rs42725112	C/T	TTCATTAAAACACAAAAATCACAAC	AACTGCTGAACAACCACCAGCAAAA
rs42823614	A/C	AGGCAAATTCTTCACCAGCTGAACC	CAGGGAAAGCCTAATTCCCACCTTC
rs42824344	C/T	GTAGCATCATTGCCCTTTAATTATC	AACTAGAAGCAAACCTGAATGTCCA
rs42880470	A/G	TCTGGAGTAGGTACTGTGGGAGCAA	CTCAATCAGAGTTGTGAATAGCCTC
rs42880522	A/G	CTGAGGCTGGCCCTGACCTGAGATA	CCACCCTTTCTTACTCTCTTTCTTC
rs42900120	G/T	GGGGAAGGGGAGGAAGGATAAATTG	GAGATTGGGACTGACATATACACAC
rs42900130	A/G	CACAGGAGATAATCCTCTGCCTCCA	TTATGGTCTTCTGTGAAAAGTACTG
rs42900481	A/G	ACTTTAGATTCAATTCTTCTTGGCT	GGGATGGAGAATCTTTGAATTTCTC
rs42961863	C/T	GGACCAGAAGTCCCTTTCCCTTGCT	ATGTGTATTTTTTAATGGTGATGACA
rs42961866	G/T	TGTTGTTTCCAGCTCTCCAATCTAG	TATTGTCCATTACTATTAAACATTC
rs42961882	A/T	CTTCTTTTTTGGTATGATTTTGGTC	CTGTCTCCTATACACTATTACAGAT
rs42968197	C/T	TGACAAGTAGATGCTTTTTATTAAA	TCATTCTATGTAAGAGACAGCTGAG
rs42968891	A/G	TTCATACCTAGATAATTGCAATTTC	TACCTAGCCTTTCCAGTCCTTTGGA
rs42968895	C/T	TCATATTCAGAGGTGGGATGTCATT	TTAAGGCTTTCAAGGCACTAATCCT
rs43089863	C/G	CATCTCTCTGAGTTGTCCTCTATTG	AGTCAGGGAGCAGGGCCTTTTTACC
rs43138398	C/T	TTGCAAGATAATTACAGTCACTTCC	TTTTCATGATCATTGGCCTTGAGCT
rs43194943	A/G	ATATCTTCTTAATATCTTCTTTTTT	TTAGGTCTGCACCATTTCTGTCCTT
rs43446022	G/C	TATGTTTCAGAGGAATTAAGTCTTGA	CTTGTCATAAATACAACAAAATGAG
rs43446601	G/T	GTTTCCTGGAATTTGGATGAAAATT	CCTTCAATGTTTATATCTGAATCTT
rs43446955	C/T	TGCTTGTTTATATCACTTTGATATA	ACTATATTAAATTATAATGCTCTTT

(Table 4.1 continued)

SNP ID	Allele Substitution	Forward Sequence	Reverse Sequence
rs43448463	A/G	AGAATGCAAAGAGGAACTAAAGAGC	TCTTGATGAGGTTGAAGGAGAAGAA
rs43448512	A/C	AGATAAACTGAGACTTTCATGACGG	AGGCTCTTGAAGGAGAAGTTCTTTG
rs43449040	A/G	CACATTGATCGCTCTAATCTTAGAG	AAAAGTGCTTAAAACTTAGACACT
rs43449194	C/T	TGAAAATGTTTCTTGCATTATTTTA	TATCAATTTCTTCATTTTGCTGTTA
rs43449209	A/G	AGTTGCTCAAGATCACACAGCATGT	TGCTGGAGCTAGGATTGAAAGCTCA
rs43449835	C/T	TAGTATCCTTTGCTAAATTTATCAT	AGTAGGTTAAAGAAGCCTTCAGGAT
rs43449896	A/C	TCCACTGGATGATCCACTGGATCAT	GAAAAAGCAAGAGAGTTCAAGAAAA
rs43451134	A/T	CATACTATATAGCACAGGAACTAT	TTCAATATCCTGGGATAAATCATAA
rs42403543	C/T	AAGGAAATGCTTTC AATTTTCACT	TTTATTATGATGCAAGCTGAAGGTT
rs42481129	A/G	TTCTCCCACACCACAGTTTAAAAGC	TCAATTCTTCGGCACTCTGCCTTCT
rs42579148	G/T	TATGACTTACCTACTGCTTTTCTTT	TATCTATGATGTCATAGAATGTAAG
rs42823610	C/T	GCCATCCAGCCATCTCATCCTCTGT	GTCCCCTTCTCCTCCTGCCCCCAAT
rs42824331	A/G	CATGGGGTCGCTGAGGGTCAGACAC	ACTGAGTGACTTCACCTTTCACCTTT
rs42725042	G/T	AGGGGAGAAGGGGACGATAGAGGAT	AGATGGCTGGATGGCATCACTGACT
rs43080446	G/T	TTAAAGGAAAGATTACTTTATACAA	TATAAAGTATTGAAACAATAGTCTA
rs43185776	C/G	TCCTATGTCATCCCCTTCTCCTCCT	CCCTCAATCCCCTCCCAGCATCAGAG
rs43178720	A/T	TGTATGTCTGTATGTACAGACATAC	GTGAAATATGTATATATGTACAGAC
rs43449906	G/T	TATATAAAATTGCATTTTAGAAAAC	TAAAGGTGATTAATGCTTTTTTAATT
rs43449868	A/G	CCTAGAGCCAGACATCCTGGAATGC	AAGTCAAGTGGGCCTTAGGAAGCAT
rs43448433	A/G	ATTGAAGAATCTCTTTCTATATTCT	AATATTCTTAGTTTTTCACATCCCCC
rs42940872	C/G	ATACAGCCAAAGGCTTTAGCAAAGT	ATGAAGCAGAAGTGTATGATTTTCT
rs43130086	A/G	AACTTAGGTGAGCTGAGGGGGCTGA	GGAAATCCACACAAGTCGCCCATGA
rs43444877	A/G	TCTGAAGAGTTCTTATCCCAAGAAA	AAAATTTTTTTTCTATTTCTTTAAT
rs43445941	G/T	AAACTCCAATACTTTGACCACCTGA	GCAAAGAAGTACTCATTAGAAAAA
rs43445971	A/G	TACATTTAGAACTGCTTACTTTCAT	TAAGTTCTTATGTAACACATAGATT
rs42900433	G/T	CTGACTCTTGGCGATCCCATGGACT	TAGCATACCAGGCTCCTCTGTCCAT
rs42961881	C/T	TGAATGCAACACTTTAACAGCATCA	CTTTAGTATTTGAAATAGCTCAGCT
rs42725037	A/G	ATATATGTTTCCTTAAGAAACAAAA	TAGACCTACCATATGTAATCTTGCA
rs43452444	C/T	AAGAAAAGGCAGTGTGCAACAGGG	GTGAGCCACGTGAGAGAGAAGGTCG

Table 4.2 Single nucleotide polymorphisms ID, allele substitutions, and upstream and downstream genomic sequences utilized for amplification and visualization of genotypes for BTA 20.

SNP ID	Allele Substitution	Forward Sequence	Reverse Sequence
rs41595713	C/T	TCTCGGTTTCCTAACACAGCCAAGAC	GTTGTCCCGAACGGGTGAGGAATGG
rs41931108	C/G	TTGGTGTGCCAAGCACATCCCCAGC	GAGGAAGGCAGGTTGTGCCCATATT
rs42476237	C/T	CCTGGCCCCACCCCTTCCTTCCTTCCC	ATTTGTGGAGAAGCACGTGGGGAAC
rs42476290	A/G	CTGAGGCCAGAATTCTTGAAAGAAT	TGTTTGCATGGTGACAGCAAAGCAT
rs42477340	C/T	CTCCCGCCTCCTTCTCTGCTCCCTC	GGCTCCCTCTCTGCTCCCTCCGGCT
rs42480445	C/T	TGGCCCCAAATGCCAAAAGGTTATC	TCATTTTTTTTCCAAGCAATCCCACC
rs42481107	A/G	AACAACACCTTCCACCGCCCCATCC	GGTCTCAGCCTAAGCATCAGCTCTT
rs42512588	C/T	GAATGGGGAGTGACTGCTTCCTAGG	CTGGGGTTTCTGTTGGGTTGATGCT
rs42520493	A/C	CATGCTGTATATCGGAGGGTCTAGG	CTGTTAAGCAGGAAATGAGAACTCC
rs42524445	C/T	GGTTCCTAAAAGTGAAATGATAATG	AGAAGAAATAGAGTGATGTGATGTG
rs42524450	C/T	TCCTTGGAAGTGGGGTTGCTCCTTC	GGCCGCCACCCCTGGCCTCAGGCGT
rs42524466	C/G	ATTTTATGTCGCAGTTTTCTCTCAC	AATCTAAGTTTAAATCTCTCAGAGG
rs42524468	A/T	AATAGACCCACAGACATAGAAAACA	ATGTATGGTTACCAAAGGGGAAAGG
rs42524472	A/T	AAAATAAATAGTAAATCACAAACAC	AATCACAGATAGGAAGAAAATGCAA
rs42524503	A/T	GTTGTATAGACAGATATCTGTCACT	ATTCTTTCCAAATGCTCTGACAGAT
rs43036576	A/G	TAATCATGAAGCCATCCTGTAGGGT	GAGCTAGGGTTTATAGCGGCTGTGA
rs42524459	C/T	ATCCACACAGTCAAAGCCTTTGGCA	AGTCAATAAAGCAGAAATAGATGTT
rs42481060	C/T	ACTGCCTCAGGCCTGGCACACAGCC	GAGAGGCCATGGGGCCCTGTGGAGC
rs41931083	C/T	GACTTCATTTCTCTCCGTGATAATC	TGCGGGGCAGGTCCCCAGGTCTGGA
rs42524449	A/G	TCTGCCCCTGCTGACCTTCAACGTG	AATAGCTCCTCTAGGACCTCCTGCG
rs42524457	G/T	GTTTATTGTGATCCACACAGTCAAA	CCTTTGGCACAGTCAATAAAGCAGA
rs42476309	C/T	GATGGTTTAGTCACTAAGTCATGTC	GACTCTTGAAACCCCATGGACTGTA
rs42236701	A/C/G	TCCACTTGATTTACATTCCAGGAT	TCTGGCTCTAGGTGAGTGATCACAC
rs41931859	C/T	GAGGAGCCTGGGCTACAGTTCATGG	GTCACAGAGAGTCGGACACAACCTGA

Statistical Analysis

The Mixed Model procedure of SAS (version 9.4, SAS Institute, Cary, NC) was utilized to evaluate potential SNP associations located on BTA 6 and BTA 20 with growth traits, carcass traits and incidence of BRD. Only the SNPs with more than one genotype were included in the analysis. The LSMEANS function, along with the pre-planned pairwise comparisons procedure, was utilized to evaluate if significant differences existed between individuals inheriting differing genotypes for SNP identified as significant for specific traits. Dependent variables in the model included BW, WW, HH, HCW, YG, MS, REA, BF, and incidence of BRD. Independent variables included sire breed, SNP genotype, site, and birth year. Sire breed (year) was fit into the model as a random nested variable to account for confounding effects of sire breeds among the four years. Significance was set at $P < 0.05$.

Results

Analyses of SNPs revealed significant genotypic effects for growth traits, carcass traits and incidence of BRD in both QTL regions. When evaluating growth traits, multiple SNP were significantly associated with BW, WW, and HH as shown in table 4.3. Specifically, four SNP (rs41595713, rs42403565, rs42571566, rs42900130) located on BTA 6 and two SNP located on BTA 20 (rs41931108, rs42480445) were significantly associated ($P < 0.05$) with BW (Table 4.3). Animals inheriting the major homozygous (TT) and heterozygous (TC) allele genotypes from rs41595713 had significantly ($P < 0.05$) higher BW than animals inheriting the minor homozygous allele genotype (Table 4.4). Animals inheriting the major homozygous (GG) allele genotype from rs42571566 had significantly ($P < 0.05$) higher BW than animals inheriting the minor homozygous allele genotype. However, there were no significant differences between the heterozygous allele genotype and the major and minor homozygous allele genotypes (Table 4.4).

Breed was also a significant ($P < 0.0001$) contributing factor for BW effects with regards to rs42571566 (Table 4.4). Animals inheriting the major homozygous (AA,TT) and heterozygous (AG,TC) allele genotypes from rs42900130 and rs42480445 had significantly ($P < 0.05$) lower BW than animals inheriting the minor homozygous allele genotype (Table 4.4). Animals inheriting the major homozygous (CC) allele genotype from rs42403565 and rs41931108 had significantly ($P < 0.05$) lower BW than animals inheriting the minor homozygous allele genotype. However, there were no significant differences between the heterozygous allele genotype and the major and minor homozygous allele genotypes (Table 4.4).

When evaluating WW, two SNP located on BTA 20 (rs41931108, rs42524450) and one SNP located on BTA 6 (rs43451134) were identified as significant ($P < 0.05$) (Table 4.3). Animals inheriting the major homozygous (CC) and minor homozygous (GG) allele genotypes from rs41931108 had significantly ($P < 0.05$) higher WW than animals inheriting the heterozygous allele genotype (Table 4.4). Animals inheriting the major homozygous (CC) and heterozygous (CT) allele genotypes from rs42524450 had significantly ($P < 0.05$) lower WW than animals inheriting the minor homozygous allele genotype (Table 4.4). Animals inheriting the major homozygous (TT) allele genotype from rs43451134 had significantly ($P < 0.05$) lower WW than animals inheriting the heterozygous allele genotype. However, there were no significant differences between the minor homozygous allele genotype and the major homozygous and heterozygous allele genotypes (Table 4.4). A single SNP marker located on BTA 6 was identified as being significantly ($P < 0.05$) associated with HH (Table 4.3). Animals inheriting the major (CC) and minor (TT) homozygous allele genotypes from rs41626155 had significantly ($P < 0.05$) higher HH than those inheriting the heterozygous allele genotype (Table 4.4).

Table 4.3 Level of significance and frequency of animals from each genotype associated with birth weight, weaning weight, and hip height.

Traits	BTA	SNP ID	Allele ⁴	Minor Genotype Frequency	Het Genotype Frequency	Major Genotype Frequency	SNP <i>P</i> - value	Breed <i>P</i> - value
BW ¹	6	rs41595713	T/C	28	170	78	0.0128	0.1473
BW	6	rs42403565	C/T	39	131	110	0.0379	0.2833
BW	6	rs42571566	G/A	28	98	124	0.0414	<.0001
BW	6	rs42900130	A/G	5	82	211	0.0438	0.2468
BW	20	rs41931108	C/G	60	122	86	0.0166	0.1875
BW	20	rs42480445	T/C	9	119	178	0.0360	0.1861
WW ²	6	rs43451134	T/A	38	14	44	0.0471	0.3190
WW	20	rs41931108	C/G	60	122	86	0.0138	0.7168
WW	20	rs42524450	C/T	38	132	74	0.0187	0.5005
HH ³	6	rs41626155	C/T	15	112	151	0.0033	0.3672

¹BW=Birth weight

²WW=Weaning weight

³HH=Hip Height

⁴Representation of the major allele is located on the left.

Table 4.4 Single nucleotide polymorphisms associated with growth traits and least square means estimate comparisons between reported genotypes for birth weight, weaning weight, and hip height.

Traits	BTA	SNP ID	Allele ⁴	Major Genotype Mean	Het Genotype Mean	Minor Genotype Mean
BW ¹	6	rs41595713	T/C	40.07±0.83 ^a	38.54±0.67 ^a	35.87±1.37 ^b
BW	6	rs42403565	C/T	38.00±1.00 ^a	38.91±0.92 ^{ab}	41.12±1.23 ^b
BW	6	rs42571566	G/A	39.99±0.68 ^a	38.92±0.70 ^{ab}	36.52±1.32 ^b
BW	6	rs42900130	A/G	38.99±0.85 ^a	38.78±1.05 ^a	46.02±2.89 ^b
BW	20	rs41931108	C/G	37.56±0.92 ^a	39.05±0.80 ^{ab}	40.62±0.98 ^b
BW	20	rs42480445	T/C	39.12±0.72 ^a	38.56±0.99 ^a	44.00±2.20 ^b
WW ²	6	rs43451134	T/A	258.87±5.73 ^a	290.86±14.04 ^b	272.50±7.04 ^{ab}
WW	20	rs41931108	C/G	272.57±12.23 ^a	258.11±11.90 ^b	271.42±12.41 ^a
WW	20	rs42524450	C/T	260.12±8.92 ^a	263.40±8.43 ^a	281.29±9.88 ^b
HH ³	6	rs41626155	C/T	113.86±0.78 ^a	112.33±0.80 ^b	116.36±1.44 ^a

^{a,b}Differing superscripts indicate a difference of means at $P < 0.05$ within rows

¹BW=Birth weight

²WW=Weaning weight

³HH=Hip Height

⁴Representation of the major allele is located on the left.

When evaluating carcass traits, multiple SNP were significantly associated with HCW, YG, MS, and REA as shown in table 4.5. A total of four SNP, three located on BTA 6 (rs42900130, rs42961882, rs43446022) and one located on BTA 20 (rs41931108), were significantly ($P < 0.05$) associated with HCW (Table 4.5). Animals inheriting the major homozygous (AA,TT) allele genotype from rs42900130 and rs42961882 had significantly ($P < 0.05$) higher HCW than animals inheriting the heterozygous allele genotype. However, there were no significant differences between the minor homozygous allele genotype and the major homozygous and heterozygous allele genotypes (Table 4.6). Animals inheriting the major homozygous (GG) allele genotype from rs43446022 had significantly ($P < 0.05$) lower HCW than animals inheriting the heterozygous allele genotype. However, there were no significant differences between the minor homozygous allele genotype and the major homozygous and heterozygous allele genotypes (Table 4.6). Animals inheriting the heterozygous (CG) allele genotype from rs41931108 had significantly ($P < 0.05$) lower HCW than animals inheriting the minor heterozygous allele genotype. However, there were no significant differences between the major homozygous allele genotype and the heterozygous and minor homozygous allele genotypes (Table 4.6). A single SNP located on BTA 20 was significantly ($P < 0.05$) associated with YG (Table 4.5). Animals inheriting the major homozygous (TT) and heterozygous (TC) allele genotypes from rs41595713 had a significantly ($P < 0.05$) higher YG than animals inheriting the minor homozygous allele genotype (Table 4.6).

A single SNP located on both BTA 6 (rs41653357) and BTA 20 (rs43036576) was significantly ($P < 0.05$) associated with MS (Table 4.5). Animals inheriting the major homozygous (AA) allele genotype from rs41653357 had significantly ($P < 0.05$) higher MS than animals inheriting the heterozygous and minor homozygous allele genotypes (Table 4.6).

Animals inheriting the major homozygous (AA) allele genotype from rs43036576 had significantly ($P < 0.05$) lower MS than animals inheriting the heterozygous allele genotype. However, there were no significant differences between the minor homozygous allele genotype and the heterozygous and major homozygous allele genotypes (Table 4.6). A single SNP marker located on both BTA 6 (rs42823614) and BTA 20 (rs42512588) was significantly ($P < 0.05$) associated with REA (Table 4.5). Animals inheriting the major homozygous (AA) allele genotype from rs42823614 had significantly ($P < 0.05$) larger REA than those inheriting the heterozygous allele genotype. However, there were no significant differences between the minor homozygous allele genotype and the heterozygous and major homozygous allele genotypes (Table 4.6). Animals inheriting the major (CC) and minor homozygous (TT) allele genotypes from rs42512588 had significantly ($P < 0.05$) larger REA than animals inheriting the heterozygous allele genotype (Table 4.6). Breed was also a significant ($P < 0.0001$) contributing factor for REA effects with regards to rs42512588 and rs42823614 (Table 4.6).

Table 4.5: Level of significance and frequency of animals from each genotype associated with hot carcass weight, yield grade, marbling score and rib eye area.

Traits	BTA	SNP ID	Allele ⁵	Minor Genotype Frequency	Het Genotype Frequency	Major Genotype Frequency	SNP <i>P</i> - value	Breed <i>P</i> - value
HCW ¹	6	rs42900130	A/G	5	82	211	0.0234	0.1176
HCW	6	rs42961882	T/A	29	115	118	0.0223	0.1624
HCW	6	rs43446022	G/C	19	48	67	0.0015	.0174
HCW	20	rs41931108	C/G	60	122	86	0.0368	0.0426
YG ²	20	rs41595713	T/C	28	170	78	0.0226	0.0510
MARB ³	6	rs41653357	A/C	31	98	115	0.0261	0.4872
MARB	20	rs43036576	A/G	24	112	150	0.0369	0.3932
REA ⁴	6	s42823614	A/C	3	50	253	0.0131	<.0001
REA	20	rs42512588	C/T	48	154	104	0.0414	<.0001

¹HCW=Hot carcass weight

²YG=Yield grade

³MARB=Marbling score

⁴REA=Rib eye area

⁵Representation of the major allele is located on the left.

Table 4.6 Single nucleotide polymorphisms associated with carcass traits and least square means estimate comparisons between reported genotypes for hot carcass weight, yield grade, marbling score and rib eye area.

Traits	BTA	SNP ID	Allele ⁵	Major Genotype Mean	Het Genotype Mean	Minor Genotype Mean
HCW ¹	6	rs42900130	A/G	357.94±4.80 ^a	343.70±6.04 ^b	374.37±24.89 ^{ab}
HCW	6	rs42961882	T/A	362.11±4.93 ^a	347.47±4.95 ^b	352.03±8.46 ^{ab}
HCW	6	rs43446022	G/C	343.27±5.38 ^a	369.25±6.43 ^b	360.39±9.08 ^{ab}
HCW	20	rs41931108	C/G	357.28±5.39 ^{ab}	348.76±4.45 ^a	364.84±5.66 ^b
YG ²	20	rs41595713	T/C	2.330±0.111 ^a	2.182±0.092 ^a	1.806±0.183 ^b
MARB ³	6	rs41653357	A/C	447.75±13.67 ^a	423.31±13.77 ^b	407.99±19.27 ^b
MARB	20	rs43036576	A/G	416.90±12.60 ^a	443.71±13.21 ^b	428.65±18.77 ^{ab}
REA ⁴	6	rs42823614	A/C	87.52±0.90 ^a	81.98±1.84 ^b	82.46±5.55 ^{ab}
REA	20	rs42512588	C/T	88.24±1.27 ^a	85.08±1.07 ^b	89.05±1.98 ^a

^{a,b}Differing superscripts indicate a difference of means at $P < 0.05$ within rows

¹HCW=Hot carcass weight

²YG=Yield grade

³MARB=Marbling score

⁴REA=Rib eye area

⁵Representation of the major allele is located on the left.

Analyses evaluating the incidence of BRD revealed that six individual SNP were identified as significant ($P < 0.05$). Three SNP were located on both BTA 6 (rs42823614, rs42968895, rs43448463) and BTA 20 (rs42477340, rs42512588, rs42524468) that were significant for incidence of BRD (Table 4.7). Animals inheriting the heterozygous (CT) allele genotype from rs42477340 had significantly ($P < 0.05$) higher incidence of BRD than animals inheriting the minor homozygous allele genotype. However, there were no significant differences between the major homozygous allele genotype and the heterozygous and minor homozygous allele genotypes (Table 4.8). Animals inheriting the major homozygous (AA) allele genotype from rs42524468 had significantly ($P < 0.05$) higher incidence of BRD than animals inheriting the heterozygous and minor homozygous allele genotypes (Table 4.8). Animals inheriting the major homozygous (AA, CC) allele genotypes from rs42823614 and rs42968895 had significantly ($P < 0.05$) lower incidence of BRD than animals inheriting the heterozygous allele genotype. However, there were no significant differences between the minor homozygous allele

genotype and the heterozygous and major homozygous allele genotypes (Table 4.8). Animals inheriting the major homozygous (AA,CC) and heterozygous (AG,CT) allele genotype from rs43448463 and rs42512588 had significantly ($P < 0.05$) lower incidence of BRD than animals inheriting the minor homozygous allele genotype (Table 4.8).

Table 4.7 Level of significance and frequency of animals from each genotype associated with incidence of Bovine Respiratory Disease.

Traits	BTA	SNP ID	Allele ²	Minor Genotype Frequency	Het Genotype Frequency	Major Genotype Frequency	SNP <i>P</i> -value	Breed <i>P</i> -value
BRD ¹	6	rs42823614	A/C	3	50	253	0.0396	0.9339
BRD	6	rs42968895	C/T	50	168	88	0.0234	0.9673
BRD	6	rs43448463	A/G	23	92	155	0.0334	0.9699
BRD	20	rs42477340	C/T	114	52	116	0.0192	0.9906
BRD	20	rs42512588	C/T	48	154	104	0.0167	0.9924
BRD	20	rs42524468	A/T	21	73	180	0.0324	0.6240

¹BRD=Bovine Respiratory Disease

²Representation of the major allele is located on the left.

Table 4.8: Single nucleotide polymorphisms associated with Bovine Respiratory Disease and least square means estimate comparisons between reported genotypes for incidence of Bovine Respiratory Disease.

Traits	BTA	SNP ID	Allele ²	Major Genotype Mean	Het Genotype Mean	Minor Genotype Mean
BRD ¹	6	rs42823614	A/C	0.0710±0.059 ^a	0.179±0.071 ^b	0.033±0.157 ^{ab}
BRD	6	rs42968895	C/T	0.028±0.061 ^a	0.121±0.058 ^b	0.057±0.065 ^{ab}
BRD	6	rs43448463	A/G	0.061±0.050 ^a	0.090±0.053 ^a	0.232±0.074 ^b
BRD	20	rs42477340	C/T	0.093±0.062 ^{ab}	0.162±0.068 ^a	0.030±0.063 ^b
BRD	20	rs42512588	C/T	0.047±0.058 ^a	0.072±0.055 ^a	0.184±0.064 ^b
BRD	20	rs42524468	A/T	0.119±0.037 ^a	0.017±0.046 ^b	0.025±0.074 ^b

^{a,b}Differing superscripts indicate a difference of means at $P < 0.05$ within rows

¹BRD=Bovine Respiratory Disease

²Representation of the major allele is located on the left.

Discussion

A total of fourteen unique SNP located on BTA 6 were significantly ($P < 0.05$) associated with growth traits, carcass traits and incidence of BRD. Six out of the fourteen unique SNP were significantly associated with growth traits including BW, WW, and HH. These results are in

agreement with reports that identified significant SNP for BW and WW on BTA 6 (Lu et al., 2013). Previous reports also identified SNP located on BTA 6 significantly associated with HH which is in agreement with the study herein (Bolormaa et al., 2014).

Five SNP were identified as being significantly associated with carcass traits including HCW, MS, and REA. These results were in agreement with reports that identified significant SNP for HCW on BTA 6 and a second report that identified significant SNP associated with REA located on BTA 6 (Lu et al., 2013; Casas et al., 2000). Previous reports also identified significant SNP for MS located on BTA 6 (Lee et al., 2012), which is in agreement with the results presented herein. The current study identified no significant SNP for YG located on BTA 6. It was previously reported that significant markers associated with BF were identified on BTA 6 (Li et al., 2004); however, the current study failed to validate these results. Three SNP identified herein located on BTA 6 were significantly associated with incidence of BRD. These results are in agreement with previous reports that identified significant QTL regions associated with incidence of BRD located on BTA 6 (Li et al., 2004).

Of the fourteen unique SNP identified on BTA 6, two were significantly associated with more than one trait. Marker rs42900130 was significantly ($P < 0.05$) associated with BW and HCW and marker rs42823614 was significantly ($P < 0.05$) associated with REA and incidence of BRD.

A total of eleven unique SNP located on BTA 20 were significantly ($P < 0.05$) associated with growth traits, carcass traits and incidence of BRD. Four out of the eleven unique SNP were significantly associated with growth traits including BW and WW. These results are in agreement with previous reports that identified significant QTL regions associated with BW and

WW on BTA 20 (Saatchi et al., 2014). However, the current study failed to validate previous reports that identified SNP on BTA 20 significantly associated with HH (Bolormaa et al., 2014).

Four SNP identified herein located on BTA 20 were significantly associated with carcass traits including HCW, YG, MS, and REA. These results are in agreement with reports that identified significant QTL regions on BTA 20 associated with HCW (McClure et al., 2010). The study herein is also in agreement with reports that identified significant SNP for YG, MS, and REA located on BTA 20 (Saatchi et al., 2014; Garcia et al., 2010). The current study was not in agreement with reports that previously identified SNP on BTA 20 that were significant for BF (Garrett et al., 2008). Three SNP identified herein located on BTA 20 were significant for incidence of BRD. These results are in agreement with previous reports that identified SNP significantly associated with incidence of BRD on BTA 20 (Casas et al., 2010).

Of the eleven unique SNP identified on BTA 20, three were significantly associated with more than one trait. Marker rs41595713 was significantly ($P < 0.05$) associated with BW and YG. Marker rs42512588 was significantly ($P < 0.05$) associated with REA and incidence of BRD. Lastly, marker rs41931108 was significantly ($P < 0.05$) associated with BW, WW, and HCW. Several SNP markers located on BTA 6 and BTA 20 were identified as significantly associated with a variety of economically important traits, as well as lowly heritable traits, such as BRD incidence. However, additional analysis and further research in populations of crossbred steers containing these markers needs to be validated before their incorporation into marker assisted selection programs.

Summary

The current study identified fourteen unique SNP located on BTA 6 that were significantly associated with BW, WW, HH, HCW, MS, REA, and incidence of BRD. Two SNP located on BTA 6 were identified as significantly associated with more than one trait. Eleven

unique SNP located on BTA 20 were identified as significantly associated with BW, WW, HCW, YG, MS, REA, and incidence of BRD. Three SNP located on BTA 20 were identified as significantly associated with more than one trait.

These results are in agreement with previous reports that identified genetic associations between growth traits, carcass traits, and BRD. These results also indicate that there may be a higher genetic predisposition to BRD than previously thought. To validate the results reported in the current study, a larger, more diverse population of animals should be utilized. Additional SNP located on BTA 6 and BTA 20 should be genotyped, as well as other regions spanning the genome. Fine mapping should be conducted in QTL regions associated with BRD in order to identify specific variations in the genome and improve the accuracy of marker assisted selection in the future.

CHAPTER V SUMMARY

The studies presented herein report the potential sources of variation in traits that may affect BRD incidence in crossbred steers. Growth and carcass traits are valuable to producers as they directly influence profits a producer makes. In the future, producers will be able to identify which animals in their herd are most susceptible to BRD with genetic testing and utilization of marker assisted selection. The objective of the first study was to evaluate on farm sources of variation and correlated effects contributing to BRD incidence in crossbred steers. Analyses revealed that incidence of BRD was lowly positively correlated to BW and lowly negatively correlated to WW, HH, sire breed and birth year. These results suggest that in the present study, the growth traits analyzed were not precursors for BRD incidence.

The objective of the second study was to evaluate single nucleotide polymorphisms (SNP) on previously described QTL regions of BTA 6 and BTA 20 for potential associations with growth traits, carcass traits, and incidence of BRD in crossbred steers sent to the feedlot from 2010-2013. Analyses identified a total of fourteen unique SNP located on BTA 6 and eleven unique SNP located on BTA 20 that were significantly associated with growth traits, carcass traits, and incidence of BRD. These results indicate that there may be a higher genetic predisposition to BRD than previously thought. Additional analysis in larger population of crossbred steers containing these markers needs to be validated before their incorporation into marker assisted selection programs.

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APPENDIX A: DNA EXTRACTION-SATURATED SALT PROCEDURE

Based on extraction procedures described in Miller et al., 1998. Nucl. Acids Res. 16: 1215.

Day 1: in 15 ml centrifuge tube

Add: 10-12 ml Lysis buffer (Appendix B) to 250 L white blood cell buffy coat; invert to mix

Spin: 7000rpm for 10 minutes at 4°C; aspirate supernatant from pellet

Add: 3ml Digestion Buffer (Appendix B); shake vigorously to re-suspend pellet

Add: 200µl 10 % SDS and 60µL RNase A (1 mg/ml); invert to mix; incubate for 1 hr at 37°C with gentle shaking

Add: 25µl Proteinase K (20 mg/ml); invert to mix; incubate overnight at 37°C with gentle shaking

Day 2:

Add: 1ml Saturated NaCl; shake vigorously by hand for 15 seconds

Spin: 2800rpm for 30 mins at 4°C; transfer supernatant to new 15 ml tube

Add: 2 volumes of 100% Ethanol (stored in freezer); invert gently to mix

Remove: DNA with soft pipette; transfer DNA into 1.5 ml snap-cap tube

Spin: at 10 setting for 10 min. in refrigerated bench-top centrifuge; aspirate off most of ethanol

Add: 1ml of 80% ethanol (keep on ice); vortex for 20 seconds; spin 5 minutes in refrigerated bench-top centrifuge; aspirate off most of ethanol

Add: 500µl of 80% ethanol (kept on ice); vortex for 20 seconds; spin 5 min in refrigerated bench-top centrifuge; aspirate off most of ethanol

Leave tubes uncovered to allow pellet to dry overnight

Add: 350µl Rehydration Buffer (Appendix B) to re-suspend DNA

Read: on spectrophotometer

APPENDIX B: BUFFER SOLUTION LABORATORY PROTOCOL

LYSIS BUFFER (1L):

7.49g NH_4Cl

2.059g Tris-HCl

pH to 7.4

DIGESTION BUFFER (1L):

1.211g Tris-HCl

23.376g NaCl

0.744g EDTA

pH to 8.0

REHYDRATION BUFFER (1L):

1.21g Tris-HCl

0.37g EDTA

VITA

Samantha Lidia Miller was born in Fort Worth, Texas in April of 1990 to her mother Tammy and grandparents Joyce and Fred. Samantha graduated from Theodore Roosevelt High School in May 2008 and started her undergraduate degree at Texas State University in the fall of 2008. Samantha graduated with a bachelor's degree in Animal Science in December of 2012.

Samantha moved to Baton Rouge, Louisiana in January 2013 and began her Master of Science degree at Louisiana State University with major professor, Dr. Matthew Garcia. After Samantha completes her Master of Science degree she plans to move back to Texas and pursue a career in animal science.