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Investigation of a Locus on Chromosome 9 for Contributions to Pulmonary Hypertension Syndrome in Broilers

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A dissertation submitted in partial fulfillment
of the requirements for the degree of
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By

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Abstract

Although the ascites syndrome in chickens has been investigated for years, it continues to inflict financial losses to the world poultry industry. It is estimated that 8% of the 361 million broiler deaths are due to ascites leading to losses of millions of dollars annually. Efforts to curb the incidence of ascites are typically designed to slow early growth. This limits the birds' ability to show its true genetic potential and impacts later yields. In 1994 lines divergent for susceptibility to ascites were established from a commercial sire line through sibling selection of birds reared at local altitude after testing siblings reared under simulated high altitude conditions. We used a whole genome SNP survey in our lines to identify regions associated with susceptibility. Seven potential regions were identified. Using microsatellite markers on chromosome 9 linked to genes with known contributions to Pulmonary Hypertension Syndrome, a survey of the lines (Generation -14) was accomplished.

This survey revealed that the selected lines changed in allele frequency for the markers as compared to each other and the line of origin. Changes were consistent with patterns of susceptibility and resistance to ascites. In addition to the research populations, it was determined that three commercial lines are also segregating for resistance related alleles from these regions. The data support the predictive nature of these loci in that, the presence of a specific genotype is associated with resistance to ascites. These microsatellite markers show utility in several different lines and therefore, could be used for marker assisted selection to improve ascites resistance. Finally, economic impact of utilizing these markers was evaluated. Significant differences were found among the genotypes of a marker for absolute breast, percentage breast and percentage leg. Therefore, if these markers have to be super-imposed in commercial selection programs, there could be compromises on traits of economic importance.

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Table of Contents

List of Tables

List of Figures

1 Review of Literature

1.1 Introduction

The poultry industry continues to be one of the fastest growing segments of the animal husbandry industry generating revenues in billions of dollars every year. The U.S, per capita consumption of broilers has exceeded 85 pounds as shown in Figure 1.1 (USDA, 2008). Poultry producers meet this enormous demand for broiler meat by applying classical quantitative genetic theory to large, relatively closed populations (Ewart, 1993) along with continuous modernization of rearing and processing environments. During the 20^{th} century, quantum changes occurred in the breeding of chickens attributed to an understanding and application of the genetics of chickens (Siegel, 2006). Thus, growth rate became the first trait to receive attention in the breeding industry during the emergence of commercial broiler production due to its economic importance and the relative ease with which it could be improved. Growth is moderately to highly heritable and can be rapidly improved through mass selection (Emmerson, 1997).

The primary breeders operate with a pyramidal structure (Figure, 1.2) where pure bred elite lines are located at the top. There are approximately 35 to 40 elite lines that are selected for various traits based on the company philosophy (Pollock, 1999). The majority of economic characteristics in commercial poultry stocks are controlled by several genes allowing a possibility of small effects and multiple correlations between them (Rishell, 1997). Generally major traits are improved by regenerating the best families in a high intensity selection while minor traits are removed by eliminating a few of the worst families through low intensity selection (Pollock, 1999).

Cobb-Vantress Incorporated analyzes and selects on over 50 phenotypic observations for each pedigree candidate at various ages (Katanbaf and Hardiman, 2010). Selections on male lines include growth rate, edible meat yield and feed conversion while the female lines are

selected for egg production, fertility and hatchability with a subtle emphasis on growth. Pre-selection of candidates at a relatively immature age prior to commercially relevant age is common in lines under intense selection for growth rate (Emmerson, 1997). In order to get maximum gains in the ultimate product, commercial poultry geneticists minimize generation interval, maximize selection intensity and utilize traits that are highly heritable. Generally, all of the permanent genetic progress happens at the elite pedigree level. Unselected pedigree birds are classified as Great Grand Parents (GGP) and are used as multipliers to produce Grand Parent progeny (GP).

Moving down the pyramid, a parent stock (P) is created by crossing two male or female lines which are usually designed to promote the expression of heterosis and protect genetic material (Anthony, 1998). These stocks are finally shipped to processors who cross a male and female parent line to produce billions of commercial broilers (B) that are used for generating meat. Thus, superior genetics of a single primary layer or broiler can be expanded more than a million-fold in the end product. Although, population genetics theory indicates that genetic variation for growth and feed efficiency will diminish with continued selection, results from long-term selection experiments provide little evidence of long-term genetic plateaus, and indicate that plateaus are only temporary when they do occur (Marks, 1991). Poultry breeders will hence continue to select for superior genetics.

Currently the average broiler market weight of 4 - 5 pounds is reached at 6 – 7 weeks. This reduction in time to market weight was primarily attributed to genetic selection involved in the pedigree elite lines (Havenstein, 2003). However, to expect a long term successful breeding program, one needs to strike a balance between economic significance and bird well being. Modern broiler survival and good health are key to efficient poultry production (Katanbaf and

Hardiman, 2010). Failure to establish check points when selecting for rapid growth and body weight has increased the incidence of numerous abnormalities as correlated responses in the modern broiler. Correlated responses occur because selection for one trait changes allele frequencies at pleiotropic loci or loci in linkage disequilibrium (Conner, 2004). They include but are not limited to increased carcass fat deposition, leg problems, reduced reproductive performance, reduced immunocompetence and increased incidence of ascites (Anthony, 1998).

1.2 Chicken Genomics

Tremendous progress in chicken genomics including a high quality draft genome sequence and a high density SNP map, enable us to utilize selective breeding with even greater efficiency. The chicken genome that was first successfully sequenced was that of a single female bird of the UCD001 inbred Red Jungle fowl line, originally developed by Abplanalp (Siegel, 2006). The jungle fowl can be described as a "Wild type" chicken serving as a base for comparing genomes of broilers or layers. The sequence contains thirty nine pairs of chromosomes including the sex chromosomes Z and W. It was estimated to be 1.2 billion base pairs and 4,000 cM in length. Unlike humans, where 1 cM approximates 1000 kb, in the chicken genome 1 cM is about 300kb and thus a third in size comparatively. A genetic linkage map based on genetic information from three chicken populations (East Lansing, Compton, and Wageningen) revealed approximately 1,965 genetic markers mapped to 50 linkage groups (Emara and Kim, 2003).

DNA variations are mutations resulting from substitution of single nucleotides (single nucleotide polymorphisms – SNPs), insertion or deletion of DNA fragments of various lengths, or duplication or inversion of DNA fragments (Rischkowsky and Pilling, 2007). DNA variations are classified as "neutral" when they cause no change in metabolic or phenotypic traits, and

hence are not subjected to positive, negative, or balancing selection. Otherwise, they are referred to as "functional". Mutations in key nucleotides of a coding sequence may change the amino acid sequence of a protein, and lead to new functional variants. Such variants may have an increased or decreased metabolic efficiency compared to the original "wild type", may lose their functionality completely, or even gain a novel function. Mutations in regulatory regions may additionally affect levels and patterns of gene expression (Rischkowsky and Pilling, 2007). The entire chicken genome however cannot be written as A's, T's, G's and C's owing to interactions between alleles at the same or different loci (Etches, 2001).

Traits of economic importance like growth rate, egg production and feed conversion are all complex and polygenic in nature and hence most genetic progress for these quantitative traits has been made by phenotypic selection using estimated breeding values without much knowledge about the number and nature of genes involved (Dekkers, 2005). By being able to study the genetic makeup of individuals at the DNA level using various genotyping techniques, molecular geneticists have a unique opportunity to investigate distinctive genotypes and incorporate them into breeding programs. Genotypic data is of great interest in selection programs as their heritability equals 1 assuming zero genotyping errors (Dekkers, 2005). It also can be obtained on both sexes and early in life, thus enabling geneticists to cull animals without having to invest feed and labor on them. Several markers are available to detect polymorphisms in the nuclear DNA. They include microsatellites and minisatellites also known as Variable Number Tandem Repeats (VNTRs), amplified fragment length polymorphism (AFLP), Restriction fragment length polymorphism (RFLP), Sequence tagged sites (STS) and Single Nucleotide Polymorphisms (SNPs).

Initially, the predominant DNA marker used in the chicken was the RFLP. However, RFLPs were relatively tedious and expensive to develop and use, leading to relatively low coverage and excessive numbers of linkage groups in the early maps (Siegel, 2006). AFLP markers are quick, inexpensive, and do not require DNA sequence information for their development, but have several disadvantages as well. So they were used only to enhance map coverage and merge smaller linkage groups (Levin et al., 1994; Herbergs et al., 1999). When large-scale sequencing and machine-based genotyping became increasingly popular in some poultry genetics laboratories, there was a growing move towards microsatellite markers (Haberfeld et al., 1991; Cheng and Crittenden, 1994). Although virtually all segregating DNA polymorphisms can be used as markers today, single nucleotide polymorphisms (SNPs) have become increasingly popular. SNPs, especially those within chicken protein coding regions (coding SNPs), have been used in genotyping for over a decade (Bumstead et al., 1994; Smith et al., 1997).

Extensive sequence data and modern SNPlotyping techniques make them the current marker of choice. Table 1.1 compares the properties of some of the important molecular markers. The industry is already using Marker Assisted Selection (MAS) to some extent, in their breeding programs. This can be used to increase the frequency of favorable alleles or to eliminate unfavorable alleles (Siegel, 2006). A promising opportunity is to use genomic selection as a method to predict the total genetic value of an animal based on data from genome wide dense marker maps. With this approach, breeders can estimate the effect of Quantitative Trait Loci (QTL) haplotypes without any need to understand the underlying molecular nature of the QTL (Meuwissen et al., 2001). Although molecular genetics continues to be the most promising tool

for the improvement of commercial poultry, it is likely to work in concert with traditional methods rather than replace them (Emmerson, 1997).

1.2.1 Microsatellites or Variable Number Tandem Repeats (VNTRs)

Microsatellites (MS) are stretches of DNA, consisting of tandemly repeating 1-5 nucleotide units. They can serve as highly informative genetic markers when Polymerase Chain Reaction (PCR) enables the detection of length variation (Powell et al., 1996). First recognized by Hamada et al., (1982), they were established as ubiquitous and abundant by Tautz and Renz (1984). PCR amplification of a MS locus from total genomic DNA is accomplished by usage of two distinctive primers, composed of short oligonucleotides that flank and define the MS locus. Amplified products from different individuals are then resolved on gels to reveal the length polymorphism (Powell et al., 1996).

MS could be classified as neutral Mendelian markers (Jarne, 1996). Their advantages include - locus specific results, high levels of polymorphism, even and random distributions across the genome, ease of isolation and evaluation, co-dominant inheritance and concomitant genotyping of large sample numbers (Hillel et al., 2003). Additionally, they can be easily transferred to resource populations segregating for the QTL of interest as allele sizes can be known with an accuracy of one base pair (Jarne, 1996). They have an advantage over other markers for tracing pedigrees as they represent single loci and avoid problems associated with multiple banding patterns (Powell et al., 1996). However, the need for sequencing or high resolution gels to characterize the products is a limitation for these markers. Even as the biological functions of MS are largely debated, they are widely used for DNA finger printing, paternity testing, construction of linkage maps, population genetic studies, individual identification, heterosis analysis and marker assisted selection (Tarik and Rabie 2009). The

ability to analyze the genetics of QTLs has been enhanced greatly by the development of detailed linkage maps based on MS in many farm species including chicken (Groenen and Crooijmans, 2003).

MS can be classified into three families: Pure (CACACACACACACACA), compound (CACACACACAGAGAGAGAGA) and interrupted (e.g. CACATTCACACATTCATT) (Jarne, 1996). Mutation rate is a major determinant of the level of variability maintained within a population. The preferred mechanism explaining the mutation model is polymerase slippage during DNA replication, resulting in an increase or decrease in the number of repeats by one or several repeats. Alleles with a large number of repeats were shown to be more vulnerable to mutation events (Weber and Wong, 1993). Unequal crossing over is another event that could contribute to mutational bias (Levinson and Gutman, 1987). Amos proposed that heterozygote instability is an important characteristic on an MS marker that makes it susceptible to mutations. Irrespective of the precise mechanism, it is accepted that recombination and DNA repair contribute to mutations in MS (Strand et al., 1993).

1.2.2 SNPs

SNPs are an abundant form of heritable genome variation. They differ from rare variations by a necessity for the least abundant allele to be more than 1 % in frequency (Anthony, 1999). Their locations within specific genes allow for alignment of chicken linkage maps with genes mapped in other species known as “comparative mapping” (Siegel, 2006). SNP detection technologies have transformed from laborious, time consuming and exorbitant processes to some of the most automated, efficient and relatively inexpensive ones. Scanning DNA sequences for unknown polymorphisms and genotyping individuals for known polymorphisms are the two major areas encompassing detection of SNPs (Kwok and Xiangning,

2003). Mutation mechanisms result either in transitions like A <-> G (purine – purine) or transverions: A <-> T (purine - pyramidine). Transitions are the most abundant forms of SNPs (Brookes, 1999). The simplest strategy to discover SNPs in a defined region containing candidate genes, to which they are often linked, is to perform direct sequencing of genomic PCR products obtained in different individuals. The number of known SNPs in chicken increased many fold over the past 10 years from just over a thousand in 2002 to the development of 60 k chip to investigate the genetic effects of selection for body weight as of 2009 (Vignal and Milen, 2002; Johansson et al., 2009).

Population genetics utilizes DNA polymorphism to study the genetic composition and inter relationships between populations. SNPs, due to their abundance, stability and ease of scoring can be exploited appropriately by population geneticists to effectively analyze genotype-phenotype relationships. For example, if a SNP is associated with disease occurrence, then it should be found at higher frequency in diseased individuals compared to non-diseased controls associated with the phenotype (Anthony, 1998). There are several molecular techniques used to reveal SNPs and their locations in the genome. They include direct sequencing of cloned ESTs and cDNAs, high resolution melts (HRM) and TaqMan® probes. The allele nomenclature is simpler in SNPs than MS as the results are typically binary (Yes/No). This enables a higher degree of automation, thereby making SNPs ideal markers for high throughput genotyping (Vignal et al., 2002).

1.2.3 Candidate genes for ascites

Several hypotheses have been put forward and tested regarding the number of genes involved in the development of ascites syndrome. Wideman and French (2000) suggested that ascites could be due to one or a few major genes. Using 15 generations of blood oxygen

saturation (Potential indicator trait for resistance to ascites) data, and estimated genotype probabilities at the putative major locus, Navarro et al., (2006) inferred that an over-dominant gene had influence on ascites. Similarly, Druyan and Cahaner (2007c) performed test crosses between Susceptible and Resistant progeny and recommended that two major complimentary genes are responsible for the observed difference in phenotype. Contrasting to these observations, other researchers have proposed that ascites is a complex polygenic trait (De Greef et al., 2001; Rabie et al., 2005; Hamal et al., 2010). Our team primarily uses SNPs and VNTRs to study variation in resource and commercial populations for multiple genes.

The candidate gene approach allows researchers to investigate the validity of an "educated guess" about the genetic basis of a disorder. In order to choose a potential candidate gene, researchers must already have an understanding of the mechanisms underlying the disorder (Kwon et al., 2000). This approach has been used more frequently in recent times instead of identifying Quantitative Trait Loci - QTLs (Thiruvenkadan, 2011). Once target genes responsible for variation in traits are identified, they are further investigated for polymorphisms like SNPs that could affect their expression and influence the phenotype (Flack, 2011). Association studies between an allele or a set of alleles and the phenotype is carried out to validate the contribution of a candidate gene. Populations diverging for the phenotype of interest can be crossed and screened for segregating markers linked to candidate genes to establish association. In chicken genomics, any marker within 1 Mb of a gene can be considered to be in linkage with the gene. Availability of a high quality genome draft for the chicken enables geneticists to screen for linked markers.

A SNP analysis was carried out for F_2 crosses between ascites susceptible and ascites resistant populations. The selection procedure and performance of these populations are

described in the ascites section of this review. The SNP analysis revealed seven chromosomal regions to be in linkage disequilibrium for ascites susceptibility. Three genes associated with pulmonary hypertension (PHS) in mice or humans were present in those regions, prompting further research. PHS, is one of the primary triggers of ascites in broilers (Wideman, 2000). The three candidate genes were a serotonin receptor (5HT2B), angiotensin II type 1 receptor (AGTR1) and angiotensin-converting enzyme (ACE).

1.2.4 Serotonin and its transporters

Serotonin, a monoamine neurotransmitter has long been suspected to play a major role in the pathogenesis of human Idiopathic Pulmonary Hypertension (IPAH).The accepted mechanism of triggering pulmonary vasoconstriction is through the interaction of serotonin with its 5HT-1B,5HT-2A and5HT-2B receptors (MacLean and Dempsie, 2009). Hypoxic pulmonary vasoconstriction is a physiological phenomenon in which pulmonary arteries constrict in the presence of hypoxia. Mice lacking the serotonin transporter or treated with serotonin transporter inhibitors were shown to be protected against hypoxic pulmonary hypertension (MacLean et al, 2000). Conversely, hypoxic pulmonary vasoconstriction was increased in mice over-expressing the serotonin transporter (MacLean et al., 2004). Bianchi et al., (2005) reported that serotonin stimulates cardiac myocyte hypertrophy through receptor independent and receptor-dependent mechanisms in mouse models.

Serotonin transporter is abundantly expressed in the lungs and located predominantly on Pulmonary Artery Smooth Muscle Cell (PASMC) (Ni and Watts, 2006). Serotonin-induced PASMC proliferation is shown to be mediated by the following cascade of events. Serotonin enters PASMC through its transporter SERT and subsequently produces reactive oxygen species thereby activating a down-stream proliferative signaling pathway (Maclean et al., 2007). In

broilers, microparticle entrapment within the pulmonary vasculature stimulates thrombocytes to release serotonin, triggering a potent pulmonary vasoconstriction resulting in ascites (Chapman and Wideman, 2002). A centralized role for serotonin in ascites was further demonstrated when Chapman and Wideman (2006) reported that injecting methiothepin, a nonselective serotonin receptor antagonist reduced Pulmonary Arterial Pressure (PAP) below baseline values and resulted in a $\geq$ 60% drop of mortality due to microparticle injection in ascites-susceptible broilers. Screening of gene expression profiles of broilers from an ascites susceptible (SUS) and ascites resistant (RES) line after inducing ascites by microparticle injection revealed a significantly higher expression of 5-HT_{1A} and 5-HT_{2B} receptors in the SUS line (Hamal et al., 2010; Wideman et al., 2007). All of these studies establish a strong association of serotonin to PHS.

1.2.5 Renin-Angiotensin system

The renin-angiotensin system (RAS) is a hormonal cascade that functions in the homeostatic control of arterial pressure, tissue perfusion, and extracellular volume. Dysregulation of the RAS plays an important role in the pathogenesis of cardiovascular disorders (Atlas, 2007). The RAS begins with the biosynthesis of renin by the juxtaglomerular cells. Renin regulates the initial, rate-limiting step of the RAS by cleaving the N-terminal portion of a large molecular weight globulin, angiotensinogen, to form the biologically inert decapeptide Angiotensin I (Ang I). Ang I is hydrolyzed by angiotensin-converting enzyme (ACE), which removes the C-terminal dipeptide to form the octapeptide Angiotensin II (Ang II), a biologically active, potent vasoconstrictor (Atlas, 2007).

Ang II plays multiple roles in the pathogenesis of hypertension through different pathways. It causes blood vessels to constrict, resulting in increased blood pressure. Similarly, it

stimulates the secretion of the hormone aldosterone to increase the reabsorption of sodium and water into the blood thereby increasing blood pressure. Finally, it acts as a potent dipsogen by aiding the brain to signal for additional water uptake, which increases blood volume, thus resulting in pulmonary hypertension (Wideman, personal communication). Lungs are the primary site for angiotensin-converting enzyme (ACE) expression resulting in high concentrations of circulating Ang II along with other components of the RAS including renin, angiotensinogen, and both subtypes of Ang II receptors (Yamazato et al., 2009). Pulmonary hypertension syndrome (PHS/Ascites) in broilers is due to a pathophysiological interplay between the lungs and heart (Wideman, 2001). Therefore, addressing both these systems will be the most effective approach to analyze PHS.

In experimental as well as clinical studies, chronic administration of RAS inhibitors has shown to be efficient in lowering blood pressure, thus making RAS inhibitors as targets for cardiovascular drug development (Dzau, 1986). Abraham (2003) suggested that individuals with a certain ACE genotype may be genetically predisposed to develop pulmonary hypertension. His group also established the function of Ang II in a pressure over loaded right ventricle in humans. Association of an angiotensin I-converting enzyme gene insertion - deletion (I/D) polymorphism (DD genotype) with predisposition for hypertension was shown in two other studies (Kim et al., 1997; Zhang et al., 2007). Most of the physiological effects of Ang II are mediated by its receptor AGTR1 (AT_1). The angiotensin receptors are a class of G protein-coupled receptor superfamily with angiotensin as its ligand. They contain 7 membrane-spanning sequences and are widely distributed on many cell types in Ang II target organs.

AT_1 has a central role in the RAS as signal transductors of the main effector hormone (Higuchi et al., 2007). Expression levels of AT_1 define the biological efficiency of Ang II with

it's over expression being a potential mechanism by which Ang II can contribute to cardiovascular disease (Ceolotto et al., 2010). AT_1 also mediates effects of Ang II on cell growth and proliferation, inflammatory responses, and oxidative stress (Atlas, 2007). Chung et al., (2009) revealed a polymorphism in the 3'-untranslated region of the human AT_1gene (1166 CC homozygote) to be associated with early onset IPAH which is a form of pulmonary hypertension. Celotto et al., (2010) reconfirmed the same genotype to be associated with hypertension and reported an increased AT_1 protein expression in those subjects. Using a rat model for pulmonary hypertension triggered by hypobaric hypoxia, Berkov, (1974) showed that an action of angiotensin II was specifically required for a significant vasoconstrictor response to hypoxia in isolated rat lungs. In a similar study, rats which received the AT_1 receptor antagonist losartan, showed a significant drop in mean pulmonary arterial pressure (MPAP) and right ventricular hypertrophy when compared to saline-treated controls. These studies suggest a direct role for endogenous ANG II acting through the type 1 receptor, in the vascular remodeling associated with hypoxic pulmonary hypertension (Morrell et al., 1995).

The chicken AGTR1 gene that codes for AT_1 receptor protein contains three exons which are distributed across 20,979 bases (Gga 9: 13,475,625 – 13,496,603). Exons 1 and 2 are non-coding, with the entire coding sequence located in exon 3 (International Chicken Genome Sequencing Consortium, 2004). The three exons produce a final mRNA transcript of 1427 bases. Exon 3 encodes a predicted 359 amino acid protein. AT_1 comparisons between Jungle Fowl, ascites-resistant, and ascites-susceptible DNA sequences revealed 24 single nucleotide polymorphisms (SNPs). Four of these SNPs affected the AGTR1 coding sequence (Burks, 2011). One of the SNP in exon 3 of the AGTR1 gene as shown by Burks (2011) is in the first position

of a proline codon at position 82 of the protein. This could result in the encoded amino acid to be changed to serine and an alteration in the secondary structure of the protein.

With components of RAS being strongly associated to PHS in several hypertension models as shown through literature and previous studies in our group, my project was designed to investigate polymorphisms in microsatellite markers linked to AT_1 and 5HT2B genes to analyze the 13 Mbp region on Gga9. Ascites susceptible, ascites resistant, relaxed selected research lines developed using a hypobaric model, along with three commercial elite lines were investigated to establish significance of the region's contribution to PHS and the merits and demerits of MAS.

1.3 Pulmonary Hypertension Syndrome (PHS) – Ascites

Selection could be considered part of the domestication practice as it directs and accelerates biological changes in animals to tailor them to suit human desires. Meat and eggs are two such needs with respect to poultry (Siegel, 1997). As a result, the genetic future of the modern broiler changed with concomitant advancements in nutrition and management providing the early foundation for vertical integration (Shrader, 1952). From being sold at 16 weeks with an average weight of 2.2 pounds and a 4.7 Feed Conversion ratio (FCR) in 1923, there has been a great change in today's commercial broiler which is typically processed at 6 weeks at 6 pounds with a FCR of 1.63 (Havenstein, 2003 ; Flock et al., 2005). Havenstein (2003) suggested that genetics was responsible for up to 90 % of the changes seen in modern commercial broilers. Poultry breeders responsible for these changes, created specialized lines of chickens to cater to human demands by decreasing generation intervals, maximizing selection intensity and utilizing highly heritable traits (Anthony, 1998). However, long term selection for production traits has shown to have resulted in increased environmental sensitivity manifested through decreased

fertility and health problems. This occurs because resources are transferred away from fitness related traits to further increase production (Waaij, 2004).

A normal, unstressed bird maintained under normal selection conditions allocates 90 % of its resources equally to growth, maintenance and reproduction, with the remaining 10 % to health. This changes dramatically when a bird is stressed with a disease challenge, where up to 80 % of available resources get allocated to health and the remainder to maintenance. In the disease challenged bird, reproduction and health do not receive any resources (Siegel, 1999). Resource allocation is age dependent. Younger birds allocate maximum resources to growth and nothing to reproduction, while a mature bird does the opposite. Selection has been shown to be another factor that influences resource allocation. An experiment using sheep red blood cells showed that, when resources available for a commercial broiler are limited, it compromises health by reallocating resources to growth for which it has been genetically programmed (Siegel, 1999).

This shift in allocation of resources from health towards growth can be illustrated through several complications in the modern broiler which include skeletal abnormalities, increased carcass fat, reduced immunocompetence, atypical meat quality, compromised reproductive performance, and ascites (Anthony, 1998). It is estimated that 8 % of the 361 million broiler deaths each year and 0.05 % of all processing plant condemnations are due to ascites with late grow-out mortalities resulting in losses worth more than 100 million dollars in the year 2002 (Pavlidis et al, 2007). A more recent study concluded that close to half of the dead-on-arrival birds showed pathological signs related to cardiovascular disorders (Ozkan et al., 2010). Economic losses due to ascites are relatively high as it tends to affect the heaviest and fastest growing birds which have had considerable amount of feed and labor invested (Lubritz and

Mcpherson, 1994). Historically associated with high altitudes and called "Altitude disease" (Riddell, 1991), ascites now has an increasingly high incidence even at modest altitudes in birds that exhibit rapid growth rates (Peacock et al., 1990).

The modern broiler being genetically programmed for fast growth creates a high demand for oxygen, from higher metabolic rates. When the demand for oxygen is not met, it eventually results in ascites as a consequence of changes in either cardiac output or pulmonary vascular resistance (Wideman and Kirby, 1995a). The pathophysiological progression of ascites is distinctive and includes the development of hypoxemia and pulmonary hypertension (See page 24). Cirrhosis of the liver, right sided congestive heart failure and transudation of ascitic fluid into the abdominal cavity are sequential events occurring subsequently (Wideman, 2000). In the United States, ascites is generally controlled by slowing early growth performance which ultimately reduces meat yield and limits realization of the true genetic potential for broilers. Consequently, ascites continues to be studied using various models for a long-term solution. This section reviews the research models used for investigating ascites. It emphasizes the genetic and physiological causes of ascites along with other components that contribute to its progress. It, concludes with a discussion of the correlation of ascites and economically important traits.

1.3.1 Genetics of ascites

Ascites has been demonstrated to be influenced by genetic factors (Lubritz and Mcpherson, 1994; Wideman and French, 2000). To visualize the genetic components of ascites, knowledge about the physiological modifications brought about in the modern broiler due to genetic selection and more importantly an understanding about organ development is required. The lungs are derived from the ectoderm while heart and most muscle are derived from the

mesoderm. Organs grow and develop at different rates over the course of an organism's life. Gene expression is influenced by the developmental stages (Siegel, 1997). Patten (1951) showed that the heart and circulatory system are among the first developed (30 - 55 hours of incubation heart grows more rapidly than the body of the embryo), while lung develops later. Artificial selection for body weight and conformation would therefore be expected to result in a greater correlated response in a heart rather than the lung creating an imbalance between the circulatory and respiratory systems (Siegel, 1997).

Selection for growth rate and muscle mass and feed conversion in broilers has resulted in development of the digestive system at the expense of heart and lung (Julian, 1993). Genetic variation in lung capacity of chickens has also been demonstrated by Vidyadaran (1990). His work indicated that Lung weight or volume contributes to a genetic susceptibility to ascites. These works are in agreement that ascites occurs due to alterations in the biological systems caused by intense genetic selection.

Moderate to high heretabilties for ascites have been reported in the literature. Lubritz et al., (1995) utilized a cold stress model to induce ascites on three male lines characterized by rapid growth rate and good feed efficiency (RG), moderate growth, good conformation, and excellent livability (MG), and maximum white meat yield and rapid growth (YD). Heritability estimates for Right Ventricle to Total Ventricle (RV: TV) were estimated at .21 ± .09, .21 ± .09, and .27 ± .08 for RG, MG, and YD, respectively. Ascites incidence had heretabilties of .36 ± .10, .11 ± .08, and .44± .09, respectively. In Cornish and White Rock breeds, Moghadam et al., (2001) estimated heritabilities of ascites at 0.12 ± 0.02 and 0.22 ± 0.01 respectively. Both of these researchers reported higher heritablities when only male performances were used for analysis indicating association of a sire component.

A cold stress model study resulted in heritabilities of 0.46 for Hematocrit Value (HCV), 0.42 for body weight, 0.47 for right ventricular weight, 0.46 for total ventricular weight, 0.45 for RV: TV, 0.32 for total mortality and 0.18 for fluid accumulation in the heart sac (Pakdel et al., 2002). Interestingly, Pakdel also reported that maternal effects significantly influenced body weight, total ventricular weight, and total mortality. Tests on lines developed using a hypobaric model showed high heretability estimates (0.30-0.55) (Anthony and Balog, 2003; Pavlidis et al., 2007.

The most recent heritability estimate on divergently selected ascites lines based on cold stress model was reported to be 0.57 by Dryuan et al., (2007a). HCV have been consistently shown as an important component indicating susceptibility to ascites. High heretabilites of 0.46-0.81 for HCV were reported by Shlosberg and Bellaiche (1998) as opposed to a low of 0.11 by Ledur et al., (2006). These reports show us that most of the estimated traits can be used as selection criteria for reducing the incidence of ascites. Consequently a few teams have embarked on short-term and long-term selection experiments to create repeatable research models for studying ascites.

1.3.2 Experimental methods to induce ascites and corresponding selection programs

Measurement of birds under ascites inducing conditions is expected to better reveal the genetic differences among birds towards ascites susceptibility than under unstressed conditions. This can be attributed to Genotype by Environment (G x E) interactions. Research teams have thus utilized surgical and non surgical procedures to induce ascites for research purposes. **Surgical methods:** Wideman and Kirby (1995a) showed that a primary increase in pulmonary vascular resistance can initiate a pathophysiological progression leading to pulmonary hypertension syndrome (PHS, ascites). This was performed by surgically clamping the left

pulmonary artery. Results found this method to be efficient in inducing ascites as birds that underwent the surgery showed 90% ascites incidence compared to 8 % non-surgery control birds Wideman and Kirby (1995a).

A slightly modified version of this procedure was performed by Wideman et al., (1997), where the primary bronchus was unilaterally occluded. Although this method was easier to perform than occluding a pulmonary artery, it was not as successful in inducing ascites. It was demonstrated that the pathophysiological progression leading to ascites triggered by pulmonary hypertension were one and the same for both models. Nevertheless both these methods were time consuming and need a high level of expertise to perform, which restricted large scale commercial application. Hence, an improvised lesser invasive method for surgically inducing ascites was devised by Wideman and Erf (2002). Under this procedure an intravenous injection of micro-particles, having a size suitable to be trapped by the pulmonary pre-capillary arterioles, was used to increase the pulmonary vascular resistance thereby triggering an acute increase in pulmonary hypertension terminating in ascites.

Results indicated that the micro-particle technique was highly effective in inducing ascites in broilers. It was suggested that this methodology could potentially replace unilateral pulmonary artery occlusion as the technique of choice for genetically selecting broilers that have a sufficiently robust pulmonary vascular capacity to resist the onset of PHS (Wideman and Erf, 2002). The effects of the consequent immune responses, either beneficial or detrimental were not evaluated. However, Wideman et al., (2003) did perform additional experiments to explore the benefits of surviving the micro-particle challenge. After a series of trials, it was shown that selected survivors exhibited improved livability and body weight gain when compared with their unselected controls under heat challenging conditions. Meat quality and blood gas values did not

differ between the groups showing that selection under this method could confer economical advantages along with resistance.

Based on the unilateral pulmonary artery occlusion surgical method to induce ascites, there was a short-term unidirectional selection program initiated by Wideman and French (1999). Eighteen sire families (n= 550) of a breeder male line were challenged by positioning silver clips to chronically clamp the left pulmonary artery. Survivors at 35 days were reared to breeding age to serve as parents for the first generation PHS-resistant line. To validate the effectiveness of the selections, sire families of the base population from the same line produced control chicks to be compared with the selected ones.

Progeny from the resistant line tolerated fast growth and cool temperatures and exhibited 50 percent lower ascites incidence compared to the base population. After another generation of selection, the lines were again challenged under cold temperatures to screen for ascites susceptibility. Ascites mortalities were 0% and 6.4% for females and males respectively as opposed to 12.3% and 43.6% in the base population (Wideman and French, 2000). Although this selection program was successful in demonstrating the genetic component for ascites, it was not carried forward.

1.3.3 Non Surgical methods

Cold stress: Cold temperatures have been shown as an important factor in inducing ascites (Wideman et al., 1998). Increases in ascites incidence under low or high altitude were shown to be more marked under cold temperatures (Julian, 2000). It has also been shown that fast growing broilers exhibit higher susceptibility to cold stress compared to slow growing broilers (Deeb et al., 2002). Several explanations for this effect were reviewed by Julian (1993). Noxious fumes causing lung damage and cold weather decreasing ventilation were hypotheses not supported by

research. Increased metabolic rate (185%) was reported to have been induced by cold stress, thus markedly increasing oxygen requirement and cardiac output resulting in ascites. Cold was also shown to increase blood viscosity (Julian, 1993). Oxygen requirement was shown as the most important mechanism of ascites induction, irrespective of the different pathways.

Using this cold-stress model Druyan et al., (2007a) created divergent lines for ascites incidence from a commercial population. From 85 sire families challenged under cold environment (after week 3) and pelleted feed, the 7 most susceptible and resistant families formed the base AS-S (Susceptible) and AS-R (Resistant) population. Within three generations of divergent selection the AS-S line had 91.3% and AS-R 4.7% ascites incidence (Druyan et al., 2007a). This model has been shown as a promising one for screening families for ascites incidence. Unfortunately, the lack of a relaxed selected control line in this model will be a limitation in revealing the Genotype X Environment interactions.

Hematocrit: A mixture of hematocrit values (HCV) and cold stress were used to develop lines that differed in ascites susceptibility (Shlosberg and Bellaiche, 1996). Blood hematocrit has also been shown as one of the minimally invasive indicators for ascites susceptibility. HCV was utilized to screen phenotype extremes from commercial lines. Very high (HH), High (HM), Low (LM) and Very low (LL) hematocrit lines were established. On testing the progeny under cold stress, ascites increased in all four lines but was significantly higher in HH compared to LL. This work suggested that HCV could be used to develop ascites resistant populations (Shlosberg and Bellaiche, 1998).

Hypobaric Hypoxia: Hypoxia induced pulmonary hypertension has been reported in several species including chickens (Mirsalimi et al., 1992). Utilizing a hypobaric model for inducing ascites, a long term divergent selection for ascites was initiated in 1995 (Anthony et al., 2001;

Balog et al., 2001; Pavlidis et al., 2007). This was facilitated through the collaboration of University of Arkansas and USDA. At mean sea level and $0^{\cdot}$ C atmospheric pressure equals 760 mm Hg. Dry air contains about 21% oxygen equating the partial pressure of oxygen to be 160 mm Hg at sea level. With increase in altitude, the partial pressure drops significantly resulting in decreased oxygen availability which is termed as hypoxia.

The selection tool utilized in this study was a hypobaric chamber capable of inducing simulated high altitude conditions (9500 feet). Three separate hatches of chicks from a commercial pedigree line which had undergone one generation of relaxed selection were transported to the University of Arkansas poultry facility to serve as the base population. The chicks represented 16 sire families of a pedigree male line (Pavlidis et al., 2007). One of the hatches was tested in the hypobaric chamber and mortality data was generated. The base population on the whole had about 67% ascites incidence under hypobaric conditions (Balog, 2003).

Sire family selections were performed using sib data to establish the 8 most susceptible and resistant families as the SUS and RES line respectively. A relaxed selected (REL) control line has been maintained across all generations of selection. At generation 9, the SUS line and the RES line were highly divergent at 95% and 7% ascites incidence at simulated 9500 feet. The relaxed selected line has been maintaining a stable ascites incidence of about 66% (Pavlidis et al., 2007). Currently 16 generations of selection have been completed on these research lines which have been excellent resource populations for several ascites related studies including a USDA supported whole genome association scan for SNPs.

1.3.4 Pathophysiology of ascites

The four chambered avian heart physically separates the low-pressure pulmonary circulation from the high pressure systemic circulation to reduce the risk of pulmonary edema and prevent intra-cardiac mixing of oxygenated and deoxygenated blood (Wideman, 2000). However, with application of quantitative genetics resulting in rapid growth rates, better feed conversion and meat yield, modern broilers tend to easily outgrow their cardio-pulmonary capacity and disrupt normal mechanisms. The increase in size of muscle mass to organ ratio surges oxygen demand. Hypoxia or shortage of oxygen has been shown as the primary stimulus of ascites in broilers. When demand for oxygen exceeds a bird's cardio pulmonary capacity additional blood supply by the heart is triggered. The augmented cardiac output has to be circulated via the lungs, resulting in pulmonary hypertension (Groves, 1997).

The structure of the avian heart, with its thin-walled right ventricle and muscular right atrioventricular valve allows PH to induce rapid heart failure (Julian, 1993). Ascites is initiated when the right ventricle (RV) is forced to develop an elevated Pulmonary Arterial Pressure (PAP) in order to meet the increased cardiac output demands. According to the equation PAP = Cardiac Output (CO) X Pulmonary Vascular Resistance (PVR), right ventricular work should increase to propel cardiac output when viscosity increases or pulmonary vasculature is inappropriately constricted. This causes right ventricular hypertrophy (Wideman, 2000). Under a wide variety of conditions healthy chickens have RV: TV ratio between 0.15 to 0.27, whereas sustained PH increases it above 0.28 (Cueva et al., 1974). Elevated RV: TV ratios and relative right ventricular weights in ascitic broilers clearly exemplify "Work hypertrophy" supporting a central role for PH in the pathogenesis of ascites (Wideman, 2000). Diffusion of oxygen across the gas exchange barriers and into the red blood cells requires time. Due to the rapid flow, the

RBC's may not reside in gas exchange surfaces long enough to achieve complete oxygen saturation. This inefficient oxygen saturation leads to hypoxemia (Wideman, 2000).

Subsequently, the onset of hypoxemia triggers an adaptive increase in hematocrit by production of erythropoietin by the kidneys, thereby increasing blood volume and viscosity (Lubritz and Mcpherson, 1994; Julian, 2000). Systemic hypotension develops concomitantly with onset of PH. The challenged right ventricle fails to elevate PAP to increase the rate of blood flow. Blood that is not pumped through the lungs congest in systemic veins thereby reducing blood flow to the left side of the heart. The result of which is a dependent reduction in cardiac output, stroke volume and mean systemic arterial pressure. The accumulated venous blood also congests the sinusoids within the liver contributing to hypoxemia-induced cirrhosis and plasma leakage through the surface of the liver. Systemic hypotension stimulates kidneys to retain solutes and water that comprise the ascitic fluid (Wideman, 2000). Figure 1.3 represents the cascade of events that contribute to the development of ascites syndrome.

The terminal stages of an ascitic broiler are readily recognized as the sequelae to right-sided congestive heart failure (Wideman, 2000). Chronic hypoxemia contributes to bilateral cardiac dilation and flaccidity. The pathology evident in terminally ascitic broiler involves deterioration of multiple organ systems. Clinical signs of an affected broiler include cyanosis of the skin of the head and body, prominent dilated veins, shrunken wattles and combs, increased respiration, decreased exercise tolerance and abdominal fluid (Julian, 1993). Druyan et al., (2009) evaluated the major physiological differences between two broiler lines divergently selected for ascites incidence. The results were different under ascites inducing conditions (AIC) and standard brooding conditions (SBC). Under AIC the susceptible line of birds has elevated hematocrit and reduced oxygen saturation in arterial blood. However, these were not different

under SBC. The only significant difference under SBC was the heart rate in the first week suggesting that it can serve as a physiological criterion for early selection. Most of the observed differences in hematology and biochemistry could be a result of PHS and not a cause. Hence, analysis of polymorphisms in DNA markers that contribute to the observed physiological differences is one of the possible approaches to identify underlying cause.

1.3.5 Indicators of ascites

Ascites tends to be a late grow-out mortality affecting the heaviest birds, before which a huge amount of feed and labor has been invested (Lubritz and Mcpherson, 1994). This results in increased financial losses for the poultry industry. Although there is a need for a long-term solution to deal with ascites losses, short term answers are equally important. Indicators that mark a vulnerable bird, when expressed at an early stage of a broiler's life could be efficiently used to eliminate potential candidates. These indicators need to be minimally invasive as harsh surgeries may affect the growth potential of a bird. Electrocardiography (ECG), pulmonary arterial pressure, elevated serum levels of troponin T, pulse oximetry and hematocrit values are considered to be good indicators of ascites (Thiruvenkadam, 2011; Wideman et al., 1998).

Negative ECG lead II S- wave amplitudes are predictive of elevated RV: TV ratio and thus can be an effective predictor of PHS (Wideman et al., 1997). Hypoxemia is one of the preliminary events in the pathophysiological progression leading to ascites, explained due to the rate of pulmonary blood flow exceeding the gas diffusion capacity (Wideman and Kirby, 1995a). Oxygen saturation is negatively correlated with ascites susceptibility at -0.5 (Druyan et al., 2007b). Onset of hypoxemia could be quantified using a pulse oximeter which directly measures the saturation of hemoglobin with oxygen (Peacock et al., 1990). Elevated serum levels of troponin T, a cardiac-specific protein, was reported to be detected during onset of PHS in

broilers. Unfortunately, proprietary components of this assay are not available for veterinary use in the US which limits its potential (Wideman et al., 1998; Maxwell et al., 1995). Finally, hematocrit values (HCV) have been shown to be positively correlated to ascites. Triggered by sustained hypoxemia, HCV has been shown successfully as a genetic predictor of ascites susceptibility (Shlosberg and Bellaiche, 1996). Concomitant use of these indicators as selection tools in commercial programs to cull birds exhibiting ascitic symptoms has largely been responsible for reduction of ascites incidence.

1.3.6 Environmental triggers and management measures

From an estimated 30 million broiler deaths in the year 2002, ascites incidence has been reduced over the years due to management techniques. Better ventilation, temperature control and lowered early average daily growth (ADG) have been deployed to control ascites (Hardiman, personal communication). Almost all of these measures compromise the genetic potential of the broiler. While long term solutions are sought by research teams, management continues to be the number one contributor for reduced ascites incidence in the United States. Water, diet, feed restriction and lighting have been reported in literature to be associated with ascites in a positive or detrimental manner.

Chickens consume 2.4 times more water than feed on a weight basis (Williams, 1996). Wideman (2000) reported that birds grown on nipple waterers tend to have lower incidence of ascites as opposed to Plasson type waterers. This was attributed to the water restricting properties of nipple waterers. Among components of water, excess sodium has been shown as a factor responsible for inducing ascites. Sholsberg et al., (1998) compared tap water with water supplemented with NaCl, NH_4Cl and $KHCO_3$ and reported that ascites mortality was significantly higher in the birds under the NaCl water group. Chicks are very sensitive to excess

sodium especially in their first three weeks of life. Excess sodium increases fluid retention resulting in increased ascites through right ventricular hypertrophy (Julian et al., 1992).

Pelleted feed increases ascites incidence as it is nutrient dense and easier to consume when compared to mashed feed (Julian, 2000). Inclusion of dietary supplements and manipulation of calories in the diet has shown to alter ascites incidence. High levels of fat, protein and amino acids have shown to increase ascites by increasing demand for metabolic oxygen (Julian, 1989). Decreased phosphorous as well as increased cobalt (500 ppm) increased ascites and right ventricular hypertrophy (Julian, 2000; Diaz et al., 1994). Wideman et al., (1995b) reported that supplementation of feed with 1% L-arginine reduced ascites losses. L-arginine, when present at higher concentrations is utilized as a substrate for production of nitric oxide that acts as a potent vasodilator to decrease pulmonary arterial pressure. The expensive nature of this product limits its large scale commercial use (Groves, 1997). Metaproterenol, a bronchodilator when tested under lowered temperatures was shown to significantly reduce ascites incidence at 17.6 % oxygen and prevent it completely at 20.6 % oxygen (Vanhooser et al., 1995).

Kleuss et al., (2011) evaluated the effect of Tryptophan (TRP) by using two different diets. The control diet contained approximately 0.22% TRP by weight (2.2 g TRP/Kg feed), while the High-TRP diet had the same components blended with sufficient additional TRP added to achieve 0.88% TRP by weight. Tryptophan is the precursor for serotonin and was expected to accelerate the development of Idiopathic Pulmonary Arterial Hypertension (IPAH), which is one of the forms of hypertension. It was seen that, pulmonary arterial pressures were higher in the High-TRP group than in the control group. Furosemide, supplemented at 0.15% or below

reduced ascites mortality without reducing body weight by acting as a diuretic and preventing fluid and sodium retention (Wideman et al., 1995b).

Newberry and Gardiner (1993) evaluated the effect of lighting patterns on growth and mortality of male broiler chickens. Two lighting treatments, either 23 h of continuous light/24-h period or an increasing photoperiod lighting system, which was 0 to 3 days-23 h light (L); 4 to 14 days- 6L; 15 to 21 days-10L; 22 to 28 days-14L; 29 to 35 days-18L and 36 to 42 days-23L were evaluated. Ascites mortality was reduced in the increasing light program as opposed to the continuous lighting without compromising final market weight. Buyse et al., (1996) found similar results for intermittent lighting schemes. Reduction in feed intake leading to improved feed conversion without compromising growth was reported as an additional economic advantage of intermittent lighting programs.

Ascites incidence through hypoxemia owing to high metabolic rates in broilers can be prevented moderately by limiting the intake energy through Feed Restriction (FR) (Julian, 2000). Balog et al., (2000) conducted an experiment to determine if the duration of FR can be used to reduce the incidence of ascites under ascites inducing and normal conditions without having a negative impact on growth. Although, FR treatments significantly reduced ascites incidence, when compared with the fully fed controls, BW was lighter for most groups.

Interestingly, the fully fed controls at normal conditions were heavier than their counterparts under ascites inducing conditions. The compromise on BW was shown to be overcome by feed restricting at an early age. This not only reduced incidence and mortality due to ascites under cold stress, but also resulted in BW similar to that of the ad libitum fed group (Ozkan et al., 2006). Recently, Ozkan et al., (2010) evaluated broilers raised with three different FR regimes under ascites inducing/normal conditions. As shown previously, all FR treatments

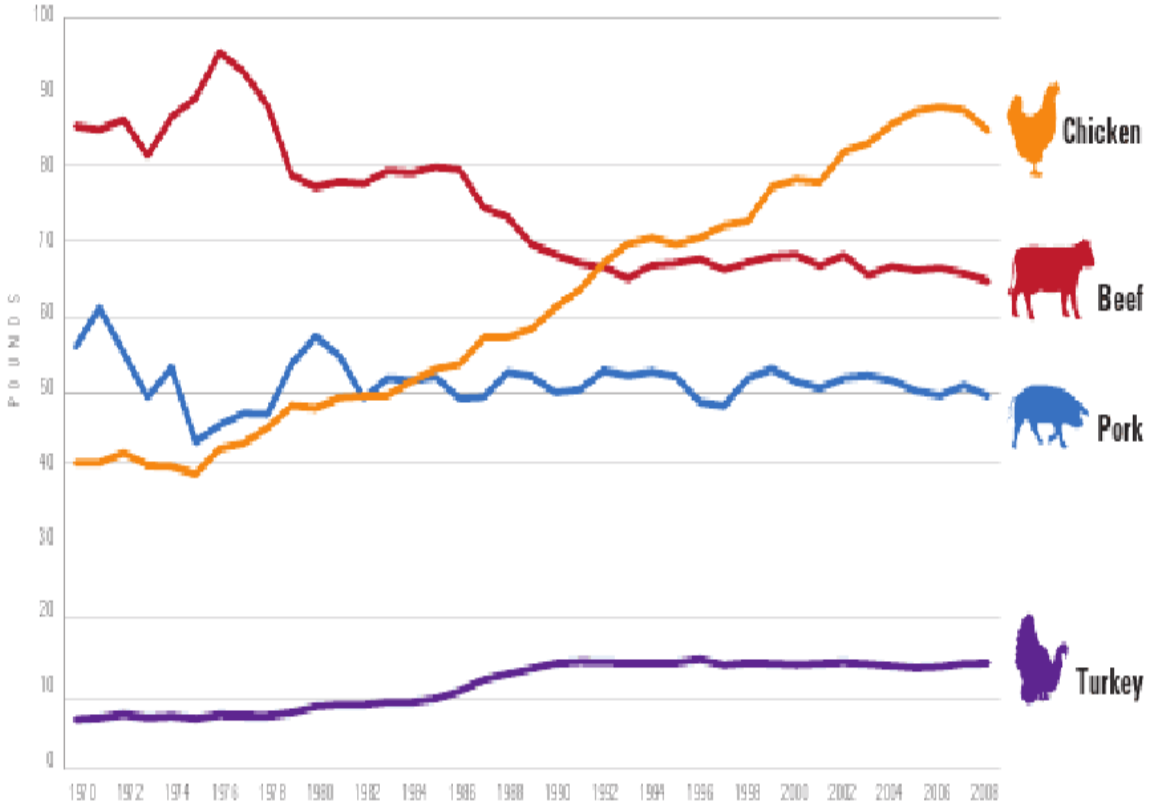
reduced ascites mortality under ascites inducing conditions but not in normal conditions. Ozkan and co workers concluded FR was effective at high altitudes and cold conditions that are often experienced in commercial farms in mountain areas.

1.3.7 Ascites and economically important traits

Although, several experiments have shown that genetic selection for ascites resistance can be successfully accomplished, correlated responses for economically important traits have not been promising. When ascites incidence is shown to have increased due to selection for rapid growth, it can be expected that the selection for ascites resistance could reduce growth rates. In fact, it was estimated that selection for ascites resistance resulted in the resistant (RES) line being approximately 163 g lighter as opposed to ascites susceptible (SUS) line at d 42 (Pavlidis et al., 2007). Pectoralis weights measured at d 42 also showed that the SUS line males were significantly heavier than the RES line males (Pavlidis, 2003).

The only improvement with respect to traits of economic importance was the fact that the RES line had significantly better Feed Conversion Ratio (FCR) compared to the SUS line (Pavlidis, 2003). However, these measurements were taken on the research lines when they were raised in pens as groups. Whether, individual FCR measurements on these lines will result in similar or different conclusions is yet to be determined. Nevertheless, with current emphasis on rapid growth, high muscle yield and improved FCR it seems unlikely that the competitive poultry breeding companies will invest to produce an ascites resistant broiler at the cost of economically important traits (Balog, 2003). For this reason, irrespective of the significance of the method used to increase ascites resistance, simultaneous estimation of the economic impact is warranted.

Figure 1.1 U.S per capita consumption of various types of meat between 1970 and 2008 Adapted from USDA links



Source: USDA Economic Research Center, Food Availability Data System, Feb. 1, 2010.
http://www.ers.usda.gov/Data/FoodConsumption/FoodAvailSpreadsheets.htm#mtpcc.

Figure 1.2 Genetic supply chain used in the poultry industry to produce live and processed product Adapted from Emmerson D

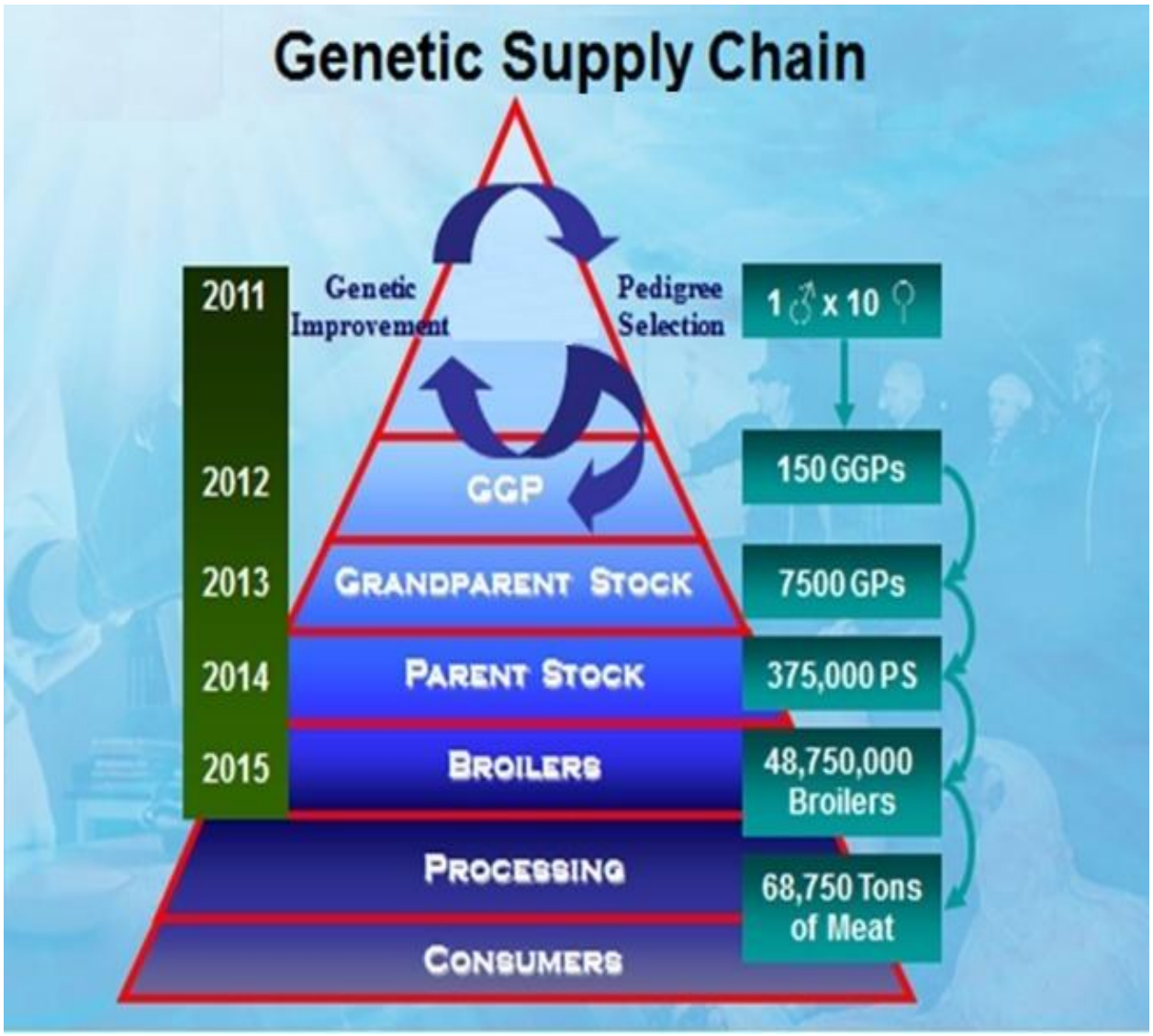


Figure 1.3 Cascade of events involved in ascites development. Adapted from Balog, 2003

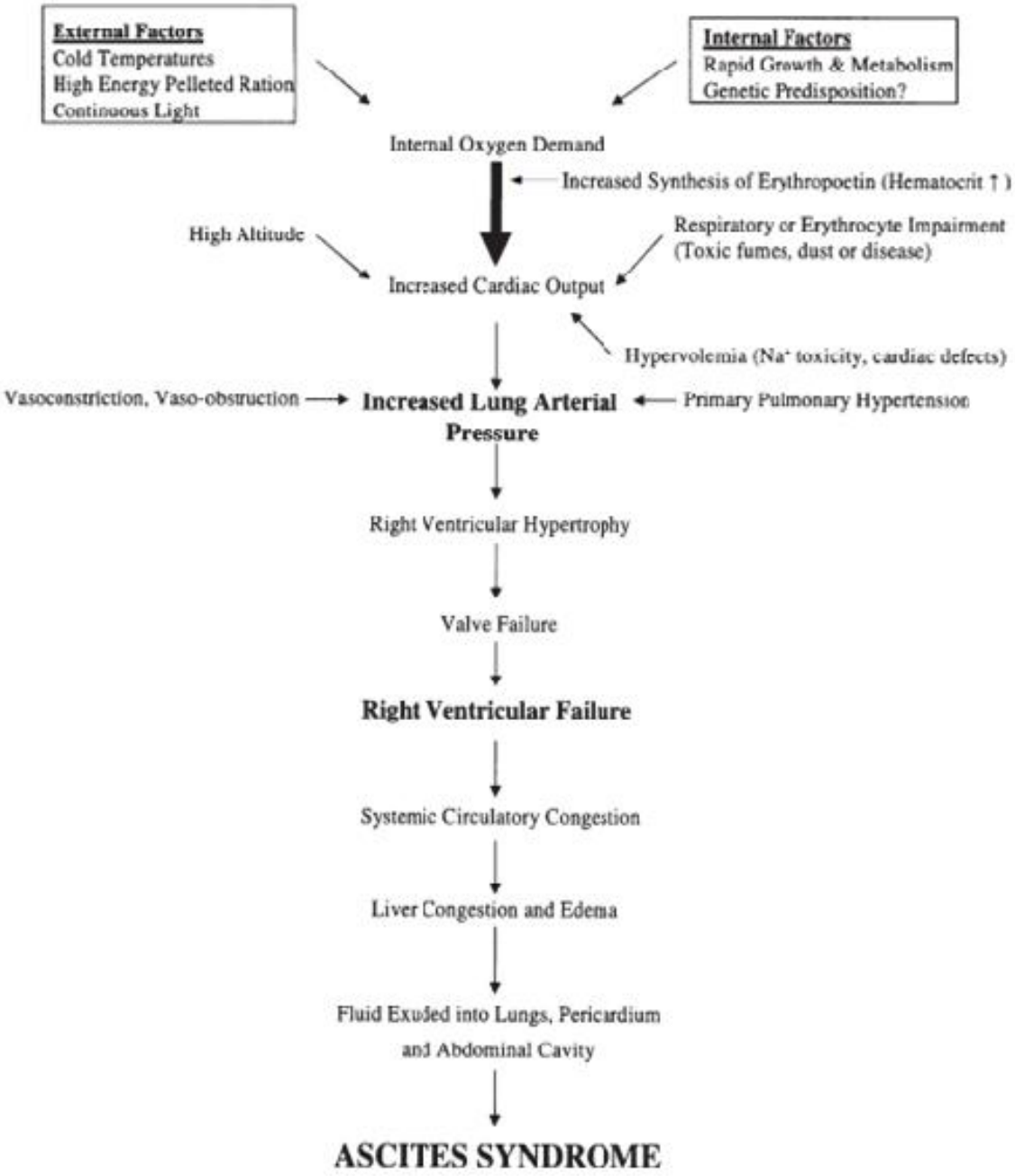


Table 1-1 Characteristics of molecular markers: Adapted from Flack, 2011

	RFLP	**RAPD**	**AFLP**	**Microsatellite**	**SNP**
Single or Multiple Locus	Multiple	Multiple	Multiple	Single/Multiple	Single
Reproducibility	***	**	***	*****	*****
Required Sample	> 10 ug DNA	~ 10 ng DNA	~ 25 ng DNA	~ 10 ng DNA	~ 10 ng DNA
Number of Alleles	2+	2	2	2 or more	2
Assay	Gel	PCR - Gel	PCR - Gel	PCR – Gel	PCR – Sequencing

Locus - Number of loci revealed by a single assay.

Reproducibility - Scored on a scale of 1* to 5* with 5* being the most reproducible.

Required sample - The amount of sample required to perform an assay.

Number of alleles - The number of alleles that can be revealed at a given loci.

Assay – Assay required for detecting polymorphic alleles.

2 Evaluation of allele and genotype frequencies of four Gga9 microsatellite markers in broiler lines divergently selected for ascites incidence

2.1 Introduction

In the United States, per capita consumption of broilers has exceeded 85 pounds (USDA, 2008). Poultry producers meet this huge demand for broiler meat by applying classical quantitative genetic theory to large, relatively closed populations (Ewart, 1993) along with continuous modernization of rearing and processing environments. From being sold at 16 weeks at an average weight of 1 Kg and a 4.7 Feed Conversion ratio (FCR) in 1923, broilers are currently processed at 6 weeks with 2.7 Kg weights and a FCR of 1.63 (Flock et al., 2005). Havenstein (2003) suggested that genetic selection involved in the pedigree elite lines was responsible for up to 90 % of the changes observed in modern commercial broilers.

However, to expect a long term successful breeding program, one needs to strike a balance between economic significance and bird well being. Modern broiler survival and good health are key to efficient poultry production (Katanbaf and Hardiman, 2010). Unfortunately, long term selection on production traits has increased environmental sensitivity manifested through decreased fitness and health problems. This occurs because resources are reallocated from fitness related traits to production traits (Waaij, 2004). The resulting health problems include increased incidence of carcass fat deposition, increased leg problems, reduced reproductive performance reduced immunocompetence and increased incidence of ascites (Anthony, 1998).

Ascites has been historically associated with high altitudes and was referred to as "Altitude disease" (Riddell, 1991). However, ascites now has an increasingly high incidence even at modest altitudes in birds that exhibit rapid growth rates (Peacock et al., 1990). It is estimated that 8 % of the 361 million broiler deaths each year and 0.05 % of all processing plant condemnations are due to ascites with late grow-out mortalities resulting in losses worth more

than 100 million dollars in the year 2002 (Pavlidis et al., 2007). A more recent study concluded that close to half of the dead-on-arrival birds showed pathological signs related to cardiovascular disorders (Ozkan et al., 2010).

Economic losses due to ascites are relatively high as it tends to affect the heaviest and fastest growing birds which have had considerable amount of feed and labor invested (Lubritz and Mcpherson, 1994). The modern broiler, being genetically programmed for fast growth, creates a high demand for oxygen, from higher metabolic rates. When the demand for oxygen is not met, the bird responds eventually resulting in ascites as a consequence of changes in either cardiac output or pulmonary vascular resistance (Wideman and Kirby, 1995a). PHS, being one of the primary triggers of ascites in broilers is used interchangeably with ascites. The pathophysiological progression of ascites is distinctive and includes the development of hypoxemia triggered by pulmonary hypertension. Cirrhosis of the liver, right sided congestive heart failure and transudation of ascitic fluid into the abdominal cavity are subsequent events (Wideman, 2000).

Several studies have shown that ascites is influenced by genetic factors (Lubritz and Mcpherson, 1994; Wideman and French, 2000; Balog, 2003). Subsequently, utilizing a hypobaric model for inducing ascites, a long term divergent selection program for ascites was initiated in 1995 (Anthony et al., 2001; Balog et al., 2001). This was facilitated through the collaboration of University of Arkansas and USDA. The selection tool utilized in this study was a hypobaric chamber capable of inducing simulated high altitude conditions (9500 feet). Sire family selections were performed using sib data to establish the most susceptible and resistant families as the SUS and RES line respectively from a commercial elite line that was tested under hypobaric conditions. A relaxed selected (REL) control line has been maintained across all

generations of selection. From a 67 % ascites incidence (9500 feet) for the base population, the SUS and RES lines were highly divergent at 95% and 7% incidence of ascites at generation 9. The unselected relaxed line has consistently maintained around 66% ascites incidence (Pavlidis et al., 2007). Currently 14 generations of divergent selection has been completed on these research lines.

Molecular tools have the potential to offer advantages in improving the accuracy of selection for disease resistance, improving the ability to screen large numbers of animals and reducing costs (Vint, 1997). Accordingly, a whole genome SNP analysis was carried out on the F_2 crosses between ascites susceptible and ascites resistant populations to identify ascites associated regions. The SNP analysis revealed seven chromosomal regions to be in linkage disequilibrium for ascites susceptibility. All seven regions were further investigated for association using VNTRs on a small subset of the SUS line (Smith, 2009). Three genes reported to be associated with pulmonary hypertension (PHS) in several species were present in those regions, prompting further research. The three candidate genes were serotonin receptor (5HT2B) (Hamal et al., 2010; Smith, 2009 and Wideman et al., 2007), angiotensin II type 1 receptor (AT_1) (Atlas, 2007; Maclean, 2007) and angiotensin-converting enzyme (ACE) (Yamazato et al., 2009).

Population genetics utilizes DNA polymorphism to study the genetic composition and interrelationships between populations (Anthony, 1999). DNA variations are mutations resulting from substitution of single nucleotides (single nucleotide polymorphisms – SNPs), insertion or deletion of DNA fragments of various lengths, or duplication or inversion of DNA fragments. The resulting variants may have an increased or decreased metabolic efficiency and disease resistance compared to the original "wild type. In addition, variants may lose their functionality

completely, or even gain a novel function (Rischkowsky and Pilling, 2007). Identifying such variants and studying the pattern involved can aid in better understanding the underlying genetics. Utilizing microsatellites (MS) is one of the more efficient ways to study polymorphisms in a population.

Microsatellites are stretches of DNA, consisting of tandemly repeating 1-5 nucleotide units. They can serve as highly informative genetic markers when Polymerase Chain Reaction (PCR) enables the detection of length variation (Powell et al., 1996). Microsatellites could be classified as neutral Mendelian markers (Jarne, 1996). Their advantages include locus specific results, high levels of polymorphism, even and random distributions across the genome, ease of isolation and evaluation, co-dominant inheritance and concomitant genotyping of large sample numbers (Hillel et al., 2003). Additionally, they can be used to transfer the chromosomal region between resource populations segregating for the QTL of interest (Jarne, 1996). A previously described MS screening approach, adapted by Smith (2009), was performed on the SUS line birds. The RES line gene pool used in the whole genome SNP association survey was not used in this study, which may or may not have influenced the outcome. Therefore, the objectives of the current study was to use MS markers present in two regions associated with ascites incidence to screen the SUS and RES lines for allele and genotype frequencies. We hypothesized that MS markers with disproportionate allele and genotype frequencies between lines divergently selected for ascites incidence could indicate regions that contribute to the development of ascites.

2.2 Materials and Methods

Genetic Stock: Birds used for this study were from generation 14 of two broiler research lines divergently selected for ascites incidence at the University of Arkansas (Pavlidis et al., 2007).

Husbandry: Floor reared pedigree replacements that were maintained under typical broiler breeder management conditions were used for this study. They were provided with ad libitum access to commercial starter feed meeting NRC requirements for the first 3 weeks. From week 4, birds were placed on a feed and photoperiod restriction program until they were 18 weeks old. Once selection was accomplished using sib data, all unselected families were culled. At week 19, selected breeders were moved to individual cages to generate the next generation progeny. Water was available ad libitum throughout rearing.

DNA: Blood samples (10 µl) were collected from 280 birds from the SUS and RES line birds at 6 weeks of age using lancets and wing vein puncture method. DNA was isolated from blood samples using an easy extraction procedure outlined by Bailes et al., (2007). Ten µl of blood was added to 400µl cold STM solution (64 mM sucrose, 20 mM Tris Cl pH 7.5, 10mM $MgCl_2$ and 0.5% Triton X-100) prefilled in 0.5 ml V shaped assay blocks. Blocks were centrifuged to pellet the nuclei and supernatant was decanted by inverting the block briefly. The pellet was resuspended in 200ul TEN (10mM Tris Cl pH 7.5, 1mM EDTA, 10mM NaCl) with 10 µg/ml pronase E (Sigma-Aldrich). Samples were placed in a 37°C shaker over night. The pronase was heat inactivated at 65°C for 20 minutes and DNAs were stored at -20°C. From each line, the DNA samples that could not recovered effectively, were excluded from the study.

Microsatellite primers: PCR primers flanking the MS repeats regions were designed to produce fragments that range from 120-180 bp. Primers were synthesized (MWG Operon Inc.) with fluorescent labels on the 5' end of the forward primer. Two MS markers from the Gga 9:15 mbp region and two from the Gga 9:13 mbp region were utilized to genotype these lines. The MS loci and primers used for this study are listed in Table 2.1.

PCR Amplification: PCR was performed in 96 well plates as 20µl reactions: 2 µl of DNA sample (20-100ng/µl), 1X Buffer (50mM TrisCl pH8.3, 1mM $MgCl_2$, and 3mg/ml Bovine Serum Albumin), 0.2 mM dNTP's, 1µM forward and reverse primer, and 3 units Taq polymerase. PCR cycle conditions were as follows: 90°C for 1 minute for an initial denature followed by 41 cycles of 90°C denature for 20 seconds, 30 seconds annealing at the corresponding annealing temperature, 72°C elongation for 1 minute, and final elongation for 3 minutes at 72°C. The primer annealing temperatures varied from 45-55°C (Table 2.1). A MJ Research PTC-100 thermo cycler or an Eppendorf Master Cycler gradient was used for all PCR reactions.

Denaturing Polyacrylamide Gel Electrophoresis: PCR products were prepared for electrophoresis by mixing 2 µl of the product with 5µl loading buffer (95% formamide, 5% 1X Tris-Borate-EDTA pH9.15 and 2% bromo phenol blue). The mix was denatured at 90°C for 3-4 minutes and rapidly cooled on ice. Two µl of this mix was loaded on a 30 x 40 cm, 0.4 mm thick 6% denaturing polyacrylamide gel (38:2 acrylamide:bisacrylamide) in 1xTEB (100 mMTris, 10 mM boric acid, 2 mMEDTA, pH 9.2) and electrophoresed at 50 Watts for 3 hours.

Gel imaging: Gels were scanned on a Typhoon fluorescence scanner 8600 (Molecular Dynamics, Amersham Bioscience, Sunnyvale, California) to estimate labeled PCR fragments. CXR ladder (Promega Corp.) was used to detect band sizes for corresponding alleles. Genotypes were assigned for each bird through designated allele names (Letters) for respective band sizes.

Statistical Analysis: Based on product size (bp), alleles for all the markers were designated letters for convenience (Table 2.2). Allelic and genotypic frequencies were calculated using observed allele counts for individuals genotyped within the two ascites lines SUS and RES. Expected allelic and genotypic frequencies for respective lines were calculated based on the allele frequency observed in the entire population according to Hardy-Weinberg equilibrium.

Disproportionate representation of alleles and genotypes in the divergent lines were established using chi-quare tests to determine P-values that represented deviation from expected counts (Microsoft Excel). For all the markers, combined frequencies of less than 0.05 at both the allelic and genotypic levels have been excluded from the study. Results were reported to be statistically significant at the P <= 0.05 level.

2.3 Results

2.3.1 Gga9:15 MS markers (PHS 001 & PHS 152)

The research lines used in this study have been divergently selected for 14 generations. At generation 14 they were highly divergent with 98 % ascites incidence for the SUS line and 7 % for the RES line (Fig 2.1). The region on Gga9 (~ 15.0 Mbp) contains the serotonin receptor (5HT2B) gene. Two linked MS markers were investigated from this region using PHS 001 and PHS 152 primer pairs. Both markers were polymorphic. PHS 001 is a two base (CA) tandem repeat MS marker. It amplified four alleles for the SUS and the RES lines. Alleles B and D were overrepresented ($P < 0.01$) in the SUS line. Contrastingly alleles A and E were overrepresented ($P < 0.01$) in the RES line (Table 2.3). Seven genotype combinations were present in the SUS and RES lines. AE, DE and EE genotypes were present in significantly higher frequencies ($P < 0.01$) in the RES line, whereas BB and BD were prominent ($P < 0.01$) in the SUS line. Genotypes AD and BE were almost equally distributed in the lines ($P = 0.528$ and 0.595) respectively (Table 2.3).

PHS 152 is a four base (AAAG) tandem repeat MS marker. It amplified two alleles for both of the lines. A and B alleles showed similar representation in the SUS and RES lines. Allele A was present in frequencies of 0.58 for SUS and 0.64 for RES line birds while allele B was 0.42 in SUS and 0.36 ($P = 0.262$ and 0.157) in RES line birds (Table 2.4). The three genotype

combinations AA, AB and BB followed expected frequencies based on their allele distributions. However, none of the three genotypes were different for the RES line birds when compared with the SUS line birds (Table 2.4).

2.3.2 Gga9:13 MS markers (PHS 009 & PHS 010)

The region on Gga9 (~ 13.0 Mbp) contains the angiotensin receptor (AT_1) gene. This region was investigated with two linked MS markers named PHS 009 and PHS 010. Both markers were polymorphic and amplified three alleles each. PHS 009 is a two base pair (GA) tandem repeat marker. Alleles A and C were overrepresented ($P < 0.01$) in the SUS line. Allele A was present at a frequency of 0.35 in the SUS line birds and 0.22 in RES line birds. Interestingly allele C, which was 39% in SUS line birds, was absent in the RES line. The B allele was found at 0.78 and 0.25 of all RES and SUS line birds respectively, which was a significant ($P < 0.01$) deviation (Table 2.5). The genotypic frequency patterns followed the allele representations and were largely divergent. Six different genotypic combinations were present in the lines with four of them (AA, AC, BC and CC) overrepresented ($P < 0.01$) in Line SUS. The remaining two genotypes (AB and BB) were prominent ($P < 0.01$) in the RES line (Table 2.5).

PHS 010 was the other marker investigated from this region. It is an (AC) tandem repeat with three alleles identified. This marker was similar to PHS 009 in terms of skewed representations between the lines, with almost all the allele and genotype combinations showing disproportionate frequencies. Among the 3 alleles present in the research lines, the A allele was present in 0.72 of all SUS line birds and absent in RES. Contrastingly, allele C was absent in the SUS line and present at 0.21 frequency in RES. Both of these results were significant deviations ($P < 0.01$) from expected counts. Allele B was overrepresented ($P < 0.01$) in the RES line (0.78) as opposed to the SUS line (0.28) birds (Table 2.6). With allele A being absent in the RES line,

genotypes AA and AB were present only in the SUS line (0.51 and 0.43) respectively ($P < 0.01$). The BB genotype was overrepresented in RES (0.59 vs 0.06). Similarly, BC was present at a frequency of 0.40 ($P < 0.01$) in the RES line and absent in SUS (Table 2.6).

2.4 Discussion

The genome wide SNP analysis of a F_2 cross of SUS and RES lines revealed SNPs at approximately 10KB spacing for a total of 2733 SNPs (Cheng et al., 2006). One thousand seven hundred and sixty three SNPs were informative in elucidating the association to right ventricle/ total ventricle weight and resistance or susceptibility to ascites. Seven chromosomal regions were regarded to contain possible QTLs for ascites. A principle objective of this study was to evaluate allele and genotype frequencies of MS markers present in 2 of the 7 significant regions in SUS and RES pure line birds. Previous MS screening approaches have been genome wide association studies that identified QTLs associated with extensive chromosomal regions. In our study, we used MS markers that are present in regions that have already been shown to be strongly associated to ascites incidence through a whole genome association scan. This approach provides supplemental evidence to further associate proven hot spots and increases robustness.

The role of a poultry geneticist is to change the genetic properties of a population by choice of the individuals used as parents. The effect of selection is a change in the array of gene frequencies (Falconer, 1996). The unequal distribution of several MS alleles between the SUS and RES lines can thus be explained as a possible effect of divergent selection. Genotypic frequencies follow the changes of allele frequencies in small populations. Genotypes present at high frequencies in a highly resistant line and low frequencies in a highly susceptible line, could be likely indicators of genes affecting resistance. The BB homozygote of PHS 010 was present at a frequency of 0.60 in the RES line and 0.06 in the SUS line (Table 2.6). Similarly, PHS 009 BB

homozygote was present at a frequency of 0.58 in the RES line and 0.06 in the SUS line (Table 2.5). Such genotypes offer an advantage to individuals in the RES line. It remains to be seen whether using resistant genotypes for Marker Assisted Selection in commercial populations will contribute to increasing resistance to ascites.

In summary, three out of the four MS markers investigated have been shown to have disproportionate allele and genotypic frequencies between ascites-susceptible and ascites resistant broiler research lines. Among the two significant regions investigated Gga9 (~ 13.0 Mbp) region with PHS 009 and PHS 010 is the most descriptive. They have certain alleles and genotypes significantly linked with selection for resistance to ascites. With more than one VNTR from this region being associated with ascites, we conclude that this region could have a strong contribution in the genetic basis of ascites. Presence of a candidate gene (AT_1) within this region lends additional support to our conclusion. PHS 009 and 010 markers can be evaluated in unrelated commercial populations to increase robustness. If these markers are informative in multiple populations, they can be used in poultry selection programs for a long term solution to reduce ascites incidence in commercial broilers.

Figure 2.1 Cumulative ascites mortality from hatch to 42 days for SUS, REL and RES lines (Generation 14) reared at simulated 9500 feet altitude

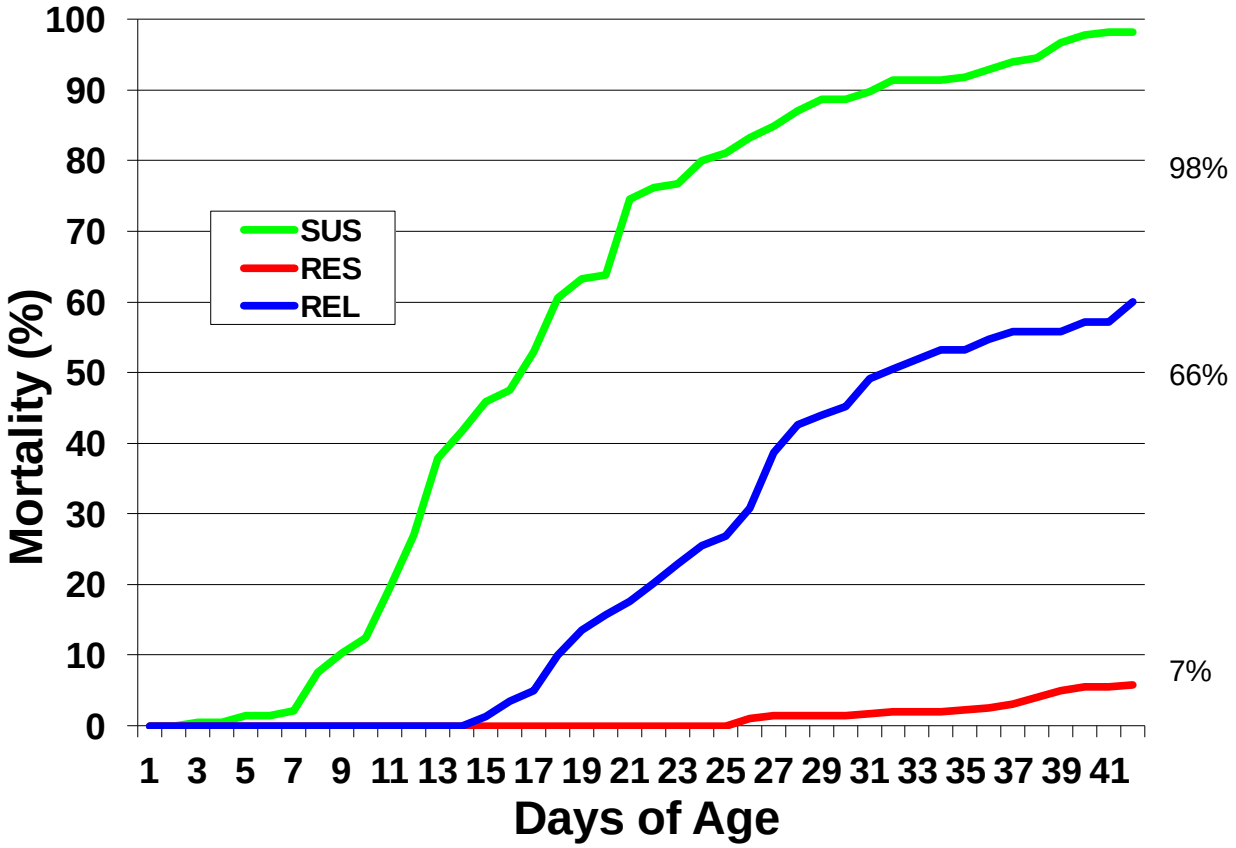


Table 2-1 Primer pairs listed with location, sequence (Forward and Reverse); VNTR, number of PCR cycles, optimized annealing temperature (°C), number of alleles amplified, and size range of those alleles (bp)

Primer Name	Locus	Primer Sequence(5'-3') Forward	Reverse	VNTR	No of PCR Cycles	Annealing Temperature	No of Alleles	Size Range (bp)
PHS 001	Gga9:15.96	GAGGCTCCATCGCTATTGA	AGTTTTTGTCTTCCCAGTCTCC	$(CA)_{17}$	43	50	5	140-160
PHS 152	Gga9:15.882	GTGACATTCATACTGGAAC	GCTATCTTGTTTGCTAATCT	$(AAAG)_{12}$	45	43	2	182,198
PHS 009	Gga9:13.42	GGGGCTATCACATCTACTTT	AGTTCAGAACCAGGAGAAGA	$(GA)_{13}$	40	52	6	137-151
PHS 010	Gga9:13.54	ACAGAATTAGGGTGGGTTTTTG	GCGGTGTGCCTGCTTTCT	$(AC)_{16}$	40	50	5	150-170

Table 2-2 Allele sizes and their corresponding designated letters for PHS 001, 009, 010 and 152 marker loci

PHS Marker	Size (bp)	Designated letter
1	160	A
	156	B
	154	C
	146	D
	140	E
9	145	A
	137	B
	143	C
	141	D
	139	E
	151	F
10	160	A
	150	B
	170	C
	158	D
	156	E
152	198	A
	182	B

Table 2-3 Chi Square analysis of allelic and genotypic frequencies at the PHS 001 locus for the SUS[1] and RES[1] broiler research lines

Variable		ALL[2]		SUS			RES			Freq	
		N	Freq	N	Freq	Exp	N	Freq	Exp	Diff	p-val[3]
Allele	A	126	0.12	49	0.09	64	77	0.15	62	-0.06	0.01
	B	232	0.23	166	0.32	119	66	0.13	113	0.18	< 0.01
	C	21	0.02	21	0.04	11	0	0	10	0.04	NC
	D	239	0.23	153	0.29	122	86	0.17	117	0.12	< 0.01
	E	406	0.4	135	0.26	208	271	0.54	198	-0.28	< 0.01
Genotype	AA	4	0.01	0	0	2	4	0.02	2	-0.02	NC
	AB	18	0.04	12	0.05	9	6	0.02	9	0.02	NC
	AD	41	0.08	23	0.09	21	18	0.07	20	0.02	0.528
	AE	59	0.12	14	0.05	30	45	0.18	29	-0.13	< 0.01
	BB	27	0.05	23	0.09	14	4	0.02	13	0.07	< 0.01
	BC	5	0.01	5	0.02	3	0	0	2	0.02	NC
	BD	68	0.13	56	0.21	35	12	0.05	33	0.17	< 0.01
	BE	87	0.17	47	0.18	45	40	0.16	42	0.02	0.595
	CD	7	0.01	7	0.03	4	0	0	3	0.03	NC
	CE	7	0.01	7	0.03	4	0	0	3	0.03	NC
	DD	22	0.04	22	0.08	11	0	0	11	0.08	NC
	DE	79	0.15	23	0.09	40	56	0.22	39	-0.14	< 0.01
	EE	87	0.17	22	0.08	45	65	0.26	42	-0.18	< 0.01

[1] Divergently selected broiler lines for ascites Susceptibility (SUS) and Resistance (RES)

[2] Total number of birds tested from both ascites lines

[3] p-value is NC (Not calculated) if overall allelic or genotypic frequencies <0.05

Table 2-4 Chi Square analysis of allelic and genotypic frequencies at the PHS 152 locus for the SUS[1] and RES[1] broiler research lines

		ALL[2]		SUS			RES			Freq	
Variable		**N**	**Freq**	**N**	**Freq**	**Exp**	**N**	**Freq**	**Exp**	**Diff**	**p-val**
Allele	A	470	0.61	188	0.58	188	282	0.64	282	-0.06	0.262
	B	296	0.39	138	0.42	138	158	0.36	158	0.06	0.157
Genotype	AA	162	0.42	60	0.37	69	102	0.46	93	-0.1	0.155
	AB	146	0.38	68	0.42	62	78	0.35	84	0.06	0.326
	BB	75	0.2	35	0.21	32	40	0.18	43	0.03	0.472

[1] Divergently selected broiler lines for ascites Susceptibility and Resistance

[2]Total number of birds tested from both ascites lines

Table 2-5 Chi Square analysis of allelic and genotypic frequencies at the PHS 009 locus for the SUS[1] and RES[1] broiler research lines

Variable		ALL[2]		SUS			RES			Freq	
		N	Freq	N	Freq	Exp	N	Freq	Exp	Diff	p-val
Allele	A	298	0.28	178	0.35	143	120	0.22	155	0.13	< 0.01
	B	560	0.53	129	0.25	268	431	0.78	292	-0.5	< 0.01
	C	200	0.19	199	0.39	96	1	0	104	0.39	< 0.01
Genotype	AA	31	0.06	26	0.10	15	5	0.02	16	0.08	< 0.01
	AB	166	0.31	56	0.22	79	110	0.40	87	-0.2	< 0.01
	AC	70	0.13	70	0.28	33	0	0	37	0.28	< 0.01
	BB	174	0.33	14	0.06	83	160	0.58	91	-0.5	< 0.01
	BC	46	0.09	45	0.18	22	1	0	24	0.17	< 0.01
	CC	42	0.08	42	0.17	20	0	0	22	0.17	< 0.01

[1] Divergently selected broiler lines for ascites Susceptibility and Resistance

[2] Total number of birds tested from both ascites lines

Table 2-6 Chi Square analysis of allelic and genotypic frequencies at the PHS 010 locus for the SUS[1] and RES[1] broiler research lines

Variable		ALL[2]		SUS			RES			Freq	
		N	Freq	N	Freq	Exp	N	Freq	Exp	Diff	p-val[3]
Allele	A	366	0.35	365	0.72	174	1	0	192	0.72	< 0.01
	B	575	0.54	139	0.28	273	436	0.78	302	-0.5	< 0.01
	C	119	0.11	0	0	57	119	0.21	62	-0.2	< 0.01
Genotype	AA	128	0.24	128	0.51	61	0	0	67	0.51	< 0.01
	AB	109	0.21	109	0.43	52	0	0	57	0.43	< 0.01
	BB	178	0.34	15	0.06	85	163	0.59	93	-0.5	< 0.01
	BC	110	0.21	0	0	52	110	0.4	58	-0.4	< 0.01
	CC	4	0.01	0	0	2	4	0.01	2	-0	NC

[1] Divergently selected broiler lines for ascites Susceptibility and Resistance

[2] Total number of birds tested from both ascites lines

[3] p-value is NC (Not calculated) if overall allelic or genotypic frequencies <0.05

3 Association of genotype to ascites phenotype for Gga9:13 microsatellite markers in commercial broilers

3.1 Introduction

During the 20th century, quantum improvements occurred in the breeding of chickens. Much of this progress has been attributed to an understanding and application of population and quantitative genetic principles. Subsequently, growth rate became the first trait to receive attention in the emerging broiler breeding industry due to its economic importance and the relative ease with which it could be improved (Siegel, 2006). Other traits including yield and feed conversion were added to selection strategies as commercial breeding became more complex. Currently, the primary breeders operate with a pyramidal structure where pure bred elite lines are located at the apex (Pollock, 1999). With an assumption that genetic diversity exists for agriculturally important traits, poultry companies identified superior individuals and used them as parents for the next generation, thus raising the overall performance of the group (Cheng et al., 2006).

A 16 week broiler in 1923 was processed at 1kg and a 4.7 Feed Conversion ratio (FCR). In 2001, broilers were processed at 6 weeks with an average weight of 2.2 Kg and a FCR of 1.63 (Flock et al., 2005; Havenstein, 2003). However, to ensure a long term successful breeding program, one needs to strike a balance between economic significance and bird well-being. Modern broiler survival and good health are key to efficient poultry production (Katanbaf and Hardiman, 2010). Unfortunately, long term selection on production traits has generally reduced fitness as resources are diverted away from fitness to production traits (Waaij, 2004).

Ascites is one of the unfavorable conditions entrenched in commercial populations owing to its correlation with rapid growth rates (Pavlidis et al., 2007). The modern broiler being genetically programmed for fast growth creates a high demand for oxygen, by higher metabolic rates. When the demand for oxygen is not met, it eventually results in ascites as a consequence of

changes in either cardiac output or pulmonary vascular resistance (Wideman and Kirby, 1995a). The pathophysiological progression of ascites is distinctive and includes the development of hypoxemia triggered by pulmonary hypertension (Wideman, 2000). Post-mortem examination of ascitic birds reveals straw-colored fluid in the abdominal cavities, enlarged hearts, pulmonary congestion, and abnormal livers and spleens (Maxwell et al., 1986).

In the United States, ascites is generally controlled by slowing early growth performance which ultimately reduces meat yield and limits realization of the true genetic potential for broilers. Consequently, ascites continues to be studied using various models for a long-term solution. Although, the environment is very important in the occurrence of ascites, it is clear that ascites and measures associated with the condition have a genetic basis (Silversides et al., 1997). Population genetics utilizes DNA polymorphism to study the genetic composition and inter relationships between populations (Brookes, 1999). DNA variations are mutations resulting from substitution of single nucleotides (single nucleotide polymorphisms – SNPs), insertion or deletion of DNA fragments of various lengths, or duplication or inversion of DNA fragments may result in variants (Rischkowsky and Pilling, 2007). Such variants may have an increased or decreased metabolic efficiency compared to the original "wild type", may lose their functionality completely, or even gain a novel function.

Molecular tools have the potential to offer advantages in improving the accuracy of selection for disease resistance, improving the ability to screen large numbers of animals and reducing costs (Vint, 1997). The industry uses Marker Assisted Selection (MAS) to some extent, in their breeding programs. This can be used to increase the frequency of favorable alleles or to eliminate unfavorable alleles (Siegel, 2006). Selection of pedigree stock through analysis of whole genome will provide accurate and rapid results, enabling significant genetic improvement.

A small drop of blood from a bird can yield tremendous genetic data for predicting the performance of the offspring. Disease resistance, animal health and welfare traits, which were previously hard to achieve, will now be feasible using molecular technology (Cobb focus, 2008).

A whole genome SNP association study was carried out for F_2 crosses between SUS and RES lines (Cheng et al., 2006; Pavlidis et al., 2007). The SNP analysis revealed seven chromosomal regions to be in linkage disequilibrium for ascites incidence. Two of those regions (Gga9:15 and Gga9:13) were further analyzed with multiple VNTRs on both SUS and RES lines. Gga9:13 was the most informative region from that study. The two VNTRs PHS 009 and PHS 010 from Gga9:13 had genotypes significantly associated with resistance to ascites (Chapter 2). Presence of a candidate gene angiotensin II type 1 receptor (AT_1) lends additional evidence for the contribution of this region (Smith, 2009). Testing this region on commercial populations will be needed before any possible utilization in MAS. Therefore, the objective of the current study was to use PHS 009 and PHS 010 MS markers and establish genotype to phenotype association in three commercial elite lines along with a random bred REL line. We hypothesized that genotypes present in high frequencies in the ascites selected RES line could have significant association to ascites resistant phenotype in the REL line and unrelated commercial lines.

3.2 Materials and Methods

Genetic Stock: Birds used for this study were from generation 14 of a relaxed selected (REL) research line (Pavlidis et al., 2007) and three commercial elite lines (A, B and C). Lines A and B are male elite lines selected primarily for growth related traits, whereas line C is a female elite line selected primarily for reproduction traits.

Husbandry: The REL line is maintained at the U of A poultry farm. One hatch was taken and placed in the hypobaric chamber for a standard trial. The commercial broilers were brought to

the University of Arkansas poultry farm at day 1 and standard hypobaric trials (Pavlidis et al., 2007) were conducted.

Hypobaric Chamber Trials: The hypobaric chamber is located at the University of Arkansas poultry research facility. It is 2.4 × 3.7 × 2.4 m^3 and equipped with identical custom stainless steel batteries fitted with trough feeders and nipple waterers. The environmental variables monitored included simulated altitude, ventilation and temperature. Ventilation was set to maintain a constant airflow at the rate of 17 m^3/min. Birds were warm room brooded and temperature was decreased weekly (Pavlidis et al., 2007). The chamber is designed to simulate an altitude of 9,500 ft above sea level which corresponds to an air pressure of around 500 mm of Hg. The photoperiod was 24 hrs of light. Each trial lasted 6 weeks.

Daily management tasks were conducted under partial vacuum with the use of an air lock which allowed for pressure equilization. Mortalities were recorded every 24 hrs in the first 3 weeks and every 12 hrs in the last 3 weeks. Dead birds were necropsied and examined for cause of death. Additionally, body weight, and heart characteristics (flaccidness, shape, size and RV to TV ratio) were evaluated to aid in the identification of ascitic birds. At the end of each trial, survivors were weighed, euthanized by cervical dislocation and scored as described above.

Molecular techniques: Extraction of DNA, Microsatellite primers, PCR amplification, Denaturing electrophoresis and Gel imaging methods were as described in Chapter 2.

Statistical Analysis: Allelic and Genotypic frequencies were calculated using observed allele counts for individuals genotyped within the two ascites phenotype groupings (Susceptible and Resistant) assigned based on hypobaric trials. Expected allelic and genotypic frequencies for respective phenotypes were calculated based on the allele frequency observed in the entire population. Marker associations with the ascitic phenotype were established using P-values that

represented deviation from Hardy Weinberg equilibrium determined using chi-square test (Microsoft Excel). For all the markers, combined frequencies of less than 0.05 at both the allelic and genotypic levels have been excluded from the study. Results were reported to be statistically significant at the 0.05 level.

3.3 Results

3.3.1 Line A – PHS 009

Based on product size (bp) alleles for both the markers were designated letters for convenience, consistent with the coding system described in Chapter 2 (Table 2.2). Line A is a male elite line for which PHS 009 amplified three alleles (Table 3.1). Alleles A and C were present at frequencies of 0.52 and 0.20 in the susceptible phenotype of Line A (A-Sus) with 0.45 and 0.15 in the resistant phenotype of Line-A (A-Res). The frequency of allele B was higher ($P = 0.049$) in A-Res. At the genotypic level there were six combinations present. Based on frequencies of the B allele in the RES line (Chapter 2) and within A-Res phenotypes in Line-A, the BB genotype was expected to be higher in birds exhibiting the A-Res phenotype. In fact, BB genotype frequency was 0.11 higher ($P = 0.039$) in the A-Res phenotype when compared to A-Sus. All other genotypic combinations were not phenotypically different (Table 3.1).

3.3.2 Line A - PHS 010

PHS 010 amplified four alleles for Line-A (Table 3.2). Allele frequencies for A and D were not different between A-Sus and A-Res phenotype birds. However, allele B was 0.16 higher ($P < 0.01$) in the A-Res phenotype. Out of the eight genotypic combinations, AA, AB and AD were present at an overall frequency of 0.77 and were regarded as the major genotypes of the general population. However, none of these had disproportionate representations between the

phenotypes. The BB genotype was present at a frequency of 0.08 in the tested population. It was 0.13 in the A-Res phenotype and absent in the A-Sus phenotype. The deviation of 0.13 between the phenotypes was highly significant ($P < 0.01$). The DD genotype was only 0.02 in the A-Res phenotype and 0.09 of A-Sus phenotype birds ($P = 0.025$) (Table 3.2). The D allele, as mentioned in Chapter 2, is not present in the SUS and RES research lines which were selected from Line-A (Table 2.7). This could be due to founder effect, random drift or artificial selection.

3.3.3 Line B – PHS 009

Line-B is also a male elite line. Based on the VNTR markers used in the current study Line-B has more genetic diversity when compared to the other two commercial lines. For PHS 009, five alleles amplified in this line (Table 3.3). Alleles A and C represented an overall frequency of 0.90. However, they were not unequal between the two ascites phenotypes. Allele D was 0.13 of in susceptible phenotype birds of Line-B (B-Sus) and 0.01 in all resistant phenotype birds of Line-B (B-Res). This was seen as a significant deviation ($P < 0.01$). Genotype CC was the only significant overrepresentation ($P < 0.01$) in B-Res birds. It was present at a frequency of 0.09 of all B-Res birds and absent in the B-Sus birds. CD was the opposite with 0.09 in B-Sus and absent in B-Res birds ($P < 0.01$). Additionally, genotype AD was also significantly overrepresented ($P < 0.01$) in the B-Sus, with a difference of 0.14 in frequency. Although, the D allele was not present in the homozygous state, both of its heterozygous combinations AD and CD were PHS birds. This is in spite of CC being highly resistant. It was seen that the D allele had a dominance effect in this population (Table 3.3).

3.3.4 Line B – PHS 010

PHS 010 amplified four alleles in Line-B (Table 3.4). Allele D was overrepresented in the B-Sus birds ($P = 0.036$), while E was higher ($P < 0.01$) in B-Res birds. Allele A was present

at a frequency of 0.50 in the overall population and did not differ between B-Sus and B-Res birds. Eight genotypic combinations were present in this population. Genotype DD which was significantly overrepresented in the A-Sus phenotype (Table 3.2) was similar to that in Line-B. It was present in 0.14 of all B-Sus birds and only 0.05 of B-Res birds ($P = 0.054$). All other genotypes did not differ between the phenotypes (Table 3.4).

3.3.5 Line C – PHS 009

Line-C is a female elite line that amplified with two alleles for PHS 009 (Table 3.5). Both these alleles showed significant deviations from expected counts. Allele A was present at an overall frequency of 0.70. It was present in 0.85 of all susceptible phenotype birds of Line-C (C-Sus) and 0.62 of all the resistant phenotype birds of Line-C (C-Res). The deviation of 0.22 was significant with $P = 0.036$. Allele F had the opposite effect in being overrepresented in the C-Res phenotype with a deviation of 0.22 ($P < 0.01$). Out of the three possible genotypic combinations, both homozygous genotypes differed significantly between the ascites phenotypes. While AA was overrepresented in C-Sus with a frequency difference of 0.29, FF was overrepresented in the C-Res birds with a deviation of 0.16. Both these deviations differed significantly ($P < 0.01$). The heterozygote AF was not statistically different among the two ascites phenotypes (Table 3.5).

3.3.6 Line C – PHS 010

Three alleles amplified for PHS 010 in this line (Table 3.6). Alleles A and B were present together at a frequency of 0.97. None of the alleles deviated significantly from expected counts. Out of the six genotypes, BB was significantly overrepresented ($P < 0.01$) in C-Res phenotype birds. The BB genotype was present at a frequency of 0.20 in the C-Res birds and 0.03 of C-Sus birds. Genotype AA did not differ between the two ascites phenotypes. However, the AB genotype was overrepresented ($P = 0.010$) in the C-Sus phenotype. It appears that the

heterozygous AB increases ascites susceptibility, even though the AA genotype did not show association to the ascites phenotype and BB was highly resistant (Table 3.6).

3.3.7 REL Line – PHS 009

The REL was genotyped to serve as a control population as it has been random mated for 16 generations. PHS 009 amplified three alleles for the REL line (Table 3.7). None of the alleles deviated significantly from expected counts with respect to phenotype. At the genotype level, BB was overrepresented in the resistant phenotype of the REL line (P = 0.037), whereas the BC genotype was significantly higher (P = 0.054) in susceptible phenotype of the REL line (Table 3.7).

3.3.8 REL Line – PHS 010

PHS 010 amplified three alleles for the REL line (Table 3.8). Alleles A and B constituted an overall frequency of 0.97. Both these alleles were randomly spread across both the ascites phenotypes. None of the four genotypic combinations differed between the phenotypes and hence no association could be deduced for this line for either of the phenotypes (Table 3.8).

3.4 Discussion

The poultry industry continues to make rapid strides in accomplishing genetic improvement through selection for traits of economic importance. There are elite lines in the major poultry companies that are selected for various traits based on the company philosophy (Pollock, 1999). Almost all of the permanent genetic progress happens at the elite pedigree level (Anthony, 1998). Thus, it is likely that the three commercial lines analyzed in this study have different traits and target goals for which they are selected. This is one of the main reasons behind the observed differences in allelic and genotypic frequencies between the three

commercial lines for PHS 009 and 010. Based on the frequencies in the divergently selected SUS and RES line, it was hypothesized that genotypes overrepresented in the RES line could be associated to the resistant phenotype of the commercial lines and vice versa.

It was seen that the BB homozygote of PHS 010 was present at a frequency of 0.60 in the divergently selected RES line and 0.06 in the SUS line (Table 2.6). The current study shows the BB genotype to be highly associated with resistant phenotype in Line A and C. Birds with BB genotype that developed ascites in a hypobaric model was 0% and 7% respectively in Lines A and C. This was considerably lower than the population averages of 40 % and 35 %. Interestingly, Line B which did not have birds with BB genotype had a higher overall incidence (50%) compared to Lines A and C. On the other hand, birds from Line B with DD genotype exhibited higher ascites incidence when compared to overall population averages. In Lines A and B, DD genotypes with susceptible phenotype was 78% and 72%. Line C, which did not have DD genotype birds had the lowest incidence among the three lines.

Similarly, PHS 009 homozygous BB was demonstrated to be present at a frequency of 0.58 in the RES line and 0.06 in the SUS line (Table 2.6). Eighty percent of all birds with the BB genotype in Line A were resistant to ascites. In commercial Lines B and C the CC and FF homozygote were significantly associated with resistant phenotypes. Although Line A served as the base population for the research lines SUS and RES, there are observed differences in frequency and types of alleles between the three populations. PHS 010 alleles D and E are present additionally and allele C is absent in Line-A. The difference in selection parameters between these lines can be one of the reasons attributed for the observed swing in allele frequencies. Founder effect or random drift could also be additional contributors.

Results from our hypobaric trial studies on the commercial lines are consistent with reports that indicate 50% susceptibility of commercial stocks under experimental protocols of high-challenge ascites-inducing conditions (Hadad et al., 2006). Cardiovascular measurements via blood oximeter machines along with examination of skin color indicating compromised oxygen circulation has aided in identification of ascites susceptible pedigree candidates (Katanbaf and Hardiman, 2010). Such phenotypic selection combined with good management practices has helped reduce ascites in recent years. The incidence of ascites in commercial flocks, under normal broiler rearing conditions is rarely higher than 5%. However, even 1% ascites mortality represents significant economic losses, because of the number of birds involved in the broiler industry and that it tends to affect fast-growing heavy birds, which have had a considerable amount of labor and feed invested (Lubritz and McPherson, 1994; Maxwell and Robertson, 1997). The disadvantage with the management approach is that, it compromises the efficiency of broiler production. Even though the breeding companies successfully improve the genetic potential for rapid growth, its full expression is not allowed at the farm level, to avoid morbidity and mortality of ascites susceptible birds (Druyan et al., 2007c). Once the underlying genetics is clearly elucidated, a managed reduction in growth rate would no longer be needed.

In summary, both the VNTR markers tested had significant association to the ascites phenotype in three commercial elite lines. For PHS 010, the BB and DD genotype birds were considerably resistant and susceptible respectively, when compared to other genotypes in two of the three lines tested. For PHS 009, BB, CC and FF genotypes were found to be significantly associated with resistance in the three lines respectively. These two markers have now been tested on multiple lines and shown to be consistently informative. They can be efficiently integrated in commercial marker assisted selection programs. Increasing the frequency of

resistant genotypes and concomitantly decreasing the susceptible genotypes is the approach that can be undertaken. However, due to the competitive nature of the poultry industry, it is unlikely that a company will produce an ascites resistant population at the expense of traits of economic importance. That is not to say that molecular management of these markers in tandem with traditional selection practices could result in the gradual movement of allele frequencies in a positive fashion. Evaluation of economic impact of using these markers is therefore required before industry recommendations can be made.

Table 3-1 Chi Square analysis of allelic and genotypic frequencies at the PHS 009 locus for susceptible and resistant ascites phenotype[1] of a Commercial elite line – A

Variable		ALL[2]		Susceptible			Resistant			Freq Diff	p-val[3]
		N	Freq	N	Freq	Exp	N	Freq	Exp		
Allele	A	180	0.48	79	0.52	72.38	101	0.45	107.62	0.07	0.314
	B	132	0.35	42	0.28	53.08	90	0.40	78.92	-0.12	0.049
	C	66	0.17	31	0.20	26.54	35	0.15	39.46	0.05	0.263
Genotype	AA	38	0.2	18	0.24	15.28	20	0.18	22.72	0.06	0.368
	AB	63	0.33	23	0.3	25.33	40	0.35	37.67	-0.05	0.549
	AC	41	0.22	20	0.26	16.49	21	0.19	24.51	0.08	0.263
	BB	25	0.13	5	0.07	10.05	20	0.18	14.95	-0.11	0.039
	BC	19	0.1	9	0.12	7.64	10	0.09	11.36	0.03	0.525
	CC	3	0.02	0	0	1.21	3	0.03	1.79	-0.03	NC

[1] Ascites phenotype obtained from hypobaric chamber trials

[2] Total number of birds tested from commercial Line-A

[3] p-value is NC (Not calculated) if overall allelic or genotypic frequencies <0.05

Table 3-2 Chi Square analysis of allelic and genotypic frequencies at the PHS 010 locus for susceptible and resistant ascites phenotype[1] of a Commercial elite line – A

Variable		ALL[2]		Susceptible			Resistant			Freq Diff	p-val[3]
		N	Freq	N	Freq	Exp	N	Freq	Exp		
Allele	A	193	0.51	85	0.56	77.61	108	0.48	115.39	0.08	0.278
	B	101	0.27	26	0.17	40.61	75	0.33	60.39	-0.16	< 0.01
	D	81	0.21	40	0.26	32.57	41	0.18	48.43	0.08	0.092
	E	3	0.01	1	0.01	1.21	2	0.01	1.79	0	NC
Genotype	AA	46	0.24	23	0.3	18.5	23	0.2	27.5	0.1	0.176
	AB	54	0.29	20	0.26	21.71	34	0.3	32.29	-0.04	0.634
	AD	45	0.24	19	0.25	18.1	26	0.23	26.9	0.02	0.783
	AE	2	0.01	0	0	0.8	2	0.02	1.2	-0.02	NC
	BB	15	0.08	0	0	6.03	15	0.13	8.97	-0.13	< 0.01
	BD	17	0.09	6	0.08	6.84	11	0.1	10.16	-0.02	0.679
	DD	9	0.05	7	0.09	3.62	2	0.02	5.38	0.07	0.025
	DE	1	0.01	1	0.01	0.4	0	0	0.6	0.01	NC

[1] Ascites phenotype obtained from hypobaric chamber trials

[2] Total number of birds tested from commercial Line-A

[3]p-value is NC (Not calculated) if overall allelic or genotypic frequencies <0.05

Table 3-3 Chi Square analysis of allelic and genotypic frequencies at the PHS 009 locus for susceptible and resistant ascites phenotype[1] of a Commercial elite line – B

Variable		ALL[2]		Susceptible			Resistant			Freq	
		N	Freq	N	Freq	Exp	N	Freq	Exp	Diff	p-val[3]
Allele	A	234	0.61	110	0.58	115.78	124	0.64	118.22	-0.06	0.45
	B	8	0.02	4	0.02	3.96	4	0.02	4.04	0	NC
	C	110	0.29	47	0.25	54.43	63	0.32	55.57	-0.08	0.157
	D	26	0.07	24	0.13	12.86	2	0.01	13.14	0.12	< 0.01
	E	6	0.02	5	0.03	2.97	1	0.01	3.03	0.02	NC
Genotype	AA	67	0.35	30	0.32	33.15	37	0.38	33.85	-0.07	0.441
	AB	7	0.04	3	0.03	3.46	4	0.04	3.54	-0.01	NC
	AC	76	0.4	32	0.34	37.6	44	0.45	38.4	-0.12	0.199
	AD	17	0.09	15	0.16	8.41	2	0.02	8.59	0.14	< 0.01
	BC	1	0.01	1	0.01	0.49	0	0	0.51	0.01	NC
	CC	9	0.05	0	0	4.75	9	0.09	4.85	-0.09	< 0.01
	CD	9	0.05	9	0.09	4.75	0	0	4.85	0.09	< 0.01
	CE	6	0.03	5	0.05	2.97	1	0.01	3.03	0.04	NC

[1] Ascites phenotype obtained from hypobaric chamber trials

[2] Total number of birds tested from commercial Line-B

[3] p-value is NC (Not calculated) if overall allelic or genotypic frequencies <0.05

Table 3-4 Chi Square analysis of allelic and genotypic frequencies at the PHS 010 locus for susceptible and resistant ascites phenotype[1] of a Commercial elite line – B

		ALL[2]		Susceptible			Resistant			Freq	
Variable		**N**	**Freq**	**N**	**Freq**	**Exp**	**N**	**Freq**	**Exp**	**Diff**	**p-val[3]**
Allele	A	192	0.5	97	0.51	95	95	0.49	97	0.02	0.773
	B	7	0.02	3	0.02	3.46	4	0.02	3.54	0	NC
	D	124	0.32	73	0.38	61.35	51	0.26	62.65	0.12	0.036
	E	61	0.16	17	0.09	30.18	44	0.23	30.82	-0.14	< 0.01
Genotype	AA	47	0.24	24	0.25	23.26	23	0.24	23.74	0.02	0.828
	AB	6	0.03	2	0.02	2.97	4	0.04	3.03	-0.02	NC
	AD	67	0.35	38	0.4	33.15	29	0.3	33.85	0.1	0.236
	AE	25	0.13	9	0.09	12.37	16	0.16	12.63	-0.07	0.178
	BD	1	0.01	1	0.01	0.49	0	0	0.51	0.01	NC
	DD	18	0.09	13	0.14	8.91	5	0.05	9.09	0.09	0.054
	DE	20	0.1	8	0.08	9.9	12	0.12	10.1	-0.04	0.397
	EE	8	0.04	0	0	3.96	8	0.08	4.04	-0.08	NC

[1] Ascites phenotype obtained from hypobaric chamber trials

[2] Total number of birds tested from commercial Line-B

[3]p-value is NC (Not calculated) if overall allelic or genotypic frequencies <0.05

Table 3-5 Chi Square analysis of allelic and genotypic frequencies at the PHS 009 locus for susceptible and resistant ascites phenotype[1] of a Commercial elite line – C

Variable		ALL[2]		Susceptible			Resistant			Freq Diff	p-val
		N	Freq	N	Freq	Exp	N	Freq	Exp		
Allele	A	267	0.7	110	0.85	91	157	0.62	176	0.22	0.013
	F	115	0.3	20	0.15	39	95	0.38	76	-0.22	< 0.01
Genotype	AA	96	0.5	45	0.69	33	51	0.4	63	0.29	< 0.01
	AF	75	0.39	20	0.31	27	55	0.44	49	-0.13	0.178
	FF	20	0.1	0	0	7	20	0.16	13	-0.16	< 0.01

[1] Ascites phenotype obtained from hypobaric chamber trials

[2] Total number of birds tested from commercial Line-C

Table 3-6 Chi Square analysis of allelic and genotypic frequencies at the PHS 010 locus for susceptible and resistant ascites phenotype[1] of a Commercial elite line – C

Variable		ALL[2]		Susceptible			Resistant			Freq Diff	p-val[3]
		N	Freq	N	Freq	Exp	N	Freq	Exp		
Allele	A	244	0.64	90	0.69	83.04	154	0.61	160.96	0.08	0.347
	B	126	0.33	38	0.29	42.88	88	0.35	83.12	-0.06	0.359
	E	12	0.03	2	0.02	4.08	10	0.04	7.92	-0.02	NC
Genotype	AA	84	0.44	27	0.42	28.59	57	0.45	55.41	-0.04	0.715
	AB	70	0.37	34	0.52	23.82	36	0.29	46.18	0.24	0.01
	AE	6	0.03	2	0.03	2.04	4	0.03	3.96	0	NC
	BB	27	0.14	2	0.03	9.19	25	0.20	17.81	-0.17	< 0.01
	BE	2	0.01	0	0	0.68	2	0.02	1.32	-0.02	NC
	EE	2	0.01	0	0	0.68	2	0.02	1.32	-0.02	NC

[1] Ascites phenotype obtained from hypobaric chamber trials

[2] Total number of birds tested from commercial Line-C

[3] p-value is NC (Not calculated) if overall allelic or genotypic frequencies <0.05

Table 3-7 Chi Square analysis of allelic and genotypic frequencies at the PHS 009 locus for susceptible and resistant ascites phenotype[1] of a random mated broiler research line REL

Variable		ALL[2]		Susceptible			Resistant			Freq Diff	p-val
		N	Freq	N	Freq	Exp	N	Freq	Exp		
Allele	A	69	0.4	40	0.43	40	29	0.35	29	0.08	0.396
	B	74	0.43	35	0.38	35	39	0.48	39	-0.1	0.337
	C	31	0.18	17	0.18	17	14	0.17	14	0.01	0.827
Genotype	AA	9	0.1	6	0.13	6	3	0.07	3	0.06	0.407
	AB	43	0.49	22	0.48	22	21	0.51	21	-0.03	0.822
	AC	8	0.09	6	0.13	6	2	0.05	2	0.08	0.21
	BB	10	0.11	2	0.04	2	8	0.20	8	-0.15	0.037
	BC	11	0.13	9	0.20	9	2	0.05	2	0.15	0.054
	CC	6	0.07	1	0.02	1	5	0.12	5	-0.1	0.076

[1] Ascites phenotype obtained from hypobaric chamber trials

[2] Total number of birds tested from research Line- REL

Table 3-8 Chi Square analysis of allelic and genotypic frequencies at the PHS 010 locus for susceptible and resistant ascites phenotype[1] of a random mated broiler research line REL

Variable		ALL[2]		Susceptible			Resistant			Freq Diff	p-val[3]
		N	Freq	N	Freq	Exp	N	Freq	Exp		
Allele	A	91	0.52	51	0.55	51	40	0.49	40	0.07	0.545
	B	78	0.45	39	0.42	39	39	0.48	39	-0.05	0.611
	C	5	0.03	2	0.02	2	3	0.04	3	-0.01	NC
Genotype	AA	22	0.25	13	0.28	13	9	0.22	9	0.06	0.559
	AB	47	0.54	25	0.54	25	22	0.54	22	0.01	0.965
	BB	13	0.15	6	0.13	6	7	0.17	7	-0.04	0.627
	BC	5	0.06	2	0.04	2	3	0.07	2	-0.03	0.646

[1] Ascites phenotype obtained from hypobaric chamber trials

[2] Total number of birds tested from research Line- REL

[3] p-value is NC (Not calculated) if overall allelic or genotypic frequencies <0.05

4 Phenotypic correlations of microsatellite markers associated with pulmonary hypertension syndrome in commercial broilers

4.1 Introduction

Selection could be considered part of the domestication practice as it directs and accelerates genetic changes of an animal to maximize efficiency in protein production in the form of meat and eggs. As a result, the genetic future of the modern broiler changed with concomitant advances in nutrition and management providing the early foundation for vertical integration (Shrader, 1952). In 1923, it took 16 weeks for an average broiler to reach a processing weight of 1 kilogram with 4.7 Feed Conversion Ratio (FCR). However, in 2001 birds averaged 2.2 kilograms at 6 weeks with 1.63 FCR (Havenstien, 2003; Flock et al., 2005).

Havenstein (2003) suggested that genetic selection has been responsible for up to 90 % of the changes seen in modern commercial broilers. Poultry breeders responsible for these changes, created specialized lines of chickens to rapidly adjust to consumer demands by decreasing generation interval, maximizing selection intensity and utilizing highly heritable traits (Anthony, 1998). However, long term selection on production traits has shown to have resulted in increased environmental sensitivity manifested through decreased fertility and health problems (Waaij, 2004). This occurs because of the negative effects of inbreeding as well as reallocation of resources from fitness traits to increase production.

This resource shift from health towards growth has resulted in several complications in the modern broiler. One such complication that continues to be investigated for long term solutions is ascites. As described in Chapters 1 and 2 ascites can have a major impact on production efficiency as it results in a late stage mortality or morbidity. A molecular approach to identify birds that are predisposed to ascites has been developed. Four microsatellite markers with disproportionate genotype and allele frequencies have been identified in divergently selected ascites research lines SUS and RES (Chapter 2). Two of those markers showed

genotype to ascites phenotype association in three commercial elite lines (Chapter 3). Before recommendations for incorporating these markers into modern selection programs are made, estimations of economic impact are needed. Studies about the correlation between ascites resistance and traits of economic importance have yielded contrasting results. Druyan (2008) reported an 84.4 % difference in ascites incidence between two lines divergently selected for ascites using a cold stress model. However, the growth rate between the lines differed only by 5% indicating little genetic correlation. Contrastingly, it was shown that long term divergent selection for ascites incidence resulted in the RES line as approximately 163 g lighter than the SUS line at d 42 (Pavlidis et al., 2007). In addition, Pectoralis weights for males measured at d 42 was greater for the SUS line than the RES line males (Pavlidis, 2003).

The only improvement with respect to traits of economic importance was the fact that the RES line had significantly better Feed Conversion Ratio (FCR) compared to the SUS line (Pavlidis, 2003). However, these measurements were taken on the research lines when they were raised in pens as groups. Whether, individual FCR measurements on these lines would have resulted in similar conclusions is yet to be determined. Nevertheless, with current emphasis on rapid growth, high muscle yield and lowered FCR it seems unlikely that the competitive poultry breeding companies will invest to produce an ascites resistant broiler at the cost of economically important traits (Balog, 2003). For this reason, irrespective of the significance of the method used to increase ascites resistance, simultaneous estimation of the economic impact is warranted. Therefore, the objectives of the current study were to elucidate the quantitative effects of selection using genotypes significantly associated with resistance to ascites, on economically important traits. Based on findings from lines selected for ascites susceptibility (Pavlidis et al.,

2007), we hypothesized that genotypes associated with resistance to ascites would be negatively correlated with traits of economic importance.

4.2 Materials and Methods

Genetic Stock: Birds used for this study were from a commercial elite line A. Line A is a male line selected primarily for growth related traits (See chapter 3 for allele and genotypic frequencies).

Husbandry: A six week trial was conducted to evaluate potential correlations between resistant genotypes and economically important traits. At hatch all chicks (n=1080) were wing banded and vaccinated against Marek's disease. They were then randomly assigned to litter floor pens and maintained under typical broiler breeder management conditions throughout the grow-out period. All birds were provided with ad libitum access to water and a commercial starter feed meeting NRC requirements for the first 3 weeks and commercial grower from week 4 onwards. Photoperiod consisted of 24 h light for the first 3 days followed by 23 h of light and 1h of dark for the remainder of the grow-out period.

Data Collection: Body weight was evaluated for all the birds that were alive on d 35 (n=975). After the weights were obtained, 400 birds from each sex were moved to feed conversion stations where the Feed Conversion Ratio (FCR) was determined. Individual FCR was monitored from d 35 to d 42 and expressed as a ratio of body weight gained to feed consumed. Feed was withdrawn the night before processing (d 42). Water was provided ad libitum even during the feed withdrawal period. On the day of processing (d 43) the birds were placed in coops and moved to the University of Arkansas processing plant. Live dock weight was measured for all the birds (n=684) that were alive on d 43. The birds were then processed under typical commercial conditions. Evisceration was done by hand and WOG (Without Giblets) weight was

obtained to calculate relative parts yield. Carcasses were subsequently deboned and individual part weights for pectoralis major, pectoralis minor, wings, drums and thighs were obtained. Relative percentage of processing parameters and organ variables were expressed as a percentage of live dock weight.

Molecular techniques: Extraction of DNA, Microsatellite primers and PCR amplification methods were as previously described in Chapter 2.

Genotyping: PCR products in 96 well plates were shipped to the DNA core lab at the University of Missouri-Columbia. Using capillary electrophoresis, samples were genotyped on an ABI 3730 DNA Analyzer. An internal ladder (600 Liz) was incorporated with each sample for accurate peak size determination. Allele calls were made using Gene Marker software (Soft genetics LLC, PA).

Statistical Analysis: Absolute weight data were log-transformed and relative weights were arc sine square roots transformed prior to being subjected to ANOVA, using the PROC GLM procedure of SAS software (SAS Institute, Cary, NC). The model included the fixed effects of genotype, source, and sex. No random effects were used. Means were compared using Duncan's MRT and declared to be different at $P < 0.05$.

4.3 Results and Discussion

Marker assisted selection will likely be applied for traits that have low heritabilities, are difficult to measure and difficult to justify being selected for in a traditional breeding program. Two genetic markers known to be associated with ascites susceptibility have been identified (Chapters 2 and 3). These markers however could be ineffective if they have a significant negative impact on traits of economic importance. Divergent selection for ascites incidence has

resulted in changes in growth, production and processing parameters whereby the SUS line showed better growth and production than the RES line (Pavlidis, 2003). This evidence suggested that genotypes associated with resistance to ascites could be negatively correlated to traits of economic importance. For both the tested markers, the genotypic means did not differ for bw35, FCR, dock weight, WOG weight, absolute and relative fat, thigh, drum and total leg weights (Tables 4.1 and 4.2). For PHS 009 genotypic differences approached significance at p= 0.06. The extreme genotypes were AA and CC with a feed conversion of 1.729 and 1.843 respectively. The AA genotype was found to be the most ascites susceptible genotype, while CC was the most resistant. The BB genotype was also relatively resistant and did not differ for FCR from AA.

Fillet (*Pectoralis* Major) and tender (*Pectoralis* Minor) weights were measured individually and added together to estimate total breast. For PHS 009 all of these three measurements were different among the genotypes (Table 4.2). BB had the highest fillet and total breast at 529 g and 638 g, respectively. This was 42 g and 43 g higher when compared to the lowest mean weights of CC. Birds with the CC genotype were different from the rest of the genotypes for both fillet and total breast weights. As shown in Table 4.3, the PHS 009 BB genotypes had a high percentage breast at 27.60 and the highest percentage leg at 26.42. For both these measurements, the CC genotype had the lowest values with 26.04 and 24.77 respectively and was shown to be different based on Duncan's grouping (Table 4.3).

Based on genetic improvement trends, Pollock (1999) predicted that BW 35 would be around 2000 grams at 2017. Interestingly, the birds used in the current study (2011) averaged 2305 g on d 35. High selection pressures have been a primary factor contributing to this better than expected genetic progress. From PHS 010, the second (AD) and third (AB) ranked

genotypes for mean BW 35 were present at a frequency of 0.5 in the tested population. It appears that selection for high body weight has favored these genotypes. Feed Conversion Ratio (FCR) is another trait for which direct selection pressure has been applied over the years. It will continue to be emphasized in selection programs as grain prices continue to rise. The low frequency of the PHS DD genotype (10%) which had the poorest feed conversion (1.785) shows that birds with this genotype are not being favored for selection. Fortunately, the PHS 010 DD genotype birds also had the highest ascites incidence (78%) and therefore could be eliminated without a negative impact on FCR. For PHS 009 AA and BC with the highest ascites incidence of 47% had the lowest FCRs at 1.729 and 1.733 respectively. Contrastingly, CC and BB with the lowest ascites incidence of 0% and 20% had the highest FCRs of 1.843 and 1.759, respectively.

Although not all of carcass without giblets (WOG) weight is sellable meat, it serves as a good estimate of a bird's usable meat. The birds did not differ for WOG weights for both markers and ranking trends were consistent with what was found for BW35 (Table 4.1). It has been shown over the years that selection for better feed conversion has indirectly reduced fat yield (Hardiman, personal communication). The PHS 010 BB genotype with numerically the highest BW35 also had the highest fat yield suggesting that a larger portion of its body weight is allocated to fat, which is not directly marketable. PHS 009 CC also had high fat content. Interestingly, both these genotype birds showed 0% ascites incidence. The PHS 009 CC genotype was ranked numerically the lowest for breast and leg measurements. This combination of low muscle and high fat contributes to the low commercial value of CC genotypes in this population. For this reason, it is present in a low frequency (~ 5%) in this population despite exhibiting 0% ascites incidence.

The definition of yield with respect to the poultry industry is the proportion of salable product recovered from the live bird after processing. In the United States, breast meat is about three times more valuable than other meat from wings and legs (Pollock, 1999). Thus, most poultry companies have a high emphasis on absolute and relative breast yield. The selected birds are therefore designed to reallocate greater portions of their energy for breast mass accumulation. With this comes increased oxygen demand, which, when not met, triggers a cascade of events resulting in ascites. Even as the PHS 010 genotypes did not differ for breast yield, the genotype (AA) with numerically the highest yield had a relatively high ascites incidence, affirming the correlation. In PHS 009 the CC genotype means were different than the other genotypes for both fillet ($p<0.01$) and total breast ($p<0.01$) Interestingly, BB genotypes had the numerically highest fillet, total breast, thigh, drum, total leg, percentage breast and percentage leg means among all the PHS 009 genotypes. This is in addition to a low ascites incidence. Therefore, accumulation of BB could not only reduce overall ascites incidence of this population, but also lead to an increase in yields.

The poultry industry is already using Marker Assisted Selection (MAS) to some extent, in their breeding programs to increase the frequency of favorable alleles or to eliminate unfavorable alleles (Siegel, 2006). Based on the results from the hypobaric trials and the processing trial, a hypothetical MAS was tabulated to better understand the gains or losses of each allele (Tables 4.4 and 4.5). Although no statistical analysis was carried out on these simulations, the tables contain good numerical estimates of the impact of allele elimination. For example, if the D allele of PHS 010 were eliminated from the population, the ascites percentage for the tested population would decrease from 40% to 37%. Relative breast and leg could increase by 0.1% and FCR would improve by .003 units.

Similarly if the A allele from PHS 009 is eliminated, ascites incidence would decrease to from 40% to 32% (Tables 4.4 and 4.5). However, relative breast meat would drop by 0.18% and FCR could worsen by 0.011 units. Although, these numbers look insignificant in the tested population, they could be highly significant with the pyramid setup where the superior genetics of one male or female is passed on to millions of broilers. These results show mixed patterns for the two markers. However, this is based on a one time elimination of certain genotypes. In reality selection against negative genotypes could be done in tandem with traditional selection in order to manage the apparent losses due to detrimental correlations of markers with traits of economic importance. Nevertheless, exact prediction of broiler performance is complicated due to several reasons. Genetic background, heterosis, mutations, random genetic drift, and gene by environment interactions, are a few factors that play key roles in the performance of a broiler. As MAS gets more and more integrated into poultry breeding schemes, extensive implementation would need more standardization.

Table 4-1 Genotypic differences in growth, production and processing trait means[1] for commercial Line-A

Marker[2]	Genotype	Ascites %[3]	N	bw35 (g)	FCR[4]	Dock (g)	WOG (g)[5]	Fat (g)[6]
PHS 009	AA	47	116	2278 ± 23	1.729 ± 0.014	2544 ± 24	1848 ± 17	38.29 ± 1.00
	AB	37	220	2326 ± 17	1.759 ± 0.011	2589 ± 18	1876 ± 13	40.87 ± 0.83
	AC	42	139	2297 ± 19	1.740 ± 0.013	2567 ± 21	1853 ± 16	38.51 ± 0.92
	BB	20	87	2317 ± 24	1.759 ± 0.020	2621 ± 28	1898 ± 21	40.94 ± 1.21
	BC	47	55	2293 ± 31	1.733 ± 0.022	2546 ± 34	1840 ± 25	41.94 ± 1.44
	CC	0	21	2288 ± 55	1.843 ± 0.039	2492 ± 61	1787 ± 46	44.38 ± 2.50
PHS 010	AA	50	146	2300 ± 21	1.759 ± 0.015	2572 ± 22	1864 ± 16	38.59 ± 0.94
	AB	37	150	2319 ± 20	1.741 ± 0.012	2593 ± 22	1882 ± 16	41.07 ± 1.05
	AD	42	167	2328 ± 18	1.758 ± 0.013	2581 ± 20	1867 ± 15	39.44 ± 0.88
	BB	0	42	2322 ± 37	1.732 ± 0.031	2611 ± 38	1885 ± 29	44.21 ± 1.76
	BD	35	66	2251 ± 28	1.730 ± 0.019	2523 ± 34	1824 ± 26	40.03 ± 1.50
	DD	78	57	2275 ± 34	1.785 ± 0.021	2539 ± 34	1834 ± 26	39.29 ± 1.61

[1]Means ± SE, [2]Microsatellite markers from Gga9:13 loci, [3]Percentage mortality based on previous hypobaric chamber ascites trials for the same line, [4]Ratio of feed consumed over weight gained between d35 and d42,

[5]Carcass without giblets, [6]Abdominal Fat + Gizzard Fat

Table 4-2 Genotypic differences in absolute breast and leg weight means[1] for commercial Line-A

Marker[2]	Genotype	Ascites %[3]	N	Fillet (g)*	Tender (g)	Breast (g)[4*]	Thigh (g)	Drum (g)	Leg (g)[5]
PHS 009	AA	47	116	520 ± 5[a]	108 ± 1	628 ± 6[a]	367 ± 4	221 ± 3	588 ± 7
	AB	37	220	527 ± 4[a]	110 ± 1	637 ± 4[a]	370 ± 3	226 ± 2	596 ± 5
	AC	42	139	516 ± 5[a]	105 ± 1	622 ± 6[a]	367 ± 4	221 ± 3	596 ± 6
	BB	20	87	529 ± 6[a]	108 ± 1	638 ± 7[a]	381 ± 5	231 ± 4	612 ± 9
	BC	47	55	510 ± 7[a]	106 ± 2	617 ± 8[a]	368 ± 6	222 ± 4	589 ± 10
	CC	0	21	487 ± 15[b]	108 ± 3	595 ± 18[b]	354 ± 10	213 ± 7	567 ± 17
PHS 010	AA	50	146	529 ± 5	108 ± 1	637 ± 6	369 ± 4	225± 3	594 ± 6
	AB	37	150	522 ± 5	109 ± 1	631 ± 5	373 ± 4	230 ± 3	603 ± 7
	AD	42	167	521 ± 5	107 ± 1	628 ± 5	373 ± 4	226 ± 3	598 ± 6
	BB	0	42	523 ± 9	108 ± 2	630 ± 10	377 ± 7	228 ± 5	605 ± 12
	BD	35	66	511 ± 8	107 ± 2	618 ± 10	362 ± 7	219 ± 4	581 ± 10
	DD	78	57	514 ± 7	108 ± 1	622 ± 8	363 ± 7	220 ± 5	582 ± 11

[1]Means ± SE, [2]Microsatellite markers from Gga9:13 loci, [3]Percentage mortality based on previous hypobaric chamber ascites trials for the same line, [4]Pectoralis Major + Pectoralis Minor, [5]Thigh + Drum, *Means within the same column and with no common superscript differ significantly ($p<0.05$)

Table 4-3 Genotypic differences in relative fat, breast and leg weight means[1] for commercial Line-A

Marker[2]	Genotype	Ascites %[3]	N	Fat%[4]	Breast%[5*]	Leg%[6*]
PHS 009	AA	47	116	1.69 ± 0.04	27.69 ± 0.21[a]	25.84 ± 0.19[a]
	AB	37	220	1.76 ± 0.03	27.48 ± 0.16[a]	25.61 ± 0.13[a]
	AC	42	139	1.69 ± 0.04	27.11 ± 0.19[a]	25.97 ± 0.19[a]
	BB	20	87	1.77 ± 0.05	27.60 ± 0.22[a]	26.42 ± 0.21[a]
	BC	47	55	1.83 ± .0.06	26.98 ± 0.30[a]	25.70 ± 0.27[a]
	CC	0	21	1.96 ± 0.12	26.04 ± 0.54[b]	24.77 ± 0.39[b]
PHS 010	AA	50	146	1.68 ± 0.04	27.77 ± 0.20	25.84 ± 0.18
	AB	37	150	1.77 ± 0.04	27.28 ± .019	26.01 ± 0.17
	AD	42	167	1.70 ± 0.03	27.07 ± 0.19	25.67 ± 0.16
	BB	0	42	1.92 ± 0.08	27.23 ± 0.37	26.06 ± 0.30
	BD	35	66	1.79 ± 0.07	27.49 ± 0.29	25.75 ± 0.28
	DD	78	57	1.73 ± 0.07	27.49 ± 0.32	25.58 ± 0.29

[1]Means ± SE, [2]Microsatellite markers from Gga9:13 loci, [3]Percentage mortality based on previous hypobaric chamber ascites trials for the same line, [4]((Abdominal Fat + Gizzard Fat)/ Dock Weight)*100, [5]((Pectoralis Major + Pectoralis Minor)/ Dock Weight) *100, [6]((Thigh + Leg)/ Dock Weight)*100, [*]Means within the same column and with no common superscript differ significantly ($p<0.05$)

Table 4-4 Projected growth and processing yield values[1] based of hypothetical elimination of alleles from the population

Marker[2]	Deleted allele[3]	Ascites %[4]	bw35[5]	FCR[5]	Dock[5]	WOG[5]
PHS 009	A	32	2305 ± 18	1.761 ± 0.014	2579 ± 21	1864 ± 16
	B	48	2288 ± 14	1.743 ± 0.009	2551 ± 15	1846 ± 11
	C	37	2311 ± 12	1.751 ± 0.008	2583 ± 13	1873 ± 9
PHS 010	A	33	2274 ± 18	1.743 ± 0.012	2547 ± 19	1839 ± 14
	B	49	2308 ± 12	1.758 ± 0.008	2571 ± 13	1860 ± 10
	D	37	2313 ± 13	1.747 ± 0.009	2587 ± 14	1875 ± 10
Actual overall population[6]		40	2305 ± 9	1.750 ± 0.006	2573 ± 10	1863 ± 8

[1]Data not subjected to statistical analysis, [2]Microsatellite markers from Gga9:13 loci

[3]Hypothetical elimination of the allele in both homozygous and heterozygous forms

[4]Projected ascites percentages, [5]Projected means ± SE

[6]Actual values for the tested population

Table 4-5 Projected absolute and relative yield values[1] based of hypothetical elimination of alleles from the population

			Absolute weights (g) [2]			Relative weights %[2, 3]		
Marker[4]	**Deleted allele[5]**	**Ascites %[6]**	**Fat**	**Breast**	**Leg**	**Fat%**	**Breast%**	**Leg%**
PHS 009	A	32	41.72 ± 0.87	625 ± 5	598 ± 6	1.81 ± 0.03	27.19 ± 0.17	25.84 ± 0.01
	B	48	38.86 ± 0.66	622 ± 4	590 ± 5	1.71 ± 0.03	27.27 ± 0.14	25.82 ± 0.13
	C	37	40.17 ± 0.57	635 ± 3	597 ± 4	1.74 ± 0.02	27.56 ± 0.11	25.84 ± 0.09
PHS 010	A	33	40.96 ± 0.85	621 ± 5	587 ± 6	1.81 ± 0.03	27.37 ± 0.17	25.77 ± 0.15
	B	49	39.38 ± 0.56	630 ± 3	594 ± 4	1.71 ± 0.02	27.38 ± 0.11	25.74 ± 0.10
	D	37	40.53 ± 0.62	634 ± 3	599 ± 4	1.76 ± 0.02	27.47 ± 0.12	25.92 ± 0.10
Actual overall population[7]		40	40.10 ± 0.45	629 ± 3	595 ± 3	1.75 ± 0.02	27.37 ± 0.13	25.82 ± 0.10

[1]Data not subjected to statistical analysis, [2]Projected means ± SE

[3]Percentages relative to dock weight, [4]Microsatellite markers from Gga9:13 loci

[5]Hypothetical elimination of the allele in both homozygous and heterozygous forms

[6]Projected ascites percentages

[7]Actual values for the tested population

5 General Synthesis

The current project was funded by Arkansas Biosciences Institute and carried out as a partial requirement for the completion of a doctoral degree and as a part of an ongoing collaborative research program at the University of Arkansas. The two long term goals of the collaborators are to more clearly elucidate the underlying genetics of ascites syndrome and establish *Gallus gallus domesticus* as an effective animal model for studying human pulmonary hypertension. Although the ascites syndrome in chickens has been investigated for years, it continues to inflict financial losses to the world poultry industry. Efforts to curb the incidence of ascites are typically designed to slow early growth. This limits the birds' ability to show its true genetic potential and impacts later yields. Research teams have thus embarked on projects aiming for a long term solution to ascites. A genome wide SNP scan of birds with different ascites phenotype revealed 7 chromosomal regions to be strongly associated with ascites. The primary objective of this project was to reaffirm two of those loci (Gga9:13 and Gga9:15) to have substantial contributions in the underlying genetics of ascites syndrome. The approach undertaken was to identify VNTR markers linked to Gga9:13 and Gga9:15 and analyze for polymorphisms.

Results of Chapter 2 showed that the most descriptive among the two tested regions was Gga9:13. All further evaluations were therefore done using VNTR markers at Gga9:13. The two VNTR markers on Gga9:13 named PHS 009 and 010 were tested for polymorphisms and association to phenotype in multiple populations. Divergent selection for ascites incidence has changed the overall ascites incidence in two populations originating from common ancestors. Screening these two divergent populations for disproportionate allele and genotype frequencies seemed to be the best approach. It was shown that both PHS 009 and 010 were highly skewed in

allele and genotype frequencies between the susceptible and resistant ascites lines. The data clearly indicates that allele frequencies have shifted in response to the corresponding divergent selection and that alleles frequent in the ascites resistant line should contribute to resistance. Even as the difference in prominence of alleles among the two lines was very clear, the argument that genetic background influences a genotype's effect on phenotype had to be accounted for. Therefore, testing the markers on unrelated commercial populations that had not been selected for ascites was carried out as the subsequent step. Chapter 3 showed that for 3 commercial lines tested, there were genotypes associated with ascites phenotype. Genotypes prevalent in the resistant line favored a resistant phenotype under ascites inducing conditions in commercial lines. Accumulating resistant genotypes in commercial elite populations should increase the overall ascites resistance.

However, the highly competitive nature of the poultry industry requires negligible compromise on traits of economic importance during production of a disease resistant broiler. On those lines, Chapter 4 evaluated the correlations between genotype and processing parameters in a commercial line. PHS 010 genotype means did not differ for any of the traits analyzed. PHS 009 means differed for fillet, breast, breast percentage and leg percentages. Accumulation of the most resistant PHS 009 genotype could result in a loss of 1.3% breast meat yield. However selecting BB which had a low ascites incidence of 20% will actually improve breast yield by 0.3%. Overall, the results of these studies provide strong evidence about the contribution of Gga9:13 towards ascites syndrome. Additionally, the data showed that there might be economic losses in producing an ascites resistant broiler. Therefore, poultry breeding companies will have to find the right balances if Marker Assisted Selection for this reason is adopted on a large scale in the future.

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